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Epidemiology of viral hepatitis infections in an area of southern Italy with high incidence rates of liver cancer

Mario Fusco[a], *Enrico Girardi*[b], *Pierluca Piselli*[b], *Raffaele Palombino*[c], *Jerry Polesel*[d], *Carmela Maione*[a], *Paola Scognamiglio*[b], *Francesco Aurelio Pisanti*[e], *Mariacarmela Solmone*[f], *Pietro Di Cicco*[g], *Giuseppe Ippolito*[b], *Silvia Franceschi*[h], *Diego Serraino*[d,*], *for the Collaborating Study Group* [i]

[a]*Registro Tumori della Regione Campania, ASL NA4 Brusciano, Naples, Italy*
[b]*Epidemiology Unit, INMI Lazzaro Spallanzani Rome, Italy*
[c]*Unit of Epidemiology and Prevention, ASL NA4 Brusciano, Naples, Italy*
[d]*Unit of Epidemiology and Biostatistics, Centro di Riferimento Oncologico IRCCS, via F. Gallini, 2, 33081 Aviano, Italy*
[e]*Clinical Pathology Unit, ASL NA4 Brusciano, Naples, Italy*
[f]*Laboratory of Virology, INMI Lazzaro Spallanzani Rome, Italy*
[g]*Medical Department, Ospedale S. Maria Della Pietà, Nola, Italy*
[h]*International Agency for Research on Cancer, Lyon, France*

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ABSTRACT

Southern Italy shows the highest rates of liver cancer for Europe, mainly related to infection with hepatitis viruses. We thus described incidence rates of liver cancer and investigated prevalence and determinants of HCV and HBV infections in 4496 individuals randomly selected from the general population of the province of Naples. 7.5% was infected with HCV and 27.6% with HBV (2.2% was HBsAg-positive). Prevalence of both infections increased with age, 23.2% of those aged 65 years or older was HCV-positive and 47.9% were HBV-positive. Intravenous drug use (odds ratio (OR) = 16.4 for anti-HCV and 4.7 for anti-HBc), history of blood transfusions (OR = 2.8 and 1.5, respectively) and surgery, and household contacts with infected people (OR = 2.1 and 1.6, respectively) increased risks for both infections. Sexual intercourse with HCV-positive individuals conveyed a 3-fold higher risk of HCV infection. This study quantified the spread of HCV and HBV in the population of southern Italy heavily affected by liver cancer.

1. Introduction

Liver cancer in all developed regions of the world is diagnosed, each year, in 8.5/10^5 men and in 3.0/10^5 women (Fig. 1).[1] In Europe, a noteworthy North-South geographic gradient exists, with rates ranging -in men- from 3.4/10^5 in the North to 11.6/10^5 in the South. The highest rates of liver cancer in industrialized countries are recorded in Italy, with 15.9

* *Corresponding author:* Tel.: +39 0434 659 354; fax: +39 0434 659 231.
E-mail address: serrainod@cro.it (D. Serraino).

[i] Members of the Collaborating Study Group: National Institute for Infectious Diseases Lazzaro Spallanzani, IRCCS, Rome: Simona Barzoni Secchia, Stefania Bellelli, Maria Rosaria Capobianchi, Fabrizio Carletti, Claudia Cimaglia, Paola Di Folco, Anna Rosa Garbuglia, Luca Iezzi, Massimiliano Scuderi; Campania Cancer Registry: Felice Avella, Letizia Gigli, Pasquale Patriciello, Pasquale Quadrano, Silvana Russospena; National Cancer Institute, Centro di Riferimento Oncologico, IRCCS, Aviano: Luigino Dal Maso.

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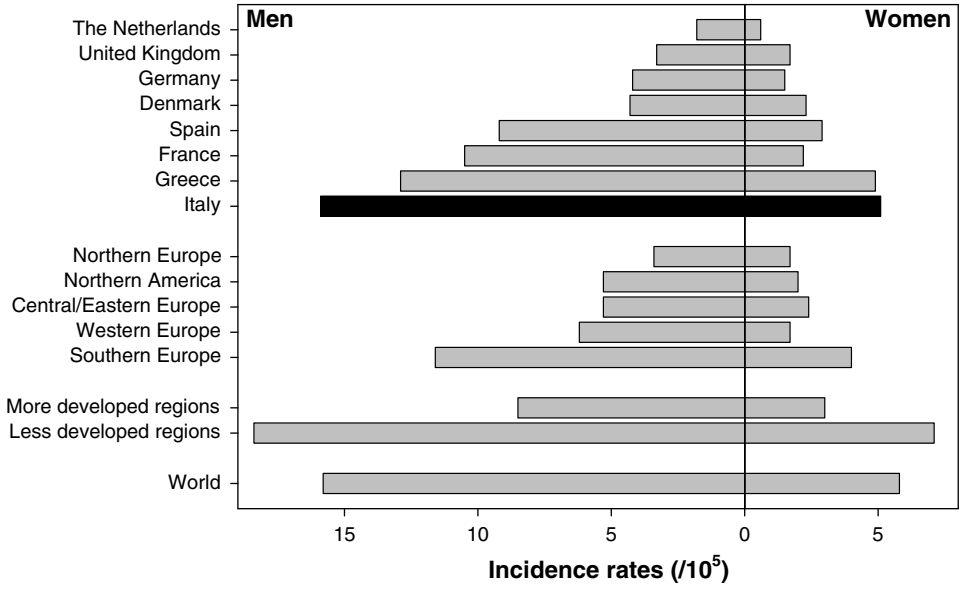

Fig. 1 – Incidence rates of liver cancer in various areas of the world (Source: GLOBOCAN 2002[1]).

cases/10^5 men and 5.1 cases /10^5 women (Fig. 1).[1] Upward trends in incidence and mortality rates have been described in the United States and in southern Europe, where the spread of hepatitis viruses (i.e. HBV and HCV) occurred earlier than in other areas.[2]

In Italy, the use of intravenous drugs and, up to the early 1990s, the use of contaminated needles in medical procedures are thought to be the major determinants of the diffusion of HBV and HCV. About 90% of liver cancer in southern Italy is estimated to be attributable to infection with HBV and HCV.[4,5] Population-based surveys showed very high prevalence of HCV (up to 26% in elderly) in southern Italy,[6–9] and a strong North-South geographic gradient - most notably among middle age and old people.[10–13]

In view of the heavy impact of liver cancer, a thorough quantification of the diffusion of hepatitis infections in southern Italy is still needed, particularly in population groups below the age of 65 years. We have thus carried out a population-based study in the province of Naples, an area covered by a population-based cancer registry - the Campania Cancer Registry (CCR). Study aims were to describe incidence rates of liver cancer and to assess prevalence of, and risk factors for, HCV and HBV infections, and to establish a cohort study of infected individuals.

2. Materials and methods

Data from the CCR were used to describe the main aspects of the incidence of liver cancer (e.g. age standardised rates to the world population) in the province of Naples.[14] In this area, we carried out, between 2003 and 2006, a cross-sectional, population-based, seroepidemiological investigation. A sample of 9996 residents (4879 men and 5117 women) stratified by health district, gender and age was obtained using a list of randomly generated numbers from the 370,846 residents 20 to 84 years of age.

Up to three invitations were sent by mail from the CCR staff to each of these 9996 individuals asking them to visit the general practitioner for study enrolment. A letter explaining the study objectives was sent to the corresponding general practitioners, including the list of patients included in the study. Thus, 394 general practitioners were informed that their patients had been invited to take part in a screening for HCV and HBV infections. For each patient, the general practitioner received a questionnaire with an identification number and a medical prescription for free screening tests.

The study protocol conformed to the 1975 Declaration of Helsinki and had been approved by the CCR Board of Ethics. Individuals who agreed to take part in the study signed the informed consent and were interviewed by the general practitioner in his/her office. Thereafter, they were referred to two public laboratories for serological testing. Those who went to a general practitioner's office but refused to participate were asked to motivate their refusal using a short questionnaire that included history of screening against HCV and medical history of liver-related conditions.

Eighteen general practitioners refused to enroll the totality of their 93 sampled patients; 619 individuals (6.2%) could not be contacted (138 had died, 458 had moved, and 23 could not be found at the given address); and 3649 declined participation. Among the remaining, 1139 completed only the short questionnaire and 4496 (48.4% of the contacted individuals) were tested and interviewed. Participants were older than non-participants (median ages: 44.5 and 39.1 years for participants and non-participants, respectively) and more often females (55.2% and 47.7%, respectively). To investigate the main correlates of HCV and HBV infections, we used a standardised questionnaire. Information collected included socio-demographic factors (e.g. sex, age, education, occupation, marital status, residence), life style, practices known to convey risk of viral transmission (i.e. blood transfusion, dialysis, organ transplantation, surgical procedures, dental therapies lasting more than 1 month, use of intravenous drugs, tattoo/piercing, history of sexual intercourse with infected partners and/or with injecting drug users, lifetime number of sexual partners,

cohabitation with HCV or HBV-infected individuals), and medical history.

2.1. Diagnostic testing

A 10 ml blood sample was collected, centrifuged and the serum distributed in six aliquots for HCV and HBV testing and biological banking. Antibodies against HCV (anti-HCV) were tested using a third generation enzyme immunoassay (Roche Diagnostics, Mannheim, Germany) on the analyser Cobas Core II (Roche Diagnostics). Sera showing anti-HCV immunoreactivity, by enzyme immunoassay, were tested for HCV RNA by means of: i) qualitative HCV-RNA assay with a lower sensitivity of 50 IU/mL (Cobas Amplicor HCV 2.0; Roche Diagnostics, Indianapolis, IN, USA) and, ii) quantitative HCV RNA by Versant HCV RNA 3.0 b-DNA assay (Bayer Diagnostic Corporation, Tarrytown, NY, USA) with a reported linear range of 615–7.7×10^6 IU/mL. Sera resulting anti-HCV positives but HCV-RNA negatives were confirmed by supplemental testing for anti-HCV antibodies by immunoblot assay (Deciscan HCV plus, Bio-Rad, Marnes La Coquette, France). HCV genotype was evaluated in all sera from subjects testing positive for HCV-RNA, and it was performed by reverse hybridisation assay (LiPA Bayer Healthcare, Tarrytown, NY, USA).

Sera were also tested for antibodies to hepatitis B core antigen (anti-HBc) and for hepatitis B surface antigen (HBsAg) by commercial immunoassays (Cobas Corell, Roche Diagnostics, Indianapolis, IN, USA).

2.2. Statistical analysis

Overall and age- and sex-specific prevalence of HCV and HBV infections were computed using proportions and corresponding 95% confidence intervals (CI). Observed prevalence of HCV and of HBV infections were standardised for sex and truncated age distribution (i.e. 20–84 years) of the population in the 11 Health Districts of the CCR.

Risk factors for HCV and HBV infections were assessed by means of multivariate statistical analysis. Multiple logistic regression odds ratios (ORs) and 95% CIs were computed adjusting for age, gender and education.[15] For examined risk factors with more than 5% of missing values, ORs were also computed for individuals with unknown values.

3. Results

Between 1996 and 2004, incidence rates for liver cancer in the CCR increased, on average each year, by 4.7% in men and 3.7% in women. Table 1 shows - for the period 1998–2002 - the 20 population-based cancer registries with the highest incidence rates of liver cancer in Europe. Nearly all of these cancer registries (16 out of 24) were located in Italy, and the CCR showed the highest rates in both men (34.8/10^5) and women (10.2/10^5).

With regard to the cross-sectional investigation, out of the 4496 tested, 336 persons turned out to be anti-HCV positives (7.5%): 246 (5.5%) were positive for HCV-RNA. Moreover, 1241 individuals (27.6%) were anti-HBc positive while 100 (2.2%) were HBsAg-positive (Table 2). The truncated age- and sex-adjusted prevalence was 6.7% for anti-HCV positivity, 27.5% for anti-HBc positivity and 2.5% for HBsAg-positivity. Infection with HCV seemed equally frequent in women and men, whereas significantly fewer women than men were anti-HBc-positive or HBsAg-positive (OR = 0.7 and 0.6, respectively) (Table 2). Ageing was associated with higher prevalence of infections, up to 23.2% for HCV and 47.9% for HBV in those aged 65 years or older. Conversely, prevalence of HBsAg-positivity peaked at 45–54 years and declined after age 64 years. Higher education conveyed reduced risks for both HCV and HBV infections (Table 2).

Table 1 – Cancer registries with the twenty highest incidence rates (/10^5) of liver cancer, Europe 1998–2002

Cancer Registry	Rank	Incidence rates/10^5		
		Males	Females	M/F
Southern Italy, Campania Cancer Registry, Naples	1	34.8	10.2	3.4
Northern Italy, Brescia	2	29.5	7.4	4.0
Northern Italy, Sondrio	3	26.0	4.1	6.3
Northern Italy, Parma	4	20.0	9.0	2.2
Northern Italy, Veneto	5	18.2	4.2	4.3
Italy, North East Cancer Surveillance Network	6	17.4	3.8	4.6
Switzerland, Ticino	7	16.3	3.2	5.1
Southern Italy, Sassari	8	16.0	6.4	2.5
France, Calvados	9	15.2	2.4	6.3
Northern Italy, Milan	9	15.2	4.0	3.8
Northern Italy, Varese	10	14.9	3.4	4.4
France, Loire-Atlantique	11	14.6	1.4	10.4
Southern Italy, Salerno	11	14.6	6.2	2.4
Southern Italy, Ragusa	12	14.5	5.0	2.9
Northern Italy, Biella	13	14.4	3.7	3.9
France, Bas-Rhin	14	13.6	2.2	6.2
Northern Italy, Turin	15	13.0	3.3	3.9
Switzerland, Valais	16	12.0	2.3	5.2
Switzerland, Geneva	17	11.7	2.1	5.6
France, Isere	17	11.7	2.1	5.6
Northern Italy, Ferrara	18	11.6	3.9	3.0
France, Haut-Rhin	18	11.6	2.6	4.5
Northern Italy, Genoa	19	11.5	3.5	3.3
Southern Italy, Syracuse	20	11.3	4.8	2.4

Source: Ref. 14.

For the two sexes separately, the strong age-related increase of the combined prevalence of infection with hepatitis viruses (i.e. anti-HCV positivity and HBsAg positivity) is illustrated in Fig. 2a, and the parallelism with increasing incidence rates of liver cancer is shown in Fig. 2b.

The risk of being HCV-positive was 2-fold significantly higher in individuals who reported household contacts (including sexual relationships) with a HCV-positive person (95% CI:1.5–2.9), and 3-fold more elevated (95% CI:1.9–5.1) in those who reported sexual intercourse with HCV-positive partners. Few subjects admitted to having used intravenous drugs, with a very high risk of HCV-infection (OR = 16.4). Some medical practices were also positively associated to an elevated risk of HCV infection. We noted higher risks associated to history of blood transfusions - particularly for transfusions received before 1993 (OR = 3.5) - or with dental therapies (OR = 1.4, 95% CI: 1.1–1.8). Moreover, clinical history of

Table 2 – Distribution of 4496 individuals according to test results and general characteristics (Naples, southern Italy, 2003–2006)

		Anti-HCV		Anti-HBc		HBsAg	
	Tested N.	Positives %	OR[a] (95%CI)	Positives %	OR[a] (95%CI)	Positives %	OR[a] (95%CI)
Sex:							
Men	2014	8.0	1[b]	32.4	1[b]	2.8	1[b]
Women	2482	7.0	1.1 (0.8–1.3)	23.7	0.7 (0.6–0.8)	1.8	0.6 (0.4–0.9)
Age:							
20–24	769	0.4	1[b]	3.8	1[b]	1.2	1[b]
25–34	793	1.5		8.1		1.5	
35–44	727	1.8	1.9 (0.9–4.0)	24.3	5.1 (3.9–6.7)	3.2	2.4 (0.3–4.4)
45–54	818	4.8	5.2 (2.8–9.4)	38.6	9.9 (7.7–12.8)	3.7	2.8 (1.6–4.9)
55–64	589	14.1	16.9 (9.7–30)	46.2	13.6 (10.4–17.7)	2.9	2.2 (1.1–4.2)
⩾65	800	23.2	31.2 (18.3–54)	47.9	14.5 (11.3–18.7)	1.1	0.8 (0.4–1.8)
Education (years)[c]:							
0–5	1345	15.4	1[b]	43.3	1[b]	2.5	1[b]
6–8	1354	4.5	0.8 (0.6–1.1)	27.0	1.0 (0.8–1.2)	3.0	1.0 (0.6–1.6)
⩾9	1661	2.9	0.7 (0.5–1.0)	14.8	0.6 (0.5–0.8)	1.4	0.5 (0.3–0.9)
History of HCV testing							
No	3040	4.2	1[b]	25.7	1[b]	1.8	1[b]
Yes	877	19.6	7.4 (5.6–9.7)	32.2	1.3 (1.1–1.5)	3.5	1.8 (1.2–2.9)
Unknown	579	6.2	1.4 (1.0–2.1)	30.7	1.2 (1.0–1.5)	2.2	1.2 (0.6–2.2)
Total (95% CI)	4496	7.5 (6.7–8.2)		27.6 (26.3–28.9)		2.2 (1.8–2.7)	

a Odds ratio (OR) estimated from multiple unconditional logistic regression and adjusted, when appropriate, for age.
b Reference category.
c The sum does not add up to the total because of missing values.

jaundice or of hepatitis was a strong correlate of HCV positivity (OR = 9.3) (Table 3).

A similar risk pattern was seen for anti-HBc positivity. Household contacts of HBV-infected people (OR = 1.6), intravenous drug users (OR = 4.7), those who were exposed to blood transfusion (OR = 1.5) or to surgical interventions (OR = 1.3) were at significantly elevated risks of being anti-HBc positives (Table 3). When the analysis was restricted to HBsAg-positive individuals, only household contacts of HBV-positive ones were at a significantly elevated risk (OR = 2.9, 95% CI:1.7–5.2) (data not shown).

4. Discussion

This study on liver cancer and infection with hepatitis viruses was conducted in the province of Naples, an area of southern Italy that shows the highest incidence rates of this cancer in Europe,[14] and where about 90% of liver cancers are attributable to infection with HCV and/or HBV.[5] The upward trends noted in men and women were in agreement with previous European descriptions of liver cancer mortality showing - in Italy between 1970 and 1996 - an average yearly increase of 4.4% per year.[3] Stable (e.g. Finland, the UK) or downward trends (e.g. Spain, Greece, Scandinavian countries) were noted in several other European countries, most notably depending on the spread of viral hepatitis infections and diagnostic improvements.[3] A recent analysis of data from all Italian cancer registries provided evidence for upward trends in liver cancer incidence rates between 1988–1997, followed by a plateau as of 2002 along with a huge geographic variability likely due to different modalities of HCV and HBV spread.[16] Whether these trends will be confirmed in Italy and in other European countries in the coming years is not clear, given the large number of immigrants from countries where HCV and HBV are common.

The results of the cross-sectional investigation confirmed the widespread diffusion of HCV and HBV infections in the province of Naples, thus confirming their etiological role in liver cancer occurrence. Hepatitis infections were widely prevalent in the older segments of the population, but in middle-aged people also, HCV was several-fold higher (i.e. 8.7% in 45–64 years old) than in other western countries.[17,18] In agreement with this observation, it is likely that high rates of liver cancer will continue to be recorded in the coming years. Compliance with this cross-sectional study was negatively influenced by the widespread HCV testing on a voluntary basis. A practice due, in the study area, to high rates of liver-related diseases (e.g. 25.3% of the 1139 participants who completed the short questionnaire refused to undergo blood testing because they had already been tested the preceding year). It is difficult to quantify the impact of non-participation on study findings, since factors affecting participation may differently influence prevalence estimates (e.g. age and sex) of HCV and HBV infections. For instance, we found that the prevalence of HCV infection in first-time tested people (4.2%) was several-fold lower than among ever tested ones, indicating a potential overestimation of the real diffusion of HCV in the area. However, other population-based investigations with higher participation rates found, in southern Italy, higher HCV prevalence (from 10.4% to 26.0%) than we did (i.e. 6.7% of HCV-positivity after standardisation).[6–8] Conversely, population-based estimates closer to ours were documented from a multicentre investigation showing a prevalence of 7.3% in southern Italy.[10]

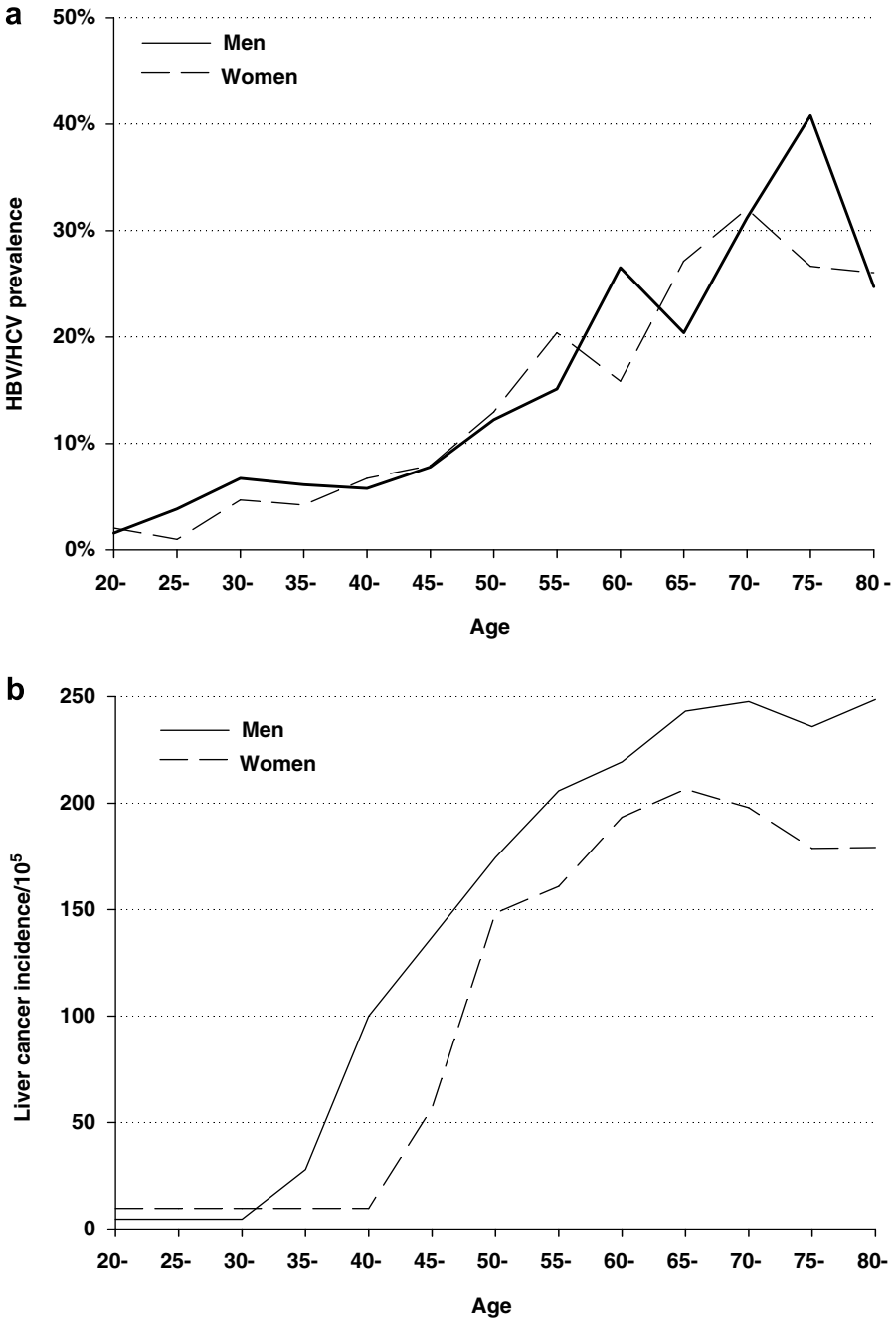

Fig. 2 – (a) Prevalence of HCV and/or HBV infections by age and sex in Naples, southern Italy; (b) Incidence rates (/100,000) of liver cancer by age and sex in Naples, southern Italy (Source: CI5 VIII[14]).

The study results showed that higher levels of education were associated with reduced risks of infection and helped to quantify the role of well-established modes of transmission (i.e. exposure to medical procedures, use of intravenous drugs, cohabitation, and sexual intercourse with infected people). Diagnostic or therapeutic procedures have represented, in the past, important modes of transmission for hepatitis viruses in Italy.[19] This particularly held true for older generations exposed to contaminated bloods or blood products up to the 1990s, when blood-screening measures reduced thereafter, to a negligible level, the risk of viral transmission. To what extent exposure to contaminated blood (e.g. dental therapies) or blood products still represents a relevant mode of transmission could not be assessed by this investigation. However, when the statistical analysis was stratified for large age groups, the results indicated that iatrogenic acquisition of infection has become rare in younger generations.

We confirmed that transmission of hepatitis infections is increased by household contacts, although we could not disentangle the role of non-sexual, intra-familial viral transmission from sexual transmission.[20,21] Information bias is also possible, as household members of subjects found to be HCV-positive or HBV-positive might have been screened with particular attention.

Table 3 – Odds ratios (ORs) and 95% confidence intervals (CIs) for hepatitis C virus or hepatitis B virus infections by selected characteristics (Naples, southern Italy, 2003–2006)

		Anti-HCV		Anti-HBc	
	Tested N.	Positives %	OR[a] (95%CI)	Positives %	OR[a] (95%CI)
Living with a person known to be infected with HCV:					
No	3045	6.4	1[b]	26.6	1[b]
Yes	644	10.7	2.1 (1.5–2.9)	28.6	1.3 (1.0–1.6)
Unknown	807	9.0	1.2 (0.9–1.7)	30.7	1.1 (0.9–1.3)
Living with a person known to be infected with HBV:					
No	3329	6.9	1[b]	26.4	1[b]
Yes	293	7.2	1.2 (0.7–2.0)	33.4	1.6 (1.2–2.1)
Unknown	874	9.8	1.3 (1.0–1.8)	30.2	1.1 (0.9–1.3)
Number of lifetime sex partners:					
⩽1	2119	8.0	1[b]	27.7	1[b]
2–4	703	6.1	0.9 (0.6–1.3)	26.0	0.9 (0.7–1.2)
⩾5	337	5.0	0.8 (0.4–1.4)	30.0	1.1 (0.8–1.5)
Unknown	1337	7.9	0.9 (0.7–1.2)	27.7	1.0 (0.8–1.2)
Sexual intercourse with HCV-positive partner:					
No	3657	6.5	1[b]	26.7	1[b]
Yes	98	29.6	3.1 (1.9–5.1)	48.0	1.4 (0.9–2.1)
Unknown	741	9.3	1.3 (1.0–1.8)	28.6	1.0 (0.8–1.2)
Use of intravenous drugs					
No	3957	7.1	1	27.3	1
Yes	18	27.8	16.4 (4.5–60)	50.0	4.7 (1.8–12.6)
Unknown	521	9.4	1.2 (0.8–1.7)	29.2	1.1 (0.8–1.3)
History of[c]:					
Blood transfusion					
No	4226	6.5	1[b]	26.5	1[b]
Yes, All	202	24.8	2.8 (1.9–4.1)	45.5	1.5 (1.1–2.1)
⩽1992	98	27.6	3.5 (2.1–5.7)	49.4	1.7 (1.1–2.5)
Surgery					
No	1711	5.5	1[b]	22.9	1[b]
Yes	2720	8.5	1.3 (1.0–1.7)	30.3	1.3 (1.1–1.5)
Prolonged dental therapies					
No	2923	6.5	1[b]	27.1	1[b]
Yes	1477	8.9	1.4 (1.1–1.8)	27.9	1.1 (0.9–1.3)
Jaundice or hepatites					
No	2844	7.2	1[b]	28.0	1[b]
Yes	43	48.8	9.3 (4.6–18.9)	46.5	1.5 (0.8–3.0)
Unknown	1609	6.8	1.0 (0.7–1.3)	27.6	1.0 (0.8–3.0)

a Odds ratio (OR) from multiple unconditional logistic regression and adjusted for age, sex and education.
b Reference category.
c In some items, the sum does not add up to the total because of missing values.

With specific regard to HBV infection, it should be noted that the standardised HBsAg (2.5%) and anti-HBc prevalences (27.5%) were higher than previously documented in southern Italy.[7,9] This observation confirmed the prominent role played by HBV infection in the development of hepatitis related conditions in the studied population, including liver cancer. Concerning risk factors for anti-HBc, our findings confirmed the role of intravenous drug use, household contacts, and medical procedures.[19]

In conclusion, this investigation quantified the heavy burden of liver cancer and the widespread diffusion of HCV and HBV infections in the general population of the province of Naples. Although the strong excess among the elderly supports a predominant (and largely disappeared) role of iatrogenic transmission, new HCV and HBV infections are still occurring in younger age groups and deserve continuing efforts to prevent liver cancer.

Conflict of interest statement

None declared.

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