

# Hepatitis viruses and HIV infection in the Naples area

## *Virus dell'epatite ed infezione da HIV nell'area metropolitana di Napoli*

Pietro Filippini, Nicola Coppola, Carlo Scolastico, Giovanni Rossi, Martina Battaglia, Mirella Onofrio, Raffaella Pisapia, Cecilia Marrocco, Caterina Sagnelli, Felice Piccinino, Evangelista Sagnelli

Department of Medicine and Public Health, Section of Infectious Diseases, Second University of Naples, Naples, Italy

### ■ INTRODUCTION

The human immunodeficiency virus (HIV) infection shares transmission routes with hepatitis B virus (HBV), hepatitis Delta virus (HDV) and hepatitis C virus (HCV) infection. Consequently, the risk factors for the acquisition of these infections are similar and markers of hepatitis virus infections are frequently detected in anti-HIV positive subjects [1].

Several studies suggest that about 80% of subjects with HIV infection show serum markers of an ongoing or previous HBV infection [1-4]; moreover, over 60% of HBsAg positive drug addicts also show HDV infection and more than one third of these are anti-HIV positive [2, 5, 6].

Also HCV infection frequently occurs in subjects with HIV infection (20-65%) [2, 3, 7, 8], especially if they have received blood transfusions or have been drug addicts (80-90%) (5, 9); the prevalence is also high in male homosexuals (8-15%) [10]. Although sexual transmission of HCV is infrequent under normal conditions (from 2 to 6%), it is 5 times as high in subjects with HIV infection [11].

The aims of our study were to evaluate the prevalence of viral hepatitis infections in anti-HIV positive patients in our geographic area, to analyse the importance of parenteral and sexual risk factors for the acquisition of these infections, to study the role of immunodeficiency on the clinical presentation of hepatitis and the role of the hepatitis viruses on the clinical presentation of HIV infection.

### ■ MATERIALS AND METHODS

*Patients:* we evaluated 189 consecutive anti-HIV positive subjects from the Naples area (130 males and 59 females) at their first observation as outpatients in our HIV day-care clinic from October 1996 to October 2001, at the time HIV infection was first identified. The median age was 32 years (range 17-57) and the CD4 lymphocyte count was  $362 \pm 333/\text{mm}^3$  (mean  $\pm$  S.D.); the patients were at different clinical stages of HIV infection and 17 had AIDS. Our patients had never been treated for HIV, HBV, HDV or HCV infections at the time they were included in the study.

*Methods:* liver function tests were performed by routine methods. The lymphocyte subsets (CD4, CD8) were determined by flow cytometry using monoclonal antibodies and FAC-Scan (Becton Dickinson, Mountain View, USA). To detect HIV replication we sought the serum p24 antigen of HIV by a semiquantitative ELISA (Abbott Lab., North Chicago, IL, USA) in subjects first observed before March 96 and by a HIV-RNA quantitative PCR assay (HIV-1 Monitor Roche, Roche Molecular System, Branchburg, NJ) in those observed later [12, 13]. The clinical staging of HIV positive subjects was performed according to the 1993 CDC classification [14]. Markers of HBV (HBsAg, HBsAb, HBcAb, HBeAg and HBeAb), HDV (Anti-HD and HD-Ag) and antibody to HCV were determined by ELISA commercial assays (Sorin Biomedica, Saluggia, Italy; Abbott Lab., North Chicago, IL, USA).

The presence of HBsAg was considered an in-

dex of ongoing HBV infection, the presence of HBcAb in HBsAg negative cases an index of previous exposure to HBV, the presence of serum HD-Ag or anti-HD was considered a marker of HDV infection and anti-HCV positivity as a marker of HCV infection.

Since no patient underwent liver biopsy, those with normal or elevated serum aminotransferase values for at least 6 months and with no sign of liver cirrhosis (see below) were considered respectively asymptomatic carriers or chronic hepatitis patients. "Clinical cirrhosis" was diagnosed on the basis of abnormalities of liver function tests and the presence of clinical signs of cirrhosis: a blood platelet count lower than 100.000/mm<sup>3</sup>, ascites, presence of porto-systemic encephalopathy, presence of oesophageal varices, presence of echographic signs characterising liver cirrhosis (coarse pattern, irregular margins, reduced capacity of the hepatic veins).

Each subject completed a questionnaire recording his/her demographic data, sexual behaviour and any exposure to parenteral risks of infection. In particular we considered as "unsafe homosexual or heterosexual activity" to have had at least three occasional partners with irregular use of condom in the last year; moreover we defined as "homosexual or heterosexual intercourse with a steady anti-HIV positive partner" to have had a steady HIV positive sexual partner with irregular use of condom in the last year. Subjects who referred unprotected sexual intercourse with an intravenous drug abuser were considered as "sexual partners of drug addicts". For 14 subjects (0.7% of cases) more than one risk factor was recorded; in these

cases the subjects determined what was their own major risk factor.

*Statistical Analysis:* the statistical analysis of the results was performed using Student's *t-test* and the chi-square test. A *p* value < 0.05 was considered as significant.

## RESULTS

The polymorph expression of serum markers associated to HBV, HDV and HCV in the 189 anti-HIV positive subjects in this study are represented in Table 1. For epidemiological and clinical evaluation, these patients were subgrouped according to the presence of hepatitis virus serum markers as reported in Table 2. Patients with no serum marker of hepatitis were included in group "HIV"; patients with chronic HBV infection, with or without HDV markers, in group "HIV+HBV"; anti-HCV positive patients, with or without HDV markers, in group "HIV+HCV"; patients with HBV+HCV co-infection, with or without HDV markers, in group "HIV+HBV+HCV" (Table 2).

The median age was similar in all the etiologic groups and subgroups, but there was a higher prevalence of males in group "HIV+HCV" (Table 2). Moreover, the prevalence of cases with a presumed acquisition time of HIV infection dating back at least 5 years was lower in group "HIV" compared to groups "HIV+HBV", "HIV+HBV+HCV" and "HIV+HCV" (32.9% vs. 71.5%, 66.6% and 71%, respectively); the difference between group "HIV" and group "HIV+HCV" was statistically significant (*p* < 0.0001).

**Tabella 1** - Chronic viral hepatitis infection in 189 anti-HIV positive subjects.

<i>HBV, HDV or HCV markers</i>	<i>No. of cases</i>	<i>%</i>
HBsAg	3	1.3
HBsAg, anti-HD or HD-Ag	4	2.1
Anti-HCV	35	18.5
Anti-HCV, HBsAg	7	4
Anti-HCV, HBsAg, anti-HD or HD-Ag	2	0.8
Anti-HCV, anti-HD or HD-Ag	2	0.8
Anti-HCV, anti-HBc	54	28.5
Anti-HBc	22	12
None	60	32
Total	189	100

**Tabella 2 - 189 anti-HIV positive subjects. Age and sex.**

<i>Etiologic group</i>	<i>No. of cases</i>	<i>Age, yrs median (range)</i>	<i>M/F</i>	<i>(% Male)</i>
"HIV"	82	30 (17-55)	1.41	(58.5)
"HIV+HCV"	91	32 (21-57)	3.13	(75.8)
"HIV+HBV"	7	34 (32-36)	6	(85.7)
"HIV+HBV+HCV"	9	30 (23-37)	2	(66.7)
Total	189	32 (17-57)	2.2	(68.7)

Of the 189 patients, 91 (48.1%) reported i.v. drug abuse, 3 (1.6%) heterosexual intercourse with a drug addict, 26 (13.7%) other unsafe heterosexual activity, 37 (19.5%) heterosexual intercourse with a steady anti-HIV positive partner, 29 (15.3%) unprotected homosexual intercourse, 1 (0.6%) homosexual intercourse with a steady anti-HIV positive partner, 1 (0.6%) blood transfusion; only one patient (0.6%) showed no risk factor for parenteral or sexual transmission. Among the 130 males and 59 females, drug abuse was reported in 60% and in 22%, respectively ( $p < 0.0001$ ); steady sexual relations with an anti-HIV positive partner in 4% and in 54% ( $p < 0.0001$ ). No difference was found between males and females for other unsafe heterosexual relations (13% males vs. 15% females).

We analysed the distribution of the hepatitis aetiologies in relation to the major risk factor reported (Table 3). Of the patients with a history of intravenous drug abuse, eighty per cent were in group "HIV+HCV", 4.4% in group "HIV+HBV", 7.7% in group "HIV+HBV+HCV" and 7.7% in group "HIV" (Table 3). Also unsafe sexual intercourse, whether homosexual or heterosexual, and sexual intercourse with a steady HIV positive partner were associated with the presence of anti-HCV in serum (10.4%, 15.4% and 26.4%, respectively).

No substantial difference in the CD4 cell count

was observed in the different hepatitis groups (Table 4). However, we observed a statistically significant association between an advanced HIV clinical stage and HCV infection ( $p < 0.005$ ); in fact, subjects in group "HIV" more frequently than those in group "HIV+HCV" were in the CDC-A clinical stage; conversely, patients in group "HIV+HCV" were more frequently in the CDC-B clinical stage; however the percentage of patients with AIDS was similar in these two groups (Table 4). The low number of patients in groups "HIV+HBV" and "HIV+HBV+HCV" does not allow statistical analysis of the results.

Among the 60 patients in whom the HIV viral load was assessed by RT-PCR we detected a lower level of viremia in the 36 patients in group "HIV" than in the 24 patients in group "HIV+HCV" ( $28.415 \pm 122.627$  vs.  $69.944 \pm 158.379$  copies/ml). This difference however is not significant to the statistical analysis ( $p < 0.2$ ).

We found 25 patients with normal ALT for at least 6 months (20 in group "HIV+HCV", 3 in group "HIV+HBV" and 2 in group "HIV+HBV+HCV") and 73 patients with biochemical signs of chronic hepatitis (66 in group "HIV+HCV", 4 in group "HIV+HBV" and 3 in group "HIV+HBV+HCV"). Signs of clinical cirrhosis were observed in 9 patients: 5 of the 91 patients in group "HIV+HCV" (5.5%)

**Tabella 3 - 189 anti-HIV positive subjects: risk factor.**

<i>Etiologic group</i>	<i>i.v. drug abuse, % 91 cases</i>	<i>Unsafe heterosexuality, % 29 cases</i>	<i>Unsafe homosexuality, % 29 cases</i>	<i>Hetero-homo sexual contact with a steady HIV partner, % 38 cases</i>
"HIV"	7.7*	82.7*	79.3°	68.4^
"HIV+HCV"	80.2*	10.4*	17.3°	26.4^
"HIV+HBV"	4.4	6.9	3.4	-
"HIV+HBV+HCV"	7.7	-	-	5.2

\* Group "HIV+HCV" vs Group "HIV" =  $p < 0.0001$ ; ° Group "HIV" vs Group "HIV+HCV" =  $p < 0.0001$ ; ^ Group "HIV" vs Group "HIV+HCV" =  $p < 0.001$

**Tabella 4** - 189 anti-HIV positive subjects: number of CD4 and HIV clinical stage.

<i>Etiologic group</i>	<i>No. of cases</i>	<i>CD4 mean ± DS</i>	<i>Stage A</i>	<i>CDC, 93 Stage B</i>	<i>Stage C</i>
"HIV"	82	349 ± 233*	72°	17°	11°
"HIV+HCV"	91	384 ± 280*	53.8°	39.6°	6.6°
"HIV+HBV"	7	179 ± 232	28.5	43	28.5
"HIV+HBV+HCV"	9	437 ± 375	44.4	55.6	0
Total	189	362 ± 333	60.8	30.2	9

\* Group "HIV" vs Group "HIV+HCV": p = n.s.; ° Group "HIV" vs Group "HIV+HCV" = p < 0.005

and 4 of the 9 in group "HIV+HBV+HCV" (57.1%). None of the 82 subjects with no marker of a hepatitis virus infection showed signs of chronic hepatitis or clinical cirrhosis. In patients with hepatitis virus infection, considered all together, no significant difference in the CD4 cell count was observed between asymptomatic carriers, chronic hepatitis patients and clinical cirrhosis patients: 350±330 CD4 cells/mm<sup>3</sup> (mean ± SD) in the 25 asymptomatic carriers, 321 ± 271 CD4 cells/mm<sup>3</sup> in the 73 chronic hepatitis patients and 276 ± 154 CD4 cells/mm<sup>3</sup> in the 9 clinical cirrhosis patients.

## DISCUSSION

Our study showed a high prevalence of patients chronically infected with hepatitis virus among anti-HIV positive subjects: HCV infection was found in 53.9% of cases and HBV infection in 8.4%, whereas only 32% of our patients had no marker of hepatitis virus infection. These prevalences are similar to those we reported in a previous study [15] and to data from other studies in Italy [2, 5].

Intravenous drug abuse was strictly associated to the presence of HCV infection, since 82.3% of anti-HIV positive drug abusers in the study were anti-HCV positive. Even unsafe sexual activity was associated to a spread of HCV in anti-HIV positive subjects. These observations confirm the importance of the role of parenteral route in the transmission of HCV infection, but even the role of sexual transmission of HCV infection in HIV positive patients should be underlined. In fact, 19.8% of the 91 patients with HIV/HCV coinfection showed only unsafe sexual activity as the major risk factor for parenteral transmission. On the other side, HCV infection was detected in

10.4% of the 29 patients who reported unsafe homosexual activity, in 15.4% of the 26 with unsafe heterosexual activity and in 26.4% of the 38 who had sexual activity with a steady anti-HIV positive partner. For full comprehension of the importance of these prevalences, we would like to recall that in the general population in southern Italy Guadagnino observed anti-HCV prevalences of 1.3%, 2.3% and 5.0%, respectively in 452 subjects under 30 years old, 215 subjects 30-39 years old and 161 subjects 40-49 years old [16]. In addition, Sagnelli found no higher risk of HCV infection for 156 spouses of HCV infected subjects than for 362 other relatives (O.R. 1.9, C.I. 95% = 0.8-4.2) [17]. Akahane showed that the rate of HCV infection among spouses correlates with the duration of marriage: no sign of HCV transmission was detected among those married for less than 10 years, whereas the possibility of transmission was observed with increasing frequency with each additional decade of marriage [18]. Considering the experience of the above-mentioned authors, we may conclude that the sexual transmission of HCV is rare in the normal population, whereas there is a helper role of HIV in the sexual transmission of HCV, probably due to immune-depression that might favour HCV replication. This was hypothesised by Eyster in 1991 [11].

Published studies on the influence of HCV infection on the outcome of an HIV related disease are conflicting and not conclusive. Indeed, the data reported by Ockenga show a more severe clinical outcome in HIV patients with HCV infection [3], whereas more recent observations claim no influence at all [8]. In our patients no substantial difference in the immunological parameters, nor in the HIV viremia levels, nor in the frequency of AIDS was observed between patients with HIV in-

fection alone and those with HIV+HCV coinfection. However, patients with HIV+HCV coinfection more frequently than those with HIV infection alone showed a CDC-B clinical stage. However, it remains unclear whether the higher prevalence of cases with oral mycosis, seborrheic dermatitis, fever, diarrhoea, weight loss and monomeric zoster, pathologies linked to a pre-AIDS stage, might be related to a longer duration of HIV infection or to some influence of HCV infection.

It is also unclear what role HIV plays in the outcome of liver disease [19-21]. A study carried out in 1997 by Soto showed a more frequent progression to cirrhosis in subjects with HIV-HCV co-infection than in those with HCV infection alone [22]; moreover the diagnosis of liver cirrhosis has been reported to be associated to a more severe degree of immunodeficiency [23]. The serum HCV-RNA levels have been reported to be higher in subjects with HCV+HIV co-infection than in those with HCV infection alone, with an inverse correlation to the CD4 count [24-26]. Our data show that a presence of a more severe viral liver disease is linked to a multiple hepatitis virus infection, regardless of the degree of immunodepression. These data agree with the results of our previous studies in anti-HIV

negative subjects with chronic hepatitis [27, 28].

In conclusion, we have shown that in anti-HIV positive patients, co-infection with the major hepatitis viruses is common, especially in drug abusers, and that the HIV infection may be a cofactor in the sexual transmission of HCV infection. Co-infection of HIV and multiple hepatitis infections seems to be associated to a more severe liver disease. Recent progress in anti-retroviral therapy and the consequent improvement in the prognosis of patients with HIV infection warrant greater attention being paid to concomitant chronic viral hepatitis.

*Key Words:* HIV, HCV, HBV, HDV, drug abuse, sexual transmission.

#### *Acknowledgements*

This study was supported by a grant from Project Viral Hepatitis "Multiple hepatitis infections: diagnostic and prognostic value of different virological patterns", n°96/B/T27 of National Institute of Health (Istituto Superiore di Sanità), Rome, Italy, and by a grant from the Ministry for University Education, Scientific Research and Technology (MURST), Rome - Italy.

### SUMMARY

In 189 anti-HIV positive subjects (130 males and 59 females; median age 32 years, range 17-57) we evaluated the prevalence of patients with hepatitis infections, the role of parenteral and sexual risk factors on the acquisition of these infections and the reciprocal influence between HIV and HCV infections. HCV infection was detected in 53.9% of cases and HBV infection in 8.4%. In only 32% of our patients no marker of hepatitis virus infection was detected. The presence of a hepatitis virus infection was associated to drug addiction; indeed in 91 drug abusers HIV/HCV co-infection was present in 80% of cases and HIV infection alone in 7.7%,  $p < 0.0001$ . On the other hand, the association between unsafe sexual activity, whether homosexual or heterosexual, and sexual activity with a steady anti-HIV positive partner with HCV infection was less evident, although the

high prevalence of anti-HCV in these cases (10.4%, 15.4% and 26.4% respectively) clearly suggests that HIV infection may improve the sexual transmission of HCV. No substantial differences in the level of immunodeficiency, nor in the HIV viral load nor in the frequency of AIDS cases were observed between patients with HIV infection alone and those with HIV/HCV co-infection. In fact, the percentage of patients with AIDS was similar in these two groups. However, we observed a statistically significant association between an advanced HIV clinical stage and the presence of HIV/HCV co-infection ( $p < 0.005$ ), since subjects with co-infection more frequently than with HIV infection alone were in the CDC-B clinical stage. The presence of a more severe liver disease was linked to a multiple hepatitis virus infection, regardless of the degree of immunodeficiency.

## RIASSUNTO

In 189 soggetti anti-HIV positivi (130 maschi, età mediana 32 anni, range 17-57) è stata valutata la prevalenza delle infezioni da virus epatitici, il ruolo dei fattori di rischio parenterali o sessuali nell'acquisizione di queste infezioni e la reciproca influenza delle infezioni da HIV ed HCV. Le infezioni da HCV ed HBV sono state evidenziate rispettivamente nel 53.9% e nell'8.4% dei casi. Solo il 32% dei pazienti non presentava alcun marker di infezione da virus epatitici. La presenza di infezione da virus epatitici era associata alla tossicodipendenza; infatti nei 91 tossicodipendenti la coinfezione HIV-HCV era presente nell'80% dei casi, mentre la sola infezione da HIV nel 7.7% ( $p < 0.0001$ ). L'associazione tra attività sessuale a rischio (rapporti sessuali non protetti, sia omo- che eterosessuali, e attività sessuale con un partner stabilmente HIV positivo) ed infezione da HCV era

meno evidente, sebbene l'alta prevalenza di anti-HCV in questi casi (10.4%, 15.4% e 26.4% rispettivamente) suggerisce che l'infezione da HIV può favorire la trasmissione sessuale di HCV. I pazienti con coinfezione HIV-HCV non presentavano nessuna differenza sostanziale per quanto riguarda il livello di immunodepressione, i livelli di viremia da HIV e la frequenza di AIDS rispetto ai pazienti con sola infezione da HIV. Tuttavia è stata osservata una associazione statisticamente significativa tra uno stadio clinico avanzato di infezione da HIV e la presenza di coinfezione da HIV-HCV ( $p < 0.005$ ), considerato che i soggetti con coinfezione più frequentemente appartenevano al gruppo B della classificazione CDC. La presenza di una malattia epatica più severa era correlata alla presenza di una infezione multipla da virus epatitici, indipendentemente dal grado di immunodeficienza.

## REFERENCES

- [1] Poles M.A., Lew E.A., Dieterich D.T. Diagnosis and treatment of hepatic disease in patients with HIV. *Gastroenterol. Clin. N. Am.* 26, 291-321, 1997.
- [2] Francisci D., Baldelli F., Papili R. et al. Prevalence of HBV, HDV and HCV hepatitis markers in HIV-positive patients. *Eur. J. Epidemiol.* 11, 123-126, 1995.
- [3] Ockenga J., Tillmann H.L., Trautwein C. et al. Hepatitis B and C in HIV-infected patients. *J. Hepatol.* 27, 18-24, 1997.
- [4] Scharschmidt B.F., Held M.J., Hollander H.H. et al. Hepatitis B in patients with HIV infection: relationship to AIDS and patients survival. *Ann. Intern. Med.* 117, 837-838, 1992.
- [5] Coppola R.C., Manconi P.E., Piro R. et al. HCV, HIV, HBV and HDV infections in intravenous drug addicts. *Eur. J. Epidemiol.* 10, 279-283, 1994.
- [6] Novick D.M., Farci P., Croxson T.S., et al. Hepatitis D virus and human immunodeficiency virus antibodies in parenteral drug abusers who are hepatitis B surface antigen positive. *J. Infect. Dis.* 158, 795-803, 1988.
- [7] Wright T.L., Hollander H.H., Xiang P. et al. Hepatitis C in HIV-infected patients with and without AIDS: prevalence and relationship to patient survival. *Hepatology* 20, 1152-1155, 1994.
- [8] Spengler U., Rockstroh J.K. Hepatitis C in the patients with human immunodeficiency virus infection. *J. Hepatol.* 29, 1023-1030, 1998.
- [9] Stevens C., Taylor P.E., Pindyc J. et al. Epidemiology of hepatitis C virus: a preliminary study in volunteer blood donors. *JAMA* 263, 49-53, 1990.
- [10] Esteban J.I., Esteban R., Viladomiu L. et al. Hepatitis C virus antibodies among risk group in Spain. *Lancet* 337, 294-297, 1989.
- [11] Eyster M.E., Alter H.J., Aledort L.M. et al. Heterosexual co-transmission of hepatitis C virus (HCV) and human immunodeficiency virus (HIV). *Ann. Intern. Med.* 115, 764-768, 1991.
- [12] Mellors J.W., Rinaldo R.C., Gupta P. et al. Prognosis in HIV-1 infection predicted by the quality of virus in plasma. *Science* 272, 1164-1170, 1996.
- [13] O'Brien W.A., Hartigan P.M., Martin D. et al. Changes in plasma HIV-1 RNA and CD4 lymphocyte counts and risk of progression to AIDS. Veterans Affairs Cooperative study Group on AIDS. *N. Engl. J. Med.* 334, 426-431, 1996.
- [14] Center for Disease Control. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR* 41, 1-9, 1992.
- [15] Sagnelli E., Filippini P., Guarino M. et al. Hepatitis C Virus infection in drug addicts from Naples, Italy: its relationship with Human Immunodeficiency Virus and other infections. *Mediterr. J. Infect. Dis.* 8, 77-80, 1993.
- [16] Guadagnino V., Stroffolini T., Rapicetta M. et al. Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in Southern Italy. *Hepatology* 26, 1006-1011, 1997.
- [17] Sagnelli E., Gaeta G.B., Felaco F.M. et al. Hepatitis C virus infection in households of anti-HCV chronic carriers in Italy: a multicentre case-control study. *Infection* 25, 346-349, 1997.
- [18] Akahane Y., Kojima M., Sugai Y. et al. Hepatitis C virus infection in spouses of patients with type C chronic liver disease. *Ann. Intern. Med.* 120, 748-752, 1994.
- [19] Collier J., Heathcote J. Hepatitis C viral infection in the immunosuppressed patient. *Hepatology* 27, 2-6, 1998.
- [20] Eyster M.E., Diamondstone L.S., Lien J.M. et al. Natural history of hepatitis C virus infection in multi-transfused haemophiliacs: effect of co-infection with human immunodeficiency virus. *J. Acquir. Immune*

*Defic. Syndr.* 6, 602-610, 1993.

[21] Pol S., Lamorthe B., Thin N.T. et al. Retrospective analysis of the impact of HIV infection and alcohol use on chronic hepatitis C in a large cohort of drug users. *J. Hepatol.* 28, 945-950, 1998.

[22] Soto B., Sanchez-Quijano A., Rodrigo L. et al. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *J. Hepatol.* 26, 1-5, 1997.

[23] Rockstroh J.K., Spengler U., Sudhop T. et al. Immunosuppression may lead to progression of hepatitis C virus-associated liver disease in haemophiliacs co-infected with HIV. *Am. J. Gastroenterol.* 91, 2563-2573, 1996.

[24] Crieber B., Rey D., Schmitt C. et al. High hepatitis

C viraemia and impaired antibody response in patients coinfecting with HIV. *AIDS* 9, 1131-1136, 1995.

[25] Eyster M.E., Fried M.W., Di Bisceglie A.M. et al. Increasing hepatitis C virus RNA levels in haemophilic patients. *Blood* 84, 1020-1023, 1994.

[26] Ghany M.G., Leisinger C., Laiger R. et al. Effect of human immunodeficiency virus infection on hepatitis C virus infection in hemophiliacs. *Dig. Dis. Sci.* 272, 1265-1272, 1996.

[27] Sagnelli E., Felaco F.M., Filippini P. et al. Multiple hepatitis virus infections in chronic HBsAg carriers in Naples. *Arch. Virol.* 142, 445-451, 1997.

[28] Sagnelli E., Coppola N., Scolastico C. et al. Aspetti virologici e clinici delle infezioni multiple da virus epatitici: dati preliminari di uno studio italiano multicentrico. *Infez. Med.* 2, 90-95, 1999.