Clinical Presentation, Outcome, and Response to Therapy Among Patients With Acute Exacerbation of Chronic Hepatitis C

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BACKGROUND & AIMS: **The slow asymptomatic progression of chronic hepatitis C (CHC) can be interrupted by an acute exacerbation, characterized by increased serum levels of alanine aminotransferase (ALT) and bilirubin and other symptoms of acute hepatitis. We aimed to provide more information about the clinical presentation of acute exacerbation of CHC.**

METHODS: **We identified 82 consecutive patients, from 2 locations in Italy, who had an acute exacerbation of CHC from January 2005 through June 2010; we followed them up for a median period of 36 months. These cases were hepatitis C virus (HCV) RNA positive, hepatitis B surface antigen-negative, and had not received anti-HCV therapy. They were matched with 82 subjects with hepatitis C without reactivation for age, sex, and HCV genotype (controls). Sixty-nine cases and 73 controls were followed up for at least 2 years. Liver biopsy specimens had been taken from 23 cases and 31 controls—once before enrollment in the study and once during the follow-up period.**

RESULTS: **HCV genotype 2 was detected in 46.4% of cases, and HCV genotype 1 was detected in 43.9%. Among cases, the mean ALT level was 1063 ± 1038 IU/dL, and the mean total bilirubin level was 15.87 ± 7.15 mg/dL. A higher percentage of cases carried the *interleukin-28B CC* genotype than controls (40.2% vs 24.4%; $P < .05$). Among cases, 43.5% had a steady increase in ALT level (>2-fold baseline value); for 56.5% of these patients, ALT levels returned to baseline values before the acute exacerbation of chronic hepatitis. Based on comparisons of biopsy specimens, 18 cases (78.3%) and 11 controls (35.5%) had increasing fibrosis, with Ishak scores increasing by more than 2 ($P < .005$); 14 cases (60.9%) and 3 controls (9.6%) had increases in necroinflammation of more than 2 points ($P < .005$). Thirty-two cases (46.4%) and 38 controls (52%) received treatment with pegylated interferon and ribavirin; a sustained virologic response was achieved in 26 cases (81.2%) and 23 controls (60.5%).**

CONCLUSIONS: **Although an acute exacerbation of chronic hepatitis is a serious medical condition, most patients achieve a sustained virologic response after treatment with pegylated interferon and ribavirin.**

Keywords: Hepatic Flare; Cirrhosis; Response to Therapy; Complication.

See editorial on page 1181.

The clinical presentation of chronic infection by hepatitis C virus (HCV) is characterized by moderate histologic lesions on liver biopsy (LB).[1,2] The course of the illness is unpredictable in single cases, but the most frequent outcomes are an indolent persistence of mild disease or a slow progression to more severe liver disease, including the development of cirrhosis.[1,3,4] About 3% of cirrhotic patients per year develop hepatocellular carcinoma (HCC).[2]

The stable persistence of an inactive or moderately active stage of the illness and a slow asymptomatic progression of chronic hepatitis may be interrupted by an exacerbation of the disease, identified in this article as "acute exacerbation of chronic hepatitis C" (CHC), an event characterized by a substantial increase in serum alanine aminotransferase (ALT) values, at times associated with an increase in bilirubin level and/or with other symptoms in common with acute hepatitis.[5–8] Reactiva-

Abbreviations used in this paper: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; HAI, Histologic Activity Index; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus; IL, interleukin; LB, liver biopsy; peg-IFN, pegylated interferon; SVR, sustained virologic response.

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tion of CHC was first described in 1996 and an annual incidence rate of approximately 10% was observed in 194 patients followed up for more than 5 years.[5] More recently, acute exacerbation of CHC has been described as associated with HCV genotype 2,[9–12] but its occurrence in patients with other HCV genotypes also has been described.[10]

At present, few articles have been published and more data are needed on the clinical presentation of acute exacerbation of CHC. In addition, to our knowledge, there is no information on the clinical course of acute exacerbation of CHC and its impact on the outcome and response to treatment of CHC.

This article reports on the data from a prospective investigation on 82 consecutive patients with symptomatic acute exacerbation of CHC and 82 pair-matched control patients who had not shown signs of acute exacerbation before being enrolled in the present study. Patients were naive to anti-HCV treatment and most of the patients were observed for at least 2 years.

Patients and Methods

Patients

Two liver units in southern Italy participated in the study, 1 in Naples and 1 in Caserta. These 2 centers have cooperated for 12 years in several clinical investigations, using the same clinical approach and laboratory methods.[13,14]

From January 2005 to June 2010, we prospectively enrolled 82 anti-HCV/HCV RNA–positive, hepatitis B surface antigen (HBsAg)/anti–human immunodeficiency virus-negative patients, naive to anti-HCV therapy (because of no indication, contraindications, or refusal), observed for a symptomatic acute exacerbation of CHC (case group). In several determinations over the years before reactivation, these 82 patients had been HCV RNA positive with normal or moderately increased serum ALT levels, suggesting an indolent, slowly progressing course of CHC. A percutaneous LB, performed at least 1 year before acute exacerbation of CHC, was available for 26 (31.7%) patients in the case group.

The diagnosis of acute exacerbation of CHC was based on an increase in the ALT value of at least 5-fold the previous basal values and on the detection of anti-HCV and HCV RNA, in the absence of other viral or iatrogenic factors known to induce liver damage.[10–12] Excluded from the study were patients with a history of alcohol intake, with serologic signs of autoimmune hepatitis, those treated in the past 6 months with drugs considered to be hepatotoxic, and those with IgM antibody to hepatitis B core antigen, hepatitis D virus (HDV), hepatitis A virus (HAV), hepatitis E virus (HEV), cytomegalovirus, or anti–Epstein–Barr virus during the acute phase of the illness. During acute exacerbation of CHC, liver function tests were performed once a week for 2 months.

Of 479 anti-HCV/HCV RNA–positive CHC patients without HCC observed in the same centers in the same period, we enrolled as a control group 82 HBsAg-negative patients who never showed signs of symptomatic acute exacerbation of CHC, with steady ALT values in 4 checks per year in the past 5 years and naive to anti-HCV therapy because of no indication, contraindications, or refusal. These patients were pair-matched by age (±5 y), sex, and HCV genotype with the patients in the case group. For 35 (42.7%) of the 82 patients in the control group, a percutaneous LB had been performed before enrollment.

Samples of plasma and whole blood were obtained for each patient in the case group at the time acute exacerbation of CHC developed and for each patient in the control group at enrollment. These samples were fractioned, stored at −80°C, and never thawed until used for this investigation.

After a 2-month observation during acute exacerbation of CHC, patients in the case group were followed up for a median period of 36 months (range, 24–72 mo), with the exception of 13 patients lost to follow-up evaluation for lack of compliance. The patients in the control group were observed for 32 months (range, 24–68 mo) after enrollment, with the exception of 9 patients lost to follow-up evaluation for lack of compliance. During this long-term follow-up period, all patients were assessed at 3-month intervals with liver function tests and at 6-month intervals with an abdominal ultrasound scan.

For patients in the case group, 3 clinical profiles of long-term biochemical outcome were established: deterioration, stationary status, and improvement. Patients were considered to have "deteriorated" if a persistent increase in ALT value of at least 2-fold was observed, compared with the values shown before reactivation; patients were considered "stationary" if after acute exacerbation of CHC their ALT values returned to the values shown before acute exacerbation of CHC, and patients were considered "improved" if they showed a stable normalization of ALT values after acute exacerbation of CHC.

An LB was suggested to patients in the case and control groups during the long-term follow-up evaluation, in accordance with international guidelines.[15,16] For patients in the case group it was performed at least 8 months after the development of acute exacerbation of CHC. Fifty-four patients naive to anti-HCV therapy agreed to undergo a second LB, 23 in the case group and 31 in the control group. The interval between the 2 biopsy procedures was 5.85 years (interquartile range, 4.2–7.1 y) for patients in the case group and 5.05 years (interquartile range, 4.18–6.89 y) for those in the control group. A second LB was not performed in 3 patients in the case group and in 4 patients in the control group because it was not indicated or refused. LBs were examined by a pathologist (G.P.) who, unaware of the clinical and laboratory data, compared the serial biopsy specimens from each patient for necroinflammation (Histologic Activity Index [HAI]) and fibrosis using the Ishak scoring system for both grading and staging.[17] Patients were considered to have deteriorated if they showed an increase of at least 2 degrees in the fibrosis or HAI scores in the second LB, to have a stationary condition if there was no change or only a minimal change (±1) in liver fibrosis or HAI scores between the 2 LBs, and to have improved if a reduction of at least 2 degrees in fibrosis or HAI scores was observed in the second LB.

Of the 69 patients in the case group and the 73 patients in the control group with a 2-year follow-up period, 32 (46.4%) and 38 (52%), respectively, received pegylated-interferon (peg-IFN) plus ribavirin treatment according to international guidelines.[15,16] Thirty-seven (53.6%) cases and 35 (47.9%) controls remained untreated because therapy was not indicated, or because of contraindications to therapy or refusal. The same treatment schedules were applied for patients in both groups. Patients with HCV genotypes 1/4 received a 12-month course with peg-IFN-alfa-2a (weekly dose, 180 μg), or peg-IFN-alfa-2b (weekly dose, 1.5 μg/kg of body weight), plus ribavirin at a daily dose of 800 to 1200 mg according to body weight. Patients with HCV genotypes 2/3 received the same treatment schedules for 6

Table 1. Initial Clinical, Epidemiologic, Biochemical, Virologic, and Genetic Characteristics of the Patients in the Case and Control Groups

	Case group (N = 82)	Control group (N = 82)
Median age (range)	53 (23–87)	51 (21–85)
Males, n (%)	62 (76)	62 (76)
Females, n (%)	20 (24)	20 (24)
Risk factors, n (%)		
Surgery without blood transfusion	37 (45.2)	30 (36.6)
HCV infection in the household	1 (1.2)	2 (2.4)
Injection drug use	31 (37.8)	29 (35.4)
Blood transfusion	2 (2.4)	2 (2.4)
Not determined	11 (13.4)	19 (23.2)
Years of HCV infection, mean ± SD	9.06 ± 7.9	8.7 ± 8.1
AST level, *IU/mL*, mean ± SD (normal value, 10–40)	672 ± 788	51 ± 55
ALT level, *IU/mL*, mean ± SD (normal value, 10–40)	1063 ± 1038	71 ± 68
Bilirubin, mean ± SD (n. v. 0.4–1.0)	15.87 ± 7.15	0.7 ± 0.4
Bilirubin level, n (%)		
>2.5 mg/dL	31 (37.8)	0
≤2.5 mg/dL	51 (62.2)	82 (100)
HCV RNA level, *IU/mL*, mean ± SD	1,289,827 ± 1,175,515	2,141,200 ± 1,384,745
Genotype, n (%)		
2, 2a, 2a/2c, 2b	38 (46.4)	38 (46.4)
1a, 1b	36 (43.9)	36 (43.9)
3	6 (7.3)	6 (7.3)
4	2 (2.4)	2 (2.4)
IL-28-B genotype, n (%)		
CC	33 (40.2)	20 (24.4)
CT	33 (40.2)	48 (58.5)
TT	16 (19.6)	14 (17.1)

SD, standard deviation.
NOTE. Differences significant to the statistical analysis: CC vs CT + TT, $P < .05$.

months. The response to treatment was analyzed according to commonly accepted international criteria.[15,16]

All the procedures applied in the study were in accordance with the standards on human experimentation of the Ethics Committee of "Azienda Ospedaliera Universitaria of the Second University of Naples" and with the Helsinki Declaration of 1975, revised in 1983. At the first observation, all patients signed an informed consent form according to the rules of the same Ethics Committee.

Methods

HCV RNA level was sought in the patients' plasma according to the method described extensively in a previous article,[18] with a detection limit estimated at approximately 40 IU/mL. HCV genotyping was performed by Line-Probe-Assay (INNO-LIPA HCV II; Innogenetics, Zwigndrecht, Belgium).

The whole-blood samples of all patients in the study were tested for interleukin (IL)-28B genotype (Roche Diagnostics, Branchburg, NJ).

HAV, hepatitis B virus, HDV, HCV, HEV, human immunodeficiency virus, cytomegalovirus, and Epstein–Barr virus serum markers were sought using the commercial immunoenzymatic assays reported in the Supplementary Materials and Method section. Liver function tests were performed applying routine methods.

Statistical Analysis

Continuous variables were summarized as the mean and standard deviation, and categoric variables were summarized as absolute and relative frequencies. Differences in the mean values were evaluated by the Student *t* test, and the chi-squared test was applied to categoric variables. A *P* value of less than .05 was considered statistically significant.

Results

Clinical Presentation of Acute Exacerbation of CHC

The demographic, genetic, clinical, biochemical, and virologic characteristics of the cases and controls recorded at enrollment are shown in Table 1. The patients in the case group had a mean age of 50 years, males predominated (76%), and the most frequent HCV genotypes were HCV genotype 2 (46.4%) and HCV genotype 1 (43.9%). The patients in the control group showed the same characteristics, reflecting their selection criteria. Patients in the case and control groups stated similar risk factors for acquiring HCV infection—surgery without transfusion and a history of injection drug use being the most frequent risk factors in both groups. The patients in the case group showed a mean aspartate aminotransferase (AST) serum value of 672 ± 788 IU/dL, a mean ALT value of 1063 ± 1038 IU/dL, and a total mean bilirubin value of 15.87 ± 7.15 mg/dL (Table 1). The patients in the control group showed an AST value of 51 ± 55 IU/mL, an ALT value of 71 ± 68 IU/mL, and a bilirubin value of 0.7 ± 0.4 mg/mL.

The IL-28B CC genotype was detected more frequently in the case group than in the control group (40.2% vs 24.4%; $P < .05$).

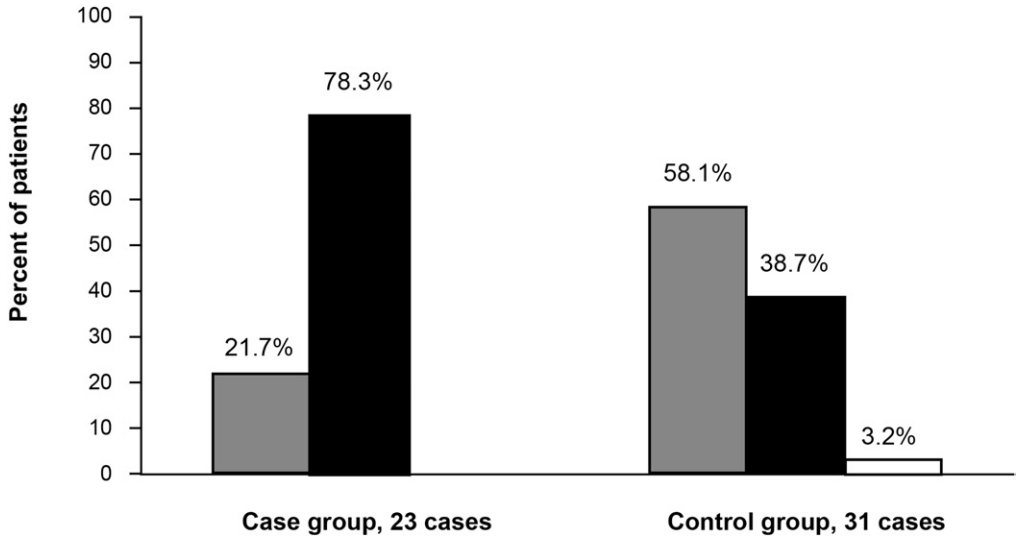

Figure 1. Changes in fibrosis score between the first and second LB of patients in the case and control groups. Fibrosis score: stationary (*grey columns*), increased ≥2 (*black columns*), decreased ≥2 (*white columns*). $P < .005$, with an increased fibrosis score of 2 or greater, case group vs control group.

Clinical Outcome

Of the 82 patients in the case group, 13 (15.8%) dropped out because of lack of compliance and 69 were followed up for at least 2 years. On the basis of the ALT profiles during and after acute exacerbation of CHC, 30 (43.5%) of these 69 patients were considered to have deteriorated, 39 (56.5%) were stationary, and none improved.

Changes in the liver fibrosis and HAI scores between the 2 LBs are shown in Figures 1 and 2, respectively. Deterioration in liver fibrosis of at least 2 points was observed in 18 (78.3%) of the 23 patients in the case group and in 11 (35.5%) of the 31 patients in the control group ($P < .005$), whereas the fibrosis scores remained stationary in 5 patients (21.7%) in the case group and 20 patients (64.5%) in the control group. Only 1 patient (3.2%) in the control group improved (Figure 2).

Deterioration of at least 2 points in the HAI score was observed in 14 patients (60.9%) in the case group and 3 patients (9.6%) in the control group, a difference significant to the statistical analysis ($P < .005$). An improvement in the HAI of at least 2 points was found only in 4 patients (12.9%) in the control group, whereas 9 patients (39.1%) in the case group and 24 patients (77.5%) in the control group remained stationary.

For a more comprehensive understanding of the impact of acute exacerbation of CHC on the clinical course of CHC, the 69 patients with a long-term follow-up evaluation are shown in Figure 3 and in Supplementary Figures 1 to 4.

Patient number 1 in Figure 3 was a 42-year-old man with HCV genotype 2a, IL-28-B CC, who showed a single episode of acute exacerbation and an ALT level decrease in less than 4 months, similar to another 50 patients (from patient 5 to patient 54 in Supplementary Figure 1). In another 6 patients (from patient 55 to patient 60), after a single episode of acute exacerbation the ALT level decrease lasted more than 5 months (Supplementary Figure 2).

Patient number 2 in Figure 3 was a 60-year-old woman with HCV genotype 3, IL-28-B CT, who developed an acute exacerbation of CHC in January 2006 with ALT levels increased up to 42-fold and a peak total bilirubin level of 21

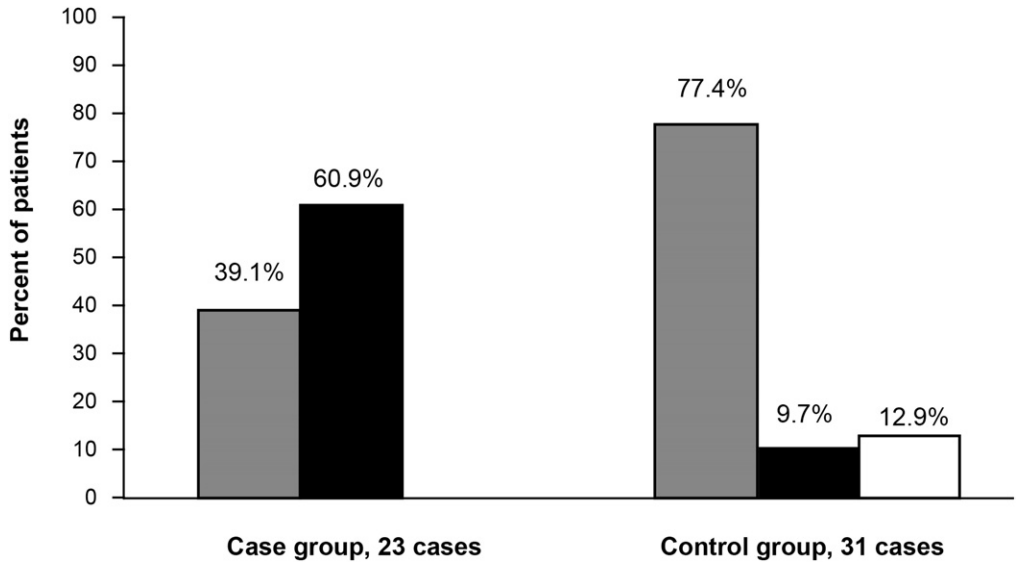

Figure 2. Changes in necroinflammation (HAI) score between the first and second LB of patients in the case and control groups. Fibrosis score: stationary (*grey columns*), increased ≥2 (*black columns*), decreased ≥2 (*white columns*). $P < .005$, with increased HAI score of 2 or greater, case group vs control group.

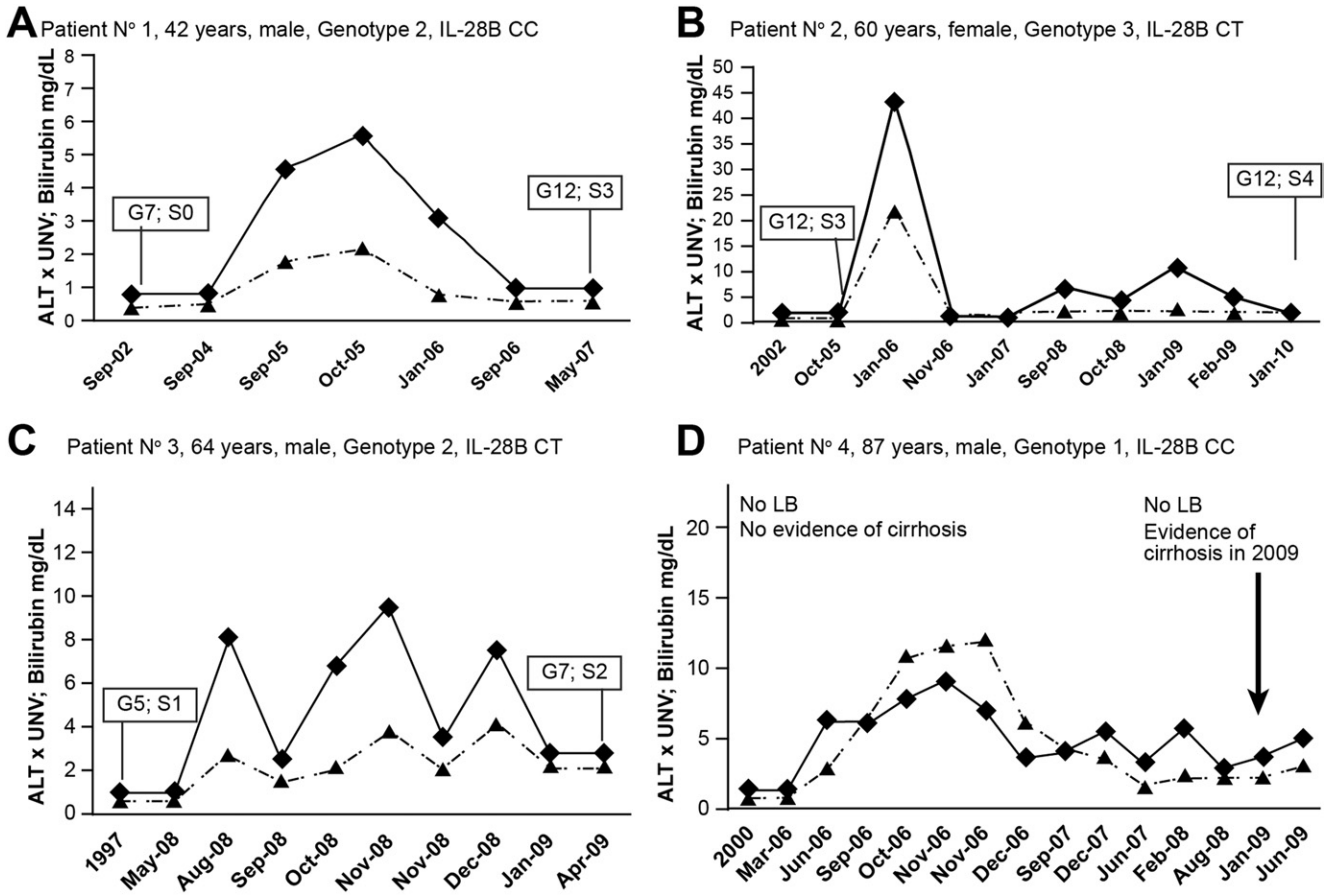

Figure 3. Serum ALT and bilirubin values throughout the observation in 4 patients (*A*–*D*) with acute exacerbation of CHC. G, grading; S, staging; UNV, upper normal value.

mg/dL during reactivation. Serum ALT and bilirubin levels returned to normal by November 2006 and remained so until September 2008 when a second episode of reactivation occurred, followed, after a partial remission, by a third episode in January 2009. The second and third episodes of reactivation were anicteric and of a lesser entity compared with the first.

Patient number 3 (Figure 3) was a 64-year-old man with HCV genotype 2, IL-28-B CC, with a first episode of acute exacerbation of CHC in August 2008 and 2 subsequent episodes of reactivation. The total bilirubin value ranged from 2 to 4 mg/dL throughout the observation period. Another 6 patients (from patient 61 to patient 66 in Supplementary Figure 3) showed a course of the disease characterized by 2 or more episodes of acute exacerbation (Supplementary Figure 3).

Patient number 4 in Figure 3 was an 87-year-old man with HCV genotype 1, IL-28-B CC, who until March 2006 had a clinical, biochemical, and ultrasound profile consistent with the diagnosis of CHC, with the ALT value near to normal and normal bilirubin level. This patient developed an acute exacerbation of CHC in June 2006 followed by another 3 episodes of reactivation of a lesser entity up to June 2009 when unequivocal biochemical and ultrasound signs of cirrhosis were documented. In addition, patients 67, 68, and 69 showed a rapid transition to cirrhosis (Supplementary Figure 4).

Antiviral Treatment

Of the 32 patients in the case group treated with peg-INF + ribavirin, 26 (81.2%) achieved a sustained virologic response (SVR) and 6 (18.8%) were nonresponders. Of the 38 treated patients in the control group, 23 (60.5%) obtained an SVR and 12 (39.5%) were nonresponders. The data on the response to treatment, according to HCV genotype and IL-28-B genotype, are presented in Table 2. The 17 patients with HCV genotypes 1/4 in the case group showed an SVR more frequently (70.6%) than the 18 patients with the same HCV genotypes in the control group (44.4%). Similarly, the 15 patients with HCV genotypes 2/3 in the case group showed an SVR more frequently than the 20 patients with the same HCV genotypes in the control group (93.3% vs 75%). Table 2 also shows that SVR was achieved by all 14 patients with the IL-28-B CC genotype in the case group, regardless of HCV genotype, and by 8 of the 11 (73%) in the control group. These differences between small groups or subgroups of treated patients are not significant to the statistical analysis.

Conclusions

This long-term follow-up study of 82 patients with acute exacerbation of CHC improves the scanty knowledge on the clinical presentation and course of symptomatic acute exacerbation of CHC and on the impact of this clinical event on the outcome and response to antiviral therapy. At the time of

Table 2. SVR to Peg-IFN + Ribavirin Treatment in 32 Patients in the Case Group and 38 Patients in the Control Group

	Case group		Control group	
	No. of patients	With SVR, n (%)	No. of patients	With SVR, n (%)
HCV genotype 1				
IL-28-B CC	8	8 (100)	6	4 (66.7)
IL-28-B CT/TT	9	4 (44.4)	12	4 (33.3)
All HCV genotype 1 cases	17	12 (70.6)	18	8 (44.4)
HCV genotype non-1				
IL-28-B CC	6	6 (100)	5	4 (80)
IL-28-B CT/TT	9	8 (88.9)	15	11 (73.3)
All HCV genotype non-1 cases	15	14 (93.3)	20	15 (75)
Total cases	32	26 (81.25)	38	23 (60.5)

the first observation, the mean AST values were as high as 16-fold the normal value, ALT was as high as 25-fold, and bilirubin was as high as 15-fold. There was a marked variability in the clinical presentation, with ALT level increased from 6 to 43-fold the normal values and serum bilirubin increased from 2 to 22 mg/dL. Also variable was the clinical course of acute exacerbation of CHC, usually characterized by a single flare, but in some cases more than 1 flare can occur. An acute exacerbation of CHC may occur at any age, as shown by the wide range of ages in the study, from 24 to 87 years. After reactivation, the ALT values slowly decreased in all patients and, by the end of a 2-year follow-up evaluation, about half of the patients returned to their baseline values before acute exacerbation of CHC, whereas for the other half, the ALT values persisted at more than 2-fold.

Nearly half of the patients with an acute exacerbation of CHC in the present study showed HCV genotype 2. This observation confirms the association between this clinical event and HCV genotype 2 shown by studies from Italy,[6,9,10] a country where the prevalence of this HCV genotype in patients with CHC is approximately 20%.[19] The reasons for this association remain unknown and warrant further investigation. The data from the present study, however, show that is frequent even in patients with HCV genotype 1 and that it rarely can occur also in patients with other HCV genotypes.

Although unexpected, the significantly higher prevalence of genotype IL-28-B CC in the case group may suggest a greater likelihood of developing acute exacerbation of CHC for patients with this genotype, an observation that deserves further consideration in more extensive studies. Most probably acute exacerbation of CHC is a consequence of a reactivation of cell-mediated immune reaction to clear HCV infection,[20–22] in some way in line with the well-known propensity of the IL-28-B CC genotype to undergo a spontaneous or treatment-induced clearance of HCV infection.[23,24]

The comparison of liver histology in sequential LBs, possible for nearly a third of the patients in each group, suggests that acute exacerbation of CHC frequently causes deterioration both in fibrosis and necroinflammation. In fact, a 2-points deterioration in fibrosis was observed in nearly three quarters of the patients in the case group and in nearly a third of the controls. Similarly, a 2-points deterioration in HAI was found in nearly 60% of patients in the case group and in 10% in the controls. The differences were both statistically significant. The data from previous long-term follow-up studies on the progression of HCV-associated liver disease suggest that the progression of liver fibrosis is indolent for nearly 2 decades after acute hepatitis C and that morbidity and mortality are more likely to emerge in the third or fourth decade after infection.[25] The rate of acceleration of liver fibrosis consequent to acute exacerbation of CHC shown in the present study is higher than that observed in the control group of this study and in previous investigations in patients who did not experience this clinical event and frequently showed an indolent course of the disease.[25] This underscores the profound implication of acute exacerbation of CHC on the progression to cirrhosis and risk of HCC.

In the present study, the patients who experienced a symptomatic acute exacerbation of CHC showed a tendency to achieve an SVR to peg-IFN + ribavirin treatment, but the high prevalence of SVR (81.2%), although impressive, deserves confirmation in more extensive multicenter studies. The frequency of patients with HCV genotype 2 and/or IL-28-B CC most probably explains the high efficacy of peg-IFN + ribavirin treatment in the case group, but an additional reason might be found in acute exacerbation of CHC itself, which, being immunologically and virologically similar to acute hepatitis, could re-establish the mechanisms that induce the high response rate to interferon observed in acute hepatitis C.[26,27]

In conclusion, acute exacerbation of CHC is a clinical event frequently associated with HCV genotype 2 and IL-28-B CC genotype, and is responsible for an unfavorable outcome in patients with CHC. However, the majority of patients with acute exacerbation of CHC obtained an SVR, most probably because of the high frequency of HCV genotype 2 and IL-28-B CC genotypes in the case group, and possibly because the reactivation of a cell-mediated immune response may favor HCV clearance. The more rapid progression to cirrhosis and the risk of HCC strongly warrant the early initiation of anti-HCV therapy for acute exacerbation of CHC patients, who in this study showed an impressive rate of SVR to peg-IFN + ribavirin.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2013.03.025.

References

1. Hoofnagle JH. Hepatitis C: the clinical spectrum of disease. Hepatology 1997;26:15S–20S.
2. Afdhal NH. The natural history of hepatitis C. Semin Liver Dis 2004;24:3–8.
3. Missiha SB, Ostrowski M, Heathcote EJ. Disease progression in CHC: modifiable and nonmodifiable factors. Gastroenterology 2008;134:1699–1714.
4. Alazawi W, Cunningham M, Dearden J, et al. Systematic review: outcome of compensated cirrhosis due to CHC infection. Aliment Pharmacol Ther 2010;32:344–355.
5. Sheen IS, Liaw YF, Lin DY, et al. Acute exacerbations in CHC: a clinicopathological and prognostic study. J Hepatol 1996;24:525–531.

6. Rumi MG, De Filippi F, Donato MF, et al. Progressive hepatic fibrosis in healthy carriers of hepatitis C virus with a transaminase breakthrough. J Viral Hepat 2002;9:71–74.
7. Hiraga N, Suzuki F, Akuta N, et al. Clinical and virological characteristics of untreated patients with CHC who develop serum alanine aminotransferase flare-up. J Med Virol 2005;75:240–248.
8. Sagnelli E, Coppola N, Marrocco C, et al. Diagnosis of hepatitis C virus related acute hepatitis by serial determination of IgM anti-HCV titres. J Hepatol 2005;42:646–651.
9. Rumi MG, De Filippi F, La Vecchia C, et al. Hepatitis C reactivation in patients with chronic infection with genotypes 1b and 2c: a retrospective cohort study of 206 untreated patients. Gut 2005;54:402–406.
10. Coppola N, Vatiero LM, Sagnelli E. HCV genotype 2 as a risk factor for reactivation of chronic HCV infection. Gut 2005;54:1207.
11. Coppola N, Pisapia R, Marrocco C, et al. Anti-HCV IgG avidity index in acute hepatitis C. J Clin Virol 2007;40:110–115.
12. Coppola N, Pisapia R, Tonziello G, et al. Improvement in the aetiological diagnosis of acute hepatitis C: a diagnostic protocol based on the anti-HCV-IgM titre and IgG Avidity Index. J Clin Virol 2009;46:222–229.
13. Sagnelli E, Imparato M, Coppola N, et al. Diagnosis and clinical impact of occult hepatitis B infection in patients with biopsy proven CHC: a multicenter study. J Med Virol 2008;80:1547–1553.
14. Coppola N, Potenza N, Pisaturo M, et al. Liver microRNA hsa-miR-125a-5p in HBV chronic infection: correlation with HBV replication and disease progression. PLoS One 2013;8:e65336.
15. Prati D, Gasbarrini A, Mazzotta F, Italian Association for the Study of the Liver, Italian Society of Infectious, Tropical Diseases, Italian Society for the Study of Sexually Transmitted Diseases. Practice guidelines for the treatment of hepatitis C: recommendations from an AISF/SIMIT/SIMAST Expert Opinion Meeting. Dig Liver Dis 2010;42:81–91.
16. European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatitis C virus infection. J Hepatol 2011;55:245–264.
17. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–699.
18. Zampino R, Coppola N, Cirillo G, et al. Abdominal fat interacts with PNPLA3 I148M, but not with the APOC3 variant in the pathogenesis of liver steatosis in chronic hepatitis C. J Viral Hep 2013;20:517–523.
19. Sagnelli E, Stroffolini T, Mele A, et al. The importance of HCV on the burden of chronic liver disease in Italy: a multicenter prevalence study of 9,997 cases. J Med Virol 2005;75:522–527.
20. Rehermann B. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. J Clin Invest 2009;119:1745–1754.
21. Sagnelli E, Argentini C, Genovese D, et al. Virological and epitope evolution of HCV infection from acute hepatitis C to subsequent episodes of HCV-related acute liver cell necrosis. Infection 2009;37:344–348.
22. Spada E, Mele A, Berton A, et al. Multispecific T cell response and negative HCV RNA tests during acute HCV infection are early prognostic factors of spontaneous clearance. Gut 2004;53:1673–1681.
23. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461:798–801.
24. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
25. Seef LB. Natural history of hepatitis C. Hepatology 1997;26(Suppl 1):21S–28S.
26. Jaeckel E, Cornberg M, Wedemeyer H, et al. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med 2001;345:1452–1457.
27. Kamal SM, Ismail A, Graham CS, et al. Pegylated interferon alpha therapy in acute hepatitis C: relation to hepatitis C virus-specific T cell response kinetics. Hepatology 2004;39:1721–1731.

Reprint requests

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Conflicts of interest

The authors disclose no conflicts.

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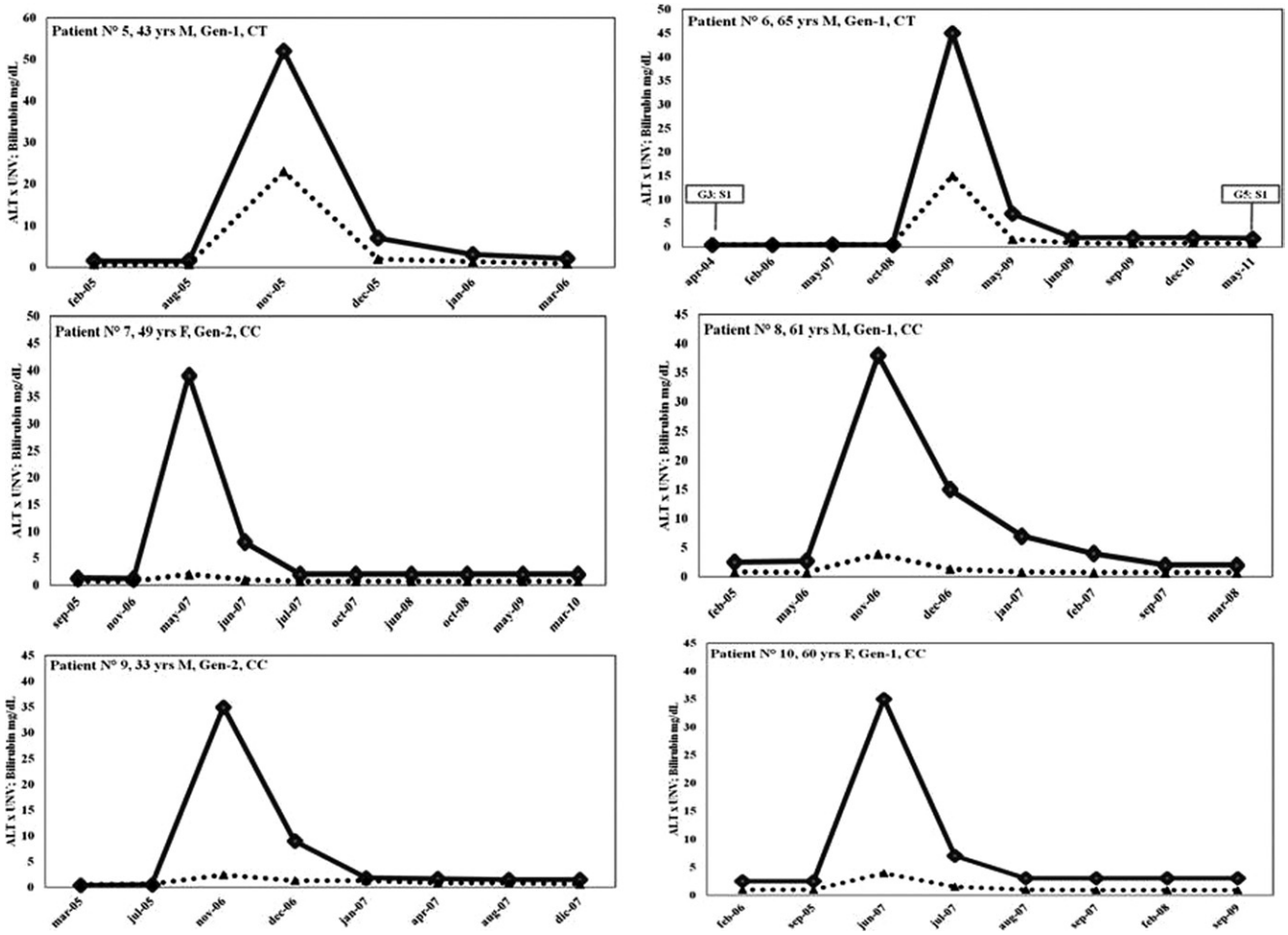
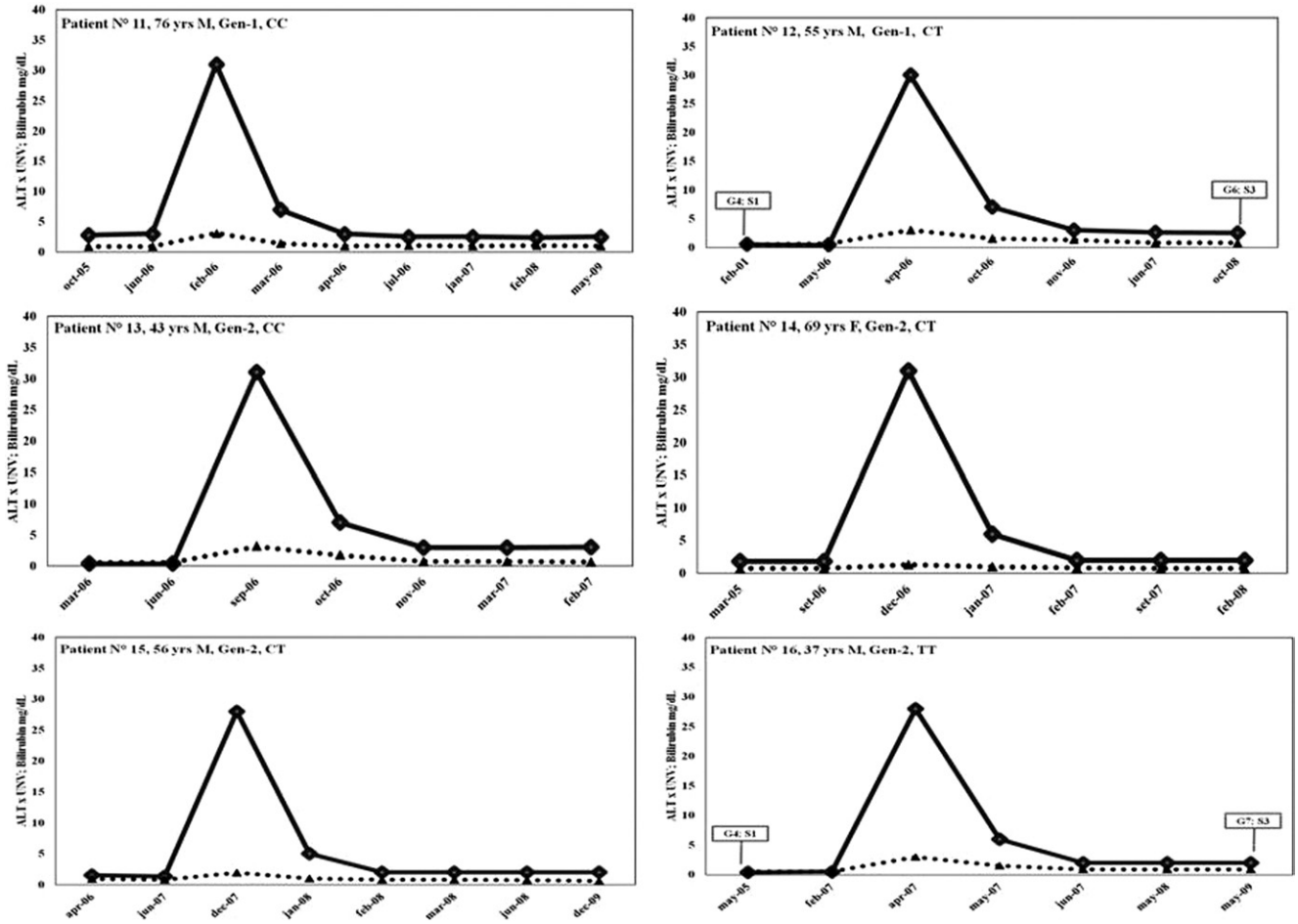
Supplementary Methods

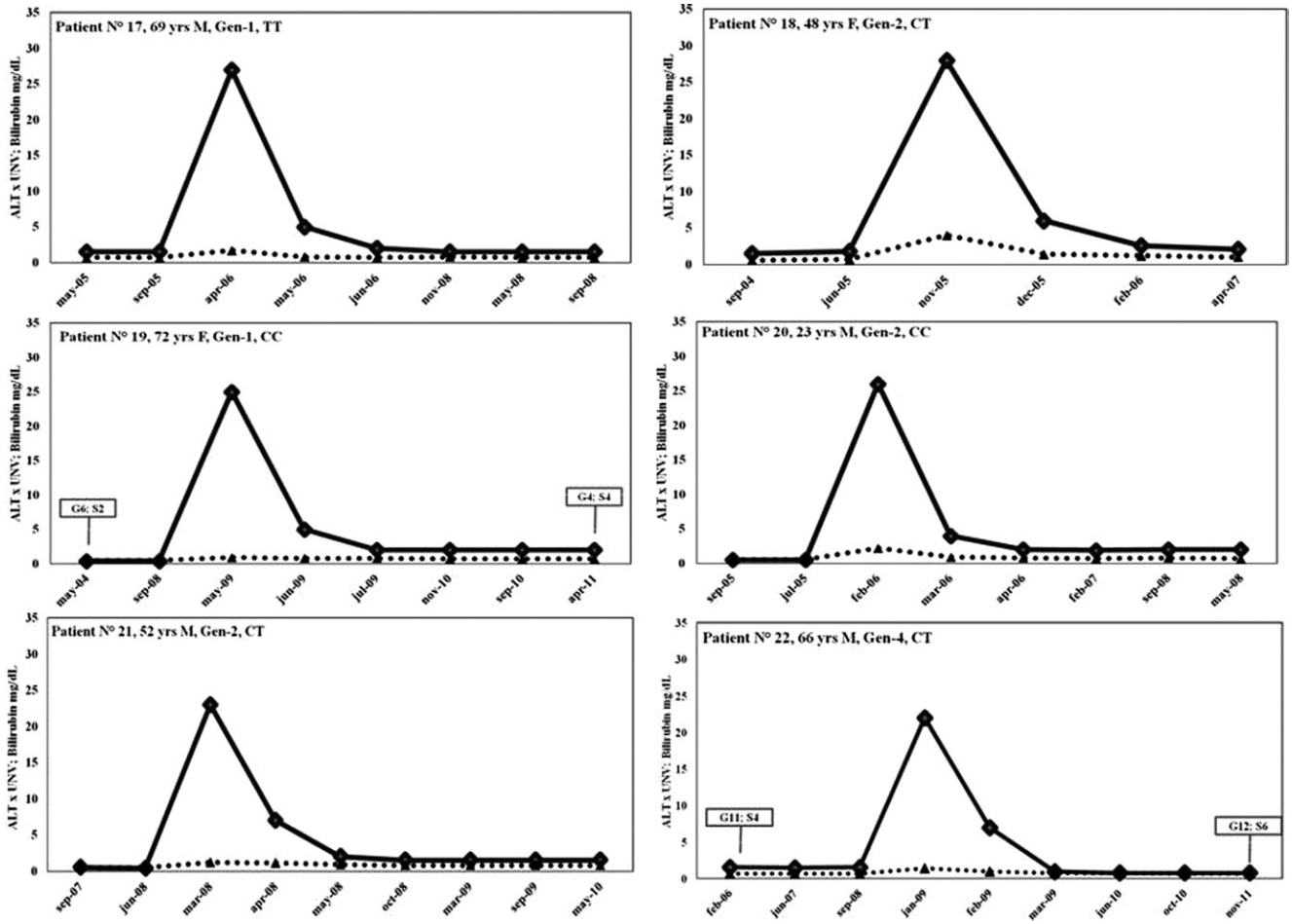
Commercial immunoenzymatic assays were used for HAV, hepatitis B virus, HDV, HCV, HEV, human immunodeficiency virus, cytomegalovirus, and Epstein–Barr virus serum markers, as follows: for HBsAg, antibody to HBsAg, anti–hepatitis B core IgM, total and IgM anti-HAV, anti–human immunodeficiency virus, anti- cytomegalovirus, and anti–Epstein–Barr virus: Abbott Laboratories (North Chicago, IL); for HBeAg, anti-HBe, anti-HDV IgG, and anti-HDV IgM: DiaSorin (Saluggia, VC, Italy); for anti-HCV: Ortho Diagnostic Systems (Neckargemund, Germany); and for anti-HEV IgM: Diapro (Diagnostic BioProbes, Sesto San Giovanni, Italy).



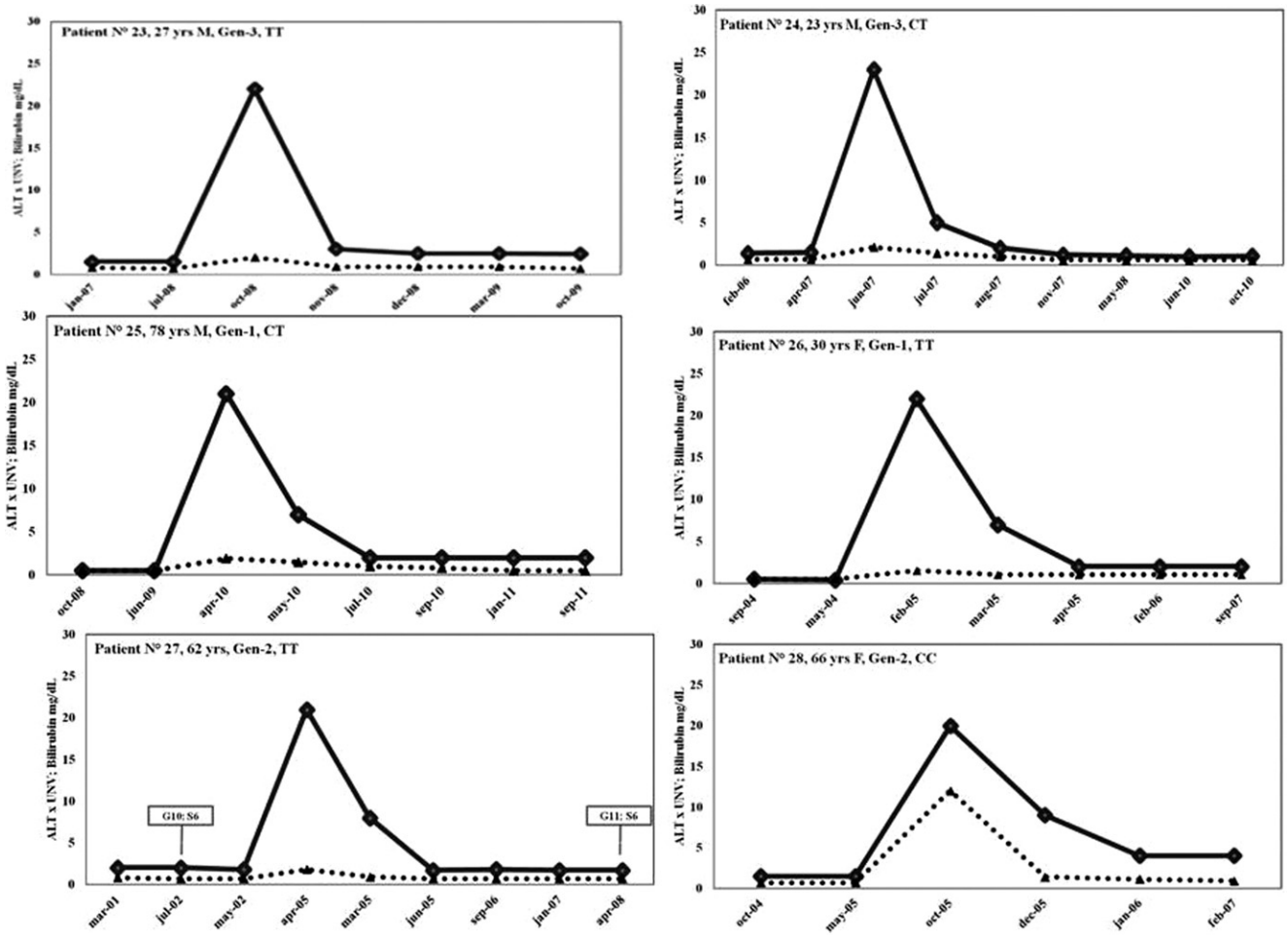
Supplementary Figure 1. Serum ALT and bilirubin values throughout the observation in 50 patients (from patient 5 to patient 54) showing a single episode of acute exacerbation lasting less than 5 months. CC, IL-28B CC; CT, IL-28B CT; F, female; gen, genotype; G, grading; M, male; S, staging; TT, IL-28 TT; UNV, upper normal value.



Supplementary Figure 1. Continued



Supplementary Figure 1. Continued



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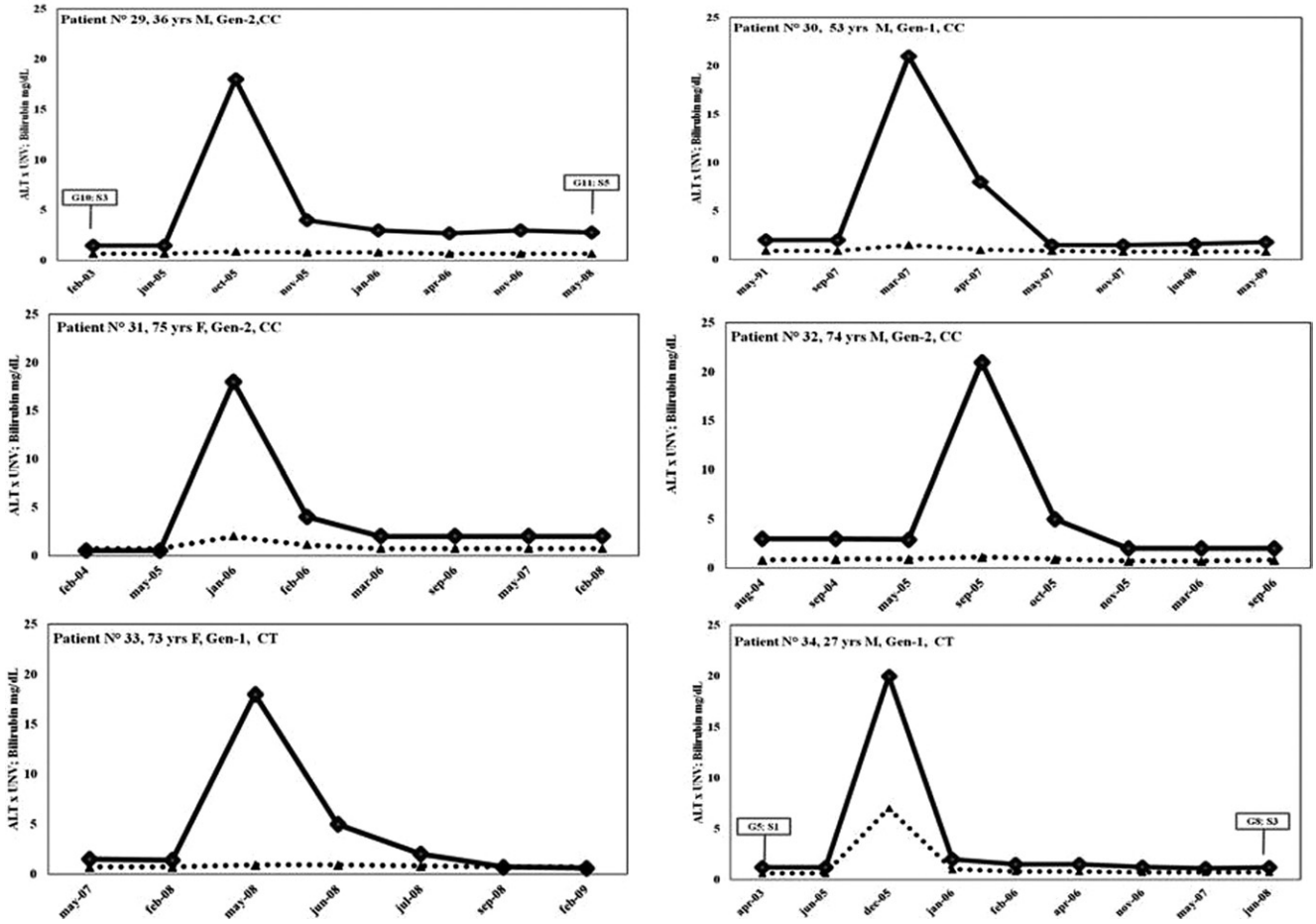

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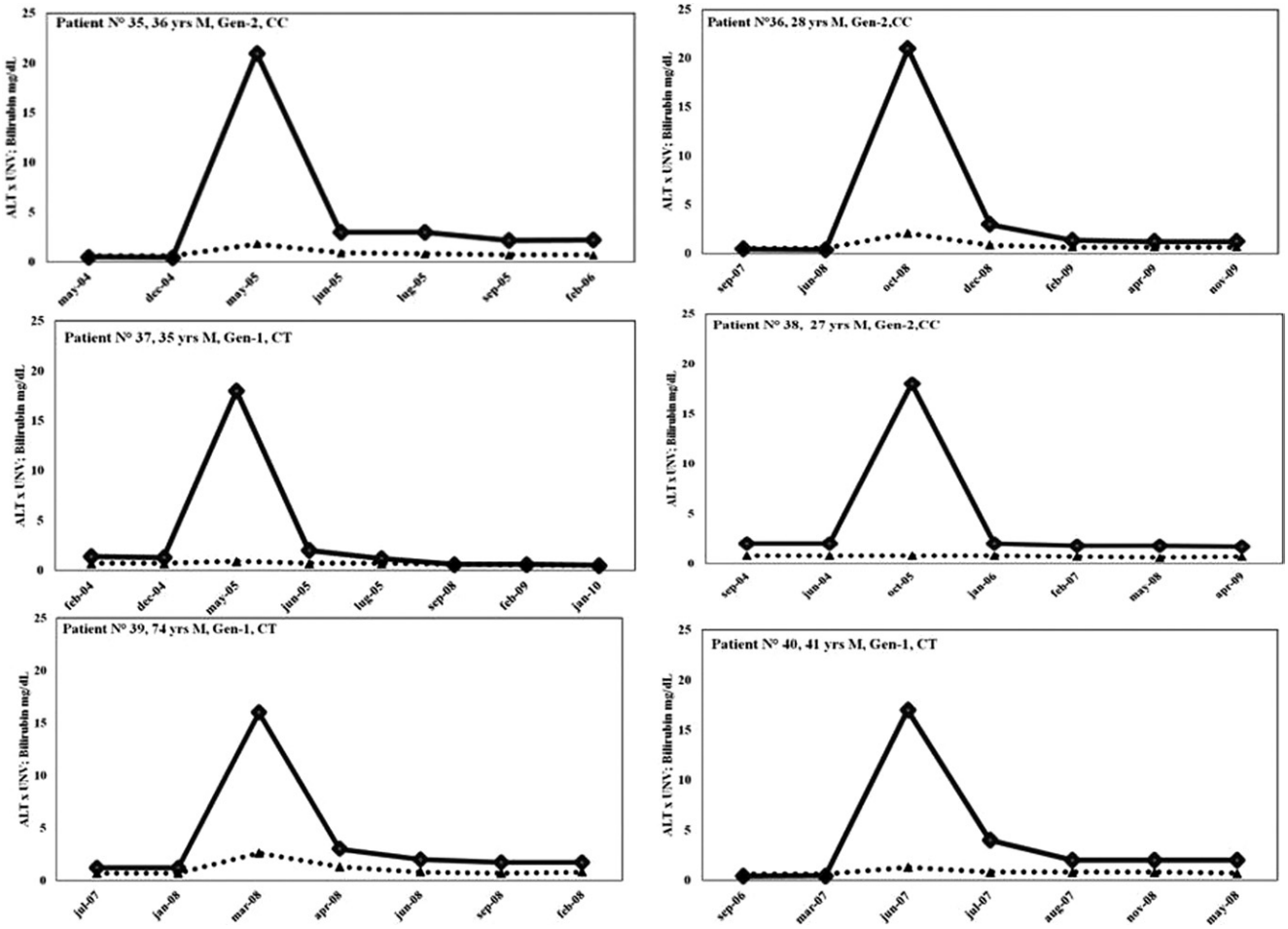

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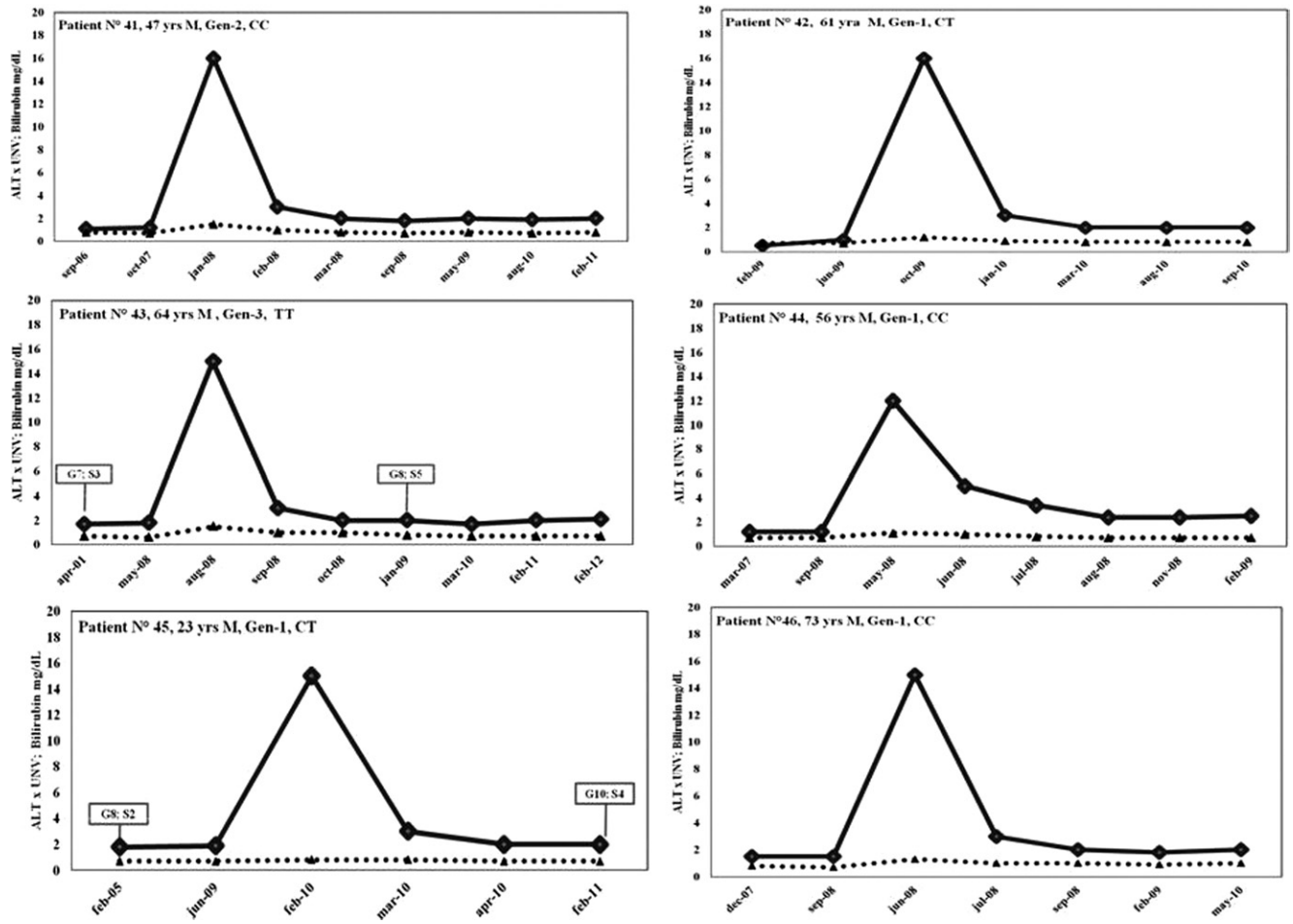

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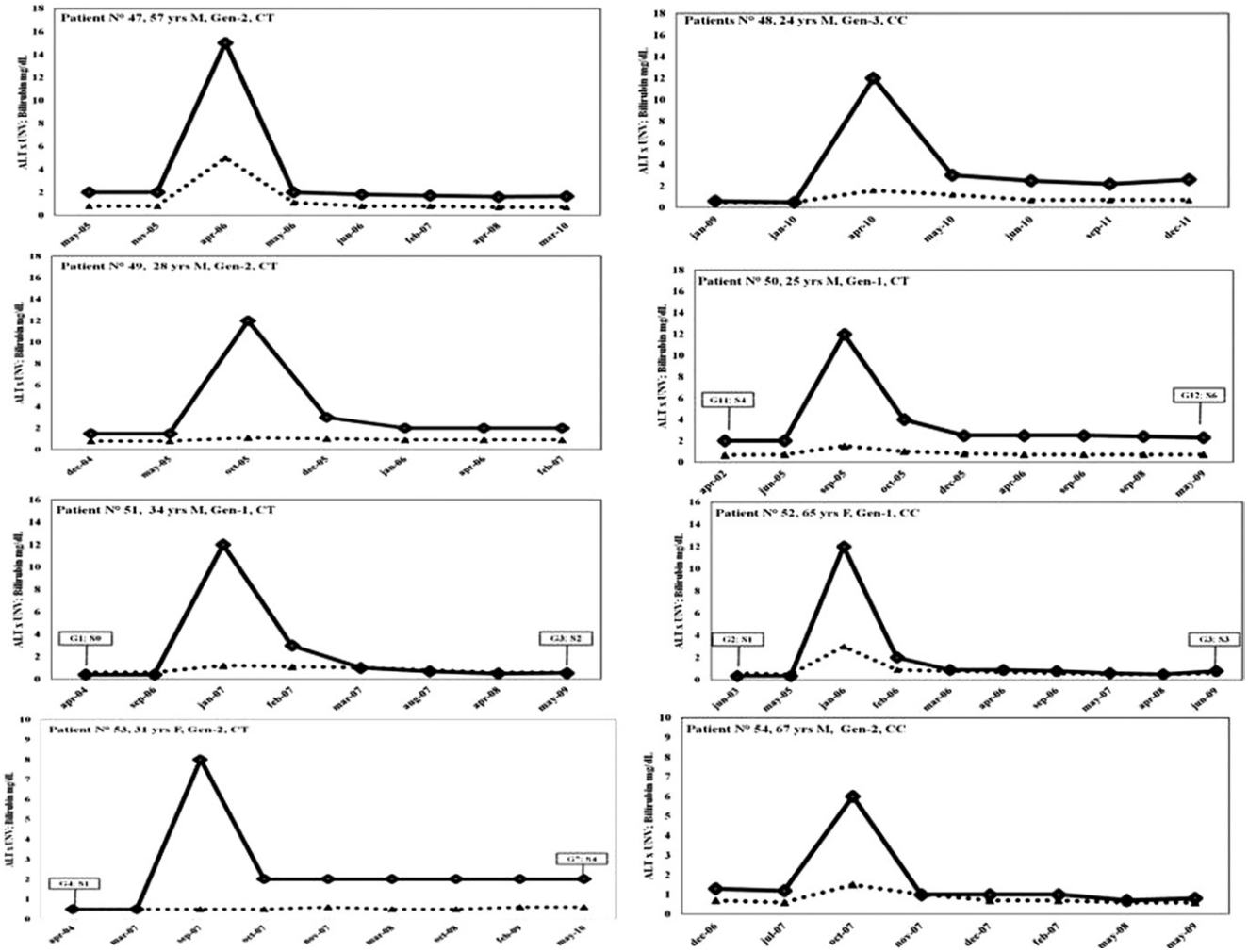

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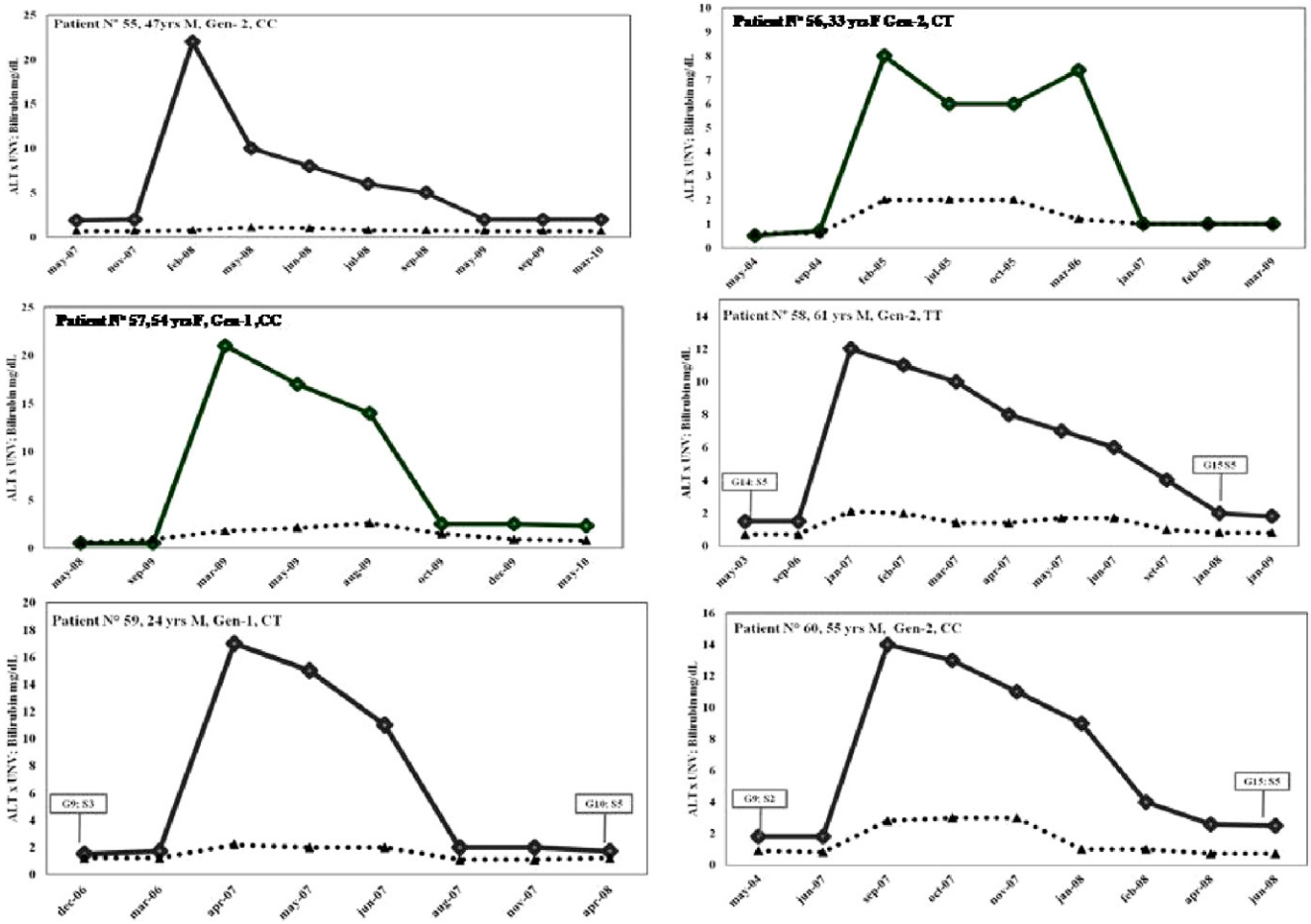
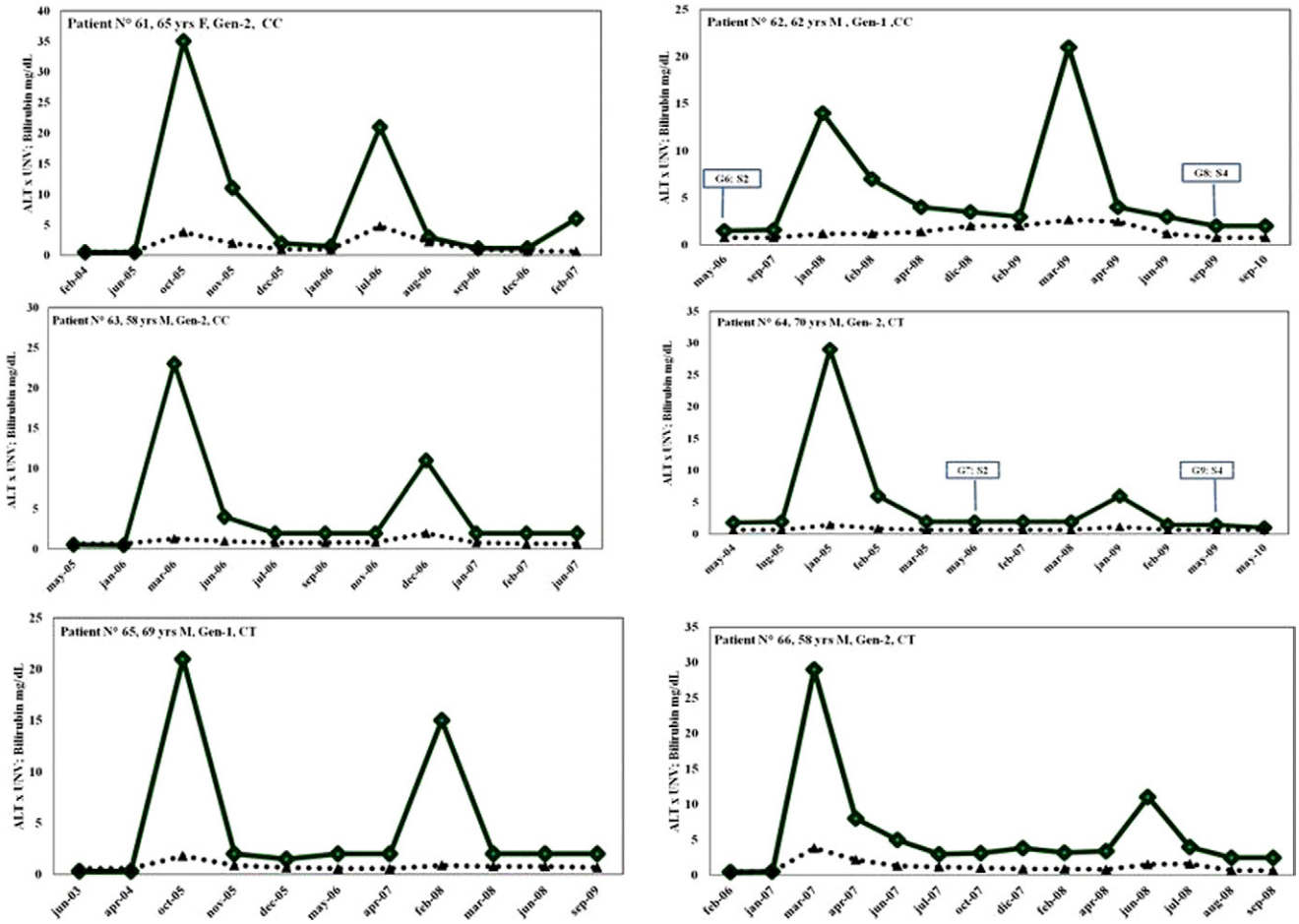
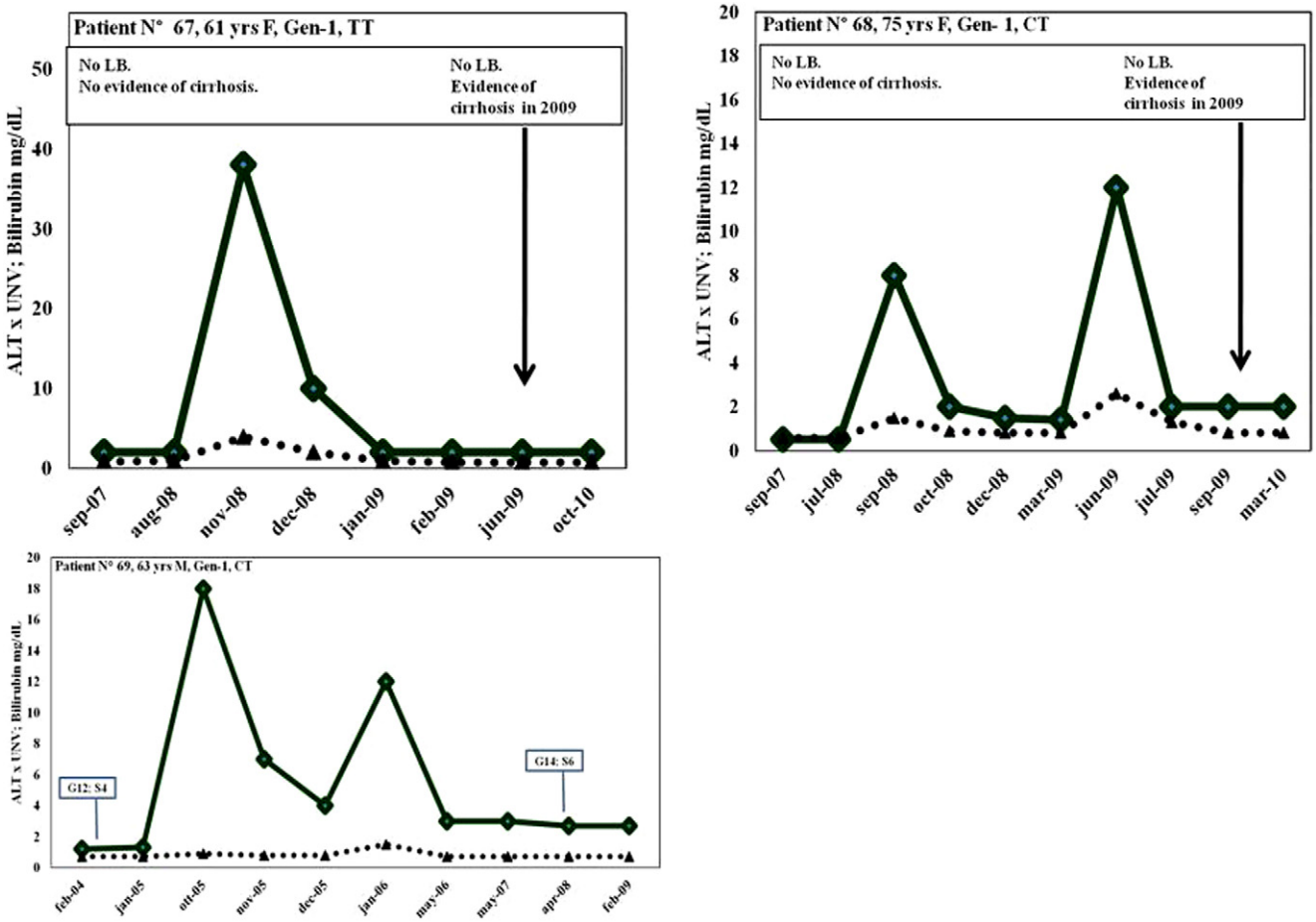


Supplementary Figure 2. Serum ALT and bilirubin values throughout the observation period in 6 patients (from patient 55 to patient 60) showing a single episode of acute exacerbation of CHC, lasting more than 6 months. CC, IL-28B CC; CT, IL-28B CT; G, grading; S, staging; TT, IL-28 TT; UNV, upper normal value.



Supplementary Figure 3. Serum ALT and bilirubin values throughout the observation period in 6 patients (from patient 61 to patient 66) who showed 2 or more peaks of acute exacerbation of CHC. CC, IL-28B CC; CT, IL-28B CT; G, grading; S, staging; TT, IL-28 TT; UNV, upper normal value.



Supplementary Figure 4. Serum ALT and bilirubin values throughout the observation period in 3 patients (from patient 67 to patient 69) who showed a rapid transition to cirrhosis. CC, IL-28B CC; CT, IL-28B CT; G, grading; S, staging; TT, IL-28 TT; UNV, upper normal value.