Antiviral Therapy 2013; **18:**541–548 (doi: 10.3851/IMP2598)

Review

An update on HDV: virology, pathogenesis and treatment

Mónica V Alvarado-Mora[1], Stephen Locarnini[2], Mario Rizzetto[3], João R Rebello Pinho[1]*

[1]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, Brazil
[2]Research & Molecular Development, Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia
[3]Division of Gastroenterology, University of Torino, Torino, Italy

*Corresponding author e-mail: monica.viviana@usp.br

Hepatitis delta is an inflammatory liver disease caused by infection with HDV. HDV is a single-stranded circular RNA pathogen with a diameter of 36 nm. HDV is classified in the genus *Deltavirus* and is still awaiting a final taxonomic classification up to the family level. HDV shares similarities with satellite RNA and viroids including a small circular single-stranded RNA with secondary structure that replicates through the 'double rolling circle' mechanism. The HDV RNA genome is capable of self-cleavage through a ribozyme and encodes only one structural protein, the hepatitis delta antigen (HDAg), from the antigenomic RNA. There are two forms of HDAg, a shorter (S; 22 kDa) and a longer (L; 24 kDa) form, the latter generated from an RNA editing mechanism. The S form is essential for viral genomic replication. The L form participates in the assembly and formation of HDV. For complete replication and transmission, HDV requires the hepatitis B surface antigen (HBsAg). Thus, HDV infection only occurs in HBsAg-positive individuals, either as acute coinfection in treatment-naive HBV-infected persons, or as superinfection in patients with pre-existing chronic hepatitis B (CHB). HDV is found throughout the world, but its prevalence, incidence, clinical features and epidemiological characteristics vary by geographic region. There are eight genotypes (1 to 8) distributed over different geographic areas: HDV-1 is distributed worldwide, whereas HDV-2 to 8 are seen more regionally. Levels of HDV viraemia change over the course of HDV infection, being significantly higher in patients with early chronic hepatitis than in cirrhosis. Chronic HDV infection leads to more severe liver disease than chronic HBV monoinfection with an accelerated course of fibrosis progression, an increased risk of hepatocellular carcinoma and early decompensation in the setting of established cirrhosis. Current treatments include pegylated interferon-α and liver transplantation; the latter of which can be curative. Further studies are needed to develop better treatment strategies for this challenging disease.

Introduction: the discovery of HDV

HDV was discovered in the mid-1970s by Mario Rizzetto and colleagues [1], who originally reported the discovery of a new antigen-antibody system in the livers of patients with chronic hepatitis B (CHB) using immunofluorescence and named it the hepatitis delta antigen. Its staining pattern was similar to hepatitis B core antigen (HBcAg) expression in hepatocytes in patients with CHB and it was initially believed to be another component of the HBV. Several years later, subsequent studies involving experimental inoculation of chimpanzees proved that this hepatitis delta antigen was in fact a component of an HBV-dependent virus [2,3], and was renamed HDV.

Classification of HDV

HDV does not resemble any other animal virus described to date. Nevertheless, there are similar infectious agents in plants, such as the viroids, virusoids and satellite RNAs viruses. Like the satellite RNA viruses of plants, HDV is coated by the envelope containing proteins derived from another virus that acts as a helper virus, and in this case, it is the hepatitis B surface antigen (HBsAg) of the HBV. HDV shares several genomic similarities with satellite RNA and viroids. The HDV RNA genome is a small circular single-stranded RNA molecule with secondary structure characteristic of agents

that replicate through the 'double rolling circle' mechanism, via linear intermediates of multimeric lengths [4]. The genomic length of HDV varies from 1672 to 1694 nucleotides and is a single-stranded circular RNA molecule, that forms a rod-like structure with at least 70% base-pairing. The HDV genome encodes only a single structural protein, the hepatitis delta antigen (HDAg). Currently, HDV is classified in the genus *Deltavirus*, but still awaiting a final taxonomic classification up to the family level [5,6].

Life cycle and replication of HDV

To replicate efficiently, HDV requires host cell elements in all stages of the replicative cycle: attachment, penetration, uncoating, viral protein synthesis, virion assembly and release. HDV also requires the presence of a helper *Hepadnavirus* to provide the protein components for its own envelope. The infectivity of HBV or HDV particles is dependent on the presence of L-HBsAg in the viral envelope. The L-HBsAg bears a receptor-binding domain within the N-terminal pre-S1 moiety, which is myristoylated at glycine 2, and this modification is indispensable for virion infectivity. Effective viral entry also requires the cysteine-rich antigenic loop within the S domain of the three-envelope proteins [7].

Following entry into the hepatocyte, viral uncoating exposes a signal within the HDAg that results in its translocation to the nucleus. The HDV RNA genome is capable of self-cleavage through its ribozyme, an event that plays an essential role in the viral life cycle [8]. The structural and functional characteristics of this enzymatic activity reveal that the HDV ribozyme belongs to a separate class of ribozymes [9].

HDV genomic replication involves three steps: RNA synthesis, RNA cleavage and RNA ligation. The first step generates oligomeric RNAs that result from the reiterative transcription of the circular templates of both polarities and is catalysed by host DNA-dependent RNA polymerases that are redirected to transcribe RNA templates. Replication of HDV RNA occurs in the nucleus through a symmetric double rolling circle mechanism with self-cleavage catalysed by ribozymes of a unique class; these ribozymes also operate *in vivo* co-transcriptionally and are stimulated by a specific RNA chaperone: the HDAg. Cleavage is mediated by *cis*-acting ribozymes: HDV harbours two structurally related self-cleaving ribozymes in its genome, one in the genomic strand and one in the antigenomic strand, contained in sequences of 85 nucleotides, which self-cleaves the linear HDV RNA into monomers. Ligation appears to be catalysed by host enzymes [10]. HDAg may modulate the self-cleaving activity of HDV RNA, but this function is dispensable, that is, the ribozyme can work perfectly without HDAg. Replication most likely involves many other non-catalytic proteins of host origin as well as the virus-encoded protein HDAg. In this process, which occurs in two steps to both the genomic and anti-genomic strands, the circular molecule of RNA is copied several times, resulting in a long strand containing multiple copies of RNA complementary to the original molecule, which is then subsequently cleaved into each of their individual copies [4,10,11].

From this rolling circle model, three forms of RNA are made: circular genomic RNA, circular antigenomic RNA and a linear polyadenylated antigenomic RNA of 0.8 kb, which is the messenger RNA containing the open reading frame of the HDAg. Evidence suggests that synthesis of the different RNA species occurs in different subcellular locations, mediated by distinct cellular polymerases: synthesis of antigenomic RNA occurs in the nucleolus mediated by RNA polymerase I, whereas synthesis of genomic RNA takes place more diffusely in the nucleoplasm by RNA polymerase II [12].

Structure of HDV

The viral particles of HDV are a little larger than the HBsAg subviral particles, being approximately 36 nm in diameter. The buoyant density of HDV in caesium chloride gradient is intermediate between HBV and HBsAg at 1.25 g/cm^3. The virus is composed of genomic RNA and the HDAg, surrounded by an envelope formed from HBV envelope proteins [13].

The genome is a circular RNA molecule of 1672 to 1694 nucleotides, depending of the viral genotype, with negative polarity, that is, the coding strand for delta antigen is complementary to that present in the viral particle. Genomic RNA has a high GC sequence and is folded back on itself with 70% of the nucleotide bases paired together, resulting in a secondary unbranched rod-like structure. This structure is similar to that found in the subviral agents of plants, as well as some introns of eukaryotic genes, supporting the hypothesis of a common origin or convergent evolution of these elements [12,14,15]. One theory on HDV origins has proposed that the HDV would have a hybrid origin between a viroid RNA and a messenger, as it has two distinct domains, one with no coding capacity, similar to a viroid, and another, which encodes delta antigen [16]. This theory was given some credence when it was found that there are similarities between the delta antigen and a protein found in higher eukaryotes, called DIPA (delta interacting protein A) [17]. This protein also has the ability to form aggregates with delta antigen and is somehow involved with the process of viral replication. Ribozymes are regarded as a feature of plant viroids, but a self-cleaving RNA sequence has recently been described in the human genome, in an

intron of the *CPEB3* gene which is structurally and biochemically related to the HDV ribozymes. Therefore, HDV might have arisen from within the human transcriptome [18].

Delta antigen is encoded by the antigenomic strand viral RNA in two different forms, a shorter (S; 22 kDa) and a longer one (L; 24 kDa); what occurs as a consequence of RNA editing of the polyadenylated antigenomic RNA during replication of the virus. This RNA editing creates a mutation that leads to the appearance of two types of RNAs: the first encodes the S protein of 195 amino acids whereas the other encodes the L protein of 214 amino acids. A UAG codon for termination of the amino acid polypeptide chain (resulting in the S form) is changed to the codon UGG for tryptophan, which allows translational read through to generate the large form with the incorporation of 19 more amino acids. This process is mediated by the cellular enzyme adenosine deaminase acting on RNA-1 (ADAR-1) [19] which catalyses double stranded RNAs as substrates, resulting in deamination of adenosine residues [20], thus UAG→UGG via a UIG intermediate.

HDAg has the ability to bind to RNA, especially the HDV RNA [21]. Four domains within HDAg have been identified: RNA binding domain in the middle third of the protein, which has two arginine rich motifs [22]; nuclear localization signal in the amino terminus [23]; coiled-coil sequence, in the first third after the amino terminus, responsible for binding and oligomerization of the protein with other proteins, such as DIPA [24]; the fourth domain present only in the L form, and this domain is prenylated [25]. The prenylation of the cysteine residue at the C terminus makes possible the interaction between HBsAg and HDAg necessary for the final formation of the viral particle. Phosphorylation of a serine residue at position 177 of the small-HDAg increases the replication of the antigenomic RNA by interacting with RNA polymerase II, whereas the sumoylation of small-HDAg enhances the synthesis of genomic RNA and messenger RNA, but not of antigenomic RNA – properties that are also ascribed to acetylation [26]. Methylation of small-HDAg by arginine methyltransferase at arginine-13 (an RNA binding domain), is essential for translocation of small-HDAg to the nucleus, and for replication of the antigenomic RNA strand to form the genomic RNA strand [12]. The role of the HBV in HDV replication seems to be restricted to the provision of the HBsAg envelope [27].

HDV genotypes

Evidence of HDV infection is found throughout the world, but its prevalence, incidence, clinical features and epidemiological characteristics vary by geographic region [28]. HDV is endemic in many populations with a high prevalence of HBV, ranging from 70% among chronic HBV carriers in the Amazon basin to 20% in Africa and less than 1% in North America [28,29].

To date, eight genotypes (1 to 8) of HDV have been described which are distributed over different parts of the world, with HDV-1 being distributed worldwide, whilst HDV-2 to 8 have a more 'local' distribution. HDV infection is uncommon in Far East Asia, most parts of Europe and the US but is endemic in central Asia, the Middle East, Turkey, some Balkan countries, such as Romania and Albania, in certain Indian populations of South America and sporadically in islands of the Pacific. Genotype 1 comprises the majority of the isolates found so far in almost all parts of the world [30]; genotype 2 and 4 are found in Japan and Taiwan and may be associated with a milder form of the disease [31], and genotype 3 is found in the Amazon region in association with a particularly severe form of the disease, which occurs in outbreaks [32,33], as in the case of the Lábrea Black Fever in Amazonas state, Brazil [34]. Genotypes 5–8 have been detected in the sera of patients of African origin [35]. Recently, genotype 8 infection was also found in Maranhão State, north-eastern Brazil [36].

HDV RNA is found in the host in the form of a quasispecies [37]. The mutation rate of the HDV is between 3×10^{-2} to 10^{-3} substitutions per nucleotide per year [38].

Natural history of hepatitis delta

HDV is a satellite virus that infects HBV carriers. Of the 350 million chronic carriers of HBV worldwide, more than 15 million have serological evidence of exposure to HDV [39]. HDV infection occurs simultaneously with HBV infection (coinfection) or its infection can occur in patients with previous HBV infection (superinfection). HDV infection is generally associated with suppression of HBV infection as has been shown by a decrease or disappearance of HBcAg in liver tissue and a decrease in HBsAg levels [2].

The clinical expression of acute HDV infection acquired through coinfection with HBV varies from subclinical enzymatic alterations to fulminant disease. Viraemia may not be detectable in subclinical and mild hepatitis delta coinfection cases and is recognized only through a delayed rise of specific immunoglobulin (Ig) M and IgG antibodies. Viraemia is early detected in severe cases by the finding of HDAg and HDV RNA in serum, followed shortly by seroconversion first to IgM and then to IgG antibodies [40,41].

The overall course of acute hepatitis D is more severe than acute hepatitis B alone, generally requiring hospitalization. Severe or fulminant hepatitis is more often observed with HBV–HDV coinfection compared with HBV monoinfection. Acute coinfection resolves

in over 95% of cases; by contrast, superinfection is frequently severe and progresses to chronicity in more than 70% of cases. Chronic infection after acute coinfection is rare and similar to the rate in monoinfected patients. Chronic infection develops in 70–90% of patients with superinfection and runs a more progressive course than chronic hepatitis B and may lead to cirrhosis within 2 years in 10–15% of patients. Active replication of both HBV and HDV may be associated with a more progressive disease pattern. Further, different HDV and HBV genotypes may contribute to various disease outcomes [42]. In a European study, HDV infection was found to increase the risk of liver decompensation and of mortality by factors of 2.2 and 2.0 compared to HBV disease alone [43].

Chronic hepatitis D has also been associated with the development of hepatocellular carcinoma, probably by an indirect mechanism of inducing inflammation, eventually leading to cirrhosis [44]. The risk of hepatocellular carcinoma amongst patients with chronic HBV plus HDV is significantly increased when compared to those patients with monochronic HBV infection [45].

Pathogenesis

The clinical picture of chronic HDV is variable. In endemic areas, benign forms of hepatic disease and asymptomatic carriage have been described. HDV disease is often severe in northern South America with subfulminant or fulminant courses. In non-endemic areas, the disease generally involves the usual bloodborne/sexual transmission risk groups and the clinical picture tends to be one of severe hepatitis. The clinical spectrum is thought to be due to differences in pathogenicity amongst the different viral genotypes and strains. It is reported that HDV-3 is associated with severe or fulminant acute hepatitis in northern South America [34]. HDV directly contributes to the severity of the liver disease, probably by inducing liver cytotoxic lesions directly [46].

In contrast, HDV-2, originally isolated in the Irabu Islands, Okinawa, Japan, is related to a less severe form of HDV infection, as most cases rarely showed biochemical alterations or severe histopathological lesions [47].

Infection with HDV-1, the genotype found in Europe and North America is more heterogeneous and is divided in two subgenotypes (1a and 1b) that are not different with respect to disease severity [48].

Liver histopathological lesions are similar to other types of viral hepatitis. Acute fulminant hepatitis D in Europe shows an extensive parenchymal collapse with disappearance of hepatocytes, except those regenerating areas resulting in a pseudoductular arrangement. Peculiar histological features have been found in outbreaks in northern South America, such as microsteatosis with some necrosis as well as portal lymphocytic infiltration. Morula cells, which are intralobular macrophages containing periodic-acid Schiff-positive non-glycogenic granules, have been shown to contain the HDAg. A characteristic lesion in many cases of chronic HDV infections is the presence of microvesicular steatosis and extensive eosinophilic necrosis. Other accompanying features included the presence of intra-acinar, mainly macrophagic, scavenger cell inflammation, intense portal inflammation, a parenchymal regeneration and ductular and arteriolar proliferation [49].

Diagnosis of HDV

One or more serological markers are used to ascertain HDV infection: HDAg, which marks an ongoing and acute infection; anti-HDV antibody (anti-HD) is the screening test for past or chronic infection. IgM anti-HD, is detectable during the 'window' phase of the infection, that is the period between the appearance of HDAg and the development of IgG anti-HD; when detected at high titres, it is indicative of chronic infection [12].

The diagnosis of HDV infection relies mainly on the demonstration of antibody tests against the HD-antigen; the IgG antibody (anti-HD) is a marker of exposure to HDV and may be seen as serological evidence of past infection. The level of IgM antibody increases transiently in acute HDV hepatitis and may be the only marker of acute infection in the window phase before recovery. As the disease proceeds to chronicity, IgM anti-HD increases and persists [12].

HDV replication is most efficiently evaluated by testing with molecular based assays for HDV RNA in serum by means of reverse transcription-PCR assays (RT-PCR). In the mid-1980s, cloning of HDV RNA provided genetic probes and reagents for the measurement of delta viraemia in serum and for its detection in the liver by means of nucleic acid hybridization methods [50]. The sensitivity of HDV RNA detection has progressively improved by using a full-length complementary DNA probe, an RNA–RNA hybridization probe and an RT-PCR technique [51,52]. The Paul-Erhlich Institute has recently released a reference standard for HDV RNA to allow standardization of HDV RNA testing.

Available studies show that viraemia levels change during the course of HDV infection [52]. By using a sensitive method for the quantitative estimation of HDV RNA in serum, viral levels were significantly higher in patients with early chronic hepatitis than in cirrhosis. Moreover, a quantitative assay, sensitive for all HDV genotypes [53], was useful to estimate viraemia variations, which occur under interferon therapy in the follow-up of chronically infected patients. In this setting, a reduction of at least 5 $\log_{10}$ copies/ml during

treatment predicted a favourable virological outcome of the therapy. Recently, other methodologies have been developed to determine HDV viral load; it must be confirmed by further studies if they are able to equally quantify all HDV genotypes [54,55].

Clinical profile and antiviral therapy

Chronic HDV infection generally leads to more severe liver disease progression, which, as discussed above, can be accompanied by an increased risk of hepatocellular carcinoma [56]

In hepatitis delta infection, two different scenarios can be recognized: coinfection with HBV and HDV and superinfection of HDV in an HBsAg-positive individual. Coinfection has been associated with more serious acute liver disease including liver failure. In the majority of cases, coinfection of HBV and HDV results in the clearance of both infections. By contrast, superinfection of HBsAg-positive individuals can lead to acute hepatitis but frequently progresses to persistent infection. HDV superinfection may lead to transient or continued suppression of HBV replication, with the majority of HDV-infected patients demonstrating very low levels of HBV DNA.

Quantification of HDV RNA is important for the clinical management of chronically infected patients undergoing antiviral therapy in order to assess efficacy, and to identify non-responders compared to those who could benefit from an extended treatment. Genotype 1 of HDV is associated with a worse outcome of disease than genotype 2. Genotype 3 is associated with severe cases in the north of South America [12].

The current management of chronic HDV-infected patients is based on accepted common practice rather than on evidence from clinical trials. Therefore, therapy should be pragmatic and individualized, and based on the clinical and virological responses during therapy.

Current treatment options include pegylated interferon-α, which has been investigated in a number of small trials with sustained virological response rates of about 20% [57–59]. Interferon therapy extended over 12 months may be of benefit in patients with a partial response. Unfortunately, many patients relapse following pegylated interferon-α withdrawal. Nucleoside/nucleotide analogues used for CHB have been tested as treatments for HDV but are ineffective when given as monotherapy [60], whereas, combination therapy of pegylated interferon and adefovir/tenofovir may be more effective. In the HIDIT-1 trial, which randomized 90 patients from Germany, Turkey and Greece, pegylated interferon led to a 28% sustained virological response; the addition of adefovir did not improve virological response but did lead to increased suppression of HBsAg concentrations, whereas adefovir monotherapy was ineffective [61]. Although combination treatment was not more effective than pegylated interferon monotherapy, combination treatment appeared to be significantly more effective compared to pegylated interferon monotherapy in reducing HBsAg levels [62,63].

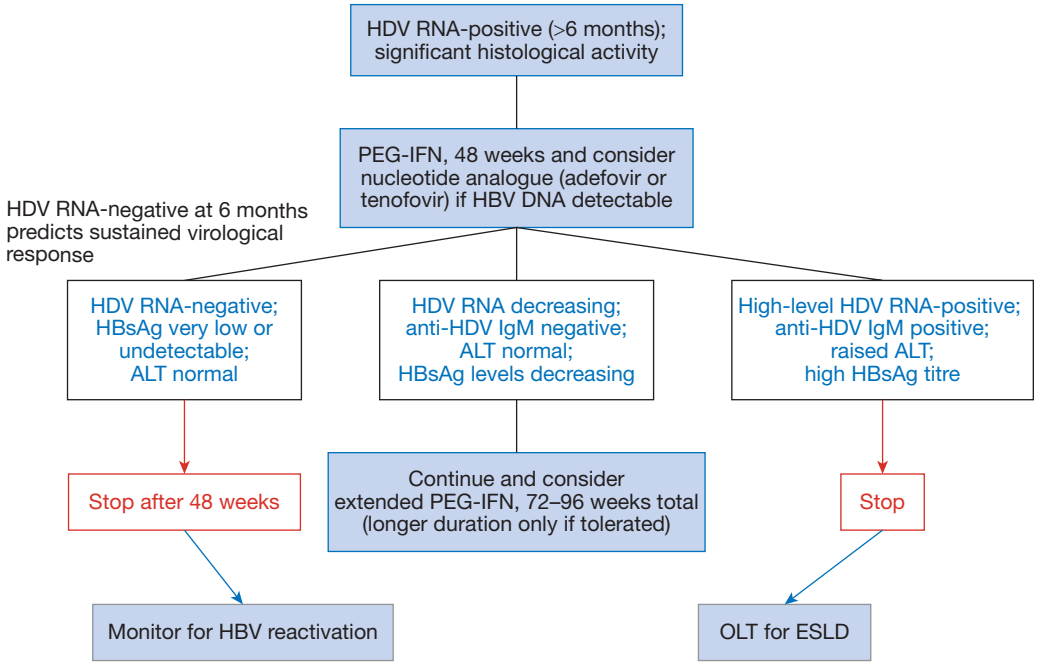
Liver transplantation currently is the only treatment option that remains as a potentially curative option [64]. The key finding in the success of this therapeutic approach has been the realization that by blocking HBsAg the clinical manifestation of HDV replication is neutralized [64]. Thus, as well as measuring HDV RNA levels, quantitative HBsAg can also provide useful guidance and patient monitoring during therapy [65]. A possible approach to antiviral therapy is shown in Figure 1.

Factors predicting the outcome of interferon treatment are not yet determined. HDV RNA correlates positively with HBsAg titre, and baseline values of both predict response to therapy [66]. HDV-1 is associated with reduced response to pegylated interferon, but it is not known if it is related to the viral HDV sequence itself or to higher viral load found in these cases. Cirrhosis associated with HDV infection was not associated with response to pegylated interferon [67]. Neither baseline liver biochemical tests are associated with treatment outcome. HDV RNA kinetics have been used to predict long-term virological outcome. Three-quarters of patients achieving an end-of-treatment response were HDV-RNA-negative after 6 months, in contrast to none of the patients who did not respond to treatment but no relation was found between undetectable RNA during treatment and relapse. HDV RNA values were lower at the end of therapy than at baseline in some non-responder patients justifying the extension of therapy in this group [68].

Three virological patterns of response to interferon have been defined: complete (negative RNA negativity after 6 months) which predicts sustained virological response; partial (lower levels but positive RNA after 6 months) which predicts rebound after discontinuation of therapy; and non-response (persistent RNA levels) throughout treatment and follow-up [69].

In conclusion, HDV is a unique in the hepatitis virology world being the only subviral or satellite agent involved as either a coinfection or superinfection with HBV. Chronic HDV infection was initially described only in the western Amazon basin, Africa and Mediterranean basin and over the last twenty years, its prevalence has overall decreased in most world regions after the implementation of hepatitis B vaccination. Nevertheless, it now appears to be re-emerging as a significant pathogen and recent reports have highlighted its current spread throughout the world. There are eight HDV genotypes (1 to 8) distributed over different geographic areas. Chronic HDV infection leads to more severe liver disease than chronic HBV monoinfection

Figure 1. A possible therapeutic outline for managing patients with chronic HDV infection



The addition of nucleotide/nucleotide analogues to increase the efficacy was proposed, particularly in patients with detectable HBV DNA, as suggested by a few studies and must be deemed as experimental. ALT, alanine transaminase; ESLD, end-stage liver disease; HBsAg, hepatitis B surface antigen; IgM, immunoglobulin M; OLT, orthotopic liver transplantation; PEG-IFN, pegylated interferon.

with an accelerated course of fibrosis leading to cirrhosis, an increased risk of hepatocellular carcinoma and early decompensation in the setting of established cirrhosis. Current treatments include pegylated interferon-α and liver transplantation; the latter of which can be curative. Further studies are needed to develop better treatment strategies for this challenging disease.

Acknowledgements

This work has been supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) 2011/50562-0 and 2011/51597-1, Alves de Queiroz Family Fund for Research, CNPq, Fundação Faculdade de Medicina and HCFMUSP.

Disclosure statement

The authors declare no competing interests.

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Accepted 29 October 2012; published online 21 June 2013