Acute non A-E hepatitis in eastern Sicily: the natural history and the role of Hepatitis G Virus

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**Abstract.** - Background. The etiology and the natural history of acute non A-E hepatitis is still undefined.

Methods. We examined the prevalence of HGV viraemia and the long-term outcome in 54 patients with non A-E acute hepatitis.

Results. HGV-RNA was found in 19 out of 54 (35%) patients. There was no difference between HGV-RNA positives and negatives in terms of age, sex, parenteral risk factors and alanine amino-transferase peak level. Gamma-glutamyl transpeptidase levels were higher in the HGV-RNA positive group. During a 3-year follow up, 10 out of 54 (18.5%) patients progressed to chronic hepatitis and 4 out of 10 (40%) had an histologically advanced disease. None of the 19 subjects infected with HGV showed a chronic evolution of liver disease.

Conclusions. Our results confirm the limited role of HGV as the etiological agent of non A-E acute hepatitis. The great majority of non A-E hepatitis cases are yet of undetermined origin and unfortunately, are characterized by a high rate of progression to chronic active hepatitis.

Key Words:

**Introduction**

Between 10 and 20 percent cases of acute hepatitis in the western world are of unknown etiology, in spite of extensive investigations for known hepatotropic viral agents. These non A-E cases show different clinical patterns and variable rates of chronicization. Several viral agents have recently been proposed to be responsible for acute non A-E hepatitis. Hepatitis G Virus (HGV) is a recently discovered parenterally-transmitted flavivirus. HGV viraemia has been detected in cases of acute, fulminant, and chronic hepatitis. Nevertheless a clear-cut correlation between this virus and acute liver damage has never been established.

A group of patients with non A-E hepatitis was submitted to a clinical and serological follow up for 3 years after the acute onset of liver disease. In the same group of patients, HGV viraemia was retrospectively tested in sera collected during the acute phase.

**Patients**

Between January 1994 and January 1997, 159 cases of acute viral hepatitis were admitted to the Department of Infectious Diseases of Catania (Eastern Sicily). Fifty-four out of 159 (33.9%) were found to be non A-E hepatitis on the basis of the absence of serum Hepatitis B surface antigen (HBsAg), IgM anti-Hepatitis B core antigen (anti-HBc), IgM anti-Hepatitis A Virus (HAV), IgM anti-Epstein-Barr Virus (EBV) capsid antigen, IgM anti-cytomegalovirus. All cases were also anti-Hepatitis C Virus (HCV) antibodies negative, HCV-RNA negative and anti-Hepatitis E Virus (HEV) IgM negative. Serum non-organ specific auto-antibodies were also negative.

Serum samples from all the non A-E cases were available within 7 days from the onset of acute disease and stored at -70°C until tested for HGV RNA. Clinical and epidemiological data both upon admission to the Hospital and during the follow-up were collected by the same group of physicians. All cases were followed-up for as long as 36-months. Patients with evidence of alanine-
aminotransferase (ALT) normalization were submitted to a six-monthly control with clinical examination and serology. Cases with persistent ALT elevation were submitted to a three-monthly clinical and serological follow up. In the latter cases, a liver biopsy was performed at the end of the follow-up.

**Methods**

HBsAg, IgM anti-HBc, IgM anti-HAV, IgM anti-EBV capsid antigen, IgM anti-cytomegalovirus and anti-toxoplasma were tested by commercially available enzyme immunoassays. A nti-HCV antibodies were tested by using a third-generation enzyme immunoassay (Ortho Diagnostic Systems, USA). HCV RNA was tested by reverse transcription and nested PCR using primers derived from the 5' untranslated region. Anti-HEV IgM antibodies were tested by an "in-house" modified enzyme immunoassay using the same antigens as in the commercially available IgG anti-HEV test (Abbott Laboratories, USA). A ntnuclear antibodies were tested by indirect immunofluorescence on rodent substrates (liver, kidneys, stomach) and on HEp2 cells at 1/40 as starting dilution.

Hepatitis G Virus RNA was measured in stored sera from non A-E acute hepatitis cases. RNA was isolated from 140 µl serum using the QIA amp HCV kit in accordance with the manufacturers' instructions. The extracted RNA was eluted with 50 ml preheated (95° C) water. cDNA synthesis was performed with 10 µl of the RNA preparation in a total reaction volume of 20 µl for 15 min at 42° C with 5 mM MgCl2, 50 mM Tris-HCl, 2.5 mM Random Hexamers (Perkin Elmer, Weiterstadt, Germany), 25 U MuLV Reverse Transcriptase (Perkin Elmer, Weiterstadt, Germany), 20 U RNase Inhibitor (Perkin Elmer, Weiterstadt, Germany), and 1 mM (each) deoxynucleotide (Pharmacia, Freiburg, Germany). The reaction was stopped by heating to 99° C for 5 min. PCR-amplification of cDNA in a final volume of 50 ml was performed by adding 30 ml reaction mix containing 1 µl of each primer, 5 µl of 10x PCR buffer (Boehringer Mannheim, Germany), 2 U Taq DNA Polymerase (Boehringer Mannheim, Germany) to the 20 µl of reverse transcription mix. Primers were derived from sequences in the 5' untranslated region and the NS5 region of the HGV genome. Digoxigenin-dUTP (1 nmol/µl) was employed for detection with "Enzynmun" assay. The sequences of the primers (as given in the HGV primer and Capture Probe Set, Boehringer Mannheim) were as follows: 5'-NCR-Region: Primer 1: 5' -Cgg-CA A -A AA -ggt-gT-ggA -TG-3' (20mer, position 101-120), Primer 2: 5' -CgA -CgA -gCC -TgA -CgT -Cgg-g-3' (19 mer, position 285-267), Capture Probe: 5'- biotin-ggt-A gc-CA C-TAT -A gg-Tgg-g-3' (19 mer, position 161-179). NS5a-Region: Primer 1: 5'-CTC -TTT -gTg-gTA -gTA -gCC -ga A -ga T-3' (25 mer, position N55a 77-101), Primer 2: 5'-CgA -Ag-A gT-CA -gA gg-gga ggg-gTAT-3' (24mer, position N55a 211-188), Capture Probe: 5'-gTA -CT g-gA gC -A gC -TCA -gAT-3' (21mer, position 152-172). A mplification involved 35 cy- cles (GeneA mp PCR system 9600, Perkin Elmer) with denaturation at 95° C (30" sec), annealing at 50° C (30" sec) and extension at 72° C (30" sec). After the final cycle, the tubes were incubated for a further 5 min at 72° C. Each series included positive and negative controls. The amplification products were detected using "Enzynmun-test DNA detection Kit" (Boehringer Mannheim, Germany). Denaturation was performed in samples tubes containing 40 µl of the PCR mixture and 360 µl of 50 mM NaOH. A ll subsequent steps were automatically performed by the ES 300 analyzer (Boehringer Mannheim, Germany). In brief, 100 µl was transferred into a streptavidine-coated tube, with 400 µl of the virus specific 5'-biotin-labeled capture probe (14 ng/ml diluted in hybridization buffer) and the mixture was left for 120 min at 37° C. A fter extensive washing the peroxidase-labelled anti-digoxi- genin polyclonal antibody was added and incubated for 30 min at 37° C. A fter washing, the enzyme substrate [1.9 mM 2,2 aminobis (3-ethylbenzthiazoline sulfoni acid), 100 mM phosphate citrate buffer (pH 4.4), with 3.2 mM H2O2] was added. The color reaction was allowed to develop for 30 min at 37° C, and optical densities (OD) were measured at 422 nm. Cut-off was de- fined as being twice the OD obtained with negative controls.
χ²-square test and t test were performed for statistical evaluation. A P value of less than 5% was considered significant.

**Results**

None of the non A-E cases showed a fulminant course of the disease.

HGV-RNA was retrospectively detected in 19 out of 54 (35.1%) patients. There was no difference between HGV positives and negatives in terms of sex, age and parenteral risk factors (Table I). Incubation time was significantly longer in the HGV negative group (p < 0.01) (Table I).

Peak ALT, aspartate-amino transferase (A ST), Bilirubin, Alkaline Phosphatase, γ-globulin levels were comparable between the two groups, whereas γ-glutamyl transpeptidase (γ-GT) was significantly higher in the HGV positive group (358 ± 101 U/L versus 116 ± 58 U/L in the HGV-negative group, p < 0.01).

Comprehensively, mean duration of ALT elevation was significantly longer in the HGV-RNA negative in comparison with the HGV-positive group (213 ± 38 days versus 41 ±11 days; p < 0.01).

During the 36-month follow-up, 10 out of 54 (28.5%) patients showed a chronic evolution of liver disease as assessed by persistent ALT elevation. None of the 19 HGV-RNA positive cases showed a progression towards chronic liver disease whereas 18 out of 19 (94.7%) became HGV-RNA negative within 36-months after the onset of acute hepatitis.

Blood exams performed 36-months after the acute onset in those 10 cases with chronic hepatitis showed a mean ALT value of 188 ± 31 U/L, mean AST of 96 ± 16 U/L, mean bilirubin of 1.8 ± 0.2 mg/dl, mean g-GT of 71 ± 28 U/L, mean albumin of 4.2 ± 1.9 g/dl, serum γ-globulins 1.9 ± 0.7 g/dl, platelet count 136.000 ± 29.000/mm³. Clinical exam showed hepatomegaly in all 10 cases and splenomegaly in 3 cases. In 3 cases portal hypertension was documented on the basis of color-doppler ultrasonography measures. One case had endoscopic evidence of esophageal varices. At the end of the 36-month follow up a liver biopsy was performed. 6 out of 10 patients (60%) showed a moderate chronic hepatitis, 3 (30%) showed a severe chronic hepatitis and one had a clear-cut liver cirrhosis. Table II gives more details on the individual histological features of the 10 chronicized cases.

**Discussion**

Little is currently known about the pathogenic relationship that exists between HGV and acute hepatitis cases of undetermined origin. A recent study by Alter et al has shown that 0.3% of patients with acute hepatitis diagnosed in the United States during the last 14 years might have been infected with HGV alone.

Table I. Epidemiological parameters in the HGV-positive and HGV-negative patients with acute non A-E hepatitis.

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<th>Parenteral Risk Factors (N.)</th>
<th>HGV-RNA Positive</th>
<th>HGV-RNA Negative</th>
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<tr>
<td>N.</td>
<td>19</td>
<td>35</td>
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<tr>
<td>Male/Female</td>
<td>9/8</td>
<td>18/17</td>
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<tr>
<td>Age (years)</td>
<td>29 ± 6</td>
<td>31 ± 8</td>
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<td>Incubation time* (days)</td>
<td>22 ± 7&quot;</td>
<td>67 ± 9&quot;</td>
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*Incubation time in cases with documented parenteral exposure; **Student’s t test: p < 0.01; ***Within 6 months from the onset of hepatitis.
We found HGV viraemia in 25% of non A-E acute hepatitis cases. This datum let us hypothesize that that HGV is a possible but not the only and not even the main agent of non A-E acute hepatitis.

Other Authors’ results are extremely variable: Frider et al detected HGV-RNA in 30% of all patients with non A-E acute hepatitis. Paraña et al reported a 16% prevalence of HGV viraemia in non A-E patients from Northeastern Brazil. Tanaka et al found serum HGV-RNA in 3 of 5 cases of acute hepatitis of unknown origin. According to Alter et al, 3 of 23 (13%) non A-C post-transfusion acute hepatitis cases had ongoing HGV infection. In a group of Chinese patients with acute non A-E hepatitis, Wu et al found a 11% prevalence of HGV viraemia. On the other hand, a number of recent reports outline a negligible role played by HGV as the etiological agent of non A-E hepatitis. Among 50 Indian patients with acute hepatitis, Wu et al found no significant difference in gender, age and other epidemiological characteristics between acute hepatitis patients with or without HGV-RNA.

In our caseload, peak ALT levels were comparable between HGV positive and negative patients. This result is in accord with other Authors’ data. Serum γ-GT was significantly higher in the HGV-RNA positive subjects with acute non A-E hepatitis. Elevated levels of γ-GT and specific bile duct lesions have previously been associated with HGV viraemia.

According to our data, HGV-positive acute hepatitis never chronicizes. In Alter’s survey, no case of chronic disease was observed among acute hepatitis patients infected by HGV alone. No evidence of chronic course was also seen by Hayashi et al among patients with acute hepatitis G. Similarly, other prospective follow-up studies showed no case of progression to chronic liver disease among patients with acute HGV-positive hepatitis. Data on persistence of HGV viraemia are less univocal: in fact long-term HGV replication could be observed in some cases in the absence of clinical evidence of liver disease, whereas in other reports HGV viraemia rapidly disappeared. In general, HGV viraemia is very unstable with a higher rate of spontaneous clearance in immunocompetent subjects in comparison with immunodeficient ones.

After a 36-months follow up, we found a 28.5% rate of chronicization among the non

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<th>Table II.</th>
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<td><strong>Individual histological features in the 10 non A-G patients who progressed to chronic liver disease</strong></td>
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<td><strong>N. patients</strong></td>
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<tr>
<td><strong>Knodell HAI score</strong></td>
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<tr>
<td><strong>Portal inflammation</strong></td>
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<td><strong>Intralobular necrosis</strong></td>
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<td><strong>Piecemeal necrosis</strong></td>
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<td><strong>Bridging necrosis</strong></td>
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<td><strong>Portal fibrosis</strong></td>
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<td><strong>Lymphoid follicles</strong></td>
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<td><strong>Micronodular steatosis</strong></td>
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<td><strong>Micronodular steatosis</strong></td>
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<tr>
<td><strong>Bile duct damage</strong></td>
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<td><strong>Councilman bodies</strong></td>
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<td><strong>Cirrhosis</strong></td>
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A-G cases. It is worrisome that 3 out of 10 patients with chronic hepatitis had a severe clinical presentation with splenomegaly and color-doppler evidence of portal hypertension. One case had endoscopic evidence of esophageal varices. A I ter et al[^4] reported a 32% progression to chronic hepatitis in a group of non A-G hepatitis cases. In a 4 year follow up study, Paran a et al.[^12] demonstrated a 16% chronicization rate among 21 patients with acute sporadic non A-G hepatitis. As far as we know, histological reports on chronically progressed non A-G hepatitis are anecdotal and controversial. Parana et al. found a mild histology in acute non A-G hepatitis progressed to chronic disease. A I ter et al.[^4] reported single cases with severe chronic active hepatitis.

The cause of acute non A-G viral hepatitis remains unknown. Presumably, epidemiological and clinical characteristics of this disease are variable according to the geographic and demographic setting. Multiple etiologic factors, which should not be necessarily virus-related, may be involved.

From our data, it is worthy of note that non A-G hepatitis is an aggressive disease with a high rate of chronicization. Further studies are required on a larger scale to better assess either epidemiological and clinical features or the natural history of this disease.

### References

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