Articoli originali

Original articles

Absence of occult HCV infection in patients experiencing an immunodepression condition

Assenza di infezione occulta da HCV in pazienti immunodepressi

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INTRODUCTION

Recently, an unusual clinical form of chronic infection by hepatitis C virus (HCV) characterized by the presence of HCV RNA in the liver and/or in Peripheral Blood Mononuclear Cells (PBMC) in the absence of circulating anti-HCV and HCV RNA has been identified [1-3].

This new entity has been defined as "occult" HCV infection and has been described in two different clinical settings: one clinical form with abnormal liver function tests is characterized by the negativity of both serum anti-HCV antibodies and HCV RNA; another clinical condition is characterized by the positivity for serum anti-HCV and no detectable HCV RNA with normal liver enzyme levels years after spontaneous, or therapy-induced resolution of HCV infection [4-8].

However, in literature there are contrasting data on this topic: Barrill et al. found that 45% of 109 anti-HCV negative hemodialysis patients with abnormal serum aminotranferases had HCV RNA in PBMC and the same group found a high occult HCV infection prevalence among family members of patients with occult HCV infection [9, 10]. De Marco et al. described in 9 subjects among 276 (3.3%) healthy people with normal alanine aminotransferase values the existence of occult HCV infection [11]. The same authors in a subsequent research found that 1.3% of 314 subjects without signs of active or previous viral hepatic infection and 28% of 7 active HBV carriers were positive for HCV-RNA in PBMC, but negative for serological and virological markers of HCV, indicating an occult infection (12). Instead, other investigators failed to demonstrate this peculiar viro-immunological condition in subjects with abnormal aminotransferases or in kidney-transplant patients [13, 14].

The present paper describes a study aiming to evaluate the presence of occult HCV infection in two settings of patients experiencing immunosoppression condition: the patients with Human Immunodeficiency Virus (HIV) infection and those with onco-hematological disease.

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PATIENTS AND METHODS

Patients

We enrolled patients belonging to a particular immunological setting: patients with HIV infection and patients with onco-hematological disease; in particular we prospectively enrolled 60 consecutive HIV-positive/anti-HCV-negative/HCV RNA-negative patients observed from January 2008 to April 2011 at the HIV Unit of the Second University of Naples, Italy (HIV group) and 32 consecutive anti-HCV/HCV RNA negative patients with an onco-hematological disease first undergoing chemotherapy from April 2007 to November 2009 at the Hematology Unit of Second University of Naples, Italy (Onco-hematological group).

At the enrollment each patient of both groups was screened for HBsAg, anti-HBs, anti-HBc, anti delta, anti-HCV; those in onco-hematological group also for anti-HIV. For each patients was performed an electronic schedule; for the patients in the HIV group the schedule collected demographic data, risk factors for acquiring HIV infection, CD4+ nadir and antiretroviral therapeutic history; for the patients in the onco-hematological group the schedule collected epidemiological, biochemical and virological characteristics, diagnosis and treatment of the onco-hematological diseases.

At enrolment the 60 patients in the HIV group were untreated or received antiretroviral treatment according to the current international guidelines. For all HIV patients a no-end follow-up was planned. A total of 51 patients were observed monthly as outpatients for at least 6 months (median 12, range 6–36), whereas 9 were lost to follow-up because of lack of compliance. No significant difference was observed between the 51 patients having a follow-up and the 9 who were lost to follow-up with regard to demographic, clinical and laboratory characteristics observed on admission.

Of the 32 patients in the onco-hematological group, 3 were HBsAg positive and received standard telbivudine or entecavir treatment, whereas 18 were HBsAg negative/anti-HBc positive and underwent a long-term Lamivudine prophylaxis according to the international guidelines [15, 16]. These 32 patients were treated with chemotherapy for 4-12 months and observed after its discontinuation for 6-24 months.

For each patient in both groups a sample of plasma and PBMC was obtained at enrolment; samples of plasma and PBMC were obtained during the follow-up in the patients in HIV group every three months, in those in onco-hematological group at months 1 and 3 during chemotherapy and then every three months after treatment discontinuation.

PBMC were isolated from 5 mL of whole blood by means of Histopaque (Sigma-Aldrich, St. Louis, MO, USA) according to a standard technique: the cells were washed three times with phosphate-buffered saline (PBS, pH 7.2), counted in a hemocytometer and immediately frozen in aliquots of $2x10^6$ cells at -80° C in the same buffer and never thawed until used for this investigation. The presence of HCV RNA in plasma and PBMC of all samples collected during the study was determined as follows: viral RNA was extracted from plasma samples or from $2x10^6$ PBMC using a microspin column (QIAamp RNA viral kit for plasma, QIAmp RNA Blood kit for PBMC, Qiagen GmbH, Hilden, Germany); the RNA was analyzed for the presence of HCV genomes by performing a real-time PCR with a wide range of linearity in a Light-cycler 1.5 (Roche Diagnostics, Branchburg, NJ, USA) [17, 18]. The detection limit in the plasma samples was around 40 IU/mL [19].

The sensitivity of our method to detect HCV RNA in PBMC samples was assessed using HCV-positive PBMC diluted in PBMC obtained from an HCV-negative patient, as described by Halfon [13]. Briefly, $2x10^6$ PBMC from an HCV RNA-positive patient quantified at $1.8x10^4$ IU/$2x10^6$ PBMC was 1:10 sequentially diluted in $2x10^6$ HCV RNA negative PBMC; in these PBMC mixtures, HCV RNA was then quantified by real-time PCR. The lowest detection limit by this method was 18 IU/$2x10^6$ cells. To avoid inter-assay variability, HCV RNA in plasma and PBMC of all patients was again determined in the same assay at the end of the study.

As a positive control of extraction of RNA from PBMC, G6PDH mRNA was sought in all PBMC samples collected (LightCycler® h-G6PDH Housekeeping Gene Set; Roche Diagnostics, Branchburg, NJ, USA) [20, 21]. Precisely, for the patients in HIV group a total of 60 and 212 samples of plasma and PBMC at enrolment and in the follow-up, respectively, were obtained; for those in onco-hematological group a total of 32, 72 and 132 samples of plasma and PBMC before, during and after chemotherapy, respectively, were obtained.

All the procedures used in the study were in accordance with the international guidelines, with the standards on human experimentation

of the Ethics Committee of the Azienda Ospedaliera Universitaria of the Second University of Naples and with the Helsinki Declaration of 1975 revised in 1983 and all patients signed an informed consent in accordance with the rules of the Ethics Committee of the Azienda Ospedaliera Universitaria of the Second University of Naples for the collection and storage of plasma and PBMC samples and for the collection and use of their data in clinical research.

RESULTS

Table 1 shows the demographic, clinical, virological and serological characteristics observed at enrolment for the 60 patients in HIV group (Table 1). Unsafe sexual intercourse was the most frequent risk factor for acquiring HIV infection (85.2%). Eleven (18.3%) patients had a CD4+ cell count lower than 200 cells/μL at enrolment, but 29 (48.3%) had a history of CD4+ cell nadir lower than 200/μL and 20 (33.3%) of AIDS. At enrolment, 50 patients (83.3%) were on HAART and 47 (73.3%) were HIV RNA-negative (Table 1). Twelve patients showed serological marker of HBV infection, one (1.6%) of active HBV infection and 19 (31.7%) of a previous infection (Table 1). Fifteen (25%) patients showed abnormal ALT serum values (Table 1). No plasma or PBMC sample of the anti-HIV-positive/anti-HCV-negative patients collected at enrollment and during the follow-up was HCV RNA-positive. All PBMC samples collected from anti-HCV-negative patients were positive for G6PDH mRNA.

Table 2 shows the demographic, clinical, biochemical and serological characteristics observed at baseline in the 32 patients in onco-hematological group. Non-Hodgkin Lymphoma was diagnosed in 18 patients, Chronic Lymphocytic Leukemia in 7, Multiple Myeloma in 6 and Hodgkin Lymphoma in 1. All patients received chemotherapy, rituximab-based in 20 (62.5%) (Table 2). The three HBsAg/HBV DNA-positive patients at the baseline were treated with telbivudine or entecavir. They became HBV DNA-negative within 6 months while still under treatment and remained so throughout the observation; the 18 HBsAg negative/anti HBc positive patients received lamivudine prophylaxis and never showed circulating HBsAg or HBV DNA. No plasma or PBMC sample collected at enrollment and dur-

Table 1 - Demographic, clinical, biochemical and serological characteristics of 60 anti-HCV-negative patients in HIV group.

Males/females	39/21
Race (Caucasian/African/Hispanic)	49/9/2
Age (year), median (range)	41 (21-64)
Patients with risk factor, N. (%)	
Unsafe heterosexual intercourse	26 (43.3)
Unsafe homosexual intercourse	16 (26.6)
Stable sexual partner of known anti-HIV-positive subject	9 (15.3)
IVDA	7 (11.6)
Vertical transmission	1 (1.6)
Accidental prick with infected needles	1 (1.6)
CD4+ (cells/μL), mean± SD	616.4±386.4
Patients with CD4+ <200 cells/μL, N. (%)	11 (18.3)
CD4+ cell nadir (cells/μL), mean ± SD	261.2±118.0
Patients with history of CD4+ nadir <200 cells/μL, N. (%)	29 (48.3)
Patients classified as CDC-C, N. (%)	20 (33.3)
Patients on HAART, N. (%)	50 (83.3)
HIV-RNA-negative patients, N. (%)	44 (73.3)
HIV viral load in 16 HIV RNA-positive patients (copies/ml), mean ± SD	76,219.38± 112,466
AST, IU/L, (normal value 10-35), mean ± SD	27.1±24.5
ALT, IU/L, (normal value 10-40), mean ± SD	34.1±27.2 (10-40)
ALP, IU/L, (normal value 53-128), mean ± SD	85.2±21.6
γGT, IU/L, (normal value 0-50), mean ± SD	54.5±61.5
Total bilirubin, mg/dl (normal value 0-1), mean ± SD	0.68±0.69 (0-1)
Patients with abnormal ALT serum value, N. (%)	15 (25)
Patients with abnormal ALT and/or γGT and/or ALP serum value, N. (%)	44 (73.3)
HBsAg positive patients, N. (%)	1 (1.6)
HBsAg negative/anti-HBc positive patients, N. (%)	19 (31.7)

Table 2 - Demographic, clinical and serological characteristics of the 32 anti-HCV-negative patients in onco-hematological group.

	Anti-HCV-negative patients
No. of patients	32
Males/Females	18/14
Age (year), median (range)	64 (21-81)
Hematological diseases:	
B-NHL, N. (%)	18 (56.3%)
HL, N. (%)	1 (3.1%)
CLL, N. (%)	7 (21.9%)
Multiple myeloma, N. (%)	6 (18.7%)
Type of immunosuppressive therapy	
Rituximab, N. (%)	2 (6.2%)
R-CHOP, N. (%)	16 (50%)
Chlorambucil+ Methylprednisolone, N. (%)	4 (12.6%)
Fludarabine+ Prednisone, N. (%)	1 (3.1%)
R-Fludarabine+ Cyclophosphamide, N. (%)	2 (6.2%)
Bortexomib+Dexametasone, N. (%)	3 (9.5%)
ABVD, N. (%)	1 (3.1%)
DVD, N. (%)	2 (6.2%)
Melphalan+Prednisone, N. (%)	1 (3.1%)
AST, n.v.x (mean ± SD)	0.71±0.28
ALT, n.v.x (mean ± SD)	0.81±0.35
Total bilirubin, mg/dL, (mean ± SD)	0.71±0.37
% Prothrombin activity, median (range)	98 (70-118)
Serological status for HBV, N. (%)	
HBsAg positive	3 (9.3%)
HBsAg -/anti-HBs+/anti-HBc +	12 (37.5%)
HBsAg -/anti-HBs-/anti-HBc +	6 (18.8%)
HBsAg negative/anti-HBc negative	11 (34.4%)
Anti-HBV prophylaxis/therapy, N. (%)	
Lamivudine	18 (56.3%)
Telbivudine	2 (6.2%)
Entecavir	1 (3.1%)
None	11 (34.4%)

Footnotes: NHL: Non-Hodgkin Lymphoma; HL: Hodgkin Lymphoma; CLL: Chronic Lymphocyte Leukemia; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; ABVD: ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine; DVD: Pegylated Liposomal Doxorubicin, Vincristine, Dexamethasone; AST: aspartate-aminotransferases; ALT: Alanine-aminotransferases; n.v: normal value; SD: Standard Deviation.

ing the follow-up was HCV RNA positive. All PBMC samples collected were positive for G6PDH mRNA.

CONCLUSION

Conflicting are the data available in the current literature about identification of occult HCV infection. Barrill et al. found a high prevalence (45% of cases) of occult HCV infection in 109 anti-HCV negative hemodialysis patients with abnormal serum aminotranferases [9]. De Marco et al. found occult HCV infection both in healthy people with normal alanine aminotransferase values and in active HBV carriers [11, 12]. Instead, other investigators did not show this peculiar viro-immunological condition in subjects with abnormal aminotransferases or in kidney-transplant patients [13, 14].

The results from this study rule out the existence of occult HCV infection in patients with strong immunosuppression due to different condition, HIV infection and onco-hematological disease. These patients represent two interesting models of investigation devoted to find occult HCV infection in immunosoppression setting. In fact, 18.3% of the patients in HIV group had severe immunodepression at enrolment, 48.3% had a history of CD4+ nadir lower than 200 and 33.3% of AIDS. In onco-hematological group, the patients suffered from a severe immunodepression for the onco-hematological disease and for the specific therapy; in fact, 62.5% of the patients enrolled received rituximab-based chemotherapy, which has been demonstrated to be able to increase the HCV replication in anti-HCV-positive patients [17, 22, 23]. Our study is in agreement with the data not identifying occult HCV infection, but are in disagreement with those form Barril and De Marco [9, 11-14]. Probably, the high prevalence of occult HCV infection observed by Barril was due to the particular immunological condition of the hemodialysis patients [9]. Moreover, we would underline that the number of patients enrolled in the De Marco studies was higher than that in our study.

In conclusion, occult HCV infection was not found in the present study in two different settings of immunodepression, the anti HIV positive anti-HCV/HCV RNA-negative patients and the anti-HCV/HCV RNA-negative patients with onco-hematological diseases. The peculiar settings of patients investigated with a

severe immunodepression induce to doubt on the existence of occult HCV infection. However, further investigations in larger case studies are needed to confirm a lacking existence of occult HCV infection in the considered settings.

Keywords: HCV infection, occult HCV infection, onco-hematological diseases, HIV infection, immunosuppressive diseases, rituximab

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SUMMARY

The aim of our study was to evaluate the presence of occult HCV infection in two settings of patients experiencing immunosuppression: patients with Human Immunodeficiency Virus (HIV) infection and those with onco-haematological disease. Sixty consecutive HIV-positive/anti-HCV-negative/HCV RNA-negative patients (HIV group) and 32 consecutive anti-HCV/HCV RNA negative patients with an onco-haematological disease first undergoing chemotherapy (Onco-haematological group) were enrolled.
HCV-RNA was sought by real time RT-PCR in plasma and Peripheral Blood Mononuclear Cell (PBMC) samples obtained at enrolment and during follow-up, in the patients in the HIV group every three months and in those in the onco-haematological group at months 1 and 3 during chemotherapy and then every three months after treatment discontinuation. No plasma or PBMC sample collected at enrolment and during the follow-up in the HIV and onco-haematological groups was HCV RNA positive. The results of this study rule out the existence of occult HCV infection in patients with strong immunosuppression due to different conditions, HIV infection and onco-haematological diseases.

RIASSUNTO

Lo scopo dello studio consisteva nel valutare la presenza di infezione occulta da HCV in due condizioni d immunodepressione, l'infezione da HIV e la malattia onco-ematologica. Sono stati arruolati 60 pazienti consecutivi HIV-positivi/anti-HCV-negativi/HCV RNA-negativi (gruppo HIV) e 32 pazienti consecutivi anti-HCV/HCV RNA negativi con patologia onco-ematologica prima dell'inizio della chemioterapia (Gruppo Onco-ematologico). L'HCV-RNA è stato cercato mediante real-time PCR nel plasma e nei PBMC ottenuti all'arruolamento e durante il follow-up, ogni tre mesi per il Gruppo HIV e al I e III mese durante la chemioterapia e poi ogni 3 mesi dopo la fine del trattamento nel Gruppo Onco-ematologico. Nessun campione di plasma e di PBMC ottenuto all'arruolamento o durante il follow-up in entrambi i gruppi è risultato positivo per HCV-RNA. I risultati di questo studio escludono l'esistenza dell'infezione occulta da HCV in due esempi di immunodepressione, l'infezione da HIV e la patologia onco-ematologica.

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