The prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection is estimated at 5% to 10% globally. In HBV/HCV coinfected individuals one virus may interfere with the replication of the other virus. In most coinfected patients HCV suppress HBV replication. Loss of HBV suppression following HCV successful treatment may lead to HBV reactivation. Reactivation of HBV has previously been described after successful clearance of HCV with IFN-based regimens. However, increases in HBV replication during treatment of HCV with pegIFN-α, which also has significant activity against HBV, are typically small and severe hepatitis appears to be rare. Treatment of HCV with the newer, interferon-free, direct-acting antiviral (DAA) agents with no activity against HBV and increased potency against HCV may increase the risk of HBV reactivation in coinfected patients. In September 2016 the American Association for the Study of Liver Diseases published an update in recommendations for treatment of hepatitis C. The guideline recommends assessment for HBV coinfection in all patients initiating DAA therapy for HCV and monitoring HBV DNA levels prior, during and after treatment (for HBsAg positive patients). On the other hand the latest European guideline on treatment of hepatitis C also published in September 2016, recommends initiating concurrent prophylactic HBV therapy if chronic hepatitis B or “occult” HBV infection is detected, although there is no interventional data in this setting to support such a strategy.

So far the incidence of HBV reactivation in HBV/HCV coinfected patients receiving DAA agents is unknown, as well as the efficacy of prophylactic HBV therapy. In this prospective multicentric cohort study we aim to determine the incidence of HBV reactivation in HBV/HCV coinfected patients receiving DAA agents for HCV infection, explore risk factors associated with HBV reactivation and evaluate the efficacy of prophylactic HBV therapy.

STUDY OBJECTIVES

Primary objective

Determine the incidence of HBV reactivation in HBV/HCV coinfected patients receiving DAA agents for HCV infection.

Secondary objective

Determine the clinical severity of HBV reactivation; explore risk factors associated with HBV reactivation during IFN free therapy: DAA class, HCV and HBV genotype, HBV infection stage (chronic, occult, resolved infection), HBsAg titer, pre-treatment DNA HBV level and liver fibrosis; and evaluate the efficacy of prophylactic HBV therapy.

HEPATITIS B REACTIVATION WITH DIRECT-ACTING ANTIVIRALS FOR HEPATITIS C TREATMENT– A PROSPECTIVE MULTICENTRIC COHORT STUDY

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STUDY PROTCOL

1. BACKGROUND

Treatment for hepatitis C virus (HCV) infection has evolved substantially over the last years[1]. Interferon (IFN)-based regimes have been replaced for direct-acting antiviral (DAA) agents since their introduction in 2013[1] . Recently, concerns have been raised regarding the occurrence of hepatitis B virus (HBV) reactivation in patients with HBV/HCV coinfection during DAA therapy[2]. In HBV/HCV coinfected individuals one virus may interfere with the replication of the other virus[1,3,4]. The mechanism of this effect is not fully understood[3]. In most coinfected patients HCV suppress HBV replication[3,5,6]. Suppression of HCV following successful treatment in coinfected patients may create a permissive environment for HBV replication, leading to clinical flare or reactivation[3]. Reactivation of HBV has previously been described after successful clearance of HCV with IFN-based regimens[7]. However, increases in HBV replication during treatment of HCV with pegIFN-α, which also has significant activity against HBV, are typically small and severe hepatitis appears to be rare[8,9]. The incidence of HBV reactivation in the setting of DAA therapy has not been well studied but it may be greater given their increased potency against HCV and lack of anti-HBV activity[10]. To our knowledge, several cases of HBV reactivation have been reported during treatment of HCV with DAAs[10-14]. In the reported cases HBV reactivation occurred regardless HCV genotype or DAAs class used. Moreover HBV reactivation occurred whatever the stage of HBV infection (chronic, occult, resolved infection) and DNA HBV level before DAAs treatment. However, a recent prospective observational cohort suggested that HBV reactivation is substantially increased in hepatitis B surface antigen (HBsAg) positive patients treated with DAAs agents for HCV[2].

Until recently, HCV treatment guidelines didn't offer specific guidance on treatment and monitoring of HCV/HBV coinfected patients receiving DAA therapy. In September 2016 American Association for the Study of Liver Diseases (AASLD) published an update in recommendations on treatment of hepatitis C[1]. The guideline recommends assessment for HBV coinfection in all patients initiating HCV DAA therapy. In patients who are HBsAg positive, HBV DNA levels should be obtained prior to DAA therapy and monitored during and immediately after treatment. Patients meeting criteria for treatment of active HBV infection should be started on therapy at the same time (or before) HCV DAA therapy is initiated and those with HBV DNA levels meeting treatment criteria should initiate HBV therapy during DAA therapy[1].

On the other hand, the latest European guideline on treatment of hepatitis C published in September 2016, recommends initiating concurrent HBV therapy if chronic hepatitis B or “occult” HBV infection is detected, although there is no scientific evidence to support this strategy[15].

The incidence of HBV reactivation in HBV/HCV coinfected patients receiving DAA agents is unknown, as is its’ the clinical course.

2. OBJECTIVES

Primary objective

Determine the incidence of HBV reactivation in HBV/HCV coinfected patients receiving DAA agents for HCV infection.

Secondary objective

Determine the clinical severity of HBV reactivation; explore risk factors associated with HBV reactivation during IFN free therapy: DAA class, HCV and HBV genotype, HBV infection stage (chronic, occult, resolved infection), HBsAg titer, pre-treatment DNA HBV level and liver fibrosis; and evaluate the efficacy of prophylactic HBV therapy.

3. STUDY METHODOLOGY

3.1. Overall design

We will perform a prospective cohort study of HBV/HCV coinfected patients treated with DAA agents for HCV infection, in order to determine the incidence of HBV reactivation, the clinical severity of HBV reactivation and the risk factors associated with HBV reactivation in this population.

Before treatment, HCV RNA level, HCV genotype and subtype should be measured. In addition, all patients should be assessed for HBV and hepatitis D virus (HDV) co-infection with HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe, HBV DNA levels and anti-HDV IgM and IgG (if indicated) before initiating HCV DAA therapy. For HBV inactive carriers two determinations of HBV DNA levels at least two weeks apart prior to DAA therapy is required. An evaluation of liver stiffness with transient elastography should be performed in all patients before treatment and/or a liver biopsy whenever indicated. Clinical evaluation, including hepatic encephalopathy, jaundice and ascites, as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST), HCV RNA and HBV DNA levels should be measured at week 2, 4, 6, 12 and

24 and until week 12 and 24 after the end of treatment. Additional tests of HBV DNA level should be made if a hepatitis flare is suspected (eg. fever, jaundice or abdominal pain).

A hepatitis flare is defined as a more than 2-fold increase of ALT on two consecutive determinations at least five days apart, from the nadir during DAAs therapy and follow-up. HBV reactivation is defined as one of the following: (1) HBsAg turning from negative to positive (2) de novo detection of HBV DNA or a ≥ 10-fold (1 $\log_{10}$) increase in HBV DNA level when compared with the baseline value before DAA therapy. In case of de novo detection or an increase in HBV DNA levels, an assessment for HBsAg and anti-HBc IgM in HBsAg negative patients should be made, as well as HBeAg and anti-HBe in HBeAg negative patients. Antiviral treatment for HBV should be given if treatment criteria for HBV are met. Other causes for hepatitis flare rather than HBV reactivation should be registered as well.

Informed consent must be obtained from all patients for the use of collected clinical data and serum samples.

3.2. Endpoints

Primary endpoint

The primary endpoint is the incidence of HBV reactivation since the start of DAA therapy and until 24 week after the end of treatment. HBV reactivation is defined as one of the following:

- HBsAg turning from negative to positive;
- De novo detection of HBV DNA or a ≥ 10-fold (1 $\log_{10}$) increase in HBV DNA level when compared with the baseline value before DAA therapy.

Secondary endpoints

Clinical severity of HBV reactivation: incidence of ALT increase (four times the upper limit of normal) and/or international normalization ratio increase (INR) (> 1,5), hospital admission, orthotropic liver transplantation and death.

Compare the incidence of HBV reactivation in patients without treatment for HBV with those receiving prophylactic HBV therapy.

3.3. Population

Inclusion Criteria

- Patients with HBV/HCV coinfection who start HCV treatment with DAA agents;

- Age ≥ 18 years.

Exclusion Criteria

- Patients coinfected with HIV;
- Concomitant use of Peg-IFN;
- Inability to give informed consent.

Sample size

There is no data in which to base our estimations of the incidence of HBV reactivation in these setting. The risk of HBV reactivation in with certain immunosuppressant drugs (B-cell depleting agents, long term corticosteroids or anthracycline derivatives) is estimated to be 10-60%.

Based on the above mentioned risk we conservatively estimated that an incidence of 20% could be expected during DAA therapy. Therefore, a sample size of 70 produces a two-sided 95% confidence interval with a width (precision) equal to 0,198.

3.4. Statistical analysis

Data analysis will be performed using the computer software Statistical Package for Social Sciences - SPSS for Mac (version 23.0). Continous variables with normal distribution will be shown as mean (standard deviation) and otherwise with median (IQR). Odds ratio will be shown with 95% confidence interval.

Differences in continuous variables between groups of patients will be analyzed by the Student's *t*-test. To explore associations of categorical data the Chi-squared test or Fisher's exact test will be used as appropriate. To estimate the effect of the predictors of reactivation a logistical regression will be preformed.

4. LIMITATIONS

One of the limitations of our study is the estimation of the incidence of HBV reactivation since there is no data in which we can support our estimations of the sample size in these setting. Another limitation is the current recommendation of the European guideline to initiate concurrent "prophylactic" HBV therapy if chronic hepatitis B or "occult" HBV infection is detected, which is one of our exclusion criteria and could limit the number of patients enrolling the study.

DATA TO COLLECT:

- Gender;
- Age;
- Race;
- IMC (Kg/m^2);
- Daily alcohol consumption (grams/day);
- Smoking habits (pack.year);
- Statin use;
- Route of transmission (blood transfusion; intravenous drug users; perinatal transmission);
- Previous treatments for HCV infection;
- Classification of treatment experienced patients (null-non responders; partial non-responders; relapsers)
- DAA treatment (class, daily dose and duration of treatment);
- Ribavirin use (daily dose and duration of treatment);
- HBV treatment (daily dose and duration of treatment);
- Before initiating DAA therapy:
    - Grade of hepatic encephalopathy (New Haven) and ascites (mild, moderate or severe);
    - Child-Pugh and MELD score for cirrhotic patients;
    - Hemoglobin (g/dL), platelet count (/uL), INR, ALT (IU/L), AST (IU/L), alkaline phosphatase (IU/L), gamma glutamyl transferase (IU/L), total bilirubin (mg/dL), conjugated bilirubin (mg/dL), serum albumin (g/dL);
    - HCV RNA level (IU/mL), HCV genotype and subtype;
    - HBsAg (titer), anti-HBs, anti-HBc, HBeAg, anti-HBe, HBV DNA levels (IU/mL and log_{10}), HBV genotype and subtype. For HBV inactive carriers two determinations of HBV DNA levels at least two weeks apart;
    - Anti-HDV IgM and IgG (if indicated);
    - Abdominal ultrasound (eg. liver, ascites, spleen);
    - Transient elastography: liver stiffness (kPa) and/or liver biopsy (Metavir fibrosis score);
- During and after DAA therapy (week 2, 4, 6, 12 and 24; and until week 12 and 24 after the end of treatment):
    - Grade of hepatic encephalopathy (New Haven) and ascites (mild, moderate or severe);
    - ALT (IU/L), AST (IU/L) and INR;
    - HCV RNA (IU/mL) and HBV DNA levels (IU/mL and log_{10});

    - In case of de novo detection or an increase in HBV DNA levels, an assessment for HBsAg and anti-HBc IgM in HBsAg negative patients should be made, as well as HBeAg and anti-HBe in HBeAg negative patients. The need of hospital admission, orthotropic liver transplantation and death should be registered in this patients;
    - If a hepatitis flare is suspected (eg. fever, jaundice or abdominal pain) additional tests of HBV DNA level should be made;
- Antiviral treatment for HBV whatever indicated (class, daily dose and duration of treatment);
- Others causes for hepatitis flare rather than HBV reactivation (eg. alcohol consumption, herbs intake or drug-induced hepatitis).

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