Ten year incidence of HCV infection in northern Italy and frequency of spontaneous viral clearance

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Background: Little is known of the incidence of hepatitis C virus (HCV) infection, and the frequency of spontaneous viral clearance in the general population is unknown. We conducted an epidemiological study in two Apennine towns in northern Italy.

Methods: Anti-HCV [ELISA and RIBA third generation] and HCV-RNA by polymerase chain reaction were tested in thawed sera from an adult general population of Loiano-Monghidoro in 1986 and 1996, obtained in the context of the Micol (Multicenter Italian Study on Cholelithiasis). In 1999, anti-HCV positive subjects and sex and age matched controls were recalled in order to identify risk factors for acquiring HCV infection and to assess the family composition of anti-HCV subjects.

Results: For 1646 subjects, sera were available from both 1986 and 1996 (mean age in 1986 43 (0.39) years). In 1986, 57 (3.46%) subjects were HCV antibody positive (HCV-Ab). Eight new cases were recorded in 1996: adult incidence was 50.3 cases/100 000 inhabitants/year. Fifty three of 63 (84.1%) HCV-Ab sera were also HCV-RNA+. Genotype 2a/2c accounted for 44% and 1b for 47.0% of cases. HCV-Ab subjects had higher serum levels of alanine aminotransferase with respect to controls (p=0.005), as did subjects infected with genotype 1 with respect to those with genotype 2 (p<0.05). Eleven of 65 (16.9%) HCV-Ab subjects spontaneously cleared HCV-Ab; 7/11 also lost HCV-RNA+ in both serum and leucocytes. Sixteen anti-HCV+ subjects belonged to families containing more than one infected member. Married couples accounted for 10 of these 16 subjects. In four of these five married couples, HCV genotype was identical in the two spouses.

Conclusions: In rural northern Italy, the adult incidence of HCV is approximately 50 cases/100 000 inhabitants/year. Our findings suggest that as many as 17% of infected subjects may spontaneously clear HCV-Ab. Interfamilial transmission seems to have a role in the spread of infection.

MATERIALS AND METHODS

Demographic and study design (fig 1)
The study involved the general population of Loiano and Monghidoro (Bologna), two small Apennine hill towns in the Emilia-Romagna region of northern Italy. The adult population of these two towns is fairly stable (totalising 3572). All subjects aged 18–69 years who were resident in Loiano and Monghidoro were called. Out of a total of 3572 citizens, 1847

Abbreviations: HCV, hepatitis C virus; HCV-Ab, HCV antibody; RT-PCR, reverse transcriptase-polymerase chain reaction; ALT, alanine aminotransferase; OD, optical density; IFN, interferon α.
In incidence and spontaneous clearance of HCV

1986

• 3572 subjects called;
• 1847 (52%) screened
• Serum samples thawed

1996

• 129 out of 1847 died
• 72 dropped out
• 1646 (89%) re-evaluated
• Anti-HCV antibody testing on all sera from 1986 and 1996

1999

New call for anti-HCV+ve subjects:
• To identify risk factors
• To acquire family composition of anti-HCV+ve subjects
Recruitment of 2 paired controls matched for sex and age

Table 1 Demographics of the study population

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<td>Sex (M:F)</td>
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Table 2 Demographics of the study population in 1996 in relation to hepatitis C virus positivity

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Ortho Diagnostic Systems, Raritan, New Jersey, USA). All anti-HCV-Ab+ samples were confirmed by third generation recombinant immunoblot assay (RIBA III; Ortho Diagnostic Systems/Chiron).

HCV-RNA and genotypes

When a serum sample was anti-HCV+ (even only once), all samples of that patient were tested for HCV-RNA by nested reverse transcriptase-PCR (RT-PCR, sensitivity <100 copies/ml). HCV RNA was tested on total RNA extracted from serum using the Estrazol kit (Bioline Diagnostici, Turin, Italy) or in leucocytes immediately isolated from blood according to standard dextran based methods in an RNAse free environment. HCV-RNA positivity was evaluated by RT-PCR with primers based on the 5’ untranslated region of the genome (RT kit; Bioline Diagnostici). HCV genotype was evaluated in all sera from subjects testing positive for HCV-RNA and, whenever necessary, on RNA extracted from leucocytes. Genotype was determined by nested RT-PCR using type specific primers (INNO-LIPA HCV II; Innogenetics NV, Ghent, Belgium) and classified according to the criteria of Simmond and colleagues.

Statistical analysis

Descriptive statistical analysis was performed for all parametric variables (HCV-Ab and/or HCV-RNA positivity/negativity, alanine aminotransferase (ALT) levels) using the Student's t test to assess significance. For subgroup analysis, univariate variance analysis was performed using the Tukey and Bonferroni restriction tests. Non-parametric variables were analysed using Pearson, Fisher, Mann-Whitney, and Kruskal-Wallis tests. SPSS version 9 for Windows was used.

RESULTS

Prevalence and incidence of HCV infection and spontaneous viral clearance (fig 2)

In 1986, the overall adult prevalence of HCV-Ab positivity was 3.46% (57/1646 subjects). With respect to age, prevalence ranged from 0.46% (1/214) in the 18–27 year age group to 7.52% (22/293) in the 48–57 year group (p<0.001). Ten years later (in 1996), eight of the 1591 subjects (three females and five males, two of whom were younger than 40 years) who had been HCV-Ab+ in 1986 had become positive. Thus the incidence of HCV infection during the 10 year period was calculated as 50.28 cases/100 000 inhabitants per year. Furthermore, between 1986 and 1996, 2/57 subjects (one male born in 1919, one female born in 1930) spontaneously cleared HCV-Ab.

In 1999, 56 of 63 subjects who had been HCV-Ab+ in 1996 returned for evaluation (six subjects dropped out and one had died from HCV related liver cirrhosis). We found that in nine (originally all antibody positive and viraemic) of 56 subjects in whom the optical density (OD) of the HCV-Ab titre had decreased between 1986 and 1996, spontaneous clearance of HCV-Ab had occurred (fig 3A). Of the remaining 47 subjects who did not clear HCV-Ab, 41 showed a relatively stable high OD (range 2.0–3.5 nm), irrespective of the absence or presence
of treatment (interferon α (IFN) in seven cases), while six showed a progressive decrease in OD without actually falling below the cut off point (fig 3B, 3C, respectively).

**HCV-RNA and genotype distribution**

In 1996 and 1999, 53/63 (84%) and 37/47 (79%) HCV-Ab+ subjects, respectively, were also positive for HCV-RNA in serum. Genotype distribution, obtained after analysing all samples positive in serum or leucocytes, is shown in fig 4.

Among the nine subjects (all HCV-RNA+ in 1986 and 1996 samples) who had lost HCV-Ab positivity between 1996 and 1999, three were still HCV-RNA positive in serum while six had lost HCV-RNA; one of these six subjects maintained HCV-RNA in leucocytes (fig 5). Thus 1.3% per year (11/65 over 13 years) of our population with chronic infection of HCV lost anti-HCV antibody and 1.1% cleared HCV-RNA during the 13 year study period. It is noteworthy that the six subjects who showed a progressive decrease in OD without actually falling below the cut off point (see fig 3C) were all HCV-RNA+ in serum; furthermore, the three subjects for whom leucocytes were available were also HCV-RNA+ in leucocytes. No association was apparent between genotype and spontaneous clearance of HCV-Ab.

Remarkably, none of the seven patients treated with IFN (three of whom were type 2a and four type 1b) lost HCV-RNA.

**Liver enzymes**

In 1996, ALT levels were abnormal in 11% (183/1646) of the tested population. Among 183 subjects with abnormal ALT levels, 27 (14.8%) were HCV-Ab+ (and 26/27 were also HCV-RNA+). Anti-HCV-Ab+ subjects had significantly higher ALT levels than controls (females, p<0.005; males, p<0.001). Among 53 HCV-RNA positive subjects, 36 (67.9%) had normal ALT levels. However, HCV-RNA positive subjects had significantly higher ALT values than those who were HCV-RNA negative (p<0.05). Genotype 1 was associated with...
abnormal ALT levels more frequently than genotype 2 (p<0.05). It is noteworthy that abnormal ALT levels were never recorded in any of the nine subjects who achieved spontaneous clearance of HCV-Ab during the course of the study. This was also the case in five of the six subjects reported in fig 3C who showed a progressive decrease in OD without actually falling below the cut off value.

**Intrafamilial spread**
Among the 63 HCV-Ab subjects, 16 (25 %) belonged to seven families (more persons living together in the same house) containing more than one infected member. Fifty four families made up the 63 HCV antibody positive subjects. Married couples accounted for 10 of these 16 subjects. In four of these five married couples, HCV genotype was identical in the two spouses. Two female members of these married couples had relatives infected with the same genotype with whom they had lived. It should be noted that three of the four subjects with genotype 2a/1b were male members of the same family.

**Risk factors**
Risk factors for acquiring HCV infection were previous blood transfusion (p<0.001) and abortion (p<0.001) in females, performed before 1970. Data obtained in the present study underscore the importance of non-sexual forms of transmission (blades, toothbrushes, etc). However, our data based on genotype seem to confirm the importance of domestic transmission of HCV infection.

**DISCUSSION**
The present study expands our knowledge of the burden and natural history of HCV infection, including genotype distribution, in the general population (as opposed to groups of patients under medical surveillance). In over 60% of the period (1986–96) covered by our study, blood donors were not patients under medical surveillance. In over 60% of the period, in the general population (as opposed to groups of patients under medical surveillance). In over 60% of the period, in the general population (as opposed to groups of patients under medical surveillance). In over 60% of the period, in the general population (as opposed to groups of patients under medical surveillance). In over 60% of the period, in the general population (as opposed to groups of patients under medical surveillance).

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