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An outbreak of hepatitis A virus infection with a high case-fatality rate among injecting drug users

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***Background/Aims*: In 2002, the first reported outbreak of hepatitis A virus (HAV) infection involving mostly intravenous drug users (IDU) occurred in Italy. We attempted a thorough evaluation of the outbreak, including epidemiological, clinical and virological analyses.**

***Methods*: We conducted an epidemiological investigation, including a case-control study, to identify the source and the modes of HAV transmission. Hepatitis B and C (HCV) viruses and human immunodeficiency virus (HIV) coinfections were clinically analysed. Sequence analysis of the VP1/2A junction of the HAV isolates was also performed.**

***Results*: Of the 47 symptomatic cases, 35 were IDUs. The only associated risk factor was contact (not related to injecting practices) with a jaundiced person (odds ratio: 5.8; 95% confidence interval: 1.3–29.9). Of the cases, 58% were anti-HCV positive and 4.7% anti-HIV positive. Three individuals died of acute liver failure: 2 were HCV-coinfected alcohol abusers, with underlying liver cirrhosis; 1 was HCV/HIV-coinfected. HAV-RNA was found in 15 of the 24 tested patients: genotype IB (8 cases) and IIIA (7 cases) were detected.**

***Conclusions*: HAV was probably transmitted through the fecal-oral route, although parenteral transmission cannot be excluded. The high fatality rate was probably due to severe underlying liver damage. The occurrence of this outbreak highlights the need for routine HAV vaccination for IDUs.**

***Keywords*: Hepatitis A; Intravenous drug use; Outbreak; Risk factors; Genotype**

1. Introduction

Injecting drug users (IDU) are at high risk of infection with bloodborne viruses, such as hepatitis B and C viruses (HBV and HCV), the human immunodeficiency virus (HIV), and, though not classically bloodborne, hepatitis A virus (HAV) [1–3]. In non-endemic countries, both large and small outbreaks of HAV infection among IDUs have occurred [4–13]. In some outbreaks, the prevalent variant was genotype IA [4,5] or IIIA [7,11]; co-circulation of different strains was also reported [7]. Nonetheless, the mode of transmission was not determined. HAV is mainly transmitted through the fecal-oral route, which, among IDUs, can be favored by poor hygiene and living conditions [4–10]. The implication of blood products in HAV transmission [14–15] suggests that the virus can also be

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transmitted through needle sharing during the viremic period, which can exceed two months [16,17].

We describe an outbreak of HAV infection with an unusually high case-fatality rate among drug users in the city of Terni, Central Italy. We attempted to identify risk factors through a case-control study, to identify the genotypes, and to evaluate the role of coinfections in disease outcome.

2. Patients and Methods

2.1. Epidemiological investigation

Terni (105,000 inhabitants) is located in an area with low HAV endemicity. Although an outbreak (probably water-borne) occurred in the late 1980s, an yearly average of only five new cases have since been reported.

Beginning in September 2002, an unexpectedly high number of cases of acute HAV infection were reported to the Local Health Unit (LHU) by the Infectious Disease Unit of Terni Hospital, by the local Drug Dependency Unit (DDU), and by general practitioners. Most cases occurred among IDUs. In January 2003, an investigation was performed to identify the source of infection and the modes of transmission and to adopt appropriate control measures. A case was defined in the presence of an acute illness clinically compatible with the disease and IgM anti-HAV antibodies.

To evaluate the role of drug-using practices and other behaviors in HAV transmission, a case-control study using a standardized questionnaire was conducted among drug users with acute infection and, as potential controls, apparently uninfected drug users consecutively attending the DDU in the same period, selected randomly. We investigated travel history, shellfish consumption, contact with a jaundiced person, sexual behavior, history of drug use, types of drugs, and drug-using practices. Information was also collected from HAV-infected non drug users, identified by the LHU.

The study was approved by the Regional Ethics Committee; written informed consent was obtained from all participants.

2.2. Prevention and control measures

In January 2003, a program was created to prevent and control infection among drug users attending the DDU, in addition to the prevention measures already in place for the contacts of cases [18]. HAV vaccination was offered, and information was provided on hygienic precautions and drug-using practices. The contacts were followed for at least 50 days after the case was diagnosed. Letters were sent to all general practitioners and pediatricians in Terni and to all DDUs and LHUs in the region (Umbria) to inform them of the outbreak and to recommend prevention and control measures.

2.3. Virological assays

All suspected cases of acute hepatitis A were tested for IgM (ETI-HA-IgMK Plus, DiaSorin, Saluggia, Italy) and total anti-HAV (ETI-AB-HAVK Plus, DiaSorin). These tests were also used to identify susceptible controls in the case-control study. Hepatitis A cases were also tested for HBsAg (ETI-MAK-2 PLUS, DiaSorin), IgM anti-HBc (ETI-CORE-IgMK, DiaSorin), anti-HCV (Ortho HCV3.0, Ortho Clinical Diagnostic, Raritan, NJ), and anti-HIV (AxSYM HIV 1/2gO, Abbott Diagnostic Division, Delkheim Germany). Immunoblot assay was used as a confirmatory test for anti-HCV (RIBA HCV 3.0, Chiron Corporation, Emeriville, CA) and anti-HIV (RIBA HIV1/HIV2, SIA, Chiron Corporation). Anti-HCV-positive samples were also tested for HCV RNA (COBAS Amplicor HCV-Monitor, Roche Diagnostic, Hoffmann-La Roche Ltd, Basel, Switzerland). Serum of IgM anti-HAV-positive patients was further tested for HAV RNA.

HAV RNA was extracted from 200 μl of serum using the QIAamp MiniElute Virus Spin Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. The RNA was recovered in 40 μl of elution buffer and a 266 bp region of the VP1/2A junction of HAV genome was amplified by RT-PCR reaction [19].

All HAV RNA-positive samples were sequenced using the BigDye 1.1 terminator kit (Applied Biosystems, Foster City, CA), following the manufacturer's instructions, and an ABI 310 automatic sequencer. Genotyping was performed by sequence analysis after alignment of the outbreak sequences with reference strains [20]. The Neighbor-Joining method implemented in the software MEGA2 [21] with 1000 bootstrap replications was applied for the phylogenetic analysis.

Samples of heroin confiscated just before the outbreak were obtained from the Terni police department and tested for HAV RNA. Two aliquots of 100 mg each were suspended in 500 μl PBS, vortexed three times for 60 s, and briefly centrifuged to sediment undissolved material; an equal volume of the suspended drug and negative human serum was mixed, and RNA was extracted, as described above. To establish the sensitivity of the method, different amounts of positive sera from the outbreak were added to the drug.

2.4. Statistical analysis

The association between HAV infection and risk factors was assessed using odds ratios (OR); 95% confidence intervals (95% CI) were also calculated. Differences in proportions were tested by Chi-square or Fisher's exact test, when necessary. A P-value <0.05 was considered as significant. All statistical analyses were conducted using STATA software (version 8.0) (Statacorp, College Station, TX, USA).

3. Results

3.1. Epidemiological characteristics of the outbreak

From September 2002 to June 2003, 47 cases of acute HAV infection were identified, with a peak between December and February (Fig. 1). The epidemic began among IDUs. Thirty-five persons (74.5%) were known to be IDUs. Contact with a jaundiced person, raw shellfish consumption and travel to highly endemic countries (India) were the other risk factors for non-IDUs (Table 1).

3.2. Case-control study

Twenty-one (60%) of the 35 IDUs with acute HAV infection and 37 (75.5%) of the 49 potential controls participated in the case-control study. All controls had been

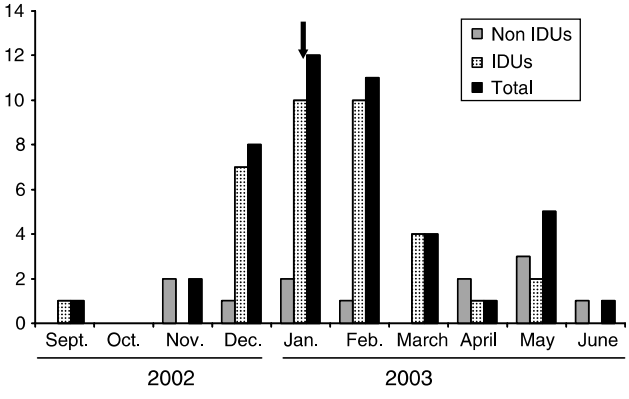

Fig. 1. Epidemic curve of acute hepatitis A virus (HAV) infection cases by calendar time and risk factors. The arrow indicates the beginning of HAV vaccination among intravenous drug users (IDUs).Terni (Central Italy), 2002-2003.