FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2013/2014

Inês de Sousa Pita

Hepatitis B inactive carriers: an overlooked population?

março, 2014

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Inês de Sousa Pita

Hepatitis B inactive carriers: an overlooked population?

Mestrado Integrado em Medicina

Área: Gastrenterologia

Trabalho efetuado sob a Orientação de:
Doutora Ana Maria Horta e Vale

Trabalho organizado de acordo com as normas da revista:
Clinics and Research in Hepatology and Gastroenterology

março, 2014

FMUP

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Inês de Sousa Pita, abaixo assinado, nº mecanográfico 200803070, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 06/03/2014

Assinatura conforme cartão de identificação:

Inês de Sousa Pita

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

Inês de Sousa Pita

CARTÃO DE CIDADÃO OU PASSAPORTE (se estrangeiro)	E-MAIL	TELEFONE OU TELEMÓVEL
13762898	mimed080936@med.up.pt	918725985

NÚMERO DE ESTUDANTE	DATA DE CONCLUSÃO
200803070	20/03/2014

DESIGNAÇÃO DA ÁREA DO PROJECTO

Gastrenterologia

TÍTULO ~~DISSERTAÇÃO~~/MONOGRAFIA (riscar o que não interessa)

Hepatitis B inactive carriers: an overlooked population?

ORIENTADOR

Ana Maria Horta e Vale

COORIENTADOR (se aplicável)

É autorizada a reprodução integral desta ~~Dissertação~~/Monografia (riscar o que não interessa) para efeitos de investigação e de divulgação pedagógica, em programas e projectos coordenados pela FMUP.

Faculdade de Medicina da Universidade do Porto, 06/03/2014

Assinatura conforme cartão de identificação: Inês de Sousa Pita

Hepatitis B inactive carriers: an overlooked population?

Inês Pita[1]; Ana Maria Horta-Vale[2]; Guilherme Macedo[2]

[1] Faculdade de Medicina da Universidade do Porto

[2] Serviço de Gastrenterologia, Hospital de São João

Corresponding author: Inês Pita, Serviço de Gastrenterologia, Hospital de São João, Al. Prof. Hernâni Monteiro 4200-319, Porto, Portugal

Phone number: 00351918725985

E-mail address: mimed08093@med.up.pt

Running title: Hepatitis B inactive carriers

Abstract

A significant portion of patients with chronic hepatitis B virus (HBV) infection are in the inactive carrier state, characterized by normal transaminase levels, little viral replication and minimal liver necroinflammatory activity. Diagnosis is made after at least one year of regular monitoring and requires lifelong follow-up to confirm that this state is maintained.

Studying the natural history of inactive carriers is currently hindered by the small number of studies with cohorts correctly diagnosed according to the latest guidelines.

When correctly defined, inactive carrier state carries a very good prognosis in the spectrum of chronic hepatitis B infection, with low rates of reactivation, hepatocellular carcinoma and progression of disease to cirrhosis. In addition, clearance of hepatitis B surface antigen is more common in inactive carriers compared to the general HBV infected population. Reactivation is more likely during the first years of follow-up and during immunosuppressive therapies: prophylactic antiviral treatment should be initiated as soon as possible in this latter case.

Current guidelines do not routinely recommend liver biopsy in inactive carriers. However, some may have significant hepatic fibrosis at diagnosis and cannot therefore be classified as such; others may develop fibrosis during follow-up and consequentially have poorer prognosis. Transient elastography appears an ideal approach for identifying such patients and for serial monitoring of liver disease in all inactive carriers.

Overall, more longitudinal studies on larger cohorts of *true* inactive carriers would be helpful for better understanding this chronic hepatitis B subpopulation.

Introduction

Infection with hepatitis B virus (HBV) affects approximately one third of the world's population during their lifetime [1]. It is estimated that 240 million people worldwide have chronic HBV infection. It is especially prevalent in Sub-Saharan Africa and East Asia, where perinatal transmission accounts for the majority of cases. Transmission can occur through body fluids such as blood, semen or vaginal secretions; the major routes of infection are perinatal, sexual and blood-to-blood contact [2].

Following acute exposure to HBV, the natural history of HBV infection goes sequentially through the following two phases:

A) the *immune-tolerant* phase is characterized by hepatitis B early antigen (HBeAg) positivity (a serological marker of viral replication), high viral replication rate (elevated HBV DNA levels) and normal alanine aminotransferase (ALT) levels. There is little to no inflammation or fibrosis of the liver. This phase tends to be more prolonged in perinatally acquired infection.

B) in the *immune-reactive* phase the host's immune system starts mounting a response against HBV: replication levels lower, with a parallel drop in serum HBV DNA, ALT levels rise and there is moderate to severe necroinflammatory activity in the liver with resultant hepatocyte lysis. This phase ends with HBeAg loss and seroconversion to anti-HBe status, which is usually associated with viral suppression by the host's immune system [1]. The likelihood of clearing the virus during this phase, evidenced serologically by hepatitis B surface antigen (HBsAg) loss, depends largely on patient's age at the time of infection, so that chronicity occurs in 80-90% of infected newborns, in contrast with 30-50% if infection is acquired in early childhood and only <5% if infected in adulthood [2].

Chronic HBV infection is defined by persistent HBsAg positivity for >6 months and clinical manifestations are varied and possibly dynamic:

C) Some patients remain in an *inactive carrier (IC) state*, defined by the European Association for the Study of the Liver (EASL) as fulfilling the following criteria: 1) HBeAg negativity; 2) anti-Hbe positivity;

3) persistently normal ALT (PNALT) levels (<40 IU/mL, with measurements at least every 3 to 4 months during 1 year); 4) serum HBV DNA levels <2000 IU/mL.

D) Others evolve to *HBeAg-negative chronic hepatitis B (CHB)*, usually associated with precore or basal core promoter mutant HBV. It is characterized by fluctuating ALT and serum HBV DNA levels that may even drop below IC cut-off levels, despite persistent viral replication and hepatic inflammation.

Differentiating between inactive carrier and CHB status is of paramount importance in clinical practice, since it has serious implications in follow-up, management and prognosis [1].

Definition of inactive chronic HBV carriers

HBA DNA and ALT cut-off levels

At the Management of Hepatitis B workshop in 2000, an arbitrary level of 20.000 IU/mL was adopted as the serum HBV DNA cut-off level distinguishing active and inactive chronic hepatitis [3]. Afterwards it was demonstrated that this cut-off would misclassify 45% and 30% of active CHB based on one or three serial HBV DNA measurements, respectively [4], which led major guidelines to adopt a lower serum HBV DNA cut-off value of 2000 IU/mL [1, 5]. Still, the EASL acknowledges that there may be some inactive carriers with viremia levels between 2000 and 20.000 IU/mL.

Debate on the value that best discriminates between active and inactive infection is ongoing. Chen et al. followed 64 inactive carriers who maintained PNALT during a mean follow-up period of 17,6 years and measured HBV DNA levels periodically (minimum of 5 measurements); 68% had at least one HBV DNA level >2000 IU/mL (excluding those obtained during the first year of follow-up) [6].

Because HBV DNA levels in HBe-negative active hepatitis can fluctuate from undetectable to >2.000.000 IU/mL [5] and some inactive carriers occasionally have HBV DNA levels between 2000 and 20.000 IU/mL, a single HBV DNA level between 2000 and 20.000 IU/mL appears to be in a “grey area”

which can correspond to both active CHB or inactive carriers. It is thus important for the clinician to be aware of the importance of *serial* HBV DNA measurements and life-long follow-up to confirm the diagnosis and that inactive carrier state is maintained.

Another inactive carrier state diagnostic criterion has recently been revised: lower ALT upper limit of normal (ULN) levels of 30 IU/L for men and 19 IU/L for women were proposed based on its 95th percentile in healthy Italian blood donor candidates [5]. Indeed, there appear to exist differences in viremia, risk of reactivation and liver histological lesions between patients with low-normal (<0,5xULN) and high-normal ALT (0,5-1xULN), as a review by Andreani recently summarized [7]. However, most of these results come from studies on chronic HBV populations with heterogeneous serological profiles and HBV DNA levels. These observations may therefore not extend to inactive carriers, especially since several prospective studies on HBe-negative carriers with PNALT failed to demonstrate significant differences in clinical outcomes between patients with higher-normal and lower-normal ALT levels [8, 9]. It remains to be determined whether ALT levels near the ULN warrant management changes for inactive carriers.

HBsAg quantification

The HBV marker HBsAg has been the foundation of HBV infection diagnosis since its discovery over 40 years ago; however, its significance in clinical practice has mostly been limited to *qualitative* status. Recently, interest has sparked regarding its quantification as an important diagnostic, prognostic and predictive tool [10].

HBsAg is the glycosylated protein of the HBV virion envelope. The DNA template for HBV replication exists in hepatocytes' nuclei as covalently closed circular DNA (cccDNA) and segments of it can become integrated in the host's hepatocyte genome. Unlike mature virions, HBsAg can also be sinthetised from integrated HBV DNA. It is produced in excess of mature virions and consequently exists in circulation both associated with virions and as subviral particles.

HBsAg levels tend to decline as disease progresses to HBe-negativity and inactivity but they re-elevate if there is reactivation to active hepatitis [11]. Several cross-sectional [12-14] and longitudinal [15-17] studies have also demonstrated a significant difference in HBsAg levels between inactive carriers versus active HBe-negative chronic hepatitis, revealing its potential usefulness in discriminating between the two. Using a HBsAg cut-off level of 650 IU/mL, Brunetto et al. found a diagnostic accuracy of 88% for diagnosing inactive carriers in a population of 209 Italian treatment-naïve HBe-negative genotype D infected patients; sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 82%, 90%, 75% and 93%, respectively [16].

In addition, HBsAg appears to have prognostic significance for both HBsAg seroclearance and disease reactivation. Lower baseline HBsAg levels and yearly HBsAg decline are independently associated with subsequent HBsAg loss [16]. In a Taiwanese study on HBe-negative treatment-naïve patients, baseline HBsAg <50 IU/mL could predict HBsAg loss during a median follow-up of 8 years with 82% sensitivity and 67% specificity [18]. In another study on Korean genotype C-infected inactive carriers, baseline HBsAg >850 IU/mL and HBV DNA >850 IU/mL could predict reactivation with 85% accuracy, 65% sensitivity, 93% specificity, 80% PPV and 86% NPV [15].

Interestingly, while HBsAg correlates strongly with serum HBV DNA in global chronic hepatitis B populations [13, 19], correlation is only weak in HBe-negative patients [12, 15, 20, 21]. It appears that HBsAg levels don't fall in the same proportion as HBV DNA levels as disease progresses. Accordingly, the serum HBV DNA/HBsAg ratio seems to be significantly higher in active CHB versus inactive HBV carriers [12, 14, 19, 20].

Indeed, it is the combination of serum HBsAg and HBV DNA levels that shows most promise as a *single-point* measurement for differentiating active CHB versus inactive carriers. A single-point measurement of serum HBsAg (cut-off <1000 IU/mL) and HBV DNA (cut-off ≤2000 IU/mL) in the above mentioned Italian study achieved an even better discriminatory power between these two stages of chronic HBV infection (94% diagnostic accuracy, 91% sensitivity, 95% specificity, 88% PPV,

97% NPV) [16]. However, Park et al. tested these cut-offs in 104 Korean treatment-naïve genotype C HBe-negative patients and obtained poorer values: 62% diagnostic accuracy, 55% sensitivity, 77% specificity, 85% PPV and 42 % NPV [15], demonstrating the need for further studies in larger cohorts of HBV infected patients with different characteristics to confirm these data.

Liver histology

Inactive carriers have by definition minimal to no necroinflammatory activity in the liver. However, because liver biopsy is seldom recommended in these patients, this is rarely confirmed histologically. Serial liver biopsies are evidently even less frequently performed, making follow-up of liver histologic changes in inactive carrier populations difficult to evaluate using this method [1].

A recent review raised the concern that in approximately 10% of inactive HBV carriers diagnosed using ALT and HBV DNA levels significant liver disease might be missed [22], based on liver biopsies conducted on Indian and French inactive carriers.

Indeed, a 2012 systematic review of 6 studies with liver histology data from inactive carriers (which included the two above-mentioned studies) concluded that mild liver necroinflammatory activity and moderate fibrosis were present in 10% and 8% of patients, respectively, when inactive carrier state diagnosis was appropriate. In contrast, studies with poor definitions of PNALT, viremia levels >2000 IU/mL and/or significant baseline liver disease found a higher proportion of moderate to severe liver changes [23].

Liver biopsy is the gold-standard for evaluation of disease activity and liver fibrosis in HBV infection; it is however invasive, observer-dependent and carries a small risk of serious complications [1], which makes its use in asymptomatic patients with modest clinical disease open to question.

Transient elastography is a recent simple and non-invasive alternative method for fibrosis assessment based on liver stiffness measured with an ultrasonographic method. It has been shown to be accurate

and reproducible in hepatitis B [24] and might supplant liver biopsy in assessing fibrosis in these patients.

Liver stiffness is significantly lower in inactive carriers (defined using previous cut-off of 20.000 IU/mL) than in HBe-negative active hepatitis patients and was similar to that of healthy controls in three European studies [25-27]. In inactive carriers and untreated HBe-negative CHB patients, its diagnostic accuracy for the presence of Ishak fibrosis score ≥3 was 90,1% (sensitivity, 93,9%; specificity, 88,5%; PPV, 76,7%; NPV, 97,3%) and 94,2% for diagnosing cirrhosis (sensitivity, 86.5%; specificity, 96.3%; PPV, 86.5%; NPV, 96.3%) [25]. The cut-offs used were those with greater areas under ROC curve (AUROCs): 7,5 pKa and 11,8 pKa for diagnosing Ishak fibrosis score ≥3 and cirrhosis, respectively. These results are similar to those proposed in a 2012 systematic review of heterogeneous chronic hepatitis B patients (7,9 and 11,7 pKa for METAVIR fibrosis score ≥2 and cirrhosis, respectively). The same review points out that transient elastography in HBV infection may yield false results under certain circumstances. On the one hand, liver stiffness readings increase with active hepatic inflammation and falsely elevated results are possible if there is substantial ALT elevation. On the other hand, falsely low values may result from the ultrasound beam missing fibrotic bands, especially considering the macronodular nature of cirrhosis in HBV infection [24].

However, there is another important aspect other than the staging of fibrosis that could reinforce the need for performing liver biopsies in these patients: the determination of the amount of HBV infected hepatocytes, which currently relies on a liver tissue sample. Nonetheless, HBsAg quantification displays some promising value in this regard as well: HBsAg levels have been shown to correlate positively with cccDNA (an accurate marker of the burden of infected liver cells) in HBe-positive CHB patients [21, 28] and has indeed displayed utility in predicting and monitoring response to therapy [10, 11, 29]. However, studies so far have found this correlation to be weaker in HBe-negative HBV infected patients [21, 28, 30]. Further studies in HBe-negative populations could shed some light on HBsAg and cccDNA kinetics during later phases of HBV infection.

Natural history of HBV inactive carriers

Among HBV-infected patients, the inactive carrier state generally has a very good prognosis; however, reactivation to active hepatitis, cirrhosis and hepatocellular carcinoma (HCC) are rare but nevertheless possible complications.

Spontaneous reactivation

Disease progression to HBe-negative active hepatitis with or without reversion to HBeAg positivity is a possible outcome for inactive carriers; this may happen spontaneously, or following immunosuppressant therapy or disease.

Spontaneous relapse of hepatitis and reversion to HBe-positivity have been extensively studied in prospective studies of HBe-negative patients. A recent review by Villa et al. has highlighted that discordant relapse rates appear to stem from different study designs and populations. In studies of non-endemic populations, incidence of reactivation ranged from 0,03 to 0,8 per 100 person-years, whereas this incidence was somewhat higher in endemic areas (1,4 to 2,8 per 100 person-years). Relapse rate also seemed to vary according to sampling method: when patients were studied after recent seroconversion to HBe-negativity during follow-up, incidence of reactivation ranged from 0,8 to 2,8 per 100 person-years, whereas those who were already HBe-negative at enrollment had inferior reactivation rates of 0,08 to 1,4 per 100 person-years. The authors conclude that longer time from seroconversion may account for this lower incidence of reactivation [22].

Accordingly, in asymptomatic HBe-negative Taiwanese patients, cumulative incidences of relapse were 10,2%, 17,4%, 19,3% and 20,2% at 5, 10, 15 and 20 years, respectively. The increases in cumulative incidence tended to be progressively lower as time went on, with approximately half of relapses occurring during the first 5 years of follow-up [31]. Both these data suggest that risk of spontaneous reactivation tends to decrease with time from seroconversion.

However, studying the rate of spontaneous relapse in *true* inactive carriers proves more difficult because only a few longitudinal studies correctly diagnose them with regular ALT and HBV DNA measurements during one year before beginning follow-up.

Incidence rates from three prospective studies in which inactive carrier state was defined after at least 6 months of persistently normal ALT are summarized in table 1, as well as data from three additional studies on asymptomatic HBe-negative patients with normal ALT.

The two factors most consistently associated with risk of relapse in HBe-negative patients are male gender [31-34] and older age at HBe seroconversion [31, 33, 34]. Other patient characteristics that seem to confer greater risk of reactivation are baseline ALT and HBV DNA levels [35], maximum ALT during follow-up, genotype C [34] and endemic geographic area [22]. It is possible that HBV primo-infection at a very young age with a longer immune-tolerant phase accounts for the higher reactivation rate in endemic populations, where transmission occurs most commonly perinatally and in early childhood [2].

A study on true inactive carriers found no statistically significant difference in the age and sex between true inactive carriers who remained so and those who reactivated; however, the latter had significantly higher baseline serum ALT and HBV DNA [35].

Non-spontaneous reactivation

Reactivation of active hepatitis in inactive HBV carriers (including HBsAg-negative anti-HBs-positive patients) has been observed during or after cytotoxic treatments for malignancies. It is most often asymptomatic, but a hepatitis flare with hepatic decompensation and even death can occur in 5% to 40% of cases [36]. It appears to be more common when corticosteroids, anthracyclines or rituximab are part of the treatment regimen [36, 37]. Reactivation has also been described in association with imunossupressive drugs used in rheumatic and autoimmune diseases, including corticosteroids, methotrexate and anti-TNFα drugs [38].

Major guidelines therefore preclude prophylactic tenofovir or entecavir for all HBsAg carriers during any cytotoxic or imunossupressive treatment and for 6 to 12 months thereafter [1, 5]. For HBV-infected patients who have cleared HBsAg and have detectable serum HBV DNA (occult HBV infection), the EASL recommends similar prophylaxis; on the other hand, if HBV DNA is undetectable, either prophylaxis with lamivudine or no treatment with a strict follow-up with ALT and HBV DNA levels every 1 to 3 months are appropriate approaches.

The natural history of inactive carriers is also influenced by human immunodeficiency virus (HIV) co-infection. In general, untreated HIV infection is associated with higher HBV replication, higher reactivation rate and reduced liver necroinflammatory activity in comparison with HBV infection alone, probably due to CD4+ cell depletion and immune system impairment. Initiation of highly active antiretroviral therapy may help immune control of viral replication, but it is also associated with hepatitis reactivation due to the immune reconstitution syndrome [39]. Still, inactive carrier state alone does not preclude any changes in HIV infection treatment according to the European AIDS Clinical Society guidelines [40].

Spontaneous clearance

The annual rate of HBsAg clearance among heterogeneous HBsAg carriers is 0,5% [5]. This is considered the best possible outcome of HBV infection; however, residual viral replication can persist and HBV DNA is detectable in the liver even decades later [41].

Incidences of HBsAg loss from three longitudinal studies in HBe-negative patients with baseline normal ALT are summarized in Table 2. Only one of them discriminated results from true inactive carriers classified after one year of PNALT and the almost two-fold higher HBsAg seroclearance rate in this population possibly reflects that [42].

Yuen et al. have extensively elucidated the natural history of disease after HBsAg clearance in a longitudinal study of 298 patients followed for a median of 3 years after HBsAg loss (96% of it

spontaneous). All became HBe-negative before clearing HBsAg and 52% of them developed anti-HBsAg. In a subset of 212 patients who had at least 4 ALT measurements, 82% maintained PNALT during the rest of follow-up; in those who didn't (18%), alternative causes for transaminase elevation other than active HBV hepatitis were present in all but 6 (2,8%) of patients (fatty liver, hepatocellular carcinoma or intake of potentially hepatotoxic traditional herbal medicines). The number of patients with detectable serum HBV DNA diminished with time from HBsAg seroclearance; however, integrated HBV DNA remained detectable in all 29 liver biopsies performed and cccDNA in 23 of them. Five patients had clinical complications of cirrhosis (all with HBsAg clearance after 47 years of age; 3 had transient elastography readings compatible with cirrhosis within 1 year of seroclearance) and 7 patients developed HCC (all were men and lost HBsAg aged > 50 years; 6 had cirrhosis on ultrasound within 1 year of seroclearance) [41].

It seems HBsAg loss is associated with improved prognosis particularly when no cirrhosis has yet developed and with younger age at HBsAg seroclearance.

Complications and survival

Studying the incidence of liver complications in inactive carriers is likewise hindered by the small number of prospective studies which correctly define this state. In two longitudinal studies of strictly defined inactive carriers followed for a median of 3,2 and 5,3 years, none developed cirrhosis or HCC (Table 3) [32, 43]. Another study on 145 true inactive carriers with a longer follow-up (mean of 8 years) found two instances of HCC and no cases of liver-related death [44].

In a larger scale sample of 1932 Taiwanese inactive carriers, annual rates of cirrhosis and HCC were 0,06% and 0,02%, respectively. Serum HBV DNA level was the most important predictor of both outcomes. Interestingly, its prognostic value for HCC was greater in HBe-negative patients with normal ALT and no cirrhosis when compared with the whole HBsAg-positive cohort; this greater association was not found for prediction of cirrhosis [45]. Another study on the same population

found older age and alcohol consumption to be predictors of HCC and liver-related death in inactive carriers; on the contrary, no statistical significant difference in outcomes was demonstrated for gender, smoking habits and high-normal versus low-normal ALT levels in inactive carriers [9].

Management of inactive carriers

Due to its general excellent prognosis, antiviral treatment is not generally recommended for HBV patients in the inactive carrier state.

Once true inactive carrier state is confirmed, regular lifelong monitoring with ALT levels every 6 to 12 months and periodic HBV DNA measurements is advised [1]. Because liver biopsy is not routinely recommended in inactive carriers, hepatic fibrosis remains uninvestigated during the follow-up of most of these patients. To this end, transient elastography appears an advantageous fibrosis assessment tool that can easily be used for baseline and even serial monitoring of liver fibrotic changes.

Decision to treat patients with ALT >2xULN and HBV DNA >20.000 IU/mL is unanimous [1, 5] but it is less firm when ALT is minimally elevated (<2xULN) and/or HBV DNA levels are between 20.000 and 2000 IU/mL. Patients with normal ALT and HBV DNA levels between 2000 and 20.000 IU/mL (the aforementioned HBV DNA level “grey area”) should be followed more closely with ALT determinations every 3 months for at least three years, because as previously discussed, relapse to active hepatitis appears more likely during the beginning of follow-up [22, 31]. Indeed, in a study of 217 HBe-negative asymptomatic patients with normal baseline ALT, time to reactivation (defined as ALT and HBV DNA elevation) after beginning of follow-up was analysed: the 10^{th} percentile was 3,4 months, which suggests that analytical assessment of inactive carriers every 3 months can detect approximately 90% of relapses [33]. When there are only small ALT or HBV DNA elevations (ALT <2xULN or HBV DNA <20.000 IU/mL), guidelines also suggest liver biopsy [5] or transient elastography [1] to assess severity of disease before decision to treat is made.

Though a rare complication, HCC is a possible life-threatening event; screening with liver ultrasound, with or without alpha-fetoprotein measurements, is recommended indefinitely [1, 5].

Discussion

HBsAg inactive carriers represent the majority of hepatitis B virus carriers who have seroconverted to anti-HBe [5], making them an especially important subpopulation of chronic HBV patients, both due to their significant number but also because of the particularities in their management. Specifically, they are not candidates for any of the current hepatitis B therapies available. The clinician's main goals when faced with an inactive carrier must be a) confirming inactive carrier status, b) monitoring for reactivation and c) screening for liver complications [5]. We will comment on each of these items separately.

A. Confirming inactive carrier status

Debate continues regarding the most recent update of lowering the **HBV DNA cut-off level** to 2000 IU/mL for diagnosing inactive carriers. One study counter-argues this cut-off alteration with the evidence that 23% of inactive carriers diagnosed after one year of PNALT had at least one HBV DNA measurement between 2000 and 20.000 IU/mL during a median follow-up of 3 years [35]. The new cut-off level would therefore leave one-fifth of these probable inactive carriers robbed of this classification while still not fulfilling the criteria for chronic active hepatitis. However, we believe that this particular subset of patients has an important risk of active disease and according to guidelines warrants different management from true inactive carriers, deserving therefore clinical distinction. Guideline recommendations for these patients vary between tighter follow-up (with ALT determination every 3 months and HBV DNA measurements every 6 to 12 months for 3 years) and liver fibrosis assessment (with a non-invasive method such as transient elastography or even liver biopsy) [1, 5]. As such, a cut-off level of 2000 IU/mL seems to us more useful clinically: even though it

is more specific for inactive carriers, it is less likely to misdiagnose patients with active disease who might benefit from antiviral treatment.

In recent years, **HBsAg quantification** has been gaining prominence as a tempting substitute for the year-long follow-up necessary to diagnose inactive carriers. As a standalone single-point test, an HBsAg level <1000 IU/mL demonstrated good discriminatory power for diagnosing inactive carrier state in a European population; diagnostic power was even stronger when associated with a same time-point HBV DNA level <2000 IU/mL [16]. Even though these cut-off levels remain to be tested and validated in different hepatitis B patient cohorts, quantitative HBsAg seems a very promising diagnostic marker.

HBsAg levels are generally correlated with viremia, but in low replicative states such as inactive carrier state, they remain proportionally higher to those of serum HBV DNA [21, 28, 30], probably reflecting HBsAg secretion from integrated HBV DNA and/or reduced host immune control over HBsAg production versus viral replication. It provides additional information about the patient's immune control over the disease and possibly about the extent of affected hepatocytes: HBsAg loss represents optimal viral suppression and lower HBsAg levels as well as higher rate of HBsAg decline during the course of the disease appear to be predictive of this outcome [16, 18].

As for the **assessment of liver fibrosis** in this subset of HBV-infected patients, it seems to us that despite being the gold-standard, liver biopsy does not constitute an optimal first-line exam in this population mainly because it is invasive, observer-dependent and carries a small risk of serious complications, making its use in asymptomatic patients with low risk of severe liver disease very questionable.

When available, liver transient elastography represents a more convenient test, both for health-care providers and patients, in diagnosing inactive carriers and even serially monitoring their degree of fibrosis. Liver biopsy should be reserved for patients with unexplained ALT elevations of >2xULN, isolated viremia of >20.000 IU/mL or abnormal liver stiffness measurements.

B. Monitoring for reactivation

There are few prospective studies of inactive carriers defined according to the most recent guidelines. Still, prognosis and outcomes are decidedly favorable in asymptomatic HBsAg carriers with PNALT levels and low viremia.

Reactivation in true inactive carriers is a very rare outcome: incidences were 0,8 and 0,4 per 100 person-years in two studies from France and Greece, respectively [32, 35]. Another Greek study revealed a surprising reactivation rate of 4,7 per 100 person-years [43]. Even though no significant differences in study design or population sampling method were reported, it is possible that in this study undisclosed alcohol consumption or unidentified advanced liver disease at baseline (no initial liver biopsies were performed) may account for this discrepant finding.

Most longitudinal studies investigating reactivation in inactive carriers fail to mention HBeAg reversion rates; in the few who do so, these are almost negligible (0 to 0,07 per 100 person-years) [32, 33].

Monitoring for relapse of active hepatitis can be accomplished with regular ALT and HBV DNA measurements. As mentioned previously, HBV DNA elevations between 2000 and 20.000 IU/mL do not necessarily correspond to disease activity: these patients benefit from more frequent follow-up to clarify this. When faced with isolated viremia in this range and PNALT, liver fibrosis stage should be investigated with transient elastography, since active liver inflammation is an unlikely confounding factor in this situation. Conversely, isolated ALT elevations <2xULN may benefit more from liver biopsy and additional studies to exclude other causes of raised transaminase levels, such as for example alcohol abuse, hepatic steatosis or HCC.

C. Liver complications

Cirrhosis and hepatocellular carcinoma are rare but possible outcomes even after years of inactive disease and even after HBsAg loss [9, 44]. Higher HBV DNA levels (within inactive carrier range), older

age and alcohol consumption were found to be risk factors for both complications in this subpopulation. It is interesting to note that HBV DNA has a proportionally greater predictor effect for HCC in inactive carriers compared to HBV chronic hepatitis in general, demonstrating the relevance of a viral-dependent, rather than fibrosis-dependent, oncogenic pathway in these patients [9, 45].

❖

In conclusion, we consider hepatitis B inactive carrier state a relatively benign condition in the HBV infection spectrum, but only if accurately diagnosed. Transient elastograhy and the promising HBsAg quantification in serum are the two most recent major advances that have contributed to more accurate diagnosis of this state and simplified its follow up, obviating the need for biopsy in many circumstances. After correct diagnosis, lifelong monitoring is warranted for early detection of eventual reactivation and hepatic complications. The goal in clinical practice is to recognize all situations which mandate a change in management (be it with tighter follow-up, further investigations or treatment), so it can be implemented as soon as possible in order to minimize liver damage and negative clinical outcomes.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

References

1. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012;57:167-85.

2. Fact sheet: hepatitis B: World Health Organization; 2013.

3. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000--summary of a workshop. Gastroenterology 2001;120:1828-53.

4. Chu CJ, Hussain M, Lok AS. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. Hepatology 2002;36:1408-15.

5. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661-2.

6. Chen YC, Huang SF, Chu CM, Liaw YF. Serial HBV DNA levels in patients with persistently normal transaminase over 10 years following spontaneous HBeAg seroconversion. J Viral Hepat 2012;19:138-46.

7. Andreani T. HBV-carriers: When is monitoring and surveillance sufficient? (point of view). Clin Res Hepatol Gastroenterol 2011;35:813-8.

8. Tai DI, Lin SM, Sheen IS, Chu CM, Lin DY, Liaw YF. Long-term outcome of hepatitis B e antigen-negative hepatitis B surface antigen carriers in relation to changes of alanine aminotransferase levels over time. Hepatology 2009;49:1859-67.

9. Chen JD, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, et al. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. Gastroenterology 2010;138:1747-54.

10. Martinot-Peignoux M, Lapalus M, Asselah T, Marcellin P. The role of HBsAg quantification for monitoring natural history and treatment outcome. Liver Int 2013;33 Suppl 1:125-32.

11. Chan HL, Thompson A, Martinot-Peignoux M, Piratvisuth T, Cornberg M, Brunetto MR, et al. Hepatitis B surface antigen quantification: why and how to use it in 2011 - a core group report. J Hepatol 2011;55:1121-31.

12. Alghamdi A, Aref N, El-Hazmi M, Al-Hamoudi W, Alswat K, Helmy A, et al. Correlation between Hepatitis B surface antigen titers and HBV DNA levels. Saudi J Gastroenterol 2013;19:252-7.

13. Kim YJ, Cho HC, Choi MS, Lee JH, Koh KC, Yoo BC, et al. The change of the quantitative HBsAg level during the natural course of chronic hepatitis B. Liver Int 2011;31:817-23.

14. Jang JW, Yoo SH, Kwon JH, You CR, Lee S, Lee JH, et al. Serum hepatitis B surface antigen levels in the natural history of chronic hepatitis B infection. Aliment Pharmacol Ther 2011;34:1337-46.

15. Park H, Lee JM, Seo JH, Kim HS, Ahn SH, Kim do Y, et al. Predictive value of HBsAg quantification for determining the clinical course of genotype C HBeAg-negative carriers. Liver Int 2012;32:796-802.

16. Brunetto MR, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, et al. Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. Gastroenterology 2010;139:483-90.

17. Martinot-Peignoux M, Moucari R, Leclere L, Cardoso A-C, Boyer N, Ripault MP, et al. Quantitative HBsAg: A New Specific Marker for the Diagnosis of HBsAg Inactive Carriage. Gastroenterology 2010;138:S-830.

18. Su TH, Liu CJ, Tseng TC, Liu CH, Yang HC, Chen CL, et al. Longitudinal change of HBsAg in HBeAg-negative patients with genotype B or C infection. PLoS One 2013;8:e55916.

19. Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. J Hepatol 2010;52:514-22.

20. Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, et al. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. J Hepatol 2010;52:508-13.

21. Thompson AJ, Nguyen T, Iser D, Ayres A, Jackson K, Littlejohn M, et al. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. Hepatology 2010;51:1933-44.

22. Villa E, Fattovich G, Mauro A, Pasino M. Natural history of chronic HBV infection: special emphasis on the prognostic implications of the inactive carrier state versus chronic hepatitis. Dig Liver Dis 2011;43 Suppl 1:S8-14.

23. Papatheodoridis GV, Manolakopoulos S, Liaw YF, Lok A. Follow-up and indications for liver biopsy in HBeAg-negative chronic hepatitis B virus infection with persistently normal ALT: a systematic review. J Hepatol 2012;57:196-202.

24. Chon YE, Choi EH, Song KJ, Park JY, Kim do Y, Han KH, et al. Performance of transient elastography for the staging of liver fibrosis in patients with chronic hepatitis B: a meta-analysis. PLoS One 2012;7:e44930.

25. Oliveri F, Coco B, Ciccorossi P, Colombatto P, Romagnoli V, Cherubini B, et al. Liver stiffness in the hepatitis B virus carrier: a non-invasive marker of liver disease influenced by the pattern of transaminases. World J Gastroenterol 2008;14:6154-62.

26. Maimone S, Calvaruso V, Pleguezuelo M, Squadrito G, Amaddeo G, Jacobs M, et al. An evaluation of transient elastography in the discrimination of HBeAg-negative disease from inactive hepatitis B carriers. J Viral Hepat 2009;16:769-74.

27. Castera L, Bernard PH, Le Bail B, Foucher J, Trimoulet P, Merrouche W, et al. Transient elastography and biomarkers for liver fibrosis assessment and follow-up of inactive hepatitis B carriers. Aliment Pharmacol Ther 2011;33:455-65.

28. Larsson SB, Eilard A, Malmstrom S, Hannoun C, Dhillon AP, Norkrans G, et al. HBsAg quantification for identification of liver disease in chronic hepatitis B virus carriers. Liver Int 2013.

29. Liaw YF. Clinical utility of hepatitis B surface antigen quantitation in patients with chronic hepatitis B: a review. Hepatology 2011;54:E1-9.

30. Lin LY, Wong VW, Zhou HJ, Chan HY, Gui HL, Guo SM, et al. Relationship between serum hepatitis B virus DNA and surface antigen with covalently closed circular DNA in HBeAg-negative patients. J Med Virol 2010;82:1494-500.

31. Chu CM, Liaw YF. Spontaneous relapse of hepatitis in inactive HBsAg carriers. Hepatology international 2007;1:311-5.

32. Martinot-Peignoux M, Boyer N, Colombat M, Akremi R, Pham BN, Ollivier S, et al. Serum hepatitis B virus DNA levels and liver histology in inactive HBsAg carriers. J Hepatol 2002;36:543-6.

33. Kumar M, Chauhan R, Gupta N, Hissar S, Sakhuja P, Sarin SK. Spontaneous increases in alanine aminotransferase levels in asymptomatic chronic hepatitis B virus-infected patients. Gastroenterology 2009;136:1272-80.

34. Chu CM, Liaw YF. Predictive factors for reactivation of hepatitis B following hepatitis B e antigen seroconversion in chronic hepatitis B. Gastroenterology 2007;133:1458-65.

35. Papatheodoridis GV, Chrysanthos N, Hadziyannis E, Cholongitas E, Manesis EK. Longitudinal changes in serum HBV DNA levels and predictors of progression during the natural course of HBeAg-negative chronic hepatitis B virus infection. J Viral Hepat 2008;15:434-41.

36. Yeo W, Johnson PJ. Diagnosis, prevention and management of hepatitis B virus reactivation during anticancer therapy. Hepatology 2006;43:209-20.

37. Yeo W, Chan TC, Leung NW, Lam WY, Mo FK, Chu MT, et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. J Clin Oncol 2009;27:605-11.

38. Xuan D, Yu Y, Shao L, Wang J, Zhang W, Zou H. Hepatitis reactivation in patients with rheumatic diseases after immunosuppressive therapy-a report of long-term follow-up of serial cases and literature review. Clin Rheumatol 2013.

39. Benhamou Y. Hepatitis B in the HIV-coinfected patient. J Acquir Immune Defic Syndr 2007;45 Suppl 2:S57-65; discussion S6-7.

40. Rockstroh JK, Bhagani S, Benhamou Y, Bruno R, Mauss S, Peters L, et al. European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of chronic hepatitis B and C coinfection in HIV-infected adults. HIV Med 2008;9:82-8.

41. Yuen MF, Wong DK, Fung J, Ip P, But D, Hung I, et al. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology 2008;135:1192-9.

42. Martinot-Peignoux M, Lapalus M, Laouenan C, Lada O, Netto-Cardoso AC, Boyer N, et al. Prediction of disease reactivation in asymptomatic hepatitis B e antigen-negative chronic hepatitis B patients using baseline serum measurements of HBsAg and HBV-DNA. J Clin Virol 2013;58:401-7.

43. Zacharakis G, Koskinas J, Kotsiou S, Tzara F, Vafeiadis N, Papoutselis M, et al. The role of serial measurement of serum HBV DNA levels in patients with chronic HBeAg(-) hepatitis B infection: association with liver disease progression. A prospective cohort study. J Hepatol 2008;49:884-91.

44. Tong MJ, Trieu J. Hepatitis B inactive carriers: clinical course and outcomes. J Dig Dis 2013;14:311-7.

45. Iloeje UH, Yang HI, Chen CJ. Natural history of chronic hepatitis B: what exactly has REVEAL revealed? Liver Int 2012;32:1333-41.

46. Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology 2007;45:1187-92.

Tables

Table 1
Incidence of reactivation of hepatitis B in Hbe-negative patients.

Author (date)	Geographic area	No. patients	% of males	Median follow-up (years)	No. Reactivation[a] (%)	Reactivation incidence (per 100 person-years)	No. Reversion
Inactive carrier state diagnosed after a minimum of 6 months of follow-up							
Martinot-Peignoux et al. (2002) [32]	France	85[b]	54	3,2	1 (1)	0,8	0
Papatheodoridis et al. (2008) [35]	Greece	85	60	3	12 (14)	4,7	n.a.
Zacharakis et al. (2008) [43]	Greece	195	n.a.	5,3	4 (2,1)[c]	0,4	n.a.
Asymptomatic HBe-negative population with baseline normal ALT							
Chu et al.[d] (2007) [31]	Taiwan	1241	53	12,3	211 (17)	1,4	n.a.
Kumar et al. (2009)[e] [33]	India	217	74	6,3	43 (19,8)	3,1	1
Tong et al. (2013) [44]	USA	146[f]	47	8	20 (13,7)	1,7	n.a.

ALT, alanine aminotransferase; ULN, upper limit of normal.
[a] Reactivation defined as ALT elevation >2xULN and/or serum HBV DNA ≥ 20.000 IU/L unless otherwise specified.
[b] 2 patients had baseline serum HBV DNA >20.000 IU/L but were not excluded from follow-up
[c] Definition of reactivation unknown.
[d] Reactivation defined as ALT elevation >2xULN *and* detectable HBV DNA by hybridization assay (sensitivity = 140.000 copies/mL)
[e] Reactivation defined as ALT elevation >2xULN *and* serum HBV DNA ≥ 20.000 IU/L or 100-fold rise in HBV DNA from previous levels.
[f] 24 inactive carriers became so after antiviral treatment.

Table 2
Incidence of HBsAg loss in HBe-negative HBsAg carriers with normal baseline ALT

Author (date)	Geographic area	No. of patients	% of males	Mean follow-up (years)	No. HBs clearance (%)	Incidence rate (per 100 person-years)
Tong et al. (2013) [44]	USA	146	47	8	13 (9)	1,1
Martinot-Peignoux et al. (2013) [42]	France	54	n.a.	10	8 (15)	1,9
Chu et al. (2007) [46]	Taiwan	1965	55	10,8	245 (12)	1,2

HBsAg, hepatitis B s antigen; ALT, alanine aminotransferase

Table 3
Liver-related outcomes in inactive carriers[a] (incidence per 100 person-years)

Author (date)	Geographic area	No. patients	% of males	Follow-up (years)	HCC incidence	Liver-related mortality incidence
Tong et al. (2013) [44]	USA	146[b]	47	8	0,17	0
Chen et al. (2010) [9]	Taiwan	1932	58	13,1	0,06	0,02
Zacharakis et al. (2008) [43]	Greece	195	n.a.	5,3	0	0[d]
Martinot-Peignoux et al. (2002) [32]	France	85[c]	54	3,2	0[d]	0[d]

HBsAg, hepatitis B s antigen; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma

[a] Inactive carriers defined as HBe-negative HBsAg carriers with normal baseline ALT and HBV DNA <2000 IU/mL unless otherwise specified.

[b] 24 patients became inactive carriers after antiviral treatment

[c] 16 patients had HBV DNA >2000 IU/mL; 2 >20.000 IU/mL

[d] Though not explicit in the text, assumed to be zero.

Agradecimentos

Agradeço em primeiro lugar à Doutora Ana Maria Horta e Vale, que muito para além da orientação científica deste trabalho, demonstrou a maior disponibilidade e confiança ao longo do último ano, mesmo face às minhas constantes incertezas.

Agradeço também aos meus amigos, pela companhia e apoio ao longo deste trabalho e durante os últimos seis anos em geral.

Por fim, uma palavra especial de agradecimento à minha família, por serem um suporte constante na minha vida e me ajudarem a não esquecer as minhas prioridades.

Clinics and Research in Hepatology and Gastroenterology

Instructions for authors

Authors are requested to submit articles online via: http://ees.elsevier.com/clinre. For technical problems with online submission, please contact our author's support service: authorsupport@elsevier.com. CLINRE publishes original articles, editorials, points of view, case reports and review articles, letters to the Editor in English language.

PUBLICATION REQUIREMENTS

Manuscripts must be presented in compliance with the following rules:

- Original articles. It is explicitly understood that, upon submission of an original article, the authors guarantee that the article is original and that it has not already been published (except for a summary of less than 200 words) or that it has not been submitted elsewhere for publication. By submitting an article for publication, the author(s) guarantee that the article is original.

The author(s) must agree that the article (even a part) cannot be reproduced in another journal or in another form of publication without the written permission of the Publisher of Clinics and Research in Hepatology and Gastroenterology. All articles are sent to reviewers who remain anonymous to the authors. " we encourage authors to indicate in a cover letter the main point(s) of interest or novelty that justify submission for publication, sug–gested as opposed reviewers of their manuscript" . Authors may indicate the name of persons they do not want to review their paper. The final decision to publish is taken by the Editorial Board.

EDITORIALS are invited comments on topically issues in liver and digestive diseases or major articles published in the journal or elsewhere. The length of an editorial should not exceed 1,500 words excluding references. Please limit reference count to a maximum of 20 references. A table and a figure can be included. Please provide a title page.

COMMENTARIES are brief comments not exceeding 1,200 words that could be accompanied by one figure aimed to discuss recent published clinical or basic papers. Commentaries must be accompanied by a title page and a very brief summary. Commentaries are reviewed by the Editors before a final decision for publication is made. Revisions may be required.

SEMINARS are classical review articles on selected clinical and basic topics of interest for the readers of the journal. Seminars must be accompanied by a title page and summary. The word limit for review articles is 5,000 words excluding the summary, references, tables, and figures. References should not exceed a maximum of 250.

MINIREVIEWS are short review articles with a word limit of 2,500 words accompanied by one or two figures aimed to explain and illustrate recent advances in clinics or basics sciences that could have impact in liver and digestive diseases. Minireviews dealing with pathophysiological aspects are encouraged.

PICTORIAL REVIEWS present short overviews of imaging aspects (body, tissue, cellular imaging...) of liver and digestive diseases. They should be accompanied by concised comments and a title page.

IMAGE OF THE MONTH is a striking clinical image that is meant to challenge and inform readers.

CLINICAL CHALLENGES can include the following: (1) case reports that contribute to a better understanding of the etiology, pathogenesis or management of a specific disorder; (2) clinical and pathological discussions of exemplary settings to highlight specific diagnostic and therapeutic approaches; (3) clinical problem-solving discussions that consider a step-by-step process of clinical decision-making. The text should not exceed 3,000 words. The use of clinical illustrative materials is mandatory.

CLINICAL, BIOLOGICAL and PHARMACOLOGICAL KEYNOTES comprised up to 1,000 words, are accompanied by one figure, and focus on topics relevant to clinical oriented information about established concepts in clinics, biology and therapy.

CLINICAL IMPLICATION OF BASIC RESEARCH are short articles, up to 1,500 words, accompanied by one figure highlighting and reviewing the findings of papers from preclinical journals. The article must be accompanied by a short summary and a title page.

SUBMISSION OF MANUSCRIPTS

Authors must submit their manuscripts electronically, by using the EES (Elsevier Editorial System) submission tool at http://ees.elsevier.com/clinre/

2.1. Technical requirements

For PC Windows:

- NT 4, 2000, XP
- Internet Explorer 5.5 and later
- Netscape 7 and later
- Firefox 0.9 and later
- Opera 7.51 and later
- Adobe Acrobat Reader 6.0 or later (download free at: http://www.adobe.fr/products/acrobat/readstep2.html)

For Macintosh:

- Macintosh: 9.x OS X
- Internet Explorer 5.x and later
- Netscape 7 and later
- Firefox 1.0 and later
- Safari 1.0 and later
- Opera 7 and later
- Adobe Acrobat Reader 6.0 or later (download free at: http://www.adobe.fr/products/acrobat/readstep2.html)

MS Word has to be used for text files.

PRESENTATION OF ARTICLES

The authors are asked to observe the rules as defined below. The Editorial Board reserves the right to send all manuscripts which do not conform to these rules back to the authors, even before submitting them to peer review. The Editorial Board strongly recommends that the authors retain a complete copy of the manuscript, including illustrations and tables.

MANUSCRIPT

- The text should be typed, double spaced throughout with a 2.5 cm margin on all sides, including title page, summary, references, tables and figure legends.
- Manuscripts are arranged as follows: title page, summaries, text (including introduction, material and methods, results, discussion), references, tables, legends to figures, each beginning on a new page.
- All pages are numbered consecutively beginning with the title page which is page 1.
- The past tense should be used for reporting the results of the experience.
- Percentages are written « % ».
- A referenced article is cited as follows in the text: name of the first author, followed by « et al. ».

TITLE PAGE

The title page includes:

- The title (in English) which must be concise but informative;
- The first name, and family name of each author;
- The name of the departments and institutions from which the study originates;
- A responsibility disclaimer whenever appropriate;
- The name, address, phone and fax numbers and email address of the author to whom all correspondence concerning the manuscript should be sent. Electronic reprints (PDF files) are sent to the corresponding author;
- The source of all grants, funding, whether for material, drugs, or other;
- A running title: less than 40 signs (letters or spaces) (except for letters).

ABSTRACT

- For original articles, case reports and review articles, an abstract of less than 250 words in English is included. For original articles, the abstract should be

structured, stating background and objective, methods, results and conclusions, underscoring the novel or distinctive facts.
- Abstracts do not contain references; abbreviations are discouraged.

ACKNOWLEDGMENTS

Acknowledgments should be placed appropriately in the manuscript, either as a note on the bottom of the page or at the end of the manuscript and should include:
- those who deserve acknowledgment but who have not contributed to the intellectual content specific to the submitted manuscript;
- those who supplied technical, material or financial assistance in preparing the manuscript;
- those who have helped in any way that might be at the origin of a conflict of interest.

CONFLICT OF INTEREST

In accordance with international practices concerning conflicts of interest, all submitted manuscripts must be accompanied by a declaration of conflict of interest (note at the end of the article).

A conflict of interest exists when an author or co-author has financial or personal interests with other persons or organisations that may influence his professional judgement concerning an essential factor (such as a patient's wellbeing or integrity of the research). The main conflicts of interest include financial interests, clinical trials, occasional business involvements and family connections.
All authors of the publication **must declare all of the relationships they have had** during the past 3 years that might be considered to have a potential conflict of interest **but only in connection to the published article.**

General rules:

1. **Where there is no conflict of interest in connection to the submitted article**, the following declaration should be added to the end of the report: No potential conflict of interest relevant to this article was reported.
2. **Where there is a conflict of interest in connection to the submitted article**, all declarations should be listed at the end of the manuscript and this in accordance with the presentation below. The initials of the author(s) concerned should be added.

For example, the following statement would be inserted at the end of the article:
Conflict of interest: C.R. Occasional involvements: advisory services: Company X; E.L. Financial interests in a company: Company Y; J.-J.E. Clinical trials: as main investigator or study coordinator.

3. **Where no conflict of interest in connection to the submitted article has been sent** by the author (or co-authors), the following statement will be added to the published article: **Conflict of interest: the authors have not declared any conflicts of interest.**

ETHICAL CONSIDERATIONS

- For studies performed on humans, the procedures respect the standards set up by the local, regional, national, or institutional Ethics Committees or are in agreement with those set out by the 1975 Helsinki declaration as revised in 1983.
- For studies performed on animals, rules concerning the use of animals and/or their care have been respected.

REFERENCES

- References are cited *in the order they first appear in the text* (using Arabic numerals in brackets' e.g. [1]).
- References (including those for abstracts) concern only published texts or those in press.
- Personal communications or unpublished data do not appear in the list of references but are mentioned in the text in parentheses.
- Theses do not appear as references.
- Journal titles are abbreviated in accordance with the list established by the National Library of Medicine. This list can be consulted at PubMed: http://www.ncbi.nlm.nih.gov/
- References must be typed double spaced and appear exactly as in the following examples (in general, all authors are listed if they are six or less; if there are seven or more, the first six are listed followed by « et al. »):

Articles (journal):

1. Naveau S, Montembault S, Balian A, Giraud V, Aubert A, Abella A, et al. Diagnostic biologique du type d'hépatopathie chez les malades alcooliques ayant des tests biologiques hépatiques anormaux. Gastroenterol Clin Biol 1999;23: 1215—24.
2. Vega KJ, Pina I, Krevsky B. Heart transplantation is associated with an increased risk for pancreatobiliary disease. Ann Intern Med 1996; 124:980—3.

Supplementary issue (journal):

3. Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89—97.

Book:

4. Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany, NY: Delmar Publishers, 1996.

Book chapter:

5. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, eds. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press, 1995:465—78.

Meeting abstracts:

6. Moal F, Aubé C, Roux J, Croquet V, Oberti F, Torrisani L, Rousselet MC, Calès P. Traitement du carcinome hépatocellulaire chez le rat (abstract). Gastroenterol Clin Biol 2001;25;607.

TABLES

- Each table should be presented on a separate sheet at the end of the manuscript (do not insert tables in the text).
- Use the "table" tool in Word (one datum per cell). All tables must be cited in the text and provide new information not repeated in the figures or text.
- Table are numbered (Arabic numerals) according to order of first citation in the text.
- The title is provided on the first line of the table and explanations or notes appear below the table. For notes, letters are employed in alphabetic order: [a, b, c]… as exponent.

FIGURES

- Artwork (graphs, drawings, color photos, black and white photos) must be furnished in separate files, **one file for each figure. If figures are compressed, use one zip file for each figure**. Authors may consult detailed instructions for submitting artwork at the following address: http://france.elsevier.com/html/index.cfm?act=inc&page=pages/author_artworks_instructions_vf.html
- All illustrations (anatomy specimen, imaging, endoscopy…) are provided in digital format (TIFF or EPS, 300 dpi minimum minimal with 10 cm, 4-color CMJN).
- All graphics (histograms, drawings, curves) are provided in digital format (TIFF or EPS, 600 dpi minimum minimal with 10 cm). Important: worksheets and graphic software (Powerpoint, PaintBrush, Excel, MacDrawPro…) should not be used.
- All illustrations and graphics are called figures. All figures are numbered in chronological order (arabic numerals) according to the order of citation in the text (figure 1, figure 2, figure 3, etc.).
- All letters, numerals, or symbols appear clearly and are large enough to be well read once printed
- Titles or explanatory notes do not appear on the illustration.

- Permission has been obtained to use an illustration already published elsewhere.

LEGENDS TO ILLUSTRATIONS

- They are typed double-spaced on a separate page. They are concise. All abbreviations used in the illustrations are defined.

MEASUREMENTS

- Lengths, heights, weights, and volume are expressed in the metric system (m, kg, L) or multiple thereof.
- Temperature is in Celsius degrees, blood pressure in millimeters of mercury.
- Hematological and biochemical measurements are in International Units.

ABBREVIATIONS AND SYMBOLS

- Use of abbreviations is discouraged unless these abbreviations are essential for improving the readability of the text.
- The abbreviated term appears written out entirely followed by the abbreviation in parentheses, the first time it is used in the text (except if it is an International Unit of measurement).

PRODUCTION, CORRECTION OF PROOFS, REQUESTS FOR REPRODUCTION

In the case of partial or complete reproduction in the manuscript of a document or an illustration that has already been published elsewhere, the written authorization of the copyright holder (publisher or author) is required.
When a manuscript that has been accepted for publication goes into production, the publisher will send the corresponding author by e-mail a formula for the transfer of copyright, which should be completed and signed by the author responsible for the article on behalf of all the authors and then returned to the publisher as rapidly as possible. The corresponding author will also receive an order form if he wants to order additional offprints in addition to the PDF file of the published article.
Electronic proofs of the article in PDF format will be sent to the corresponding author. Corrections are limited to typographical errors.
Authors are responsible for returning the corrected proofs to the publisher within 48 hours after reception at all times of the year.
In the case of further delay, the publisher reserves the right to start printing without the author's corrections.
After publication, all requests for reproduction should be addressed to the publisher.

Instructions for authors are available on our website: www.em-consulte.com/produit/CLINRE