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Clinical features and effect of antiviral therapy on anti-liver/kidney microsomal antibody type 1 positive chronic hepatitis C☆

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***Background/Aims*: Anti-liver/kidney microsomal antibody type 1 (anti-LKM1), a serological marker of type 2 autoimmune hepatitis, is also detected in a small proportion of patients with hepatitis C. This study aimed to evaluate clinical features and effect of antiviral therapy in patients with hepatitis C who are anti-LKM1 positive.**

***Methods*: Sixty consecutive anti-LKM1 positive and 120 age and sex-matched anti-LKM1 negative chronic hepatitis C patients were assessed at diagnosis and during follow-up. Of these, 26 anti-LKM1 positive and 72 anti-LKM1 negative received antiviral therapy. Anti-LKM1 was detected by indirect immunofluorescence and immunoblot. Number of HCV-infected hepatocytes and intrahepatic CD8+ lymphocytes was determined by immunohistochemistry.**

***Results*: At diagnosis anti-LKM1 positive patients had higher IgG levels and more intrahepatic CD8+ lymphocytes (p 0.022 and 0.046, respectively). Viral genotypes distribution and response to therapy were identical. Hepatic flares during antiviral treatment only occurred in a minority of patients in concomitance with anti-LKM1 positivity.**

***Conclusions*: Immune system activation is more pronounced in anti-LKM1 positive patients with hepatitis C, possibly representing the expression of autoimmune mechanisms of liver damage. Antiviral treatment is as beneficial in these patients as in anti-LKM1 negative patients, and the rare necroinflammatory flares are effectively controlled by corticosteroids, allowing subsequent resumption of antiviral therapy.**

***Keywords*: Hepatitis C virus; Anti-Liver Kidney Microsome antibody (anti-LKM1); Hepatic immunohistochemistry; Interferon treatment; Hepatitis flare**

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Abbreviations: LKM1, anti-liver kidney microsome type 1; HCV, hepatitis C virus; SMA, anti-smooth muscle antibody; ANA, anti-nuclear antibody; E2, viral envelope protein 2; CLD, chronic liver disease; IAIHG, International Autoimmune Hepatitis Group; CYP2-D6, cytochrome P isoform 2D6 IFN standard interferon; Peg-IFN, pegylated interferon.

1. Introduction

Hepatitis C virus (HCV) infection not only is the cause of chronic liver disease and its sequelae [1], but is also associated to a variety of immunopathological abnormalities and disorders such as mixed cryoglobulinemia [2], non-Hodgkin's lymphoma [3] and occurrence of non-organ specific autoantibodies, which are detected in 25–30% of HCV-infected patients, the most frequent reactivity being anti-smooth muscle antibody (SMA), anti-nuclear antibody (ANA) and anti-liver/kidney microsomal antibody type 1 (anti-LKM1) [4–8].

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Autoantibody production appears to be the result of a non-specific activation of the immune system during the course of chronic HCV infection. The viral envelope protein E2 of HCV binds CD81 on the B cell surface and engages the CD19–CD21 co-stimulating complex, lowering the activation threshold of the B cell [9]. In addition, the direct infection of B cells might also play a role in the development of autoimmune phenomena [10,11]. Finally, a further hypothesis based on molecular mimicry between viral and host antigens has been proposed for the appearance of anti-LKM1 antibody [12].

Anti-LKM1, the serological hallmark of type 2 autoimmune hepatitis, is also detected in a limited proportion of HCV-infected patients. The anti-LKM1 immuno-morphological pattern on liver/kidney rat sections is one and the same in type 2 autoimmune hepatitis and in HCV-related chronic hepatitis. On the other hand, the immunodominant epitopes are different in the two conditions: autoimmune anti-LKM1 mainly react with linear [13,14] whereas HCV-related anti-LKM1 is directed to conformational epitopes [15,16].

Genetic predisposition, in particular the presence of HLA-*DRB1*0701*, appears to be important for the development of anti-LKM1 in Italian [17] and Spanish patients with chronic hepatitis C [18].

The target of anti-LKM1 is the isoform 2D6 of cytochrome P450 family, it is located in the microsomal fraction of the hepatocyte, but it may also be exposed on the plasma membrane, thus accessible to the immune systems effectors [19]. Anti-LKM1 reactivity therefore may play a potential pathogenetic role in the initiation and perpetuation of hepatic damage.

At variance with ANA and SMA positivity, whose presence does not affect the response to antiviral therapy [8,20], we and others have previously reported severe transaminase flares in anti-LKM1 positive patients with chronic hepatitis C on interferon [7,21–25], an observation suggesting a potential autoimmune-mediated mechanism, exacerbated by a pro-inflammatory cytokine, in addition to HCV-induced liver damage.

The aim of our retrospective analysis is to describe the features of anti-LKM1 positive patients with HCV infection compared to sex- and age-matched population of anti-LKM1 negative patients with hepatitis C in order to evaluate the impact of anti-LKM1 positivity on disease presentation, follow-up and response to antiviral treatment.

2. Patients and methods

2.1. Patients

Sixty consecutive patients with anti-LKM1 positive HCV-related chronic liver disease (CLD) and 120 age and sex-matched patients with HCV-related CLD negative for anti-LKM1 and all the other non-organ specific autoantibodies were enrolled between 1992 and 2006 (median age 52 years, male percentage 41.7%, median follow-up 60 months). All patients were assessed at liver disease diagnosis. The liver disease was assumed to start from the first determination of increased levels of serum transaminases together with the detection of anti-HCV antibodies and HCV-RNA. Prior to 1989, altered transaminases were used to date the duration of the disease. A presumable source of infection (major surgery intervention, blood transfusion and intravenous drug use) was identified in about one third of the subjects, irrespective of the anti-LKM1 status. For the remaining patients we hypothesize a iatrogenic transmission (non-disposable needles and syringes and dental therapy), as already reported for the Italian population [26]. Two additional subjects were referred to our Unit at the time of a hepatitis flare with positive anti-LKM1 antibodies during antiviral treatment. Their baseline data are unknown and were considered only for the follow-up and matched with 4 more controls. Clinical, immunological and serological features of 10 anti-LKM1 positive patients were reported in detail in previous publications [20–22]. All patients were positive for anti-HCV (Ortho HCV version 2.0/3.0 ELISA, Ortho Clinical Diagnostics, Inc. Raritan, NJ, USA or MEIA/CMIA, Abbott Diagnostic, Abbott Park, IL, USA) and HCV-RNA detected by nested RT-PCR (Beckman Analytical, Milan, Italy), Versant HCV RNA 3.0 (bDNA) or Bayer Versant HCV RNA qualitative (TMA) assay (Bayer Diagnostics, Berkeley, CA, USA). None of the anti-LKM1 positive patients or the anti-LKM1 negative controls tested positive for hepatitis B surface antigen (HBsAg). HCV genotype was determined by core region PCR amplification with specific antisense primers (HCV Gen-Eti-K DEIA; Sorin Biomedica). To assess the possibility of concomitant type 2 autoimmune hepatitis, the International Autoimmune Hepatitis Group (IAIHG) score for the diagnosis of autoimmune hepatitis [27] was calculated for each anti-LKM1 positive patient.

Conventional laboratory tests of liver damage and function were performed at presentation and at each follow-up visit. Autoantibodies were searched at presentation and additional determinations were performed every three months during antiviral treatment and every six months after the end of therapy. Liver biopsy for histological examination was performed at presentation.

Twenty-six anti-LKM1 positive patients (43%) and 72 controls (60%) received at least one course of antiviral treatment; 10 anti-LKM1 positive patients (38%) and 19 controls (25%) were given more than one course of therapy, with a mean of 1.5 and 1.3 treatments administered to each patient respectively (p not significant). Thirty-nine and 95 courses were overall administered to anti-LKM1 positive patients and controls respectively, with an equal distribution of different molecules (standard interferon alone, standard interferon plus ribavirin, pegylated interferon plus ribavirin) and schedules between the two groups.

Sustained virological response was defined as the absence of detectable serum HCV RNA at 24 weeks after the end of treatment [28].

2.2. Autoantibody testing

Sera were tested for the presence of non-organ specific autoantibodies (anti-nuclear antibodies, anti-smooth muscle antibodies, anti-LKM1) by indirect immunofluorescence on cryostat sections of rat liver, kidney and stomach specimens at a serum dilution of 1:40 which were titred to extinction as previously reported [20]. Anti-LKM1 positive sera were assessed by an in-house immunoblot assay using human liver microsomes as antigenic source, as described elsewhere [29]. Anti-CYP2D6 reactivity was confirmed using a commercial immunoblot (Euroline Liver profile, Euroimmune, Labordiagnostika, Lubeck, Germany) based on purified CYP2D6. Detection of anti-neutrophil cytoplasmic antibodies was performed at a serum dilution 1:20, as described elsewhere [30].

2.3. Liver histology

Liver biopsy specimens were obtained percutaneously using 1.4 or 1.2 mm needles in 133 patients. Liver fragments were divided into two parts. The first part (at least 20 mm, available for all 133 patients) was fixed in formalin, embedded in paraffin and evaluated by the pathologist who scored inflammation and fibrosis as minimal-mild and