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II INTERNATIONAL THEORETICAL COURSE ON VIRAL HEPATITIS AND THE HUMAN HOST

March 12nd to 15th, 2012
Rebouças Convention Center
São Paulo - SP, Brazil

MEDICINA
USP

Department of Gastroenterology
University of São Paulo School of Medicine
São Paulo - SP – Brazil
2012

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Av. Dr. Enéas de Carvalho Aguiar, 470
05403-000 São Paulo, SP - Brazil
Phone/Fax: 55.11.3062.2174; 55.11.3061-7005
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COURSE ORGANIZERS

Dr. João Renato Rebello Pinho
Department of Gastroenterology
School of Medicine
University of São Paulo, São Paulo

Prof. Dr. Flair José Carrilho
Department of Gastroenterology
School of Medicine
University of São Paulo, São Paulo

Dr.Suzane Kioko Ono
Department of Gastroenterology
School of Medicine
University of São Paulo, São Paulo

Dr. Eduardo Luíz Rachid Cançado
Department of Gastroenterology
School of Medicine
University of São Paulo, São Paulo

Prof.Dr. Alberto José da Silva Duarte
Instituto Adolfo Lutz
São Paulo

Dr. Mónica Viviana Alvarado-Mora
Department of Gastroenterology
School of Medicine
University of São Paulo, São Paulo

Dr. Maria Cássia J Mendes Corrêa
Infectious Diseases Research Unit, School of Medicine - ABC Foundation
Department of Infectious Diseases
School of Medicine
University of São Paulo, São Paulo

Dr. Esper Georges Kállas
Departament of Clinical Medicine
School of Medicine
University of São Paulo, São Paulo

Dr. Mário Guimarães Pessôa
Department of Gastroenterology
School of Medicine
University of São Paulo, São Paulo

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Prezados Amigos,

É uma grande honra apresentar o programa do **II International Theoretical Course on VIRAL HEPATITIS AND THE HUMAN HOST**. O curso será realizado de 12 a 15 de março de 2012, no Centro de Convenções Rebouças, em São Paulo, Brasil.

Durante este evento, pretendemos discutir os pontos mais importantes sobre:

1) Epidemiologia e evolução dos vírus da Hepatite B (HBV), Hepatite C (HCV) e da hepatite Delta (HDV);
2) Abordagens filogenéticos para estudar os vírus das hepatites e a população humana infectada pelos mesmos;
3) Avanços no tratamento antiviral da infecção pelos vírus das Hepatites B, C e Delta em pacientes monoinfectados e co-infectados com HIV;
4) Aplicação dos novos métodos de sequenciamento e sua aplicação para o estudo das hepatites;
5) Imunogenética e os vírus das hepatites.

Para este curso, convidamos palestrantes de África do Sul, Argentina, Austrália, Brasil, Canadá, Estados Unidos da América, Itália, México e Nova Zelândia. Esta será uma excelente oportunidade para estabelecer contatos entre os pesquisadores e estudantes que irão participar do evento.

Estavamos esperando por você, seja bem-vindo!

João Renato Rebello Pinho
Maria Cássia J Mendes Corrêa
Esper Georges Kallás
Eduardo Luís Rachid Cançado
Alberto José da Silva Duarte
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SCIENTIFIC PROGRAM

Monday - 12/03/2012

08:15 - 08:30	Welcome and Introduction - *Organizing Committee*
08:30 - 09:00	Chair: *Stephen Locarnini*
	Virology of HBV and HCV - *João Renato Rebello Pinho*
09:00 - 09:15	Discussion
09:15 - 09:45	Chair: *Carla Osiowy*
	Hepatitis B Virus – Tracing Human Migrations In And Out Of Africa - *Anna Kramvis*
09:45 - 10:00	Discussion
10:00 - 10:15	Coffee Break
10:15 - 10:45	Chair: *Flair José Carrilho*
	Prevalence and epidemiological patterns of Hepatitis B and C infection in Brazil - *Leila Beltrão Pereira*
10:45 - 11:00	Discussion
11:00 - 11:30	Chair: *Alberto José da Silva Duarte*
	Epidemiological update of Hepatitis B, C and Delta in Latin America - *Mónica Viviana Alvarado Mora*
11:30 - 11:45	Discussion
11:45 - 14:00	Lunch
14:00 - 14:30	Posters session
14:30 - 15:00	Chair: *Arturo Panduro*
	Demographic distribution of viral hepatitis in the Northern Hemisphere - *Carla Osiowy*
15:00 - 15:15	Discussion
15:15 - 15:45	Chair: *João Renato Rebello Pinho*
	Phylogeography of human hepatitis viruses - *Remco Bouckaert*
15:45 - 16:00	Discussion
16:00 - 16:15	Coffee Break
16:15 - 16:45	Chair: *Maria Cássia Jacintho Mendes Corrêa*
	Epidemic history of Hepatitis C virus in Brazil - *Elisabeth Lampe*
16:45 - 17:00	Discussion
17:00 - 17:30	Chair: *Anna Kramvis*
	Hepatitis Delta Virus: 35 years later - *Mario Rizzetto*
17:30 - 17:45	Discussion
17:45	Close

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Tuesday - 13/03/2012

08:30 - 09:00	Chair: *Maria Cássia Jacintho Mendes Corrêa*
	HBV and HCV: Predictive factors of Disease progression - *Maria Lúcia Cardoso Gomes Ferraz*
09:00 - 09:15	Discussion
09:15 - 09:45	Chair: *Tânia Reuter*
	Genetic markers associated with different evolutions of hepatitis C Infection - *André Lyra*
09:45 - 10:00	Discussion
10:00 - 10:15	Coffee Break
10:15 - 10:45	Chair: *Deborah Taylor*
	Immunogenetics of Hepatitis C - *João Renato Rebello Pinho*
10:45 - 11:00	Discussion
11:00 - 11:30	Chair: *Stephen Locarnini*
	HLA genes and hepatitis B infection - *Arman Bashirova*
11:30 - 11:45	Discussion
11:45 - 14:00	Lunch
14:00 - 14:30	Posters session
14:30 - 15:00	Chair: *André Lyra*
	Clinical and epidemiological aspects of hepatocellular carcinoma in Brazil - *Flair José Carrilho*
15:00 - 15:15	Discussion
15:15 - 15:45	Chair: *Maria Lúcia Gomes Ferraz*
	Viral Hepatitis and Hepatocellular Carcinoma - *Carla Osiowy*
15:45 - 16:00	Discussion
16:00 - 16:15	Coffee Break
16:15 - 16:45	Chair: *Anna Kramvis*
	Clinical applications of Molecular Sequencing of Hepatitis - *Lilly Yuen*
16:45 - 17:00	Discussion
17:00 - 17:30	Chair: *Ana de Lourdes Candolo Martinelli*
	Hepatitis B: Vaccine Development and Global Control - *Marta Heloisa Lopes*
17:30 - 17:45	Discussion
17:45	Close

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Wednesday - 14/03/2012

08:30 - 09:00	Chair: *Anna Kramvis*
	Update on the treatment of Hepatitis delta - *Mario Rizzetto*
09:00 - 09:15	Discussion
09:15 - 09:45	Chair: *André Lyra*
	Current Treatment of Hepatitis C with Interferons - *Tânia Reuter*
09:45 - 10:00	Discussion
10:00 - 10:15	Coffee Break
10:15 - 10:45	Chair: *Maria Cássia Jacintho Mendes Corrêa*
	Current concepts in the management and treatment of hepatitis B in HIV - Infected Patients - *Marina Nuñez*
10:45 - 11:00	Discussion
11:00 - 11:30	Chair: *Paulo Abrão Ferreira*
	Viral Hepatitis and HIV: Update on Management in Brazil - *Maria Cássia Jacintho Mendes Corrêa*
11:30 - 11:45	Discussion
11:45 - 14:00	Lunch
14:00 - 14:30	Posters session
14:30 - 15:00	Chair: *Maria Cássia Jacintho Mendes Corrêa*
	New paradigms in the management of HIV and hepatitis C virus co-infection - *Marina Nuñez*
15:00 - 15:15	Discussion
15:15 - 15:45	Chair: *Mario Guimarães Pessôa*
	Guidelines for the Treatment of Viral Hepatitis B and C in Brazil - *Suzane Kioko Ono*
15:45 - 16:00	Discussion
16:00 - 16:15	Coffee Break
16:15 - 16:45	Chair: *Stephen Locarnini*
	Implementing the New HCV Treatment: Direct-Acting Antiviral (DAA) Therapy - *Mario Guimarães Pessôa*
16:45 - 17:00	Discussion
17:00 - 17:30	Chair: *Marina Nuñez*
	Hepatitis B treatment in 2012 - *Suzane Kioko Ono*
17:30 - 17:45	Discussion
17:45	Close

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Thursday - 15/03/2012

08:00 - 08:30	Chair: *Remco Bouckaert*
	Origin of HBV and their arrival in the Americas - *Nelson Jurandi Rosa Fagundes*
08:30 - 08:45	Discussion
08:45 - 09:00	Chair: *Stephen Locarnini*
	Bayesian approaches for the study of human populations - *Remco Bouckaert*
09:00 - 09:15	Discussion
09:15 - 09:30	Coffee Break
09:30 - 10:00	Chair: *João Renato Rebello Pinho*
	Bioinformatics methods for the analysis of hepatitis virus - *Stephen Locarnini*
10:00 - 10:15	Discussion
10:15 - 10:45	Chair: Mario Rizzetto
	HBV therapy: is drug resistance inevitable? - *Mário Guimarães Pessoa*
10:45 - 11:00	Discussion
11:00 - 11:30	Chair: *Arman Bashirova*
	Persistent growth of a human plasma-derived hepatitis C isolates in cell culture - *Deborah Taylor*
11:30 - 11:45	Discussion
11:45 - 13:00	Lunch
13:00 - 13:30	Chair: *Deborah Taylor*
	Variations in genes of innate immunity in hepatitis C infection - *Arman Bashirova*
13:30 - 13:45	Discussion
13:45 - 14:15	Chair: *Esper Georges Kallás*
	Innate immunity and hepatitis C virus: eluding the host cell defense - *Deborah Taylor*
14:15 - 14:30	Discussion
	WORKSHOP OF HEPATITIS B GENOTYPE F
	Chair: *João Renato Rebello Pinho and Stephen Locarnini*
14:30 - 15:00	**Distribution of HBV/F in South America** - *Mónica Viviana Alvarado Mora*
15:00 - 15:30	**HBV genotype F Arctic Circle indigenous HBV data** - *Carla Osiowy*
15:30 - 16:00	**Distribution of HBV/F in Central and North América** - *Arturo Panduro*
16:00 - 16:30	**HBV genotype F treatment** - *Sebastian Marciano*
16:30 - 17:00	Discussion
17:00 - 17:15	Concluding Remark

SUMMARY OF THE LECTURES

L1 - HEPATITIS B (HBV) AND HEPATITIS C (HCV) VIRUSES

João Renato Rebello Pinho

Laboratório de Hepatologia e Gastroenterologia Tropical "João Alves de Queiroz e Castorina Bittencourt Alves", Instituto de Medicina Tropical, Departamento de Gastroenterologia da Faculdade de Medicina da Universidade de São Paulo.

Hepatitis B (HBV) and *C (HCV) viruses* are major human pathogens and are involved with acute and chronic hepatitis up to diseases with severe clinical pictures such as liver cirrhosis and hepatocellular carcinoma. Although the clinical pictures of the diseases developed after infection with each one of them share many common features, their etiological agents are strikingly different.

HBV is classified in the *Hepadnaviridae* family, that comprehends other similar agents that have been previously identified in birds (*Avihepadnavirus)* - found in ducks, herons, storks, cranes; and in mammals (*Orthohepadnavirus)* –ten human *HBV* genotypes (A- J), closely related viruses found in chimpanzee, orangutan, gibbon and wooly monkey, as well as in the woodchuck and in arctic and ground squirrel. Some *HBV* genotypes are further divided in subgenotypes: A1–A6 in *HBV*/A, B1–B9 in *HBV*/B, C1–C16 in *HBV*/C, D1–D7 in *HBV*/D, and F1–F4 in *HBV*/F (some subgenotypes further divided in F1a, F1B, F2a and F2b).

HCV is classified in the *Flaviviridae* family that comprehends three different viral genuses (*Flavivirus, Pestivirus* and *Hepacivirus). HCV* is the prototype of this last genus and it is further classified in different genotypes 1 to 6, that are further divided in subtypes 1a to 1g; 2a to 2f, 2i to 2m, 3a, 3b, 3g, 3k; 4a, 4c to 4f,4k , 4l, 4n, 4o, 4r; 5a; 6a to 6q and 6v. *GB virus B* was identified in captive tamarins and is most closely related to *HCV* and is currently classified in the same genus. In this genus, we found also the recently discovered *Canine Hepacivirus (CHV)*, identified in liver and respiratory samples from affected dogs in five respiratory disease outbreaks in four shelters from the USA as the most genetically similar animal virus homolog of *HCV* that may provide new insights into the origin and evolution of *HCV* and into a model system with which to probe the pathogenesis, prevention, and treatment of diseases caused by *Hepacivirus* infection. Other closely related viruses; such as *GBV-A* (isolated from several New World monkeys), *GBV-C* (from humans and chimpanzees) and *GBV-D* (from bats), are nowadays classified in *Pegivirus* genus.

Recombinants viruses are more frequent for *HBV*. The most striking example of intergenotype recombination is represented by the B2 and B3 subgenotypes, as they are composed of a genotype B backbone mixed with the core gene and sometimes the core promoter of genotype C. Recombinant genomes involving other combinations of genotypes have been described, including recombination between human genotypes and primate sequences, as the genotype C/gibbon recombinants, that had previously been classified as subtype C4. A large-scale data and automated phylogenetic analysis detected a total of 24 phylogenetically independent potential recombinants (different genotype combinations or distinct breakpoints). Instances of intergenotype recombination were observed in all human and ape HBV variants, providing evidence for the occurrence of past, extensive recombination events in the evolutionary history of the currently classified genotypes of HBV and potential changes in its global epidemiology and associations with human disease.

Recombination in *HCV* is a rare event and the number of well-documented cases is still very low. The first recombinant strain of *HCV* was found in St. Petersburg, Russia, in 2002, an intergenotype recombinant between subtypes 2k and 1b, called CRF1_2k/1b. Different isolates were found derived from the same recombination event in different world countries (Ireland, France, Cyprus, Azerbaijan, Uzbekistan, and Russia). At least, other eight different intergenotypic recombinants have also been described, comprising all *HCV* genotypes except genotype 4, with wide geographical distribution. All CRFs but one (CRF_3a/1b) are formed by a 5′-end of genotype 2 and 3′-end of a different genotype, in which only subtype 1b appears in more than one RF. Genotype 2 and subtype 1b are usually found in older patients and are not usually related to the recent epidemic spread linked to increased usage of intravenous drugs and it is not possible to know if this pattern derives from adaptive features or is simply due to chance.

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L2 - HEPATITIS B VIRUS – TRACING HUMAN MIGRATIONS IN AND OUT OF AFRICA

Anna Kramvis

Hepatitis Virus Diversity Research Programme Department of Internal Medicine University of the Witwatersrand, Johannesburg. South Africa

Hepatitis B virus (HBV) replicates via reverse transcription of the pregenomic RNA by a virus-encoded polymerase that lacks proof reading ability. Thus sequence heterogeneity is a feature of the virus, which has been classified into at least nine genotypes and 32 subgenotypes. The genotypes and/or subgenotypes have a distinct geographic distribution. An estimated 65 of the world's 360 million chronic carriers of hepatitis B virus (HBV) reside in Africa. Genotypes A, D and E of HBV circulate in Africa and show a geographical distribution within Africa. The high number of subgenotypes of both genotypes A and D circulating in Africa,

as well as the geographical clustering of the different subgenotypes, suggest a long endemic history of these genotypes in Africa. On the other hand, the low genetic diversity of genotype E and the absence of subgenotypes in this genotype intimate a more recent introduction into the African population, possibly by iatrogenic means. This presentation will compare the molecular and functional characteristics of the different subgenotypes/genotypes prevailing in Africa, relative to those prevailing outside Africa. The influence of subgenotypes/genotypes of HBV on the natural history of HBV infection and how they can be used to track human migrations and interventions, will be discussed.

L3 - EPIDEMIOLOGICAL UPDATE OF HEPATITIS B, C AND DELTA IN LATIN AMERICA

Mónica Viviana Alvarado-Mora

Laboratory of Tropical Gastroenterology and Hepatology "João Alves de Queiroz and Castorina Bittencourt Alves", Institute of Tropical Medicine, Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo, SP, Brazil

There are several etiological agents of viral hepatitis, including hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis delta virus (HDV), the three major agents involved in chronic infections around the world.

Hepatitis B virus (HBV) is classified in the *Hepadnaviridae* family, *Orthohepadnavirus* gender. HBV genome is a partially double strand circular DNA with approximately 3200 bp [1,2] and has four open reading frames[3, 4]. About two billion people worldwide were infected with HBV and about 350 million people are chronic carriers. HBV infection is associated with 500.000 to 1.2 million of deaths each year representing the tenth leading cause of deaths in world[5]. The incidence of hepatocellular carcinoma (HCC) is increasing in the world, with a mortalitity rate of 300,000 to 500,000 people each year in the world[6]. Vaccination against hepatitis B is available since 1982 with more than 95% effective in preventing HBV infection. In 2009, 177 countries reported that they had included the hepatitis B vaccine in their national childhood immunization programs. The percentage of children up to one year old who received three doses of the vaccine was around 70% in the world in 2009, and particularly 86%in the Americas[7]. About 45% of the world population live in areas where chronic HBV infection is highly endemic (> 8%), 43% live in areas of intermediate endemicity (2-7%) and 12% live in areas of low endemicity (<2%). In Latin America, a strong decrease in HBsAg prevalence was found between 1990 and 2005, changing this region from an intermediate to low endemicity[8]. Epidemiological data suggest that 7 to 12 million Latin Americans are infected with HBV. The routes of transmission in Central and South America are highly variable. The highest prevalence was reported for groups of people aging from 20 to 40 years old, supporting the horizontal transmission in adults and the most common route of infection. The regions with high prevalence (>8%) are found in the Amazon Basin region, the northern parts of Brazil, Colombia, Peru and Venezuela[9], where it is estimated that over 30% of patients in South America, are located. HBV is classified into nine genotypes, from A to I, and genotypes F and H are the most common genotypes in Latin America.

HCV is a positive sense, single-stranded RNA virus with a genome of 9400 bp . It contains a large open reading frame that encodes a precursor polyprotein of about 3,000 amino acids. The virus represents the genus *Hepacivirus* of the family *Flaviviridae*[10]. According to the World Health Organization (WHO), over 170 million people are nowadays HCV infected worldwide, corresponding to a 3% of the world's population, substantially impacting the public health all over the world[11]. In Latin America, the overall prevalence of HCV is 1.23%, varying from 1.7% to 3.4% in the different countries , with an overall distribution similar to other regions of the world[12]. The main risk factor is injecting drug use, but some countries, blood transfusions are always an important risk factor for infection, but it also decreasing its importance in new acquired cases. As across the world, the prevalence tends to be higher in men than in women, and the most prevalent age group is of individuals over 40 years. Injection drug use is not as large a problem in Latin America as compared with the USA and Europe and yet the prevalence in most of the countries studied is remaining flat or increasing. This suggests that other risk factors play a major role in new infections[13]. The number of diagnosed and treated patients is low, thereby increasing the burden of complications such as liver cirrhosis or HCC[13]. Also in Latin America the most prevalent genotype is 1 (1a and 1b), although 1b is mostly found among older members of the population who have a history of blood transfusion[14].

HDV is associated with HBV, as a primary co-infection with HBV or a superinfection in an HBV carrier. It has a negative-sense circular RNA genome with about 1700 nucleotides that only expresses delta antigen[15] and represent the genus *Deltavirus.* The interpretations of epidemiological studies on infection should take into account the fact that it requires the presence of HBV. HDV is widely distributed and associated with fulminant hepatitis epidemics in areas with high prevalence of HBV. Several studies performed in the 1980s showed the precence of HDV infection in South America. HDV usually induces a severe disease but its clinical manifestations are very broad, ranging from asymptomatic cases to fulminant hepatitis[16,17.] The virus is found worldwide but is not uniformly distributed, as determined by seroprevalence studies of anti-HD in HBsAg-positive patients[18]. HDV-3 has been isolated in the northern area of South America only (Amazon Basin of Brazil, Peru, Colombia and Venezuela)[19]. For HDV/3, studies in the Amazon region on prevalence of HBV and HDV showed that family members are reservoirs for transmission of infection by HDV[20]. In this way, the chances of contamination from an extra-familial source are expressed by highly divergent isolates[21] and the sequence similarity in most families units indicate a single source of infection providing evidence that HDV infection is probably mostly transmitted within the families[22]. Recently, it was determined that HDV/3 spread exponentially from early 1950s to the 1970s in South America. It was suggest that the measures implemented to control HBV transmission resulted in the control of HDV/3 spreading in South America, especially after the important raise in this infection associated with a huge mortality during the 1950s up to the 1970s[19]. Furthermore, in a recent study, it was reported the presence of HDV-8 infected individuals in Brazil who have not been in Africa, what may reflect the close relation with HDV genotypes' geographic distribution and human migrations[23]. Finally, it was reported 1.2% of anti-HDV positive among HIV/HBV co-infected patients in Southeast Brazil showing that this group is at potential risk for HDV infection[24].

HBV, HCV and HDV infections are detected throughout Latin America in frequency levels that would place some areas as hyperendemic for HBV, especially those found in Amazon region. Novel strategies to increase HBV immunization in the rural population and to strengthen HCV surveillance are reinforced by these results.

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L4 - DEMOGRAPHIC DISTRIBUTION OF VIRAL HEPATITIS IN THE NORTHERN HEMISPHERE

Carla Osiowy

Public Health Agency of Canada, National Microbiology Laboratory, Bloodborne Pathogens and Hepatitis Section

This lecture will focus on the prevalence and molecular epidemiology of hepatitis B, C and D in the Northern Hemisphere (North America, Europe, Russia and the Far East), with an in-depth discussion of circumpolar Arctic regions. The prevalence of viral hepatitis infection throughout most populations of the northern hemisphere is generally considered low (<2%), except for the Far East, in which hepatitis B infection is endemic (≥8% prevalence), therefore permitting high hepatitis D infection rates, depending on the region and population investigated. The 8 HBV genotypes (A-H) are observed in countries in which immigration is common, although generally genotypes A and D predominate in North America, Europe and Russia, while genotypes B and C predominate in the Far East. The HCV strains circulating in North America, Europe and Russia are usually genotypes 1 and 3 and are mostly associated with injection drug use as a risk factor for infection, while genotypes 1 and 6 are predominantly observed in China and Southeast Asian countries, respectively. Within indigenous populations of the circumpolar Arctic, HBV infection was historically considered endemic; however, the implementation of universal vaccination programs in most regions has resulted in decreased incidence and a reduction in the rate of chronic HBV infection. Concomitant with traditionally endemic rates of HBV infection in Arctic regions, a high prevalence of HDV infection has also been observed in specific Greenlandic and Russian settlements, although it is largely not observed in Alaska or the Canadian Arctic. Hepatitis C infection among circumpolar indigenous peoples is considered uncommon, but with changes in lifestyle and remote to urban migration, prevalence rates are increasing. The distribution of unique HBV genotypes and their association with clinical outcomes in northern indigenous peoples will be discussed further in the lecture.

L5 - PHYLOGEOGRAPHY OF HUMAN HEPATITIS VIRUSES

Remco Bouckaert*, Alexei Drummond

Bioinformatics Institute, University of Auckland, Auckland. New Zealand

Now that DNA sequence information and sampling location for many hepatitis virus populations have become readily available, phylogeographical analysis becomes an important tool in assessing the historical spread, population connectivity and public health impact of the virus. Research aimed at understanding the geographic context of evolutionary histories is burgeoning across biological disciplines.

Bayesian Markov chain Monte Carlo and related computational tools have been the primary source of advances in phylogenetic and phylogeographic approaches. The Bayesian method offers a framework for inference, visualization and hypothesis testing of phylogeographic history. One promising development involves reconstructing phylogeographic history assuming the discrete locations

where the samples originate restrict the location of internal nodes. This way, geographical locations can be modeled as discrete traits and standard phylogenetic techniques applied. This enables the reconstruction of timed viral dispersal patterns while simultaneously reconstructing the evolutionary history in time from molecular sequence data.

An alternative approach is to model the geographical movement as a random walk on a continuous landscape. This method allows one to infer continuous phylogeographic diffusion using random walk models while accommodating phylogenetic uncertainty. By accommodating branch-specific variation in dispersal rates, the most restrictive assumption of the standard Brownian diffusion process can be relaxed which typically results in increased statistical efficiency in spatial reconstructions of overdispersed random walks.

These techniques have already been applied successfully in the analysis of dispersal of hepatitis B, C and E. The purpose of this talk is to introduce phylogeographical analysis through Bayesian MCMC and survey the application of these methods to hepatitis viruses.

L6 - EPIDEMIC HISTORY OF HEPATITIS C VIRUS IN BRAZIL

Elisabeth Lampe

Laboratório de Hepatites Virais, Instituto Oswaldo Cruz - FIOCRUZ, Rio de Janeiro, Brasil.

Hepatitis C virus (HCV) subtypes 1a, 1b and 3a are the most prevalent strains in Brazil, but very little is known about the epidemic history of these subtypes in the country. A total of 231 HCV NS5B gene sequences (subtype 1a=89, subtype 1b=56, and subtype 3a=86) isolated in Brazil between 1995 and 2007 were analyzed in the present study. Sequences (328-pb) were subjected to phylogenetic analyses and statistical tests of phylogenetic mixing by sampling location and risk group. Our results revealed important variations in the pattern of HCV transmission among subtypes. Transmission of subtype 1a was characterized by dissemination of one major Brazilian lineage with a random virus exchange between different geographical regions but not between IDU and non-IDU populations. Transmission of subtype 1b was characterized by concurrent dissemination of multiple HCV lineages with a restricted virus exchange between country regions and risk groups. Transmission of subtype 3a was characterized by simultaneous spreading of multiple HCV lineages and random phylogenetic mixing by risk group and sampling location. Epidemic histories of major subtypes 1a, 1b and 3a Brazilian clades were estimated using a Bayesian coalescent approach. Our results indicate that all major HCV Brazilian clades probably start to circulate in the country during the second half of the 20th century and displayed roughly similar epidemic histories characterized by an initial phase of exponential expansion and by reduction of growth rates since 1980-1995. This suggests that the expansion of HCV may have been effectively controlled in Brazil.

L7 - HBV AND HCV – PREDICTIVE FACTORS OF DISEASE PROGRESSION

Maria Lucia Gomes Ferraz

The natural history of hepatitis B (HBV) and C (HCV) virus infection is vary heterogeneous and is mediated by variables associated to the host and each of the virus (Seeff, 2002; Thran & Martin, 2004). In HCV infected patients different patterns of hepatic fibrosis progression have been observed. Most of the studies regarding fibrosis progression in hepatitis C are based on the *fibrosis progression rate*, obtained dividing the grade of fibrosis (METAVIR) by the estimated time of infection (Poynard et al., 1997). This índex has shown an almost linear correlation between grades of fibrosis and duration of infection, and has permited to indentify three different patterns of fibrosis progression: slow, intermediate and rapid (Poynard et al., 2000). Many different factors associated to fibrosis progression in hepatitis C have been identified: male gender, excessive alcohol intake, more advanced age at the time of infection, co-infection with hepatitis B and HIV, steatosis and insulin resistance, among other factors. And more recently, genetic patterns have been identified as associated with more rapid fibrosis progression rates. On the other side, hepatitis C fibrosis progression seems to be not related to viral factors, such as genotype and viral load. The influence of these variables is more related to the response to treatment, than to the natural history of hepatitis C. In hepatitis B, the natural history of the disease and the progression of hepatic fibrosis is strongly associated to the host immune response, because histological damage is mainly associated to cellular immune response against viral antigens expressed in the membranes of infected hepatocytes. Studies evaluating long-term complications of chronic HBV infection (liver cirrhosis and hepatocellular carcinoma) have also demonstrated the important role of HBV viral load. Iloeje et al, in a study including 3500 HBsAg-positive patients in Taiwan, have demonstrated that the progression to cirrhosis is strongly associated to HBV viral load (Iloeje et al., 2006). Additionally to these aspects, other factors associated to the host also have impact in the course of chronic HBV infection, such as alcohol abuse and HIV and HCV co-infection. The correct identification of predictive variables related to disease progression in hepatitis B and C is very important for the clinical management of the patients, permitting, whenever possible, to reduce or eliminate the modifiable factors.

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L8 - GENETIC MARKERS ASSOCIATED WITH DIFFERENT EVOLUTIONS OF HEPATITIS C INFECTION

André Castro Lyra

Departamento de Gastroenterologia, Universidade Federal da Bahia, Salvador - BA, Brasil

Several clinical studies have reported that African-American HCV patients have a lower response rate to IFN-α therapy compared to

Caucasians. Ethnic differences in HCV antiviral therapy response could be explained, in part, by host genetic heterogeneity. We have evaluated the influence of ethnic ancestry as determined by genetic polymorphism analysis on the response to antiviral therapy of subjects with HCV and its relationship with *IL28B* polymorphisms. Among HCV genotype patients with C/C genotype, genomic ancestry did not interfere with therapy response. Among patients with rs12979860 T/T genotype, African genetic contribution was greater in the non-response/relapse group, whereas Amerindian and European genetic ancestry contribution were higher in the SVR group. Among HCV type 1 patients with rs8099917 T/T, African genetic contribution was significantly greater in the non-response/relapse group; Amerindian and European ancestry genetic contribution were greater in the SVR group. Other genetic polymorphisms in *SOCS3*, *MxA*, *OPN* and *IFNG* genes may also influence the therapeutic response and may add power to predict SVR if associated with *IL28B*. We analyzed 181 HCV genotype 1 patients, including 52 who had achieved SVR. The protective genotypes frequencies among the SVR group were as follows: G/G *SOCS3* (rs4969170) (62.2%); T/T *OPN* (rs2853744) (60%); T/T *OPN* (rs11730582) (64.3%) and G/T *MxA* (rs2071430) genotype (59.7%). We did not find any significance in *IFNG* analysis. Patients who had ≥ 3 of the protective genotypes from *MxA*, *SOCS3* and *OPN* had a greater than 90% chance of achieving SVR (P<0.0001). The C/C *IL28B* genotype was present in 58.8% of subjects in the SVR group. The SVR rate increased to 85.7% (P < 0.0001) and 91.7% (P < 0.0001) by analyzing C/C *IL28B* with either the T/T OPN genotype at rs11730582 or the G/G *SOCS3* genotype, respectively.

L9 - IMMUNOGENETICS OF HEPATITIS C

Fernanda de Mello Malta[1], Fernanda Romano Bruno[2], Karina Carvalho[2], Ana Catharina Seixas Santos Nastri[1,3], Thais Rodrigues[2], Flair José Carrilho[1], Esper Kallás[2], João Renato Rebello Pinho[1]

[1]Department of Gastroenterology,[2] Clinical Immunology and Allergy Branch, Department of Internal Medical,[3] Department of Infectious and Parasitic Diseases, University of São Paulo School of Medicine, São Paulo, Brazil

Two different aspects of the interactions among hepatitis C virus and the human host have been recently studied by many different groups around the world. First, genome-wide association studies in genetically homogeneous populations pointed the association between single nucleotide polymorphisms (SNPs) in the IL28B gene and spontaneous and treatment-related resolution of HCV infection. The strongest associations were detected for SNPs rs12979860 and rs8099917. Second, interactions between HCV and host immune defense, especially the innate immune response, are crucial to determine the disease evolution and might also impact the response to current therapeutic strategies, most of them based in modulating viral immunity. The immunological mechanisms associated with viral clearance or chronic persistence are still controversial. The profiles and functions of Natural Killer (NK) and Dendritic cells (DC) differ in the different forms of evolutions of HCV infection. We have studied the frequency of IL28 polymorphisms and their association with different outcomes of HCV infection in the highly miscegenated Brazilian population, with mixed Caucasian, Amerindian and African origins in 154 individuals from 4 different groups: 40 patients with natural HCV clearance, 58 patients with SVR, 14 non-responder patients and a control group of 42 individuals without HCV infection. All individuals were genotyped as CC, CT or TT at rs12979860 and as TT, GT and GG at rs8099917 with real-time PCR. The CC genotype at rs12979860 was associated with greater probability of spontaneous HCV clearance (62% CC, 30% CT and 8% TT). Individuals homozygous for the T allele at rs8099917 were also more likely to spontaneously clear the virus (83% TT and 17% GT). These latter findings are very similar to what was encountered in the group of patients with SVR (84% TT and 16% GT). At the SNP rs12979860 the frequencies found for this group were 43% CC, 48% CT and 9% TT, This data corroborates the findings in the group of non-responders, in which the presence of the G allele at rs8099917 is associated with increased likelihood of non response to treatment (29% TT, 57% GT and 14% GG). The genotype GG was only found in this group. No individual homozygous for C at rs12979860 was found in this group. In the control group, the frequencies of genotypes were: 57% TT and 43% GT (no GG genotype) at rs8099917 and 41% CC, 52% CT and 7% TT at rs12979860. In this study, a subgroup of patients was selected to further analyze the innate immune response in patients with chronic HCV genotype 1 infection (n=29), individuals with spontaneous clearance of HCV (n=28) and healthy control subjects (n=26). Multiparametric flow cytometry was used to determine the frequency and phenotype of NK and DC cells. Additionally, we evaluated cytotoxicity and INF-γ release capacity of NK cells. Results: No differences were observed regarding the frequency of DC cells, but the frequency of $CD86^+$ mDCs was significantly higher in individuals with chronic hepatitis C (CHC) compared to healthy controls (HC) (p=0.0370). A correlation positive was observed between $CD86^+$ mDCs frequency and HCV viral load. Among the analyzed NK cells populations, $CD56^{dim}$ cells were predominant and showed increased expression of NKG2A and KIR3DL1/DS1 receptors when compared to $CD56^{bright}$ and $CD56^{neg}$ cells. $CD57^+CD56^{dim}$ and $CD57^+CD56^{bright}$ cells showed marked cytotoxicity. Comparing the three different groups, individuals with current chronic HCV infection had the highest frequency of $CD56^{dim}$ (p=0.0462) and $CD56^{neg}$ (p=0.0389) cells with features of more differentiated cells with cytotoxic profile. A negative correlation was observed between the frequency of $CD56^{dim}$ $CD107a^+$ cells and the viral load in individuals with chronic infection (r -0.6848). The conclusions of this study are: 1) Considering the IL28 polimorphisms, our results are similar form those previously published. Despite the particular and high miscegenation of our population, our results are similar to other studies with more genetically homogeneous individuals. 2) The presence of previous or current HCV infection does not affect the frequency, phenotype and activation of DC cells. Interestingly, we found a negative correlation between $CD56^{dim}$ cells expressing CD107a and HCV viral load in chronic infected individuals. These data suggest that a particular population of NK cells may help in the viral load control.

L10 - HLA GENES AND HEPATITIS B INFECTION

Arman A. Bashirova

Cancer and Inflammation Program, Laboratory of Experimental Immunology, Frederick, MD. United States of America.

Hepatitis B virus (HBV) clearance by the host largely depends on successful adaptive immune response rather than innate response, which the virus generally evades. Studies suggest that the major mechanism of HBV elimination is the strong CD8+ T cell response in the liver, including direct killing of infected cells and antiviral activity of interferon-γ.

Establishment of an early CD4+ T cell response is thought to be critical in induction of proper CD8+ T cell response for the HBV clearance. This is supported by epidemiological data emphasizing the role of polymorphism in human leukocyte antigen (HLA) class II region in HBV infection outcome. HLA class II molecules present viral peptides to CD4+ T cells and therefore variation in these molecules may contribute to the quality of the CD4+ T cell response. Variation near *HLA-DP* showed the strongest effect on persistent HBV infection in a genome-wide association study (GWAS) in a Japanese population, where the two major hits are single nucleotide polymorphisms (SNPs) located in 3' untranslated regions (UTRs) of *HLA-DPB1* and *DPA1*. A replicative study demonstrated that the SNP in *HLA-DPA1* strongly associates with both resistance to HBV infection and viral clearance in a Chinese cohort. However, the *HLA-DPB1* SNP had only a marginal effect on HBV persistence/clearance in a cohort of European and African Americans (EA and AA, respectively). Sequencing analysis revealed a different SNP located in the *HLA-DPB1* 3'UTR that was not tested in the GWAS that demonstrated a strong association with the HBV clearance in both EA and AA, the effect being stronger than any of individual *HLA* alleles in our cohorts. The variant also associates with the DPB1 protein and mRNA expression level, which may explain its association with viral clearance. In conclusion, consistent, significant effect of variations in the *HLA* class II loci on HBV infection outcomes across different studies implicate critical role of the CD4+T cell response in the HBV immunity.

L11 - CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF HEPATOCELLULAR CARCINOMA IN BRAZIL

Flair J Carrilho*, Luciana Oba Onishi Kikuchi, Aline Chagas, Regiane Saraiva, Denise Paranaguá-Vezozzo

Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo, Brazil

Hepatocellular carcinoma (HCC) is the 5th most common cancer in the world. Cirrhosis is the main risk factor for HCC development. Hepatitis C virus, hepatitis B virus, alcohol abuse and non-alcoholic steatohepatitis are important causes of chronic liver injury that progress to cirrhosis. In Brazil, two large multicenter retrospective studies were performed to investigate clinical and epidemiological aspects of HCC. The first study performed in 1997 and included 287 patients. HCC was found in cirrhotic livers in 71.2% of cases. Chronic alcoholism was present in 36% of cases, chronic hepatitis B in 35% and hepatitis C in 25%. In 2010, a national survey was performed and data from 1,405 patients were included. Cirrhosis was present in 98% of cases. Hepatitis C virus was the main etiology (54%). Differences in HBV prevalence was found among regions, and it was more prevalent in the Northern than in the Southern regions. Most patients (43%) were diagnosed in early stages and chemoembolization was the most common initial therapy employed (36%). Liver transplantation was performed in 242 patients (19%). After modifications in priority policy, the number of patients with early HCC submitted to liver transplant has increased in the last five years in Brazil.

L12 - VIRAL HEPATITIS AND HEPATOCELLULAR CARCINOMA

Carla Osiowy

Public Health Agency of Canada, National Microbiology Laboratory, Bloodborne Pathogens and Hepatitis Section

Hepatitis B and C infections account for over 80% of cases of hepatocellular carcinoma (HCC), the 3rd most important cause of death due to cancer. This lecture will focus on the hepatitis B and C virological aspects associated with the development of HCC. Viral hepatocarcinogenesis is often associated with chronic inflammation and regeneration due to modulated host intracellular signalling or immune-mediated pathogenesis. Both HBV and HCV express proteins having pro-oncogenic properties, and HBV DNA integration into host chromosomes is a common observation in HBV-associated HCC. Recent evidence has shown that HBV variability is an important contributing factor in the development of HCC. Differing pathogenic potential has been observed among specific HBV genotypes, depending on the population studied. Genotype C is associated with an increased progression to terminal chronic liver disease in Asian patients, while genotypes F and A have been highly associated with the development of HCC in Alaska Natives and Black Africans, respectively. Current studies have highlighted specific genomic variants of HBV as one of the key intrinsic viral features associated with liver disease progression and HCC development, regardless of HBV genotype. The double mutation within the X coding region, overlapping with the basal core promoter (BCP) region of the HBV genome (A1762T/G1764A), has been shown to be an independent risk factor for development of HCC. Further mutations within the BCP, upstream regulatory regions, and deletions within the PreS-coding region have also been associated with progression to cirrhosis and HCC. An investigation of these mutations and different genotypes within a homogeneous population, and their association with differing clinical outcomes, including HCC, will be discussed further in the lecture.

L13 - CLINICAL APPLICATIONS OF MOLECULAR SEQUENCING OF HEPATITIS VIRUSES

Stephen Locarnini

Head, Research & Molecular Development, Victorian Infectious Diseases Reference Laboratory, Australia

The emergence of antiviral drug resistance in hepatitis B and hepatitis C has a number of important consequences including progression of liver disease, development of hepatic flares, and increased risk of decompensation, as well as the possibility of transmission of drug-resistant variants in clinical settings such as mother-to-baby and iatrogenic transmission.

Antiviral therapy during late pregnancy may reduce the risk of HBV transmission in HBeAg-positive highly viraemic pregnant women. We have measured the impact of lamivudine (LMV) therapy in reducing maternal viraemia thereby reducing transmission risk and also investigated for the emergence of LMV-resistant HBV, comparing conventional population based PCR methods to the much more sensitive ultra deep pyrosequencing (UDPS). UDPS was able to detect the multi-

drug resistant rtA181T variant present at baseline (2.1%) and the LMV-resistant rtM204V variant at end-of-therapy which conventional PCR failed to detect. Thus, the more sensitive UDPS revealed the presence of emerging as well as pre-existing genotypic resistant variants present at frequencies well below the level of population-based sequencing methods. Thus, in specialised settings such as mother-baby transmission scenarios, conventional population based PCR sequencing technologies appear inadequate for appropriate patient management.

Molecular analysis has emerged as an important tool for tracing virus transmission pathways, particularly for the blood-borne viruses such as HIV, HBV and HCV. A number of reports have now described the use of this approach to establish and follow the transmission links between cases of clinically-acquired (iatrogenic) hepatitis C. Very recently, the VIDRL was involved in detecting possible pathways of transmission when an HCV-infected anaesthetist was epidemiologically linked to an outbreak of acute HCV infection in a group of women undergoing termination of pregnancy (TOP) procedures. Over forty cases of HCV transmission have now been confirmed by virological analysis, whilst phylogenetics conclusively established the link between those women undergoing TOP and the HCV-positive anaesthetist. Clustal sequence alignments of HCV core and NS5B identified a number of "signature" substitutions within those regions found only in the anaesthetist and infected women. Phylogenetic analysis confirmed this close relatedness between the women on the surgical TOP list and the attending anaesthetist by establishing a common genetic lineage. Epidemiological investigations are still ongoing to determine the exact mechanism of HCV transmission in this outbreak

L14 - TRATAMENTO ATUAL DA HEPATITE C COM INTERFERONS

Tânia Reuter

Ambulatório HIV/AIDS/Hepatites Virais, Universidade Federal do Espírito Santo, Vitória, Espírito Santo-ES

Hepatitis C treatment in the acute or chronic presentation are an important and actual issue. Peg INF and ribavirin is still the standard-of-care (SOC) with some new recommendations for use the DAA.

For chronic hepatitis C treatment, guidelines differ in some. EASL/AASLD guidelines recommends PegIFN for all genotipes. On the other hand, Brazilian guidelines recommend it for genotype 1 and for genotype 2/3 only in presence of advanced fibrosis.

Some new tools have been used. First, some *IL28b* polimorfisms at the host gene (CC,CT and TT) are associated with diferent SVR in patients with HCV genotype 1, 2 and 3. In the 2 last genotypes it seems be important only in patients who still have HCV RNA detectable after week 4 of treatment.

Another one is the response guided therapy. Duration therapy now should be tailored to the on-treatment virological response at weeks 4 and 12 and eventually in week 24. Shortened treatment for 24 weeks (genotipes 1 and 4) or 12-16 weeks (for genotipe 2/ 3) can be considered in patients with RVR and low baseline viral load but this rule is not aplicable at Brazilian guidelines. However, if negative predictors are present, evidence for equal eficacy of shortened treatment is insuficient. On the other hand, prolonged duration (72 weeks) is sugested in slow/partial responders patients.

A special actual aproach is for re-treatment hepatitis C. The EASL/AASLD considers triple therapy with DAA drugs for genotype 1 re-treatment, non-response or relapse with. At this time, the Brazilian guidelines recommendatios for re-treatment are only to prolong the duration of therapy without use these new antiviral drugs: 48 week of pegylated IFN for genotypes 2 and 3 and 72 weeks for genotype 1 associated with weight aesed ribavirin (15 mg/kg/day) . For null responders, re-treatment is not recommended.

L15 - VIRAL HEPATITIS AND HIV: UPDATE ON MANAGEMENT IN BRAZIL

Maria Cassia Mendes-Correa

Infectious Diseases Department - Medical School - São Paulo University - São Paulo; Infectious Diseases Research Unit - ABC Foundation Medical School - Santo Andre - Brazil

Since 1980, when the HIV epidemic began until June 2011, 608,230 cases of AIDS have been reported in Brazil, according to the latest data from Brazilian Ministry of Health[1]. In 2010, 34,218 new AIDS cases were reported and the disease incidence rate of AIDS in Brazil was 17.9 cases per 100 000 inhabitants[1]. Regarding the mode of transmission among people over 13 years of age, sex prevails. Among women, 83.1% of reported cases were related to heterosexual transmission in 2010. Among men, 42.4% of cases were due to heterosexual contact, 22% due to homosexual and 7.7% due to bisexual relationship. The remainder occurred by parenteral or vertical transmission[1]. Among patients with parenteral mode of transmission, we highlight the cases of transmission among injecting drug users.Hepatitis C then arises as an important co-morbidity in this group of patients co-infected with HIV and a history of parenteral transmission. The prevalence of HBV and HCV among HIV infected patients in Brazil varies considerably according to different regions, since Brazil is a country of continental dimensions. In Brazil the prevalence of HCV among HIV-infected varies according to geographical region from 4% to 53%[2-14]. In Brazil, HBV infection among HIV infected patients occurs with a prevalence ranging from 0.4% to 8.5%[15-20]. The majority of cases of hepatitis B among HIV infected people occurs among men who have sex with men. Among all cases of hepatitis B reported in our country from 2007 to 2010, 6% occurred among patients infected with HIV[21]. Among all hepatitis C reported cases in our country in the same period, 12% occurred among those infected with HIV[21]. The presence of HIV determines major impact on the natural history of hepatitis B and C. Different researchers around the world have conducted important studies regarding clinical presentation of liver disease among this population. In Brazil, most of the studies in this population evaluates clinical, immunological and epidemiological characteristics of these patients. Similarly, studies on the therapeutic aspects of viral hepatitis B and C have also been conducted in this population. These studies are important and relevant since they help us to better understand particular aspects of these co-infections among HIV-infected population in Brazil. Among these studies we highligth the ones which analyze the clinical impact of the presence of IL28B polymorphisms in the clinical evolution of liver disease in this population.

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L16 - NEW PARADIGMS IN THE MANAGEMENT OF HIV AND HEPATITIS C VIRUS COINFECTION

Marina Nuñez

Department of Internal Medicine, Section on Infectious Diseases, Wake Forest University, Health Sciences, Medical Center Boulevard, Winston Salem. United States of America.

With the availability of the HCV protease inhibitors telaprevir and boceprevir we have entered a new era in the treatment of hepatitis C virus infection. Triple therapy which includes pegylated interferon, ribavirin and a HCV protease inhibitor is currently the standard of care for chronic genotype 1 HCV infection. Much higher proportion of patients achieves sustained virological response with these new treatment modalities. The downside is the increase in side effects, although a shorter treatment duration in many cases, especially in treatment naïve patients, offsets the worsened safety profile. Selection of resistance mutations in patients failing treatment is another important limitation. The success with HCV triple therapy is predicted by the same factors predicting response to interferon although the addition of new agents mitigates the negative factors. In treatment-experienced patients a prior null response and advanced liver fibrosis diminish significantly the effectiveness of the treatment. While the two HCV protease inhibitors approved are great advance in the field, special patient groups including those infected with non-1 genotypes, renal insufficiency, HIV-co-infection, liver transplant, end-stage liver disease or intolerance to current treatments are currently underserved. There are multiple oral agents currently under development with activity across HCV genotypes, different resistance patterns, increased safety and tolerability and more user-friendly. Of great interest, interferon-sparing regimens are being developed, which seem to offer hope even for null responders.

L17 - GUIDELINES FOR THE TREATMENT OF VIRAL HEPATITIS B AND C IN BRAZIL

Suzane Kioko Ono

Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo, SP, Brazil

Over the past years, several new antivirals for the treatment of chronic hepatitis B became available. Despite the potent action of these drugs the development of new antivirals and strategies to treat hepatitis B are still the major goal.

Lamivudine is a nucleoside analogue reverse transcriptase inhibitor widely used in Brazil, but because its high resistance rate development, the Brazilian Health ministry included the new antivirals as the first line treatment for chronic hepatitis B. The new oral antivirals included were entecavir, tenofovir and adefovir. Interferon based therapy is also an option. Indication of therapy can vary according to HBeAg status, presence or not of cirrhosis and resistance to previous nucleos(t)ide therapy. We will review the latest options for therapy of chronic hepatitis B, including combination strategies that could be an approach to improving the response rate of treatment.

During the past decade the treatment of chronic hepatitis C was based on pegylated interferon associated with ribavirin with modest sustained virological response on clinical practice daily basis. Recently, the new

antivirals boceprevir and telaprevir were approved and it is expected their availability in the public health system in Brazil to improve treatment of chronic hepatitis C. We will discuss the most common advert events and support therapy. In addition, approaches such us rapid and early virological response and variation of duration of treatment will also be discussed.

L18 - CURRENT CONCEPTS IN THE MANAGEMENT AND TREATMENT OF HEPATITIS B IN HIV-INFECTED PATIENTS

Marina Nuñez

Department of Internal Medicine, Section on Infectious Diseases, Wake Forest University, Health Sciences, Medical Center Boulevard, Winston Salem. United States of America.

Current approved treatments for chronic hepatitis B include interferon-α andnucleos(t)ide analogues (NRTI). Interferon treatment entails a finite course and provides higher HBeAg clearance rates. NRTIs are often given indefinitely and most often achieve HBV DNA suppression although it may take up to 48 weeks with high levels of HBV replication at baseline. Selection of resistance mutations is the main limitation of NRTIs in the treatment of chronic HBV infection. Antiviral potency, resistance profiles and safety should drive the selection of NRTI in HBV treatment. Maintenance of durable undetectable HBV DNA is an important end-point in the treatment of chronic hepatitis B since HBV DNA levels predict the development of liver complications. Prevention of HBV-related liver disease encompasses the identification of HBsAg+ subjects in populations at risk, their treatment when indicated, and screening of hepatocellular carcinoma.

L19 - ORIGIN OF HBV AND ITS ARRIVAL IN THE AMERICAS

Nelson Fagundes

Departamento de Genética, UFRGS, Porto Alegre, RS

In spite of many efforts, the origin of hepatitis B virus in the Americas in not fully understood. In one hand, the strong geographic structure shown by the global pattern of HBV lineages suggest an ancient co-evolutionary scenario between the virus and human populations, but on the other hand, estimates based on the molecular clock suggest a very recent origin for the Native American genotype F, which could be reconciled with a very recent introduction from Europe (or Africa). In this presentation, I will discuss how HBV genetic diversity correlates with human demographic history in the Americas, ranging from the early settling of the New World in the Pleistocene until the much more recent events of admixture among Native Americans, Europeans and Africans since the Colonial period. I will discuss how evolutionary rates estimated for pedigrees may underestimate the time of the most basal splits in HBV phylogenies. More specifically, there is a clear excess of non-synonymous substitutions in the most recent branches of HBV phylogeny, suggesting that purifying selection is currently in action to reduce long-term genetic variation in this virus. In terms of the evolutionary rate, this result suggest that all rates estimated based on pedigree data or recent dated samples will show many non-synonymous substitutions, and will therefore overestimate the evolutionary rate and underestimate the coalescence time for more ancient nodes in HBV phylogeny. Recent studies on the tempo and mode of evolution for human mtDNA show the same pattern observed for HBV and may be particularly useful to understand how HBV rate could be corrected. However, the apparent contradiction between (older times suggested by) geographic structure and (recent times suggested by) the molecular clock will only be solved by gathering more data to allow more internal calibration of the HBV phylogeny. Financial support: CAPES, CNPq

L20 - BAYESIAN APPROACHES FOR THE STUDY OF HUMAN POPULATIONS

Remco Bouckaert*, Alexei Drummond

Bioinformatics Institute, University of Auckland, Auckland. New Zealand

Considerable progress in the field of population genetic inference has been made during the past decade, following parallel increases in computer processing speed and available gene sequence data. Inferring population distributions of hepatitis in together with its genealogy is important in understanding the population dynamics of the virus. A number of methods for inferring current and past population sizes from genetic data have been developed since J.F.C. Kingman introduced the n-coalescent in 1982. The coalescent is a stochastic process that describes how population genetic processes determine the shape of the genealogy of sampled gene sequences. Earlier coalescent methods for inferring demographic histories required a demographic model and each demographic model has one or more demographic parameters. Past population dynamics are reconstructed by estimating the demographic parameters, typically by Bayesian methods.

The Bayesian skyline plot is coalescent-based method without dependence on a pre-specified parametric model of a demographic history. This Markov chain Monte Carlo algorithm allows inference of population dynamics of hepatitis viruses while are the same time inferring its phylogenetic history.

Hepatitis-infected populations can be studied at various levels, from the population of viruses in a single person, to the spread of the disease within a small community in a village, city or country, to the global epidemic dynamics.

The Bayesian skyline plot can be applied and is appropriate for performing population analysis at these various levels. This talk outlines the ideas behind the coalescent for population analysis, and Bayesian techniques for demographic analysis in particular the Bayesian skyline plot. Further, it gives an overview of their application to the study of hepatitis in human populations.

L21 - BIOINFORMATICS METHODS FOR THE ANALYSIS OF HEPATITIS VIRUS

Stephen Locarnini

Head, Research & Molecular Development, Victorian Infectious Diseases Reference Laboratory, Australia

Antiviral treatment has been available for hepatitis B for over a decade, and is now becoming available for hepatitis C. However, antiviral

resistance has emerged as the single most important factor in treatment failure when using direct acting antivirals (DAA) for these infections. For hepatitis C, antiviral resistance may even emerge within days to weeks of treatment initiation, depending on viral genotype and antiviral agent. Since antiviral resistance associated viral mutations (both primary and secondary) selected under one agent may affect either positively or negatively the efficacy of subsequent rescue therapies with other DAAs, their rapid and accurate detection is of considerable importance in the management of patients.

SeqHepB is composed of a hepatitis B virus (HBV) genome sequence analysis program (www.seqhepb.com) and a database which can be used to correlate large numbers of patient clinical, routine pathology diagnostic data, viral mutational sequence information, and *in vitro* antiviral sensitivity and cross-resistance phenotypic data in an integrated and structured way for subsequent patient monitoring. The HBV genome sequence analysis component was designed as a simple diagnostic tool to provide a drug-resistance testing service for clinicians managing patients on antiviral therapy. The ability to correlate clinical, routine pathology and viral molecular biological data from a single source can also facilitate new opportunities for research into the pathogenesis and natural history of chronic hepatitis B in the era of antiviral drugs and associated resistance. A similar approach has now been adopted for hepatitis C by developing a SeqHepC system. As well as a diagnostic tool for HCV drug resistance testing, it is possible to integrate patient clinical, pathological and viral molecular biological data for rapid identification of novel patterns of HCV DAA-resistance associated mutations, which can then be confirmed by *in vitro* drug susceptibility phenotype testing. SeqHepB and SeqHepC will enable virologists and physicians to individualise patient management and use the approach of response guided therapy in "real time", as well as to cope with the current explosion of DAA-associated viral mutations, to conduct cross-sectional retrospective or prospective studies on virus-infected individuals undergoing antiviral therapy, including the development of next generation combination therapies.

L22 - HBV THERAPY: IS DRUG RESISTANCE INEVITABLE?

Mário Guimarães Pessoa

Departamento de Gastroenterologia, Faculdade de Medicina, Universidade de São Paulo –SP, Brasil

Each class of antiviral agents has advantages and disadvantages. The oral agents have demonstrated a relatively good safety and tolerability profile, as well as high rates of efficacy with a good durability of response. The barrier to resistance is variable among agents.

The advantages of choosing peginterferon over the oral agents are the finite course of therapy; the higher rates of e-antigen loss in the first year of treatment, as is the rate of surface antigen loss. The latter observation is particularly true for HBeAg-positive patients who have genotype A infection. However, disadvantages of peginterferon include the need for subcutaneous administration, frequent adverse events, and contraindication in certain patient groups such as those who have decompensated cirrhosis or are immunosuppressed. Exposure to oral anti-HBV agents presents a risk of evolving drug resistance. Cumulative rates of resistance differ between agents, with higher rates reported with the use of first generation agents. In nucleos(t)ide-naive patients, treatment with lamivudine was associated with relatively high rates of resistance: 24% at Year 1, rising to 70% by Year 5. Reported rates of resistance were lower with use of the second-generation drugs adefovir and telbivudine. Data on telbivudine are limited to 2 years of follow-up, at which point resistance was reported in up to 22% of naïve patients. The cumulative rate for adefovir was 29% at Year 5. The resistance profile of the third-generation agents entecavir and tenofovir is different. For tenofovir, 3-year follow-up of naive patients found no evidence of emergent resistance. For entecavir, the rate of resistance in comparable populations remained low: 1.2% at Year 6 of therapy. In trials evaluating the use of entecavir and tenofovir in HBeAg-negative chronic hepatitis B, between 87% and 100% of patients sustained undetectable HBV DNA over the first 3 years of therapy, suggesting that drug resistance seems no longer to be an issue in the naïve population, at least in the short to medium term follow up. However, the potential for drug resistance to emerge over many years of treatment cannot be discarded.

L23 - VARIATIONS IN GENES OF INNATE IMMUNITY IN HEPATITIS C INFECTION

Arman A. Bashirova

Cancer and Inflammation Program, Laboratory of Experimental Immunology, Frederick, MD. United States of America.

Resolution of viral hepatitis depends on complex interplay between innate and acquired immune responses. It has been known that hepatitis C virus (HCV) is a potent inducer of the innate response, in contrast to hepatitis B virus (HBV). Indeed, genetic association data suggest that HCV clearance depends more on innate factors, whereas HBV outcome is largely influenced by acquired immunity. Two examples of innate factors contributing to HCV immunity include killer-immunoglobulin-like receptors (KIRs) and IL28B, as polymorphism in these genes associate with the infection clearance. KIRs are receptors expressed on natural killer (NK) cells. KIR ligation with HLA class I molecules can regulate NK cell function through inhibitory and activating signaling. KIRs are encoded by a multi-gene family which exhibit extensive gene content and allelic variation. Given the extensive polymorphism of *HLA* class I and *KIR* loci, variation in the potency of NK cell activity can be expected among individuals. Inhibitory KIR2DL3 in combination with its group 1 HLA-C (HLA-C1) ligands associates with HCV resolution in Caucasians and African Americans who are expected to have been exposed to low infectious doses. Such a protective effect can be explained by diminished inhibitory signaling in NK cells conferred by this specific compound genotype. Variation near to the *IL28B* gene demonstrated a genome-wide significant effect in both spontaneous and treatment-induced HCV clearance. The finding was replicated in a number of studies with two linked single nucleotide polymorphisms (SNPs) located 3 and 8 kb upstream showing the strongest effect, which appears to be independent of race, viral load and genotype. The *IL28B* gene encodes interferon λ, a type III interferon, which exhibits a strong antiviral effect similar to type I interferons. Expression of type III receptors is limited to the liver and epithelial cells, favoring their use as antiviral drug due to lower cytotoxicity compared to type I intereferons, whose receptors are ubiquitously expressed. Despite convincing evidence for the role of the variations near the *IL28B* gene in the HCV clearance, functional consequences of the identified SNPs are not clear. There are a number

of SNPs in linkage disequilibrium with the identified variants, both synonymous and non-synonymous, that can potentially influence the cytokine function or expression level, but this has not been proven yet. However, the genetic information on *IL28B* polymorphisms has predictive value for both treatment-induced and spontaneous HCV clearance and may direct individualized treatment management.

L24 - DISTRIBUTION OF HBV GENOTYPE F IN SOUTH AMERICA

Mónica Viviana Alvarado-Mora

Laboratory of Tropical Gastroenterology and Hepatology "João Alves de Queiroz and Castorina Bittencourt Alves", Institute of Tropical Medicine, Department of Gastroenterology, University of São Paulo School of Medicine, São Paulo, SP, Brazil

Hepatitis B virus (HBV) infection is a public health problem affecting about 2 billion people and more 350 millions are chronic carriers of the virus around the world[1]. HBV has a partial double stranded DNA genome of around 3,200 nt. It replicates through a retrotranscription step and contains four partially overlapping open reading frames encoding the DNA polymerase; surface antigens; core and HBe antigen; and X protein[2]. A genetic classification based on the comparison of complete genome HBV genomes identified nine genotypes[3], that are further subdivided in subgenotypes. HBV genotypes and subgenotypes have distinct geographical distribution and it is currently discussed if they are associated with different prognosis considering the severity of liver diseases in different populations. Genotype A is globally distributed and is the main genotype found in Europe, North America, Africa and India[4]. Genotypes B and C are the most frequent in Asia[5]. Genotype D is found predominantly in the Mediterranean region but has a worldwide distribution[6]. Genotype E is widely distributed in West Africa and has rarely been found in other continents, except for few cases in individuals with African background[7]. Genotypes F and H are thought to be indigenous to America since they have been found in the native population, mainly in Central and South America.[8,9] Genotype G has an apparent low prevalence in the world but it is was reported in many countries in Europe and Americas[10]. Recently, phylogenetical analysis characterized a new genotype I in Vietnam and Laos[3]. HBV genotype F was primarily found in indigenous populations from South America and is divided into four subgenotypes (F1 to F4) showing a genetic divergence of around 4.3% - 6.1%[11]. Subgenotype F1 is further divided in F1a (found in Costa Rica and El Salvador[8]) and F1b (found in in Alaska[12], Argentina and Chile[13]). Subgenotypes F2 and F3 co-circulate in the north of South America: F2a is more common in Brazil[14] and Venezuela; F2b was described only in Venezuela[11]; F3 is common in Colombia, Venezuela and Panama[9]. Subgenotype F4 is associated with the central and south areas of South America: Bolivia[15], Argentina[16] and the southern area of Brazil. Recently, it was observed that HBV/F3 had three amino acid substitutions in the genome that were different from the other HBV/F subgenotypes and they are found in the genotype H[9]. Thus, the clustering observed in the phylogeny of HBV genotype F and the presence of specific lineages in particular regions seem to imply that a far deep historical association has taken place. However, the rigorous standard HBV geographic distribution has changed in recent years, especially in areas of the world where human immigrations had occurred and thus, it is likely that the geographical relation of human populations and HBV genotypes will become even more heterogeneous in a near future.

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L25 - THE DISTRIBUTION AND CHARACTERISTICS OF HEPATITIS B GENOTYPE F IN THE ARCTIC

Brenna Simons*, Brian McMahon, Stephen Livingston

Alaska Native Tribal Health Consortium, Liver Disease and Hepatitis Program, Anchorage, Alaska 99508 USA

Five hepatitis B virus (HBV) genotypes are present amongst chronic HBV carriers in Alaska (A, B, C, D, and F); genotype D is the most predominant followed by F. A significant association has been shown between hepatocellular carcinoma (HCC) and genotype F in Alaska Native children and genotype C in Alaska Native adults. The incidence of HCC in persons <20 years of age was 3 per 100,000, but due to universal screening and vaccine programs (initiated in 1983), no cases of HCC in persons <20 years of age have occurred since 1999. In a comparison of

genotypes B, D and F HBV viral isolates, genotype F exhibited distinct genetic mutations and deletions within the precore, core and PreS regions of the HBV virus that were not identified in genotypes B and D. In a nested case-control study of chronic HBV carriers infected with genotype F, no significant associations were measured at the time of HCC diagnosis with basal core promoter (BCP), precore (PC) or core mutations. However in longitudinal studies amongst genotype F HBV carriers, mutations in BCP were not present at baseline, but found to develop 4-5 months prior to HCC diagnosis, 5 years after baseline. In contrast, genotype F isolates from a control-matched group of HBV carriers that did not develop HCC at baseline exhibited identical mutations in BCP, PC and core regions of HBV described in the HCC group. While HBV genotypes D, A and B are found in other Arctic regions, genotype C is found only in Northwest Alaska and Western Siberia and genotype F only in Southwest Alaska. Comparing the clinical outcomes of genotype F amongst geographic areas and populations may shed light on the mechanism by which genotype F promotes amplified disease progression and incidence of HCC.

L26 - DISTRIBUTION OF HEPATITIS B VIRUS (HBV) GENOTYPE F IN CENTRAL AND NORTH AMERICA

Arturo Panduro*, Montserrat Maldonado, Yadira Aguilar-Gutierrez, Laura V. Sanchez, Sonia Roman

Hepatitis B virus genotype F is known to be an Amerindian genotype with a distinct subgenotype distribution throughout Latin America. Originally HBV genotype F was identified among the indigenous population of South America, until strains from Central America formed a distinct clade, described as a new genotype, HBV genotype H.

The aim of the present study was to describe the distribution of HBV genotype F and H in Central and North America to date, to analyze the clinical characteristics of patients infected with HBV genotype H and to describe the strong association of this genotype with the descendents of Aztec (Mexicans) population. The first complete genome of HBV genotype H described were two strains from Nicaragua and one from the US, plus 10 partial genomes from Mexico. Since then, more than 90 sequences have been reported to the Gen Bank, most of them from México.

An epidemiological study in native Mexican population (Nahua and Huichols) has shown a high endemicity of HBV infection. The predominant HBV genotype was H followed by A, D and G genotypes. When the epidemiological study was extended to chronic carriers in mestizo Mexican population from different geographical regions of the country, HBV genotype H still was predominant with a similar distribution as in native populations. However, mixtures of HBV genotypes were detected in which HBV genotype H/D and H/A were the most predominant in cirrhotic patients, HBV genotype A and H in acute cases, whereas HBV genotype G in men who have sex with men. In both chronic carriers and native population, occult hepatitis B is detected with a predominance of the HBV genotype H. No cases of HBV infection associated to HCC were detected. The Mexican genome as in the rest of Latin American is a mixture of Amerindian, white and black populations. Historical and genetic population studies indicate that the Aztecs or Nahuas are the main ancestors of the Mexican population including the population from Central America such as Guatemala, Nicaragua, Honduras and El Salvador. This geographical region belonged to the Aztec empire before the conquest by the Spaniards. Furthermore the Aztecs arrived in the last migration from Asia to America more than 2000 years ago. These findings appear to explain the origin and predominance of HBV genotype H in this specific region of Latin America and suggest that HBV genotype H detected in North America and other continents come mainly from Mexico. Whereas, HBV genotype F found in North and Central America appears to come from different Amerindian ethnic groups from South America that have migrated to these locations. Contrary to the association of HBV genotype F to cirrhosis and HCC in other countries (Alaska and Spain), in Mexico we have found a high frequency of occult HBV/H in both chronic carriers and native populations but not HCC. Furthermore, mixtures of HBV genotypes is a common finding among cirrhotic patients, suggesting that the progression of liver disease may be more aggressive in these patients than those that are mono-infected with HBV genotype H. The presence of regions with high endemicity among the native population, the rapid clearance of the virus among both natives and mestizo, and the high frequency of occult hepatitis B infection may suggest that patients with HBV genotype H have a better outcome of the disease than other genotypes, even than with HBV genotype F. This may be due either to the extraordinary adaptability of the Mexican genome to the HBV genotype H or to the genomic characteristics of this particular.

L27 - GENOTYPE F HEPATITIS B VIRUS: CLINICAL CHARACTERISTICS AND RESPONSE TO THERAPY

Adrián Gadano

Liver Unit, Hospital Italiano de Buenos Aires, Argentina

From the 8 different genotypes in which Hepatitis B virus has been classified, genotypes A, D and F are the most prevalent in South America. Genotype F has been shown to be predominant in many countries, not only in aboriginal communities but also in some urban areas as it has been shown in Venezuela. Moreover, different subgenotypes have been identified. Phylogenetic analysis of HBV genotype F complete genome has demonstrated that the most prevalent subgenotype seems to be the F1b in Chilean and Argentinian patients. The knowledge of the behavior of this genotype in terms of epidemiology, clinical outcome and response to treatment, is mandatory in the region. A recent study demonstrated that genotype F HBV is responsible for most acute symptomatic infections in Buenos Aires city (65% of cases) while in the same area is responsible for 36% of chronic HBV infections. Little is known about the response of HBV genotype F patients to therapy. In a recent study performed in Buenos Aires we could observe that patients with genotype F chronic hepatitis B experienced high rates of response when treated with pegylated IFN (48% HBeAg loss or seroconversion). These rates were comparable to those reported in the same study for patients infected with genotype A.

POSTER PRESENTATIONS

PO01 - ALTERAÇÕES ENDÓCRINAS VISTAS NA INFECÇÃO CRÔNICAS PELO VÍRUS C - REVISÃO DE LITERATURA

Leonardo Biscaia, Carlos Eduardo Brandão-Mello
leonardo-biscaia@hotmail.com

Universidade Federal do Estado do Rio de Janeiro, Universidade Federal do Paraná

Revisar a literatura sobre as alterações endócrinas prevalentes relacionadas à infecção crônica pelo vírus C. Realizou-se pesquisa nos bancos de dados do PubMed (Medline) e Scielo buscando-se pelos seguintes descritores: HCV, hepatitis C virus, chronic C hepatitis cruzando-se com os seguintes unitermos: tireóide, adrenal, prolactina, GH, hGH, testosterona, ossos, testículo, ovário, pituitária e hipotálamo, tanto em português quanto em inglês; também se procurou nos Anais dos Congressos Brasileiros de Hepatologia. Não houve restrição em relação a data ou língua de publicação, desde que houvesse resumo disponível para consulta; para a análise dos artigos, deu-se preferência a artigos de revisão, publicados em inglês ou português, os mais recentes, os mais abrangentes e os com textos completos disponíveis. A infecção crônica pelo vírus C provoca diversos efeitos no sistema endócrino, sendo as mais prevalentes o diabete melito do tipo 2 e resistência à insulina, várias manifestações tireoideanas e alterações ósseas. Em outras glândulas ou hormônios, a infecção pelo HCV provoca, de modo geral, diminuição da secreção e/ou resistência à ação hormonal; no entanto, os efeitos nos demais hormônios e glândulas não são tão bem estudados ou conhecidos. **Conclusão:** Os efeitos endócrinos mais prevalentes da infecção pelo vírus C envolvem a resistência insulínica/diabete melito, tireopatias e alterações ósseas. Mais estudos são necessários para esclarecer as alterações endócrinas pelo vírus C.

PO02 - ANALYSIS OF HEPATITIS C VIRUS INTRA-HOST DIVERSITY ACROSS THE CODING REGION BY ULTRA-DEEP PYROSEQUENCING

Michael Lauck[1], **Mónica V. Alvarado-Mora[2]*,** Ericka A. Becker[1], Dipankar Bhattacharya[4], Rob Striker[3,4], Austin L. Hughes[5], Flair J. Carrilho[2], David H. OConnor[1], João R. Rebello Pinho[2].
monica.viviana@usp.br

1. Department of Pathology and Laboratory Medicine, University of Wisconsin - Madison, Madison, WI, United States of America. 2. Laboratory of Tropical Gastroenterology and Hepatology, São Paulo Institute of Tropical Medicine and Department of Gastroenterology, School of Medicine, University of São Paulo - São Paulo, SP, Brazil. 3. W. S. Middleton Memorial Veteran's Association Hospital, Madison, WI, United States of America. 4. Departments of Medicine and Medical Microbiology & Immunology, University of Wisconsin - Madison, WI, United States of America. 5. Department of Biological Sciences, University of South Carolina, Columbia, South Carolina, United States of America.

According to the World Health Organization, 130?170 million people are persistently infected with Hepatitis C virus (HCV) and are at risk of developing severe liver disease and hepatocellular carcinoma. HCV is an enveloped RNA virus, containing a single-stranded positive strand RNA genome of approximately 9600 nucleotides. HCV replication is characterized by a rapid rate of virus production, and a corresponding high degree of genetic diversity in circulating viremia. This is due to the lack of efficient proofreading by the HCV RNA-dependent RNA polymerase. DNA sequencing has been radically altered with the development of second-generation pyrosequencing techniques. While targeted sequencing has been used to analyze differences in HCV variability in HCV-monoinfected and HIV-HCV-coinfected subjects as well as to determine antiviral resistant mutations against protease inhibitors, no study has employed second generation sequencing techniques to examine HCV subtype 1a heterogeneity across the entire coding region. Here, we combined pyrosequencing with a transposon-based fragmentation method to perform genome-wide ultra-deep sequencing of four HCV-1a genomes allowing analysis of viral sequence heterogeneity and identification of minor variants conferring pre-existing HCV-specific drug resistance. Plasma samples were obtained from four treatment-naïve, anonymously selected patients after qualitative and genotypic testing at the University of Wisconsin Hospital and Clinics. Viral RNA in the plasma was isolated using the Qiagen QIAamp MinElute virus spin kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. We designed four overlapping PCR amplicons of approximately 2.5kb each to amplify nucleotide positions 16 through 9302 of the HCV genome, including all but 4bp of the coding region. Fragmentation was performed according to the manufacturer?s protocol. Libraries were then subjected to emulsion PCR, enriched DNA beads loaded onto a picotiter plate, and pyrosequenced with a Roche/454 GS Junior sequencer using titanium chemistry (454 Life Sciences, Branford, CT). All four HCV-1a genomes were sequenced in a single GS Junior run. We obtained between 29,567 and 37,627 sequence reads for each of the four HCV genomes resulting in an average coverage depth between 916x and 1125x across the coding region. This deep coverage creates a high-resolution view of the viral population, revealing both the number and frequency of mutations within the quasispecies. Patient A showed a pre-existing resistance mutation in the NS5B region (V499A) at a frequency of 98.7%. Patient B and D showed low-level drug-resistance mutations in the NS5A (Q30R) and the NS3/4A (I170V) region that were present at frequencies of 2.5% and 1%, respectively. We report the presence of low-level drug resistance mutations that would most likely have been missed using conventional sequencing methods. The approach described here is broadly applicable to studies of viral diversity and could help improve the efficacy of direct-acting antiviral agents (DAA) in the treatment of HCV infected patients. FAPESP 2011/50562-0

PO03 - AVALIAÇÃO DA PERFORMANCE DE TESTES MOLECULARES PARA DETECÇÃO E QUANTIFICAÇÃO DO DNA DO VIRUS DA HEPATITE B (HBV DNA)

Portilho, M.M., Marques, V.A., Miguel, J.C.; Silva, E.F.; Scalioni, L.P.; Sousa, P.S.F.; Lewis-Ximenez, L.L., Lampe, E., Villar, L.M.
moyra.portilho@hotmail.com

Laboratório de Hepatites Virais - Fundação Oswaldo Cruz, Rio de Janeiro

O objetivo deste estudo é avaliar o desempenho de três métodos

moleculares qualitativos em comparação com um teste comercial quantitativo para detecção do HBV DNA. Amostras de soro foram obtidas de 55 indivíduos atendidos no Laboratório de Hepatites Virais (IOC/FIOCRUZ) e submetidas a três metodologias qualitativas in house: semi-nested PCR e one round PCR para amplificação do gene S do HBV, e one round PCR para amplificação do gene C do HBV. Estas amostras também foram submetidas ao teste Cobas Amplicor HBV Monitor (Roche Diagnostics, EUA) para quantificação do HBV DNA Como resultado, o semi-nested PCR apresentou maior sensibilidade (48/55), seguido pelo método quantitativo comercial (46/55), one round PCR para o gene S (31/55) e one round PCR para o gene C (22/55). A carga viral média das amostras detectadas pelo teste comercial foi igual a 85.574 cópias de HBV DNA/mL (42 - 22.670.600 cópias de HBV DNA/mL). O semi-nested PCR amplificou três possíveis casos de hepatite B oculta (anti-HBc reagente isolado) enquanto que o teste comercial quantitativo amplificou duas amostras. Estes resultados indicam que o semi-nested PCR seria o método mais eficiente para detecção do HBV DNA, podendo inclusive detectar casos de hepatite B oculta devido a sua alta sensibilidade. Estudos posteriores com maior número de amostras serão conduzidos a fim de comprovar esta hipótese.

PO04 - AVALIAÇÃO DE METODOLOGIAS DE EXTRAÇÃO DO RNA DO VÍRUS DA HEPATITE C (HCV) EM AMOSTRAS DE SANGUE COLETADOS EM PAPEL DE FILTRO (SCPF) UTILIZANDO PCR EM TEMPO REAL

Marques, B. L. C.; Scalione, L. P.; Silva, E. F.; Lampe, E.; Villar, L. M.
miss.marques@gmail.com

Fiocruz/ IOC

O diagnóstico molecular da infecção pelo HCV é realizado em amostras de soro obtidas por punção venosa, entretanto estudos recentes demonstram que o SCPF representa um bom espécime alternativo para estudos em áreas remotas devido a facilidade da coleta, armazenagem e transporte. O objetivo do presente estudo é avaliar dois métodos comerciais de extração de RNA viral em amostras de SCPF detectados por PCR em tempo real in house. Uma amostra de sangue venoso HCV negativa foi artificialmente infectada com uma alíquota de soro HCV positiva contendo 3,22 x 107 cópias/mL, e uma diluição seriada foi realizada (concentrações entre 106 a 100 cópias/mL). Posteriormente, 75?L de sangue foram transferidos para círculos de 12mm de diâmetro de papel de filtro (Whatman 903), e secos por 4 horas em temperatura ambiente. Os dois métodos de extração foram QIAamp Mini Kit extraction (Qiagen), onde o tempo de incubação da amostra foi aumentado (1h a 56°C para 4h a 56°C) e volume de eluição foi diminuído (150?L para 30?L); e Dried Blood Spot Genomic DNA Isolation Kit (Norgen Biotek), onde o protocolo do fabricante foi seguido. O cDNA foi obtido utilizando a enzima SuperScript III e iniciadores randômicos, e posteriormente submetido ao PCR em tempo real (Icycler, BioRad), empregando a metodologia Taqman com iniciadores e sonda desenvolvidos para detectar a região 5? não codificante do vírus. Não foram obtidas amostras reagentes utilizando o DBS Genomic DNA Isolation Kit enquanto que o HCV foi detectado até a concentração de 105 cópias/mL utilizando QIAamp Mini Kit. Concluímos que é possível detectar o HCV RNA em amostras de SCPF utilizando o QIAamp Mini Kit e que este será utilizado nos estudos posteriores em amostras obtidas de indivíduos HCV infectados para avaliação do método em amostras obtidas da população em geral.

PO05 - AVALIAÇÃO DE UM TESTE RÁPIDO PARA DETECÇÃO DE ANTICORPOS CONTRA O VIRUS DA HEPATITE C (ANTI-HCV) EM DIFERENTES FLUÍDOS BIOLÓGICOS

Scalioni, L.P., [1]Cruz, H.M., [1]Marques, B.L.C.; [1]Marques, V.A.; [1]Portilho, M.M.; [2]Villela-Nogueira, C.A.; [1]Lewis-Ximenez, L.L.; [1]Lampe, E.; [1]Villar, L.M.
le_scali@hotmail.com

[1]Laboratório de Hepatites Virais-IOC/Fiocruz; [2]Hospital Universitário Clementino Fraga Filho-UFRJ

O diagnóstico da hepatite C é feito através de testes imunoenzimáticos (EIA) e moleculares, entretanto os testes rápidos baseados no principio imunocromatografico podem ser bastante úteis em situações de emergência, onde há necessidade de liberação dos resultados em curto intervalo de tempo. O objetivo deste estudo é avaliar o desempenho de um teste rápido (Oraquick HCV Rapid Antibody Test, Orasure) para detecção de anticorpos anti-HCV em amostras de sangue total, soro humano e fluido oral. Foram recrutados 81 indivíduos reagentes para anti-HCV por EIA e HCV RNA por PCR, e 39 indivíduos EIA HBsAg/anti-HCV negativos que forneceram amostras de soro, sangue total e fluido oral. Este último foi obtido com o coletor do próprio teste, enquanto que os outros dois fluidos foram obtidos por punção venosa. Os três fluidos foram submetidos ao teste rápido conforme as instruções do fabricante. O teste apresentou valores de concordância de 94,48%, 98,12% e 100% para amostras de fluido oral, sangue total e soro, respectivamente. Estes resultados demonstram que o soro seria o fluido mais apropriado para este teste, entretanto os outros dois fluidos também poderiam ser empregados com grande eficiência. Nenhum resultado falso positivo foi observado para nenhum tipo de amostra demonstrando a alta especificidade do teste. Conclui-se que o Oraquick HCV Rapid Antibody Test pode ser empregado com boa eficiência em diferentes tipos de amostras biológicas o que pode facilitar o acesso ao diagnóstico, especialmente em situações de emergência ou em áreas distantes do laboratório.

PO06 - AVALIAÇÃO DO DESEMPENHO DE DIFERENTES TESTES RÁPIDOS PARA DETECÇÃO DO MARCADOR HBSAG EM AMOSTRAS DE SORO.

Helena Medina Cruz, Juliana Custódio Miguel, Elisangela Ferreira da Silva, Jaqueline Correia de Oliveira, Leticia de Paula Scalioni, Moyra Machado Portilho, Brunna Lemos Crespo Marques, Gabriela Cardoso Caldas, Vanessa Salete de Paula, Cristiane Alves Villela-Nogueira, Flavio Augusto de Pádua Milagres, Lia Laura Lewis Ximenez, Elisabeth Lampe, Livia Melo Villar.
leninhamedina@gmail.com

Fiocruz-IOC

O diagnóstico e acompanhamento de indivíduos infectados com o

vírus da Hepatite B são feitos pela detecção dos marcadores virais desta infecção em amostras de soro utilizando ensaios imunoenzimáticos. O uso de outras formas de diagnostico tais como o uso de testes rápidos, traria vantagens fornecendo a determinação de indivíduos infectados dos não infectados em poucos minutos. O objetivo desse estudo foi avaliar a aplicabilidade de três diferentes testes rápidos comerciais para a detecção do marcador HBsAg em amostras de soro. Foram selecionados 411 indivíduos que fornecera amostras de soro, as quais foram testadas pelo EIE comercial ETI-MAK 4 (Diasorin, Itália) e aquelas amostras HBsAg reagentes foram submetidas a detecção do HBV DNA (Cobas Amplicor HBV monitor test, Roche Diagnostics). Três testes rápidos para detecção do HBsAg foram avaliados: T1 (Vikia HBsAg, Biomerieux), T2 (Imuno-Rápido HBsAg, Wama) e T3 (HBsAg teste rápido, Doles). Cada amostra teve seu resultado determinado por dois diferentes tecnologistas de acordo com os critérios de interpretação de resultado fornecido pelo fabricante e, os resultados inválidos foram repetidos em duplicata. O HBsAg não foi observado no soro de 380 indivíduos e foi detectado em 31 indivíduos pelo EIA sendo que o HBV DNA também foi detectado em 20 destes indivíduos. A detecção do HBsAg nos testes rápidos demonstrou sensibilidades de 100% no T1, 96,77 no T2 e 90,32% no T3 enquanto as especificidades foram 100% no T1, 96,5% no T2 e 96,8% no T3 quando comparados com o EIA. A concordância kappa dos testes foram: T1- 100%, T2- 79,3% e T3- 76,9. Ao avaliarmos os resultados em relação a detecção do HBV DNA, podemos verificar que os testes rápidos puderam detectar o HBsAg inclusive em amostras HBV DNA reagentes indicando boa concordância com o EIE empregado. Concluímos que os testes rápidos para detecção do HBsAg em amostras de soro apresentam boa eficiência sendo possível seu emprego como alternativa ao diagnóstico convencional em situações de emergência ou em laboratórios com recursos escassos.

PO07 - COMPARATIVE GENOMIC ANALYSIS BETWEEN AFRICAN AND BRAZILIAN HBV ISOLATES: THE ROLE OF AFRICAN COUNTRIES IN THE DISSEMINATION OF HBV/A/A1 IN BRAZIL

Lago, BV; Valente, FL; Soares,CC; Mello, FCA; Gomes, SA
babi_v_l@hotmail.com

Fundação Oswaldo Cruz

It is estimated that 2 billion people have been infected with hepatitis B virus (HBV) worldwide and more than 400 million people are at risk of developing cirrhosis and hepatocellular carcinoma due to chronic infection. Based on a genomic sequence divergence in the entire genome exceeding 7,5%, HBV strains have been classified into 8 genotypes, denoted A (HBV/A) to H (HBV/H). It have been established that HBV/A1 is one of the most prevalent genotype in Africa, especially in southern and eastern coast. In previous studies we have shown that HBV/A1 is the main genotype circulating in Brazil. Studies conducted in isolated Afro-Brazilian communities demonstrated that these communities have almost exclusively HBV/A1, suggesting that it was introduced by the slave trade. The aim of this study is to compare HBV/A1 isolates from different African regions with Brazilian isolates. in order to investigate, throughout genetic identity, which African countries have contributed to dissemination of HBV/A1 in Brazil. A comparison among samples from African countries and Brazilian isolates may help to establish possible routes of HBV/A1 spread. For this purpose, 50 samples, previously classified as HBV/A1 by RFLP analysis, from different Brazilian regions were selected. Until this moment, 22 samples were amplified for HBV complete genome by PCR assay. Sixteen HBV/A1 samples were successfully sequenced for HBV entire genome directly from PCR products. Sequences were compared with HBV/A1 samples available in GenBank/NCBI. Five samples were cloned into pUC19 vector and transformed in chemically competent E. coli strains.

Phylogenetic analysis demonstrated that Brazilian sequences are more closely related to Asian/East African sequences than with sequences from other African regions (genetic distance values: 0,02 versus 0,03). These preliminary results suggest that HBV infected slaves brought to Brazil came mostly from the East African coast during the slave trade. However, further studies are necessary to confirm this hypotesis and to estimate which African countries have contributed to the spread of HBV/A1 in Brazil

PO08 - DETALHAMENTO DA OCORRÊCIA DE HEPATITE A NO MUNICÍPIO DE PELOTAS-RS DE 2007 A 2011.

GILL, P.E.[1]; Júnior Ramires, O.V.[1]; Vieira, C.O.[2]; Gomes, M.R.R.[3]; Azevedo, A.L.S.4
pablu_espinosa@hotmail.com

[1]Universidade Católica de Pelotas, acadêmico do curso de Ciências Biológicas; [2]Instituto Federal Sul Riograndense, estudante do curso Técnico em Química; [3]Enfermeira da Secretaria Municipal de Saúde de Pelotas; 4Universidade Católica de Pelotas, Professora do curso de Enfermagem. Descrever a ocorrência de

Hepatite A no Município de Pelotas nos anos de 2007 a 2011. Foi realizado um estudo retrospectivo, com dados obtidos a partir dos registros de notificação e investigação de Hepatite A no Sistema de Informação de Agravos de Notificação (SINAN), acessados através do Programa de Educação pelo Trabalho (PET-Saúde/VS) da UCPel que visa implementar a inserção de estudantes no Departamento de Vigilância em Saúde da Secretaria Municipal de Saúde (SMS). No que diz respeito à ocorrência de Hepatite ?A? na cidade de Pelotas, em 2007 foram constados apenas 01 caso, já em 2008 foram 42 casos, em 2010 foram 14 casos de Hepatite ?A? e, em 2011, apenas 02 casos, foram notificados até o presente. O ano com maior número de casos foi em 2009 que tivemos 230 pessoas contaminadas. O detalhamento dessa ocorrência revela 57,8% dos casos pertencentes ao sexo masculino, e o restante (42,2%), ao sexo feminino. Quanto à faixa etária, 70,6% dos casos concentram-se na faixa de até 14 anos; 26,6% na de 15 a 34 anos; 2,1% na de 35 a 54; e 0,7% na de mais de 55 anos.Conclui-se que no ano de 2009 a epidemia de Hepatite A foi mais agravante do que nos outros anos, com maior incidência de casos na faixa etária de RN a 14 anos. Sendo mais frequênte a doeñça em pessoas do sexo masculino.

PO09 - DETECÇÃO DE MUTAÇÕES ASSOCIADAS À RESISTENCIA NOS GENES NS3 E NS5B DE HCV A DROGAS EXPERIMENTAIS EM PACIENTES VIRGENS DE TRATAMENTO DO SUL DO BRASIL.

Luanna L. Vidal[a], Fabiana N. Germano[a], Ana M. Martinez[b], Jussara Silveira [b], Gabriel S. Gôvea a, Breno Rodrigues a, Marcelo A. Soares a,c, André F. Santos a
luannalvidal@gmail.com

[a]Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil [b]Faculdade de Medicina, Universidade Federal de Rio Grande, Rio Grande, Brasil; [c]Divisão de Genética, Instituto Nacional de Câncer, Rio de Janeiro, Brasil

A combinação de interferon peguilado e ribavirina é atualmente usada no tratamento da infecção por HCV, levando a uma resposta virológica sustentada (SRV) em mais de 50% dos pacientes com infecção crônica. Entretanto, a maioria dos pacientes infectados pelo HCV genótipo 1 não atingem SRV. Investigações de fármacos com alvos nas enzimas virais resultaram no desenvolvimento de 29 novos compostos, conhecidos como antivirais de atuação direta (DAA) que atuam contra a protease e a polimerase virais. Experimentos in vitro mostraram aquisição de mutações de resistência para todas as drogas. Neste trabalho, objetivamos caracterizar polimorfismos naturais associados à DAA em pacientes da região Sul do Brasil. Amostras de plasma foram obtidas de pacientes virgens de tratamento do Hospital Universitário de Rio Grande. RNA viral foi extraído, submetido a RT-PCR e o cDNA usado para amplificar as regiões NS3 (protease/helicase) e NS5b (polimerase). Os produtos de PCR foram sequenciados e alinhados. Análise de mutações foi realizada através da tradução de aminoácidos e análises filogenéticas foram realizadas usando o método de Neighbor-Joining para atribuição do genótipo de HCV. Sequências virais de 49 pacientes virgens de tratamento foram amplificadas, das quais 22 apresentaram mutações associadas à resistência aos DAA. Mutações foram encontradas para dois inibidores de NS3/NS4A, um já aprovado (telaprevir; VX950) e outro em estudos clínicos (TMC435330), e para 5 inibidores de NS5b em estudos pré-clínicos (A782759, A837093, benzothiadiazine, thiophene-2 carboxylic acid e benzimidazoles). A maioria dos pacientes foi infectada pelo genótipo 1a (32), seguido pelos genótipos 3a (12) e 1b (05). A mutação mais frequente foi V138I (33%), seguida por V36L e M71V (18% cada). Algumas mutações encontradas não foram associadas a um genótipo específico, como M71V e V138I. Entretanto outras, como as mutações V36L e I482L, foram encontradas apenas em pacientes do genótipo 3a. Contudo, ao obter sequências referência de banco de dados, observamos que os demais genótipos(2,4 ao 7) também apresentam tais mutações, mostrando que essas mutações não representam uma assinatura desse genótipo. Desta maneira, alguns isolados virais apresentaram polimorfismos naturais associados à resistência nas regiões genômicas NS3 e NS5b que podem prejudicar o futuro tratamento desses pacientes com DAA.

PO10 - DISTRIBUTION AND MOLECULAR CHARACTERIZATION OF HEPATITIS C VIRUS (HCV) GENOTYPES IN PATIENTS WITH CHRONIC INFECTION FROM PERNAMBUCO STATE, BRAZIL

Mónica Viviana Alvarado-Mora[1]*, Izolda Maria Moura[2], Livia de Souza Botelho[1], Edmundo Lopes[3], Flair José Carrilho[1], João Renato Rebello Pinho[1]
monica.viviana@usp.br

[1] Laboratory of Tropical Gastroenterology and Hepatology, Department of Gastroenterology, School of Medicine, University of São Paulo, São Paulo SP, Brazil. [2] Central laboratory of Clinical Hospital, Federal University of Pernambuco (UFPE), Recife, PE, Brazil. [3] Department of Clinical Medicine, Federal University of Pernambuco (UFPE), Recife, PE, Brazil.

Hepatitis C virus (HCV), first identified in 1990, is a single strand RNA virus in the family Flaviviridae. HCV is a public health problem throughout the world and, 3% of the world population is infected with this virus. It is estimated that 3-4% millions individuals are being infected every year. The sequencing of HCV isolates has identified 6 genotypes with more than 70 subtypes. Sequencing of NS5B region has been standardized and used for identification of HCV subtypes for epidemiological applications. In Brazil, it has been estimated that around 1.5% of Brazilian populations is anti-HCV positive and the Northeast region showed a higher prevalence among the Brazilian regions. Objective: The aim of this study was to characterize the HCV genotypes circulating in Pernambuco state (PE), Brazil located in the Northeast region of the country. This study was carried out in Pernambuco state and including 85 anti- HCV positive patients (63 from Recife and 22 from other regions of Pernambuco) collected between 2004 and 2011. Forty-three were males and 42 were females, with age ranging from 26 to 65 years old. Fifty-eight patients were HCV monoinfected, 25 present antibodies to Schistosomiasis (HCV/EHE) and 2 were HCV/HIV coinfected. Fifty-three patients were treatment naïve and 32 have been submitted to treatment with pegylated interferon (Peg-INF) and Ribavirin. Furthermore, fibrosis stage indexes were obtained from liver biopsies performed in 51/85: F0= 3 (5.88%), F1=14 (27.4%), F2=18 (35.3%), F3=12 (23.5%) and four patients (7.84%) had inconclusive result. To perform the phylogenetic analysis, HCV RNA extraction was carried out from 140?l of serum using QIAamp® viral RNA kit (QIAGEN). The reverse transcriptase reaction was performed using the enzyme Reverse Transcriptase Moloney Murine Leukemia Virus (MMLV) and random primers. A fragment of 380bp of HCV NS5B region was amplified by Nested PCR for genotyping analysis. Viral sequences were characterized by phylogenetic analysis using reference sequences obtained from the Genbank (n=224). Sequences were aligned using Muscle software and edit in the SE-AL program. Phylogenetic analysis was conducted using Bayesian Markov chain Monte Carlo simulation (MCMC) using BEAST v.1.5.3. The maximum clade credibility (MCC) tree was obtained from summarizing the substitution trees and then it was removed 10% of burn-in using Tree Annotator v.1.5.3. From 85 samples, 63 (74.1%) were positive to NS5B fragment and successfully sequenced. Subtype 1b was the most prevalent in this population (42- 66.7%), followed by 3a (16-25.4%), 1a (4-6.3%) and 2b (1-1.6%). Twelve HCV/EHE patients were infected with subtype 1b and seven with subtype 3a, respectively. Discussion: Brazil is a large country with many different population backgrounds; a large variation in the frequencies of HCV genotypes

is predictable throughout its territory. Subtype 1b is associated with a higher rate of chronic active hepatitis or cirrhosis, and with a poorer response to convention treatment than genotypes 2 or 3. In Pernambuco, previously studies reported subtypes 1a, 3a and 1b in hemodialysis patients and reported subtype 1b and 3a as prevalent in patients from this state. Conclusion: This study reports HCV genotypes from Pernambuco state where subtype 1b was found to be the most prevalent such as other Brazilian regions. Also, phylogenetic analysis showed that although some sequences from Pernambuco were together in the same cluster in the phylogenetic tree, the values of the posteriori probability are not high, suggesting the presence of the different HCV strains circulating within this population to the present. FAPESP 2011/50562-0

P011- EFEITO LIMITADO DA INFECÇÃO POR HCV NOS NÍVEIS DE EXPRESSÃO DE APOBEC3G E 3F EM INDIVÍDUOS MONOINFECTADOS E COINFECTADOS PELO HIV

Fabiana N. Germano, Nívea D. Amoedo, Naylê O. Silva, Adriana O. Afonso,Rossana Basso, Jussara Silveira, Hector N. Seuánez, Ana Maria B. Martinez, Marcelo A. Soares
fngermano@yahoo.com.br

Universidade Federal do Rio de Janeiro (UFRJ); Instituto Nacional de Câncer (INCA); Universidade Federal do Rio Grande

O vírus da imunodeficiência humana (HIV) e o vírus da hepatite C (HCV) compartilham algumas rotas de transmissão, e por isso a coinfecção é um fenômeno comum. Os dois vírus interagem sinergicamente e exacerbam o curso das infecções nesses pacientes. Entre os membros da família APOBEC em humanos, a APOBEC3G (hA3G) e a APOBEC3F (hA3F) desempenham um importante papel nos mecanismos de defesa antiviral inata do hospedeiro. Sendo assim, nosso objetivo foi investigar os níveis de expressão de mRNA de hA3G e hA3F em PBMC de pacientes monoinfectados por HCV ou HIV e coinfectados pelos dois vírus. Sangue venoso de 33 pacientes foi colhido (4 HCV+; 6 HIV+ virgens de tratamento; 10 HIV+/HCV+ em tratamento antirretroviral e 13 doadores saudáveis) e as PBMCs foram isoladas pelo gradiente de Ficoll. O RNA total foi extraído por TRIzol, retrotranscrito em cDNA e os níveis de mRNA de hA3G e hA3F foram quantificados por PCR em tempo real, utilizando sondas TaqMan®. Entre os indivíduos infectados, 55% eram homens, a média de idade foi de 46,1 anos, e as principais vias de transmissão relatadas foram a intravenosa (21,4%) e a hemodiálise (28,6%). O teste t de Student mostrou que houve um aumento significativo da expressão de hA3G e hA3F na infecção pelo HIV quando comparado aos níveis de doadores saudáveis (p=0,024 e p=0,026, respectivamente). Da mesma forma, os níveis de expressão de mRNA dessas APOBECs foram mais elevados na infecção por HIV do que na infecção por HCV (p=0,05 e p=0,04, respectivamente). A coinfecção HIV/HCV mostrou uma tendência de aumento dos níveis de mRNA de hA3G e hA3F em relação aos controles (p=0,053 e p=0,051, respectivamente), mas estes não foram maiores quando comparados aos monoinfectados por HIV. Os pacientes infectados pelo HIV não-tratados apresentaram níveis mais elevados de expressão de hA3G e hA3F se comparados a doadores saudáveis ou àqueles coinfectados por HIV/HCV em tratamento antirretroviral. Por outro lado, a monoinfecção por HCV não alterou a expressão dessas APOBECs em PBMCs. A coinfecção, ao nível celular, não parece explicar os efeitos sinergísticos dos dois vírus observados em pacientes coinfectados.

PO12 - EFEITOS DA COINFECÇÃO HIV-HCV NAS QUASIESPECIES DE HCV EM PACIENTES INFECTADOS ATENDIDOS EM UM SERVIÇO DE DST/AIDS NO SUL DO BRASIL

Germano FN[1,2], Pose PS[1], Silveira J[3], Basso R[3], Seuánez HN[1,2], Martinez AMB[3], Soares MA[1,2]
fngermano@yahoo.com.br

[1]Programa de Genética, Instituto Nacional de Câncer. [2]Departamento de Genética, Universidade Federal do Rio de Janeiro. [3]Faculdade de Medicina, Universidade Federal do Rio Grande

A infecção pelo vírus da hepatite C (HCV) atinge cerca de 3% da população mundial, e constitui a causa líder de hepatocarcinoma e transplante de fígado. Estima-se que 180 milhões de pessoas sejam portadores crônicos desse vírus. A coinfecção HIV-1 e HCV é bastante comum em diferentes grupos de risco, visto que os dois vírus compartilham rotas de transmissão. Sabe-se que as interações moleculares entre os dois vírus exacerbam vários aspectos de ambas as doenças, no entanto os padrões de evolução das quasiespécies de HCV nesses pacientes ainda não são bem compreendidos. Nosso objetivo foi caracterizar a evolução das quasiespecies em pacientes coinfectados, portadores de dois genótipos distintos de HCV, 1 e 3. Doze pacientes coinfectados atendidos no serviço de HIV/Aids do Hospital Universitário da Universidade Federal do Rio Grande (HU/FURG) assinaram um termo de consentimento livre e esclarecido para participação na pesquisa. O RNA viral foi extraído do plasma dos pacientes, retrotranscrito em cDNA e submetido a PCR utilizando oligonucleotídeos para amplificar um fragmento da região HVR-1 do gene viral E2. Os produtos de PCR foram clonados e dez sequências de cada amostra foram analisadas filogeneticamente juntamente com sequências-referência de HCV, usando o método de neighbor-joining com correção de Kimura 2-p no programa MEGA5. A diversidade das quasiespecies foi calculada por distância par a par das sequências clonais no MEGA5. Dos 12 pacientes, cinco eram portadores do genótipo 1 e sete eram portadores do genótipo 3. Nenhuma evidência de infecções múltiplas ou vírus recombinantes foi encontrada. Pacientes infectados com genótipo 1 apresentaram uma maior diversidade nucleotídica quando comparada àquelas de pacientes infectados pelo genótipo 3 (0,229 ± 0.16 versus 0,200 ± 0,08, p = 0,002).Sabe-se que pacientes infectados com HCV genótipo 1 geralmente têm pior prognóstico comparado com pacientes portadores de outros genótipos. Nossos resultados mostram uma maior diversidade de quasiespecie como um dos possíveis fatores relacionados com tal diferença no desfecho clínico da infecção.

PO13 - GENOTYPE DISTRIBUTION OF HEPATITIS C VIRUS (HCV) BASED ON SAMPLES RECEIVED FROM LABORATORIES FROM SANTA CATARINA, PARANÁ AND SÃO PAULO FROM 2007 TO 2010

Marchi MFS, Bonamigo LF, Mielle Filho A, Remualdo VR
mfsmarchi@gmail.com

Fundação Universidade Regional de Blumenau (FURB), Laboratório Genolab

The Hepatitis C Virus (HCV) is an enveloped, single-stranded RNA virus of the family Flaviviridae and genus Hepacivirus. Until this moment six genotypes have been identified, these genotypes differ from each other an average of 31% and 33%, there also have been established various subtypes of the HCV. Few Brazilian studies have analyzed the frequency of genotypic strains of the HCV, whereas the literature shows that the knowledge of the genotype has an implication in both the duration of the treatment and the prognosis of the disease. We conducted a retrospective, cross-sectional study, using as database records from the Genolab Laboratory, a reference laboratory in genetic analysis, based on blood samples sent by laboratories located in São Paulo, Paraná and Santa Catarina from January, 01, 2007 to December, 31, 2010. The following variables were included in the study: gender, age, place of origin and HCV genotype. Blood samples of 587 patients were reviewed, 287 from São Paulo, 203 from Santa Catarina and 77 from Paraná. We?ve found a higher prevalence of genotype 1 (corresponding to 76,9% of the blood samples from São Paulo, 56,4% of the samples from Santa Catarina and 48,7% of the samples from Paraná), with statistically significant intervals in each state ($p<0.001$), followed then by genotype 3 (19,6% of the blood samples from São Paulo, 42,1% of the samples from Santa Catarina and 43,4% of the samples from Paraná) and in a minor percentage, genotype 2 (3,5% of the blood samples from São Paulo, 1,5% of the samples from Santa Catarina and 3,5% of the samples from São Paulo). The results we have found in this research are consistent with the literature on national HCV genotype dissemination, with a similar proportion of gender and age distribution. However, further studies are essential to a better understanding of the population infected by HCV in order to improve the treatment of these individuals.

P014 - HEPATITE DELTA: SITUAÇÃO DOS COMUNICANTES DE PORTADORES NO MARANHÃO.

Lena Maria Barros Fonseca, Michele Soares Gomes Gouvêa, João Renato Rebello Pinho, Max Diego Cruz Santos, Adna Gesarone Carvalho e Adalgisa de Souza Paiva Ferreira
lenafonsecamaria@ibest.com.br

Universidade Federal do Maranhão - UFMA

Descrever a situação sorológica dos comunicantes familiares dos portadores de co-infecção HBV/HDV identificados no Maranhão. Foram realizados testes sorológicos (HBsAg, anti-HBc total, anti-HBs e anti-HDV) pelas autoridades de vigilância epidemiológica do Estado em 25 comunicantes dos portadores da co-infecção HBV/HDV citados anteriormente. Entre os indivíduos avaliados: 4 (16%) eram HBsAg positivos; destes, 2 (8%) eram anti-HDV positivos, 8 (32%) tinham anti-HBc e anti-HBs positivos, 8 (32%) tinham apenas o anti-HBc positivo e 5 (20%) tinham todos os marcadores negativos. Os dois pacientes com sorologia positiva para HBV e HDV eram provenientes do município maranhense de Urbano Santos. Os resultados confirmam a informação de que há infecção pelo HDV no Maranhão, reforçando a idéia de que deve ser realizado um estudo de base populacional para determinar o estado real da infecção, especialmente na região onde foram descritos a maioria dos casos

P015 - HEPATITE "B": DADOS EPIDEMIOLÓGICOS DA CIDADE DE PELOTAS-RS, NOS ANOS DE 2007 A 2011

Júnior Ramires, O.V.[1]; Gill, P.E.[1]; Vieira, C.O.[2]; Gomes, M.R.R.[3]; Azevedo, A.L.S.[4]
junior-ramires@hotmail.com

[1]Universidade Católica de Pelotas, acadêmico do curso de Ciências Biológicas; [2]Instituto Federal Sul Riograndense, estudante do curso Técnico em Química; [3]Enfermeira da Secretaria Municipal de Saúde de Pelotas; 4Universidade Católica de Pelotas, Professora do curso de Enfermagem.

Descrever os dados epidemiológicos da Hepatite B no municipio de Pelotas-Rs, nos anos de 2007 a 2011. Através de um estudo retrospectivo dos anos de 2007 a 2011 foi analisado dados epidemiológicos de hepatite B, no Sistema de Informação de Agravos de notificação (SINAN), acessados através do Programa de Educação pelo Trabalho (PET-Saúde/VS) da UCPel. No que diz respeito à ocorrência de Hepatite "B" na cidade de Pelotas, em 2007 foram constados 26 casos, já em 2008 foram 30 casos, em 2010 foram 23 casos de Hepatite ?B? e, em 2011, o número de casos notificados, se manteve igual ao de 2010 com 23 casos notificados. O ano com maior número de casos foi em 2009 que tivemos 51 pessoas contaminadas. O detalhamento dessa ocorrência revela 58,0% dos casos pertencentes ao sexo masculino, e o restante (42,0%), ao sexo feminino. Quanto à faixa etária, 3,76% dos casos concentram-se na faixa de RN até 14 anos; 47,37% na de 15 a 34 anos; 30,07% na de 35 a 54; e 18,80 % na de mais de 55 anos. Observou-se que no ano de 2009 a incidência de casos de hepatite B na cidade de Pelotas-RS, foi mais abundante do que nos outros anos, sendo esta de maior ocorrência na faixa etária de jovens e adultos entre 15 a 34 anos. Foi analisado também, que pessoas do sexo masculino tiveram maior n° de casos de Hepatite viral.

PO16 - TRANSIENT ELASTOGRAPHY, ULTRASONOGRAPHY AND VIRAL GENOTYPES IN CHRONIC HEPATITIS B PATIENTS FOLLOWED UP IN SÃO PAULO, BRAZIL – PRELIMINARY RESULTS

Livia de Souza Botelho[1], João Paulo Moreira[1], Denise Paranaguá-Vezozzo[1], Luciana Kikuchi[1], Aline Chagas[1], Regiane Saraiva S. M. Alencar[1], Suzane Kioko Ono[1], Nairo Massazaku Sumita[2], Raymundo Soares de Azevedo[2], João Renato Rebello Pinho[1], Flair José Carrilho[1], **Mónica Viviana Alvarado-Mora[1]***
monica.viviana@usp.br

Departments of Gastroenterology[1] and Pathology[2], University of São Paulo School of Medicine, São Paulo, SP, Brazil

Chronic hepatitis B virus (HBV) infection is the most common cause of hepatocellular carcinoma (HCC) worldwide. HBV is an enveloped, partially double-stranded DNA virus with approximately 3,200 nucleotides that contains four overlapping open reading frames (ORFs). Liver ultrasonography (US) is currently utilized to evaluate the degree of liver diseases from chronic hepatitis B (CHB) and to detect the presence of liver cirrhosis (LC) and HCC. Transient elastography (TE) is another non-invasive method to evaluate the fibrosis stage in clinical settings. As most HCCs arise after LC, it was suggested as the most important risk factor for HBV-related HCC. There is also growing evidence suggesting that viral genotypes may influence the clinical outcome of HBV infection including the risk for HCC. In Brazil, to our knowledge, there are not any previous studies reporting HBV genotypes found in HCC cases. The aim of this study is to characterize the viral genotypes circulating among HCC, LC and CHB patients. Thirty-two HBV/HCC patients and a control group 59 of HCC-free HBsAg positive patients with chronic liver disease were enrolled for this study. Fibrosis stage was determined in the control group using ultrasonography (US) and transient elastography (TE). US was carried out by an experienced clinician to determine the presence of LC. For TE, prediction of advanced fibrosis is presence of liver stifness > 9 kPa, which provided a sensitivity of 79.5% and specificity of 80%. Only patients with LC diagnosis by both methods were considered in our analysis. Statistical analysis was performed using Minitab v15.1.10. For HBV genotyping, a fragment of 1,306 bp partially comprising the DNA polymerase and the HBsAg genes (S/POL) was amplified, sequenced and genotyped by phylogenetical analysis using reference sequences representing each genotype obtained from the GenBank. They were aligned using Muscle software and edited with the SE-AL software. Bayesian phylogenetic analyses were conducted using the Markov Chain Monte Carlo (MCMC) simulation implemented in BEAST v.1.5.3. The maximum credibility tree (MCCT) was constructed with the TreeAnnotator v1.4.8 after discarding 10% of the sampling and then visualized with the FigTree v1.1.2 software. The 59 HCC-free HBsAg positive patients were classified as cirrhotic (n = 39) and non-cirrhotic (n = 20) by US and as cirrhotic (n = 35) and non-cirrhotic (n = 24) by TE. The two methods agreed significantly in the diagnosis of liver cirrhosis in 31 cases [p<0.001]. For the US exam, it was found that there is a statistically significant difference on spleen size (> 20cm) [p <0.001], portal vein caliber (> 12mm) [p <0.001], echogenicity [p = 0.009] and irregular liver surface [p <0.001] between chirrotic and non-cirrhotic patients. HBV DNA was amplified from 12 HCC and 29 non-HCC patients sequences have already been obtained. The frequency of genotypes among HCC patients was A1 (5 - 41.6%), C2 (2 - 16.6%), D3 (2- 16.6%), A2 (1- 8.3%), F2a (1- 8.3%) and D1 (1- 8.3%) and among HBV/HCC-free patients was found A1 (21- 72.4%), D3 (3- 10.3%), F2a (2- 6.9%), C2 (1- 3.4%), B2 (1- 3.4%) and A2 (1- 3.4%). Transient elastography is a promising non-invasive method for detection of cirrhosis in patients with chronic liver disease. Although there is not a statistical significant difference, genotype A1 was more frequent in the HCC-free patients while genotypes C2 and D3 were more frequent in the HCC group. This analysis will be followed with a larger number of patients. FAPESP 2011/50562-0

PO17 - HEPATITIS E VIRUS INFECTION IN KIDNEY TRANSPLANT PATIENTS IN BRAZIL

A.M. Passos[1]*, T.P. Heringer[2], M.L.G. Ferraz[2], C.F.H. Granato
anampassos@gmail.com

[1]Clinical Virology Laboratory, Division of Infectious Diseases, [2]Hepatitis Section, Division of Gastroenterology, Federal University of Sao Paulo, Sao Paulo, Brazil. anampassos@gmail.com

Hepatitis E virus (HEV) infection has recently been added as an emerging cause of chronic hepatitis in organ transplantation. Specific antibody for HEV (anti-HEV) has been found among different population groups in Brazil and a single acute case has been confirmed. However, no cases of acute or chronic HEV infection in immunocompromised patients have yet been investigated or identified, although HEV genotype 3 infection seems common among Brazilian swine livestock. The present study investigated HEV cases among kidney transplant patients in Brazil. Serum samples from 96 kidney transplant patients who presented with unexplained elevations of liver-enzyme levels in an outpatient clinic in Southeastern Brazil were retrospectively analyzed. ELISA and RT-PCR were performed for screening of HEV. A fragment from the HEV ORF2 genome region was subsequently amplified and submitted to direct sequencing using ABI PRISM Big Dye Terminator. N-J algorithm was implemented for the phylogenetic analysis. We identified 3 confirmed cases of hepatitis E. The subjects did not convert to anti-HEV IgG antibody, HEV-RNA was amplified from serum and sequencing analysis classified the strains within genotype 3. The HEV strains isolated from the kidney transplant patients were closely related to a human isolate from China and, in special, to a swine strain previously characterized in Brazil, both genotype 3b. Laboratory tests showed negative results for diagnostic markers of hepatitis A, B and C viruses. No risk factors, neither recent travel to regions endemic for hepatitis were found. Elevated liver enzymes persisted for at least 7 months in one of the cases, characterizing chronic infection. This was the first report of HEV infection in solid-organ transplant recipients in Brazil. The results of this study indicate that kidney transplant patients in Brazil may be at risk of HEV infection and chronic hepatitis E, which should be further investigated as cause of abnormal liver tests of unknown origin in this setting.

PO18 - HIGH PREVALENCE OF HEPATITIS B VIRUS GENOTYPE A1 IN PATIENTS WITH CHRONIC INFECTION FROM PERNAMBUCO STATE, BRAZIL

Izolda Maria Moura[2], Edmundo Lopes[2], João Renato Rebello Pinho[1], Flair José Carrilho[1], **Mónica Viviana Alvarado-Mora[1]***
monica.viviana@usp.br

[1]Laboratory of Tropical Gastroenterology and Hepatology, Institute of Tropical Medicine, Department of Gastroenterology, School of Medicine, University of São Paulo, Sao Paulo-Brazil; [2]Federal University of Pernambuco, Recife - Brazil.

Infection with hepatitis B virus (HBV) is a worldwide health problem, infecting about two billion people, with more than 350 million chronic carriers. HBV has been classified into nine different genotypes, designated from A to I, that represent genetically stable viral populations that share a common ancestor but show diverse evolutionary history. They emerged in specific human populations and migrated with their hosts to other areas in the world, leading to their current geographical distribution. In Brazil, the prevalence of HBV varies throughout the country and is especially high in the North and Northeast regions. Some studies showed that genotypes A, D and F are the most frequent around the country. The aim of this study was to characterize for the first time the HBV subgenotypes circulating in patients with chronic hepatitis B from Pernambuco, a Brazil state located in the Northeast region of the country. We included 68 patients with chronic infection. A fragment of 1306 bp comprising part of the DNA polymerase and the HBsAg (S/POL) was amplified and sequenced. The sequences obtained were genotyped by phylogenetic analysis using reference sequences from each genotype obtained from GenBank (n=267). They were aligned using Clustal X software and edited with the SE-AL software. Bayesian phylogenetic analyses were conducted using the Markov Chain Monte Carlo (MCMC) simulation implemented in BEAST v.1.5.3. The maximum clade credibility (MCC) tree was obtained by summarizing the 10,000 substitution trees and then it was removed 10% of burn-in using Tree Annotator v.1.5.3. The frequency of subgenotype found was A1 (78.7%), F2a (12.1%), A2 (6.2%) and F4 (3.0%). Subgenotype A1 was the most prevalent in this study and most of subgenotype A1 sequences grouped within the same cluster with high posterior probability in the phylogenetic tree. Isolates belonging to subgroup A1 have been mostly identified in African populations and their descendants. This genotype has been reported in several studies related to the presence of Afro-descendants in Brazil. The high prevalence of subgenotype A1 and the results of the phylogenetic analysis strongly suggest that these sequences originated from a unique lineage. This lineage was introduced into this community possibly between XVI and XVII centuries when the slaves came from West Africa. This finding agrees with the origins of Brazilian population, which is a mixture of European-descendants, Indigenous people and African-descendants. FAPESP 2011/50562-0

PO19 - IDENTIFICAR OS ASPECTOS SOROEPIDEMIOLÓGICOS E MOLECULARES DAS INFECÇÕES PELOS VÍRUS DAS HEPATITES B E C EM HSH (TRAVESTIS, TRANSGÊNEROS E GAYS) EM CAMPO GRANDE, MS.

Rezende, G.R.[1]; Zanini, P.B.[1]; Pires, F.R.[1]; Castro-Souza, L.[1]; Stief, A.F.[1]; Pinto, C.S.[3]; Bertolacci, L.[1]; Puga, M.A[1]; Mousquer, G.J.; Castro, L.S.; Bandeira, L.M.; Tanaka, T.S.O.1; Teles, S.A.2; Iglecias L.M.4; Lindenberg A.S.5; **Motta-Castro, A.R.C.[1]**
arcm.castro@hotmail.com

Departamento de Farmácia Bioquímica, Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande-MS; 2 Faculdade de Enfermagem, Universidade Federal de Goiás (UFG), Goiânia, GO, Brasil; Secretaria de Saúde Mato Grosso do Sul3 Laboratório de Análises Clínicas NHU/UFMS4; Centro de Referência em Doenças Infecciosas e Parasitárias-CEDIP5

Identificar os aspectos soroepidemiológicos e moleculares das infecções pelos vírus das hepatites B e C em HSH (travestis, transgêneros e gays) em Campo Grande, MS.Um formulário foi aplicado em 193 HSH, a fim de obter informações sobre comportamentos de risco. As amostras de sangue coletadas foram submetidas à detecção dos marcadores HBsAg, anti-HBc Total e anti-HCV, utilizando imunoensaio enzimático (ELISA) e immunoblot como confirmatório para infecção pelo HCV. Dos 193 HSH investigados, 77,2% não são profissionais do sexo e 22,8% relataram ser. A maioria apresentou pouco ou nenhum conhecimento a respeito das formas de transmissão das hepatites B e C. O uso de droga ilícita foi relatado por 37,3% e 62,7% relataram nunca ter usado. Com relação a idade da primeira relação sexual, 75,6% tiveram a primeira relação antes dos 17 anos de idade, e 24,4% a partir dos 18 anos de idade. O início precoce da vida sexual e o número de parceiros sexuais na vida são elementos relevantes para o risco de aquisição de infecções. Dentre os profissionais do sexo, a maioria (59%) relatou ter tido mais do que 7 clientes na última semana. Quanto ao uso de preservativo, 75% relataram sempre usar com clientes e 25% fazem uso irregular. A prevalência global da infecção pelo HBV foi de 16,2% (IC 95%: 10,9?21,4) com índice de 1,0% (IC 95%: 0,6? 1,5) para o HBsAg. Em 30,8% dos participantes foi encontrado anti-HBs isolado, marcador de vacinação prévia contra hepatite B, indicando baixo índice de cobertura vacinal. A prevalência do anti-HCV foi de 1,6% (IC 95%: 1,0?2,1). Estes resultados preliminares indicam a necessidade de implementação das políticas de prevenção, promoção e atenção integral à saúde a essa população de difícil acesso.

P020 - IDENTIFICATION OF NOVEL RECOMBINANTS OF HEPATITIS B VIRUS GENOTYPES F AND G IN HUMAN IMMUNODEFICIENCY VIRUS-POSITIVE PATIENTS FROM ARGENTINA AND BRAZIL

Natalia M. Araujo[1], Oscar C. Araujo[1], Cristiane A. Villela-Nogueira[2], Letícia C. Nabuco[2], Edinete M. Silva[3], Raymundo Parana[4], Fernando Bessone[5], Selma A. Gomes[1], Alan Kay[3]
nmaraujo@ioc.fiocruz.br

[1]Laboratory of Molecular Virology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. [2]Hepatology Division, Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. [3]Centre de Recherche en Cancérologie de Lyon, INSERM U 1052 /CNRS UMR 5286, Lyon, France. [4]Department of Hepato-Gastroenterology, Prof. Edgard Santos University Hospital, Federal University of Bahia, Salvador, Brazil. [5]Department of Gastroenterology and Hepatology, University of Rosario School of Medicine, Rosario, Argentina.

Hepatitis B virus (HBV) genotype G (HBV/G) infection is almost always detected along with a co-infecting HBV strain that can supply HBeAg, typically HBV/A. In this study, we describe in two human immunodeficiency virus (HIV)-positive patients from Argentina and Brazil the first report of HBV/G infection in Argentina and co-circulation of HBV/G, HBV/F and G/F recombinants in the Americas.Full-length genomes and precore/core genomic region were amplified by PCR and cloned into pUC19 and pCR4 vectors, respectively. Clones were screened by colony hybridization using a radioactive probe specific for the 36-bp insertion of HBV/G. Several clones were sequenced to determine the complete genomic sequences. HBV isolates carrying the 36-bp insertion were the most prevalent in both patients, since more than 99% of colonies hybridized to the probe specific for this insertion. Phylogenetic analyses of full-length genomes and precore/core fragments revealed that F4 and F1b were the co-infecting subgenotypes in the Brazilian and Argentinian patients, respectively. Moreover, bootscanning analysis provided evidence of recombination in several clones from both patients, with recombination breakpoints located mainly at the precore/core region. These data should encourage further investigations on the clinical implications of HBV/G recombinants in HBV/HIV co-infected patients.

PO21 - INFECÇÃO PELO VÍRUS DA HEPATITE B EM MULHERES PROFISSIONAIS DO SEXO EM CAMPO GRANDE, MATO GROSSO DO SUL

Gina JonassonMousquer[1]; Paula Guerra Murat[1]; Grazielli Rocha de Rezende[2]; Fernanda Rodas Pires[1]; Lisie Souza Castro[1]; Marco Antônio Moreira Puga[1]; Tayana Serpa Ortiz Tanaka[1]; Danilo Yamamoto Thomaz[1]; Sheila Araújo Teles[3]; **Ana Rita Coimbra Motta-Castro**[1].
arcm.castro@hotmail.com

Departamento de Farmácia Bioquímica, Universidade Federal de Mato Grosso do Sul (UFMS)1, Campo Grande-MS; [2]Hospital Dia, NHU/UFMS, Campo Grande, MS; [3]Universidade Federal de Goiás (UFG).

Este estudo teve como objetivo estimar a prevalência da infecção pelo HBV e identificar comportamentos associados ao risco de infecção em mulheres profissionais do sexo em Campo Grande, MS, entre novembro de 2009 a dezembro de 2010. Para seleção da amostra foi utilizada a técnica Respondent-drivensampling. As 402 participantes foram submetidas à entrevista e coleta de amostras sanguíneas para detecção dos marcadores sorológicos HBsAg, anti-HBs e anti-HBc total utilizando imunoensaio enzimático (ELISA). Além disso, foi administrada a vacina contra a hepatite B utilizando os esquemas acelerado (0, 1 e 2 meses) ou convencional (0, 1 e 6 meses). A idade mediana das participantes investigadas foi de 25 anos, a maioria sem parceiro fixo (86,2%) e com 5 a 9 anos de estudo (54,5%). O consumo de álcool foi relatado por 88,5% das prostitutas e 68,6% possuíam tatuagem/bodypiercing. A idade média da primeira relação sexual foi de 15 anos, a maioria (87,7%) relatou fazer uso regular de preservativo com os clientes e 54,9% relataram ter até sete clientes por semana. A prevalência global para a infecção pelo vírus da hepatite B foi de 9,3% (IC: 95%: 5,3 ? 13,9) e positividade de 0,7% (IC: 95%: 0,6 ? 2,5) para o HBsAg. Foi encontrada uma baixa cobertura vacinal (29,6%) nessa população e 61,5% (247/402) das mulheres profissionais do sexo estudadas eram suscetíveis para a infecção pelo HBV. Com o intuito de avaliar a adesão e resposta vacinal contra hepatite B, 230 mulheres profissionais do sexo foram vacinadas utilizando os esquemas acelerado e convencional. Quanto à adesão vacinal, das 230 que receberam a primeira dose, somente 82 (35,7%) receberam o esquema vacinal completo. Os achados soroepidemiológicos indicam que medidas preventivas, como ações de educação em saúde e de vacinação contra hepatite B, são necessárias para o controle e prevenção desta infecção na população estudada.

PO22 - MANIFESTAÇÕES DERMATOLÓGICAS NUMA CASUÍSTICA DE DOENÇA HEPÁTICA, EM UM HOSPITAL DE REFERÊNCIA, BELÉM, PARÁ, BRASIL: RESULTADOS PRELIMINARES

Heloisa Marceliano Nunes, Manoel do Carmo Pereira Soares, Naiara Chaves Maia, Fernando Costa Araújo, Simone Regina Souza da Silva Conde, Carla Andrea Avelar Pires, Olglaize do Socorro da Costa Souza Elizabete Maria de Figueiredo Brito
heloisanunes@iec.pa.gov.br

Instituto Evandro Chagas, Fundação Santa casa de Misericórdia do Pará, Universidade Federal do Pará

Detectar e descrever as principais lesões dermatológicas, numa casuística de doença hepática em hospital de referência para doenças do fígado, Belém, Pará, Brasil. Entre outubro e dezembro de 2011, pacientes atendidos no ambulatório de Fígado da Fundação Santa Casa de Misericórdia do Pará, diagnosticados com doença hepática, virgens de tratamento, foram individualmente examinados por médico dermatologista. Após leitura e assinatura de Termo de Consentimento Livre e Esclarecido, houve a documentação dos achados de interesse clínico, dermatológico e laboratorial, utilizando roteiro de investigação previamente elaborado para a pesquisa, imagens fotográficas, amostras de soro coletadas e analisadas para os marcadores sorológicos das hepatites virais. O projeto foi aprovado por Comitê de Ética e Pesquisa em Seres Humanos (CAAE- 0034.0.072.000-11). Foram incluídos no estudo 22 indivíduos, com frequência de 59,1% para o gênero feminino. As hepatopatias mais prevalentes foram: hepatite C em 45,45% (10/22); esteatohepatite em 13,63% (3/22); esteatohepatite com cirrose, hepatite B e cirrose biliar primária apresentaram individualmente prevalência de 9,09%; cirrose alcoólica, hepatomegalia e esplenomegalia com ascite foram encontradas em 4,54% (1/22) dos casos, respectivamente. As

lesões dermatológicas mais significativas estiveram presentes em 86,36% (19/22) dos examinados, entre esses 63,15% (12/19) apresentavam duas ou mais lesões associadas, 21,05% mostraram alterações de unhas e 5,26% (1/19) alterações de pelos. As lesões de pele mais prevalentes foram xerose intensa e telangiectasia, encontradas em 36,84% (7/19) e 26,31% (5/19), respectivamente. Entre os 19 examinados também foram detectados dois casos (10,52%) de ceratose seborréica e tipo Felt; dois casos (10,52%) de hanseníase, de herpes simples e de úlceras cutâneas e um caso de Síndrome de Sjögren, de vitiligo, granuloma, neurofibroma, ceratose actínea e candidíase cutânea. Dois pacientes apresentaram distrofia ungueal e dois de onicomicose. A foliculite foi encontrada em 5,26% (1/19) dos examinados.Alterações dermatológicas primárias ou secundárias podem influenciar no diagnóstico e no manejo clínico da doença hepática.

PO23-MOLECULAR ANALYSIS OF HEPATITIS B VIRUS AND HEPATITIS C VIRUS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA.

Oscar C. Araujo[1], José J.F. Barros[1], Kycia M.R. Ó[2], Letícia C. Nabuco[2], Marcia T.B. Moares[1], Cristiane A. Villela-Nogueira[2], Natalia M. Araujo[1].
araujo.orc@gmail.com

[1]Laboratory of Molecular Virology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. [2]Hepatology Division, Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Hepatocellular carcinoma (HCC) is globally the fifth most common cancer in men and the eighth in women. Due to the poor prognosis, HCC ranks as the third leading cause of cancer mortality worldwide. The major risk factors for developing HCC are chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Several mutations in these viruses have been associated to hepatocarcinogenesis. In this study, we describe the clinical and epidemiological profile of patients diagnosed with HCC, and investigate the prevalence and genomic features of HBV-DNA and HCV-RNA. At this moment, 47 patients with HCC of any etiology from the University Hospital Clementino Fraga Filho were enrolled in the study. A database was made using a questionnaire containing demographic, epidemiological and clinical data of patients. HBV-DNA and HCV-RNA were extracted and Pre-S/S and X/precore/core (HBV) and core (HCV) regions were amplified by PCR and RT-PCR, respectively. Nucleotide sequences were determined and phylogenetic analysis was conducted using MEGA version 4.1. The median age of the patients was 65 (39-84 years old; 59.6% male). Cirrhosis was present in 87.2% of the patients. HCV was the main etiology (70.2%), followed by HBV (17%). Alcoholism and NASH accounted for 14.9% each. HBV-DNA was detected in 7 of 8 (87,5%) HBsAg positive samples and in 4 of 39 HBsAg negative samples, indicating a prevalence of 10,3% of occult HBV infection. Up to now, four HBV samples were genotyped and all of them were genotype A (three subgenotype A1 and one A2). Pre-S deletions were found in 25% (1/4) of the samples, and A1762T and G1764A mutations in 100% (2/2). The G1896A mutation, which has been associated with a reduction in the risk of HCC, was not found in two analyzed samples (0/2). HCV-RNA was detected 31 of 33 (93.9%) anti-HCV positive samples. Sequencing analysis for HCV genotyping and evaluation of mutations previously associated to HCC (amino acids 70 and 91 in the core region) are in progress. Specific mutations in HBV and HCV isolates may be useful biomarkers of disease progression and early HCC detection.

PO24 - NOTIFICAÇÕES DE CASOS DE "HEPATITE C" NA CIDADE DE PELOTAS-RS NOS ANOS DE 2007 A 2011.

Júnior Ramires, O.V.[1]; Gill, P.E.[1]; Vieira, C.O.[2]; Gomes, M.R.R.[3]; Azevedo, A.L.S.4
junior-ramires@hotmail.com

[1]Universidade Católica de Pelotas, acadêmico do curso de Ciências Biológicas; [2]Instituto Federal Sul Riograndense, estudante do curso Técnico em Química; [3]Enfermeira da Secretaria Municipal de Saúde de Pelotas; 4Universidade Católica de Pelotas, Professora do curso de Enfermagem.

O objetivo deste trabalho, é determinar os dados epidemiológicos de Hepatite "C" no municipio de Pelotas-RS nos anos de 2007 a 2011. O trabalho foi realizado através de dados obtidos no Sistema de Informação de Agravos de Notificação (SINAN), no qual foi possivel a realização de um estudo retrospectivo de casos notificados de Hepatite C. Na cidade de Pelotas-RS, foram contatados 77 casos de Hepatite C no ano de 2007, já em 2009 foram 104 casos, e em 2010 88 casos e em 2011 65 casos. O ano com maior número de casos foi em 2008 que tivemos 118 casos de pessoas contaminadas com o vírus. A pesquisa também revela que 58% pertencem ao sexo masculino e 4% ao sexo feminino. Quanto a faixa etária, 17,20% dos casos encontra-se na faixa de 15 a 34 anos, 51,55% na faixa de 35 a 54 anos, e 31,25% na faixa etária de maiores de 55 anos. Não obteve casos notificados na faixa etária de RN a 14 anos. Hepatite C foi detectada em maior número no ano de 2008, a mesma obteve uma ocorrência significativa em pessoas de faixa etária entre 35 a 54 anos. Pessoas do sexo masculino demonstraram uma frequência mais elevada de casos da doença.

PO25 - NOTIFICAÇÕES DE CASOS EPIDEMIOLÓGICOS DE PACIENTES CO-INFECTADOS COM O VÍRUS HIV/AIDS E HEPATITE A, B E C, NA CIDADE DE PELOTAS-RS NOS ANOS DE 2007 A 2011.

GILL, P.E.[1]; JÚNIOR RAMIRES, O.V.[1]; VIEIRA, C.O.[2]; GOMES, M.R.R.[3]; AZEVEDO, A.L.S.[4]
pablu_espinosa@hotmail.com

[1]Universidade Católica de Pelotas, acadêmico do curso de Ciências Biológicas; [2]Instituto Federal Sul Riograndense, estudante do curso Técnico em Química; [3]Enfermeira da Secretaria Municipal de Saúde de Pelotas; 4Universidade Católica de Pelotas, Professora do curso de Enfermagem.

Este trabalho tem por objetivo descrever os dados epidemiológicos da cidade de Pelotas-RS, nos anos de 2007 a 2011 de pessoas contaminadas com o virus HIV/AIDS, que também são portadoras dos virus A, B e C da Hepatite. Este trabalho foi realizado na Secretaria Municipal de Saúde de Pelotas-RS, através do Programa de Educação pelo Trabalho (PET-Saúde/VS) da UCPel, e foi realizado um estudo retrospectivo com dados obtidos através dos registros do Sistema de Informação de Agravos de Notificação (SINAN). Nos anos de 2007 a 2011, foi possivel a constatação

de que o virus da Hepatite A, foram 289 notificações, sendo que 6 desses casos foram confirmados com HIV/AIDS. No virus da Hepatite B, foram 133 notificações, sendo que 8 delas também foram confirmados com HIV/AIDS. Já no virus da Hepatite C foram 452 notificações, com 146 casos confirmados com o virus HIV/AIDS e 306 descasrtados. Verificou-se que pessoas infectadas pelo virus HIV/AIDS, nos anos de 2007 a 2011 na cidade de Pelotas-RS, apresentaram maior incidência de Hepatite C do que as demais analisadas. Sendo que é bastante significativa a relação entre individuos contaminados com ambos os vírus.

PO26 - PADRONIZAÇÃO DE TÉCNICAS PARA QUANTIFICAÇÃO E GENOTIPAGEM DO VÍRUS DA HEPATITE B

GUSATTI, Carolina de Souza[1,2]; COSTI, Cintia[2]; **GRANDI, Tarciana[1,2]**; SILVEIRA, Carolina[1]; VAINSTEIN, Marilene Henning[1]; SILVA, Márcia Susana Nunes[2]; ROSSETTI, Maria Lucia Rosa[1,2]
tarcianagrandi@gmail.com

[1]Programa de Pós-Graduação em Biologia Celular e Molecular, Universidade Federal do Rio Grande do Sul (UFRGS); [2]Centro de Desenvolvimento Científico e Tecnológico, Fundação Estadual de Produção e Pesquisa em Saúde (CDCT/FEPPS)

A hepatie B é uma doença infecto-contagiosa que tem se transformado em um grande problema de saúde publica devido sua vasta distrbuição geográfica e disseminação mundial. Atualmente, estima-se que 350 milhões de pessoas em todo mundo sejam portadores crônicos do vírus da hepatite B (HBV) e que destes, aproximadamente 2% morram, anualmente, das consequências desta doença. Os níveis de carga viral e, em menor escala, o genótipo viral podem ser importantes fatores a serem considerados no inicio do tratamento para hepatite B. Diante disso, o objetivo deste trabalho foi o desenvolvimento de testes moleculares para a detecção/determinação da carga viral do HBV, utilizando PCR em Tempo Real e a genotipagem viral, através de sequenciamento de DNA.

Até o momento, este trabalho encontra-se em fase de padronização das técnicas empregadas. Foi realizada a construção de iniciadores e de sonda para a quantifcação por PCR em tempo real, baseando-se no alinhamento de sequencias depositadas no GenBank (NCBI) e de sequêcias já genotipadas do Rio Grande do Sul (Becker et al., 2010), utilizando os programas ClustalX e BioEdit. O consenso formado foi usado no desenho dos iniciadores/sonda (TaqMan®MGB), utilizando o programa PrimerExpress. Os iniciadores resultantes foram: HBVQF: 5'-TTG TCC TGG YTA TCG YTG GAT GTG-3' e HBVQR: 5'- GAT GAG GCA TAG CAG CAG GAT G-3', que amplificam um produto de 72 pb e a sonda: 6-FAM TGCGGCGTTTTATCAT MGB NFQ. O desenvolvimento de padrões, úteis na quantificação, foi realizado por técnica de clonagem, utilizando parte do gene S viral (genótipo D, prevalente no Rio Grande do Sul) inserido-o no plasmídeo pUC18 e transformando-o em E. coli competentes via eletroporação. Para a genotipagem, os iniciadores selecionados foram HBMF2: 5?-GTC TAG ACT CGT GGT GGA CTT CTC TC-3? e HBMR2: 5?-AAG CCA NAC ART GGG GGA AAG C-3?, que amplifcam um fragmento de 485 pb correspondente a parte do gene S viral.Os teste empregados terão sua acurácia verificada pela comparação com métodos de referência. Após a conclusão desta fase do trabalho, estas metodologias poderão ser ferramentas úteis no auxílio do início e monitoramento do tratamento de portadores crônicos de hepatite B. Apoio: Capes, PADCT 2010/FEPPS.

PO27 - PADRONIZAÇÃO E IMPLANTAÇÃO DA TÉCNICA DE PCR EM TEMPO REAL PARA O DIAGNÓSTICO DA HEPATITE B

Ana Paula de Torres Santos[1,2] , José Eduardo Levi[3], Isabel Takano Oba[1],Samira Julien Calux[1], Angela Maria Miranda Spina[1], Marcilio Figueiredo Lemos[1], & Regina Célia Moreira [1]
anapaula.ts@bol.com.br

1.Núcleo de Doenças Sanguíneas e Sexuais do Centro de Virologia do Instituto Adolfo Lutz. 2.Laboratório Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. 3.Laboratório de Virologia do Instituto de Medicina Tropical da Faculdade de Medicina da Universidade de São Paulo.

O objetivo desse trabalho foi padronizar a técnica de amplificação quantitativa em tempo real (PCR em tempo real) do DNA do vírus da Hepatite B; comparar a técnica com métodos comerciais e implantar a técnica como rotina de diagnóstico no laboratório de Hepatites do Centro de Virologia do Instituto Adolfo Lutz. A população de estudo foi constituída por 171 amostras de soro analisadas pelo teste comercial Amplicor® Roche, provenientes de diferentes regiões do Estado de São Paulo, com resultados que variaram de 60 a 2,59X108 UI/mL, estocadas a -20°C, no período de 2009 a 2011. Os genótipos foram inicialmente caracterizados, e nossa casuística foi constituída por genótipos A (51), C (7), D (65) e F(10) 38 amostras sem genótipo do HBV. Para o controle da quantificação, uma curva padrão foi contruída em todas as reações, utilizando-se padrões internacionais produzidos pela Organização Mundial de Saúde, em diluições seriadas. Para a extração do DNA das amostras e do padrão, foi utilizado kit comercial (QIAGEN®), seguindo procedimentos do fabricante. Os testes foram realizados empregando-se o método do TaqMan PCR, em um equipamento ABI 7300 (Applied Biosystems, Foster City, CA). Foram testados três conjuntos de primers e sondas e o escolhido foi o descrito por DROSTEN et al. (2000), por apresentar melhores resultados. A técnica padronizada apresentou resultados satisfatórios quando comparada com o método comercial utilizado (IC Pearson=0,61). Foi possível identificar amostras com valores baixos de carga viral, o teste mostrou 100% de especificidade, além de apresentar ótima performance na detecção de todos os genótipos. Outras análises estatísticas são necessárias para a sua efetiva implantação na rotina diagnóstica em nosso laboratório. Projeto FAPESP n°09/53086-4.

PO28 - SIMULTÂNEA DETECÇÃO, CARACTERIZAÇÃO GENOTÍPICA E QUANTIFICAÇÃO DA CARGA VIRAL DO VÍRUS DA HEPATITE B (VHB) POR SEQUENCIAMENTO E PCR EM TEMPO REAL EM AMOSTRAS PROCEDENTES DA AMAZÔNIA OCIDENTAL

Holanda, SCN[1]; Braga, WSM[2]; Castilho, MC[2]; Oliveira, CMC[2]
sanmile_cristina@hotmail.com

1-Universidade do Estado do Amazonas – UEA; 2-Fundação de Medicina Tropical do Amazonas - FMTAM/HVD

Neste trabalho propomos detectar e identificar os genótipos do VHB, pelas técnicas de PCR convencional e seqüenciamento direto, respectivamente e determinar a carga viral pela PCR em tempo real baseado no sistema TaqMan. Foram analisadas amostras de pacientes HBsAg positivos, procedente da Amazônia Ocidental brasileira. A extração de DNA foi realizada utilizando Qiamp DNA Blood Mini Kit. O DNA foi amplificado simultaneamente pela PCR convencional e PCR em tempo real. Na PCR semi-nested convencional amplificou-se um fragmento de 1200 e 600pb, respectivamente e na PCR em tempo real um fragmento de 220pb, correspondentes as regiões pré-S1/S2 e S do genoma VHB. Fragmentos de 600pb foram seqüenciados, utilizando Big Dye Terminator Cycle Sequencing Kit, v3.1 no ABI 3130 xL. O alinhamento das sequencias foi realizado no programa BioEdit e a análise filogenética no MEGA 5.05. Concomitantemente foi feita à construção de uma curva padrão externa para o procedimento de quantificação de carga viral do VHB por PCR em tempo real. Foi analisado um total de 62 amostras. 58% (36/62) foram DNA-VHB positivas. De 22/36 (61,1%) seqüências analisadas, o genótipo A representou 68,1%, seguido de 27,2% do genótipo F e 4,5% genótipo D. A amplificação do DNA-VHB foi possível em 36 (58%) das amostras analisadas. Os 26 (42%) pacientes cujo DNA-VHB não foi detectável, provavelmente apresentavam baixa carga viral, pois 10 (16,1%) pacientes, eram co-infectados com o vírus da hepatite D (VHD) e 2 (3,2%) com HIV. De acordo com a literatura, o genótipo A é o mais prevalente no Brasil e o genótipo F na Amazônia, porém, nosso estudo evidencia a prevalência do genótipo A na região.

PO29 - THE INFLUENCE OF HOST GENETIC DIVERSITY IN PATIENTS WITH CHRONIC HEPATITIS C AND THE SPONTANEOUS VIRAL CLEARANCE.

Juliene Antonio Ramos[1,2]; **Fabio Braz Leão[1]**; Luisa Hoffmann[2]; Elizabeth Valentin de Souza[2], Ana Lucia Ramos[3]; Cristiane Vilella Nogueira[3]; Turan Peter Ürményi[2]; Rosane Silva[2]; Edson Rondinelli[1,3].
fbleao@hotmail.com

[1]Instituo Federal de Educação Ciência e Tecnologia do Rio de Janeiro, IFRJ. [2]Laboratório de Metabolismo Macromolecular Firmino Torres de Castro, Instituto de Biofísica Carlos Chagas Filho da UFRJ. [3]Departamento de Clínica Médica da Faculdade de Medicina da UFRJ.

Hepatitis C is a health problem in Brazil with 3 million people infected. The evolution of the infection and the response to treatment varies according to HCV quasispecies and/or host genetic factors. The aim of this work is to investigate the role of the polymorphisms in the cytokines genes. We evaluated the influence of host genetic diversity in the response to treatment in patients with chronic hepatitis C and the spontaneous viral clearance. The diversity of the host was studied by analysis of SNPs in cytokine genes related to TH1 (IFN-γ, TNF-α), TH2 (IL-4, IL-10) response and the activation of the antiviral state (IL-28B) in patients with chronic hepatitis C and in patients with spontaneous viral clearance. In the study of genetic diversity of the host, we found that the polymorphisms rs8099917 and rs12979860 of the gene for IL-28B were associated with treatment response in patients with HCV and with spontaneous viral clearance. We also found in patients with spontaneous viral clearance the difference in genotype distribution for the polymorphisms of -1082 IL-10 and +33 of the IL-4 when compared with patients who progressed to chronicity. Thus, we can see the host sides can influence the response to treatment and that genetic polymorphisms in cytokine genes are associated with response to treatment and spontaneous viral elimination..

PO30 - PERFORMANCE EVALUATION OF AUTOMATED REAL TIME HBV QUANTIFICATION ASSAY OF ROCHE COBAS AMPLIPREP/COBAS TAQMAN (CAP/CTM) AND ABBOTT M2000 COMPARED TO AN "IN-HOUSE" METHOD CARRIED OUT AT ALBERT EINSTEIN HOSPITAL

Nair H Muto, Gregorio TF Dastoli, Juliana NM Rodrigues, Ozires PS Ramos, João Renato R Pinho, Roberta Sitnik.

Molecular Diagnostic Department, Clinical Laboratory, Albert Einstein Hospital, SP, Brazil

Hepatitis B virus (HBV) is a member of the Hepadnaviridae virus family which can cause acute or chronic hepatitis. According to World Health Organization, an estimated two billion people have been infected with HBV worldwide and more than 350 million have chronic long-term liver infection. Molecular diagnostic assays for the accurate detection and quantification of HBV DNA are very important for patient management. The Molecular Pathology Department at Albert Einstein Hospital has provided an "in-house" Real Time PCR assay for HBV viral load for 5 years. However, due to the increasing demand for HBV molecular tests in our laboratory, there was a need to replace our method by an automated process with similar or better performance. Two automated systems (Roche Cobas Ampliprep/Cobas Taqman - CAP/CTM and Abbott m2000 HBV test) were evaluated regarding accuracy, linearity and intra and inter-assay precision. Accuracy was tested comparing the results of 35 samples previously quantified by our method and resulted in a log difference of 0.33 and 0.5 log for Abbott and CAP/CTM, respectively. Linearity was determined using dilution series of a high viral load sample, while intra and inter-assay variations were determined using three pools of samples (high, medium and low) and a negative plasma sample obtained from the blood bank. The regression line of linearity presented an excellent correlation of R=0.996 for CAP/CTM and R=0.979 for Abbott, and both systems had an intra and inter-assay precision with coefficient of variation less than 10%, as expected. Based on the results obtained, our evaluations demonstrated that both systems had a great performance and are able to replace our manual method.

PO31 - ANALYSIS OF RESULTS FROM AUTOMATED REAL TIME HCV QUANTIFICATION ASSAYS (ROCHE COBAS AMPLIPREP/COBAS TAQMAN AND ABBOTT M2000) COMPARED TO COBAS TAQMAN 48

Nair H Muto, Juliana NM Rodrigues, Gregorio TF Dastoli, Vanessa FD Castro, Ozires PS Ramos, João Renato R Pinho, Roberta Sitnik.

Molecular Diagnostic Department, Clinical Laboratory, Albert Einstein Hospital, SP, Brazil

Hepatitis C is an infectious disease caused by the Hepatitis C virus (HCV), which mainly affects the liver. About 80% of exposed patients develop a chronic infection. For those patients, HCV treatment protocols require viral load monitoring for antiviral therapy management. Therefore, the quantification of HCV viral load represents a key parameter for therapy evaluation and different standardized quantification assays are commercially available. According to Matsuura et al (2009), the automated Roche Cobas Ampliprep/Cobas Taqman (CAP/CTM) and Abbott m2000 HCV methods are considered to be more effective at predicting sustained viral response compared to the manual Cobas Amplicor HCV Monitor test, v.2.0, thus providing a higher clinical value for the management of therapeutic responses to chronic hepatitis C. In order to improve the quality of HCV test provided by our laboratory, we evaluated the performance of automated systems CAP/CTM and Abbott m2000 in comparison with the Cobas TaqMan 48 which was routinely used in our service. We had previously replaced the Cobas Amplicor Monitor by Cobas TaqMan 48 which allows automated Real-Time amplification and detection, but still needs a manual extraction. The analysis of the automated processes CAP/CTM and Abbot m2000 included linearity, intra and inter-assay precision and accuracy. Linearity was determined using dilution series of a high viral load sample, while intra and inter-assay variations were determined using three pools of samples (high, medium and low) and a negative plasma sample obtained from the blood bank. The regression linearity showed an excellent correlation of R=0.997 for CAP/CTM and R=0.999 for Abbott and both systems had an intra and inter-assay precision with coefficient of variation less than 10%, as expected. Accuracy was established using 30 serum samples previously quantified by Cobas TaqMan. The mean of the log differences obtained for CAP/CTM and Abbott m2000 was 0.05 log and 0.29 log. Considering that Cobas TaqMan and CAP/CTM method are provided by the same manufacturer, we expected a better accuracy value for CAP/CTM but, in our hands, the performance of the three methods was similar. Based on these results, the use of fully automated system appears as a good option, as one can obtain the same results but with less handling when compared to the other system that needed sample manual extraction.