

No detection of occult HBV-DNA in patients with various rheumatic diseases treated with anti-TNF agents: a two-year prospective study

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Abstract

Objectives

The widespread use of tumour necrosis factor (TNF)-targeted therapies in patients with rheumatic, digestive and dermatologic diseases has been associated with reports of reactivation of HBV replication and ensuing hepatitis flares both in asymptomatic HBsAg carriers and in subjects with occult HBV infection. The aim of our work was to investigate in a two-year prospective study the potential for HBV reactivation in patients with inflammatory joint diseases undergoing anti-TNF treatment from a southern Mediterranean area.

Methods

Fifty-seven consecutive outpatients attending the Academic Unit of Rheumatology at the University of Palermo (12 with rheumatoid arthritis, 17 with psoriatic arthritis and 28 with ankylosing spondylitis) were enrolled in the study. HBV-DNA was tested by a standard quantitative assay in HBsAg-positive subjects and by an ad hoc highly sensitive PCR in HBsAg-negative patients performed at baseline and then every six months on the anti-TNF agent.

Results

Occult HBV-DNA was never detected in the 54 HBsAg negative subjects, regardless of their anti HBs/HBc status. All HBsAg positive patients, who were started on prophylactic lamivudine, remained HBV-DNA undetectable throughout the anti-TNF treatment.

Conclusion

Even in an area of previously high HBV endemicity, where occult HBV infection is likely to have a high prevalence, treatment of rheumatological patients with anti-TNF drugs is safe in terms of its potential to reactivate HBV. Prophylaxis with lamivudine is sufficient to prevent reactivation in HBsAg carriers.

Key words

HBV-DNA, anti-TNF-alpha, rheumatic diseases

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Introduction

Persistence of hepatitis B virus (HBV) infection, by far the most common chronic viral infection affecting the liver in the world with over 400 million subjects infected, causes in most patients an "overt" infection, characterised by HBsAg detectable in sample serum and variably amount of HBV DNA (>2000 UI/ml). In a smaller proportion of patients, HBV persists in the nucleus of hepatocytes as a covalently closed, circular DNA (cccDNA) determining an "occult" infection (OBI), characterised by HBsAg negativity and a small amount of HBV DNA (<200 UI/ml). On the basis of the HBV antibody profile, OBI can be distinguished as seropositive (anti HBc and/or antiHBs positive) or seronegative (anti HBc and anti HBs negative) (1).

Reactivation of HBV replication in patients undergoing immunosuppressive therapy is a well recognised and frequently reported complication of considerable clinical importance (2-6).

Patients with occult hepatitis B virus infection undergoing deep immunosuppression, because of cytotoxic chemotherapies, are potentially at risk of HBV reactivation, but the true risk is still undefined. However, the OBI reactivation takes place as an acute hepatitis-like syndrome, indistinguishable from *de novo* acute infection (7-9). Although most of these reports have come from the fields of oncology and transplantation, there has been a growing number of cases reported in patients with rheumatic disease undergoing immunosuppressive therapy (10).

The emergence of tumour necrosis factor (TNF)-targeted therapies as a therapeutic option for patients with rheumatic, digestive and dermatologic diseases has been associated with reports of liver damage in those with HBV infection. In 2003, Michel *et al.* reported acute liver failure requiring urgent transplantation in a patient who was positive for HBV surface antigen (HBsAg+) and who had been treated with infliximab for adult-onset Still disease (11). On the contrary, reactivation is an infrequent event in antiHBc positive patients as described by Vassilopoulos *et al.* (12) and Caporali *et al.* (13), and only rare cases were ob-

served (14, 15), supporting the suggestion that using anti-TNF agents in these patients is safe and an antiviral prophylaxis is not necessary.

Despite the recent findings, the risk of HBV reactivation after exposure to pharmacological TNF-blockade is still unknown. In particular, despite a close monitoring of liver enzymes and HBV-DNA levels in patients with a previous HBV infection receiving anti-TNF treatment, some individuals might have occult HBV infection owing to HBsAg escape mutants or low levels of viremia.

For these reasons, the virological status for HBV must be assessed before and during any immunosuppressive therapy in order to use appropriate prophylaxis measures. The HBsAg positive patients must be treated with antiviral therapy. The HBsAg negative patients may be evaluated for the presence of occult HBV infection by HBVDNA detection, which is the only reliable diagnostic marker of OBI. Moreover, the anti-HBc positivity should be considered a surrogate marker to identify potential OBI (1).

The aim of our work was to investigate in a two-year prospective study the potential for HBV reactivation in HBsAg negative patients from a Southern Mediterranean area with inflammatory joint diseases undergoing anti-TNF treatment utilising a home-made high sensitive assay specific for HBV DNA (16). This was felt to be specifically relevant, due to the fact that the previously high endemicity rate for HBV left a high background rate of up to 16% of OBI in the general hospital population in this region (17).

As a secondary aim, we also evaluated in this setting the efficacy of long-term prophylaxis in asymptomatic HBsAg carriers with lamivudine, a first-generation nucleoside analogue.

Materials and methods

Patients

Fifty-seven consecutive patients, 12 with rheumatoid arthritis (RA), 17 with psoriatic arthritis (PsA) and 28 with ankylosing spondylitis (AS) attending the Unit of Rheumatology at the University of Palermo from January to December 2008, were enrolled in the study. All patients fulfilled the international criteria

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for diagnosis. The study was approved by the institutional review board of our medical centre, and a written informed consent was obtained from subjects enrolled.

Patients included in the study were non-responder to oral non-steroidal anti-inflammatory drugs and/or disease modifying anti-rheumatic drugs (DMARDs) and/or low dose prednisone (<10 mg/day). Thirty-four patients were female and 23 were male, the mean age was 49 years $\pm$ 11.32 (range 24–72). Mean disease duration was 4.6 (range 6 months–10 years) for RA patients, 3.8 years (range 2–8 years) for PsA patients and 4.1 years (range 2–10 years) for AS patients. Disease activity, evaluated at baseline by the disease activity score in 28 joints (DAS 28) in patients with RA and by the Bath ankylosing spondylitis disease activity index (BASDAI) in patients with PsA and AS, was respectively 5.2 ± 1.2 and 4.9 ± 1.5 .

All RA patients and 12 out of the 17 PsA patients had been treated with methotrexate for at least two years at a dose comprised between 10 and 20 mg per week and prednisone (<10 mg/day) and continued this kind of treatment during the follow-up. Patients were assigned to receive an anti-TNF alpha treatment according to the Italian guideline for each disease. Four RA, 4 PsA and 16 AS patients received 5 mg per Kg infliximab. Eight RA, 13 PsA and 12 of the AS patients received etanercept 50 mg per week. At baseline, the median values of ALT and AST were within the normal range (Table I). Patients were monitored before starting treatment (baseline) and every 6 months during therapy in a two-year prospective study.

Methods

Routine baseline laboratory assessment including routine chemistry, liver enzymes and inflammation pointers (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) determination was performed at baseline and then every 3 months for 2 years.

Virological assay

Serological markers of HBV (HBsAg, total anti-HBc, anti-HBs HBeAg, anti-

HBe and IgM anti-HBc) were tested by commercial enzyme immunoassays (EIAs) (Dia Sorin, Saluggia, Italy). HBV DNA was quantified by bDNA (Versant HBV 3.0, Siemens medical Solutions Diagnostics Europe, Dublin, Ireland; range 357–17 857 000 IU/ml, 2000, 108 copies/ml).

HBV-DNA assays for OBI detection were performed at baseline and 6–24 months after starting anti-TNF therapy according to our recently published paper (16). Briefly, total DNA was extracted using the phenol method, with a few *ad hoc* modifications. One hundred μ l of serum was treated with lysis buffer and proteinase K at 55°C for 150 min, and treatment with Tris-saturated phenol (pH 8) and chloroform/isoamyl alcohol, DNA was precipitated with absolute cold ethanol with glycogen (20 ng/ml), and incubated at -20°C overnight. The pellet was washed with 70% alcohol, air dried and dissolved in 10 μ l H₂O. Total DNA extracts from four independent extractions were tested for four different HBV genomic regions (preS/S, preC/C, Pol and X) by nested PCR with HBV-specific primers (17). The sensitivity was between 0.1 and 0.05 IU/ml, as compared with the reference standard serum, and between 15 and 150 virus genome equivalents/ml, as compared with the pUC.HBV D plasmid. Guideline procedures were

strictly adhered to minimise the risk of carryover contamination.

Results

Fifty-seven consecutive patients affected by rheumatic diseases treated with anti-TNF agents were included in the prospective study. Of the patients, 12 were affected by RA, 17 by PsA, and 28 by AS. Of the patients, 24 were treated with infliximab and 33 with etanercept. Of the 57 patients, 24 were also treated with weekly methotrexate (MTX), 40 with non-steroidal anti-inflammatory drugs (NSAIDs), and 34 with prednisone $\leq$ 10 mg/day. The demographic and clinical features of the patients are reported in Table I.

Fifty-three patients were HBsAg negative. Thirty-eight of them had no markers of previous HBV infection, while 15 were HBV experienced (3 anti-HBc positive, 8 anti-HBs positive and 4 anti-HBc/anti-HBs positive) (Fig. 1). All patients had no previous exposure to HCV. No patients were lost to follow-up. Testing for occult HBV in samples collected before and during immunosuppressive treatment showed that no patients presented detectable HBV DNA for the four genomic regions. No cases of HBV reactivation occurred during the 2 years of follow-up, but an increase in ALT/AST was documented in 4 patients on methotrexate therapy.

Table I. Baseline characteristics of patients included in the study (n=57).

Characteristics of patients	Value
Men : women	34:23
Age, mean $\pm$ SD yrs	49 $\pm$ 11.32
Diagnosis	
Rheumatoid arthritis	12
Psoriatic arthritis	17
Ankylosing spondylitis	28
Disease duration, median yrs	
Rheumatoid arthritis	4.6 (6 m–10)
Psoriatic arthritis	3.8 (2–10)
Ankylosing spondylitis	4.1 (2–10)
DAS 28, mean $\pm$ SD	5.2 $\pm$ 1.2
BASDAI score, mean $\pm$ SD	4.9 $\pm$ 1.5
ALT/AST median mU/ml	23/19
Tumour necrosis factor α inhibitor	
Infliximab (RA 4, PsA 4, AS 16)	24
Etanercept (RA 8, PsA 13, AS 12)	33
Methotrexate (dosage 10–20 mg/week) (RA 12, PsA 12)	24
Prednisone (dosage $\leq$ 10 mg/day)	34

DAS 28: disease activity score in 28 joints; BASDAI: Bath ankylosing spondylitis disease activity index; RA: rheumatoid arthritis; PsA: psoriatic arthritis; AS: ankylosing spondylitis.

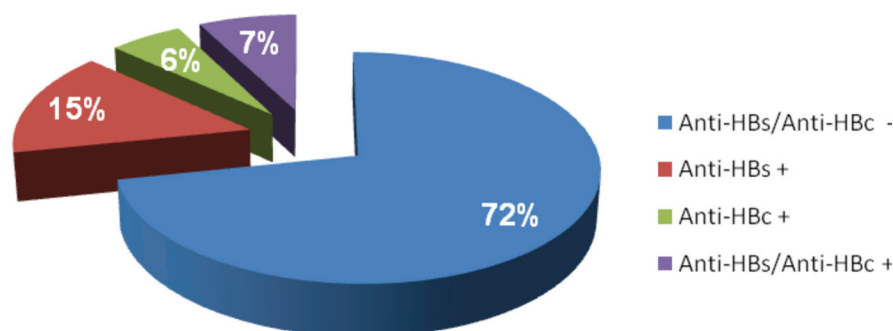


Fig. 1. Virological characteristics of HBsAg negative patients. Results are expressed as percentages.

Normalisation of aminotransferases was observed within two weeks after methotrexate discontinuation in all these patients, without the need for any other modification in therapy.

Four patients out of the 57 enrolled were HBsAg positive with normal ALT (asymptomatic HBsAg carriers) at baseline. They were started on lamivudine 100 mg/day as prophylaxis one month before starting anti-TNF- α therapy, and are currently on treatment in accordance with current guidelines. In all the patients, HBV DNA remained undetectable by the bDNA assay throughout follow-up.

Discussion

It is known that when the immune system is made unresponsive, HBV can reactivate overcoming the host control system, with a significant incidence in morbidity and mortality. This effect was well studied over the past 30 years in patients with "overt" HBV infection undergoing chemotherapy courses for haematological or neoplastic diseases or transplantation (4).

The availability of effective nucleotide analogues that suppress HBV replication is a valuable aid to prevent viral reactivation during immunosuppressive treatment. In particular, for patients who receive a short-term chemotherapy lamivudine prophylaxis is suggested, while tenofovir or entecavir should be given for long-term immunosuppressive therapy (18, 19).

Although at a lower rate than occurs with cancer chemotherapy, in patients with rheumatic diseases who have either HBsAg or anti-HBc in serum, simple immune-suppression may induce reappearance of HBV replication and disease activation.

The risk of HBV reactivation in patients with rheumatic disease under immunosuppressive therapy is less well defined and the studies were principally carried out in RA patients (11, 21-25). Differently from patients receiving chemotherapy for definite periods, these patients usually receive long-term immunosuppressive therapy, which increases the risk of viral reactivation (26) and it is possible that a significant proportion of chronically infected patients remains undiagnosed, due to the usual absence of clinical symptoms (27).

Moreover, in the last few years, more RA patients received anti-TNF- α , because they were resistant to conventional disease-modifying anti-rheumatic drugs (DMARDs), making the remission of RA an increasingly realistic aim.

As hypothesised for mycobacterial infection, TNF could be a key cytokine in controlling HBV infection, and raised levels of TNF and TNF receptor super family member 16 (also called p75) have been found in the serum and liver of patients with HBV infection (13). A pivotal role for TNF in the suppression of HBV replication that involves a synergistic effect with interferon has also been suggested (28, 29), and TNF appears to be essential in clearing HBV (30, 31). It seems reasonable to suggest, therefore, that pharmacological blockade of TNF- α might facilitate the replication of HBV by allowing the virus to escape endogenous antiviral defense mechanisms blocking the proliferation of HBV-specific cytotoxic T lymphocytes and restoring the helper T lymphocyte ability to suppress the HBV specific immune response (32,33).

Vassilopoulos *et al.* (12) have recently reported the results from a study of 131

patients with rheumatic diseases treated with anti-TNF agents, of whom 19 had undergone HBV vaccination, 19 were positive for anti-HBc antibodies (indicative of previous HBV infection) and 14 were HBsAg+ (a marker of chronic HBV infection). Antiviral prophylaxis was administered to HBsAg+ patients before anti-TNF therapy; after a mean follow-up of 2 years, only one HBsAg+ patient had experienced HBV reactivation, owing to the emergence of a lamivudine-resistant viral strain.

Although less frequently than observed in HBsAg positive patients, HBV reactivation can also occur in HBsAg negative patients if an occult infection is present. Indeed, according to the results of the Pubmed search, of the 255 with rheumatic disease treated with anti-TNF α agents, HBV reactivation occurred in 33 of 87 HBsAg+ patients compared with only 9 of 168 of those who were HBsAg negative/anti-HBc positive.

Unlike other studies that evaluated only anti-TNF treated patients with rheumatoid arthritis, in the present 2-year prospective study also patients suffering from psoriatic arthritis and ankylosing spondylitis were included. The study of markers for major hepatotropic viruses in this group of 57 patients affected by rheumatic disease before and during the therapy with conventional and anti-TNF drugs showed that four patients were infected with HBV and none with HCV. The HBsAg positive patients were treated with a preemptive lamivudine therapy and no increase in viral load nor clinical signs of viral reactivation was observed.

In the HBsAg negative patient an occult HBV infection was investigated by the presence of HBV DNA in serum sample obtained before and during anti-TNF therapy.

To our knowledge, this study is the first in which the occult HBV DNA was investigated by home-made nested PCR assay highly sensitive and specific for the four HBV genomic regions in patients with rheumatic disease.

In oncohaematological patients undergoing chemotherapy, this highly sensitive serum HBV DNA testing at baseline had shown in 18 out of 75 patients the occult infection with a 28% predic-

tive ability to forecast HBsAg seroconversion. The HBV reactivation was observed also in 6 out of 57 occult HBV DNA negative, showing a 90% ability to forecast persistent HBsAg negativity (16). In contrast with this previous study, utilising the same procedure, occult HBV-DNA was not detected in all HBsAg negative patients with various rheumatic diseases under anti-TNF agents.

A possible explanation for this different result may be attributed to the lower mean age of the group study (49 vs. 60.9) and the different pathology. Indeed, epidemiological studies have demonstrated the relation between HBV infection and oncohaematological disease (34). The HBV reactivation observed in oncohaematological patients with or without occult HBV DNA detectable in the serum may be due to the chemotherapeutic agents, including anthracyclines and corticosteroids that stimulate HBV replication (35, 36). However, the data in the present study are consistent with the literature in which only few cases of OBI reactivation are reported.

Although the number of enrolled subjects is limited, some considerations may be made.

- a) even in an area of previously high HBV endemicity, where occult HBV infection is likely to have a high prevalence, especially in onco-haematological patients, HBV reactivation in patients with rheumatic diseases is rare.
- b) treatment of rheumatological patients with anti-TNF drugs is thus safe in terms of its potential to reactivate HBV.
- c) in this setting, serological markers of previous exposure to HBV (anti-HBc and/or anti-HBs, in the absence of HBsAg) do not indicate a higher likelihood of OBI, and hence are not an indication to pre-emptive prophylaxis with nucleoside analogues.
- d) long-term prophylaxis with lamivudine is sufficient and safe to prevent reactivation in HBsAg carriers.

The choice of antiviral agent in the prophylaxis of HBsAg+ patients who require treatment with anti-TNF agents should be made according to existing international guidelines that suggest

that lamivudine alone is sufficient for patients receiving immunosuppressive therapy for less than 6 months (37).

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