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Acute Hepatitis C in South America: transmission routes, clinical features and outcome predictors

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Abstract

Introduction: There is a considerable paucity of data of acute hepatitis C (aHC) infection; no large series have been reported in South America.

Aims: To identify clinical characteristics and risk factors for aHC in a cohort of South American patients. To evaluate factors associated with aHC evolution towards chronicity (in an untreated cohort) and response to antiviral treatment (in a treated cohort).

Methods:A retrospective clinical record analysis of all patients ≥16 years old registered with diagnosis of acute hepatitis C was performed in thirteen hepatology units.

Results:64 patients fulfilled inclusion criteria.The majority were middle-aged (median age 46 years) females; most of them were symptomatic at diagnosis (79.6%).Five patients had liver failure: three had severe acute hepatitis andtwo patients required liver transplantation. Nosocomial exposure was the most prevalent risk factor detected.Seroconversion provided diagnosis in 27 patients and in the remaining the diagnosis was stablished by HCV RNA detection in the presence of an acute hepatitis syndrome without alternative diagnosis. Evolution was assessed in 46 patients. In the untreated cohort, spontaneous resolution occurred in 50% of the patients and was associated with AST levels (OR: 1.13, CI 95% 1-1.27; p=0.04 for each time above the upper limit of normal value). In the treated cohort, SVR was associated with nosocomial transmission (p=0.03) and early treatment initiation (p=0.05).

Conclusion:this is the largest aHC cohort evaluated in our region; the most prevalent suspected transmission source was of nosocomial origin, thus stressing the importance of revising universal precautions measures to prevent HCV infection.

Key words: hepatitis C virus; nosocomial transmission; spontaneous resolution; South America.

Abbreviations: HCV: hepatitis C virus; USA: United States of America; aHC: acute

infection by hepatitis C; anti-HCV: IgG antibodies for hepatitis C virus; HCV RNA: hepatitis C virus ribonucleic acid; Peg-IFN: pegylated interferon.

Core tip: There is a considerable paucity of data regarding acute hepatitis C risk factors and evolution, especially in developing regions such as South America. We present hereby the largest South American aHC cohort. The most prevalent transmission source was of nosocomial origin, thus stressing the importance of revising precautions to prevent HCV transmission. A severe evolution was observed in three patients. Spontaneous resolution was associated with necro-inflammatory activity, whereas treatment response was associated with a nosocomial transmission and early treatment onset. In a setting of scarce availability of direct acting antiviral therapy, early Peg-IFN based treatment may still have a role.

Introduction

Hepatitis C virus (HCV) infection has long been considered a major public health issue; affecting not only developed countries such as USA (estimated prevalence 1.3% of the population) and those comprisingWestern Europe (estimated prevalence 2.4%) but also developing regions such as Tropical and Southern America (1.2-1.6% respectively)[1]. Since HCV progresses to chronic infection in the majority of cases (approximately 75-85%)[2], risk factors for the development of fibrosis, cirrhosis and hepatocellular carcinoma have been the focus of major research efforts. Acute hepatitis C (aHC) represents an entirely different scenario; asit is seldom symptomatic (acute hepatitis syndrome occurs in 15% of patients), thus, there is a considerable paucity of data that precludes precise knowledge of its risk factors and evolution[3].

aHCis defined as an acute necro-inflammatory process of the liver caused by HCV and characterized by an increase in the aminotransferases level of at least ten times the upper normal value[4].There is no specific test to diagnose aHC; the current gold-standard is to document anti-HCV seroconversion. However, an anti-HCV prior to the acute hepatitis is seldom availablein the general population, except in cases in which early post exposure follow up is performed[5]. The alternative diagnostic approach (and currently the most frequently used strategy) is to consider aHCdiagnosis when there is a likely recent source for HCV transmission, an acute hepatitis syndrome (elevation of aminotransferases ten times from baseline) and HCV RNA is detected with or without certainty of anti-HCV seroconversion; with a throughout exclusion of other acute hepatitis etiologies (viral, toxic or metabolic) and chronic liver disease. These criteria are considered valid both in monoinfected and HIV co-infected patients[6-9]. aHC represents a substantial etiology of acute hepatitis; it accounts for approximately 20% of cases of acute hepatitis, and approximately 30,000 new cases occur every year in the United States alone[6]. Unfortunately, precise data in developing regions such as South America is scarce. The lack of general data is considered to be multifactorial, due to the elevated amount of unrecognized early infections and the insignificant number of acute

infections in controlled clinical settings (as were in the past, aHC after blood transfusions)[10].

Accurate identification of patients with aHCand a more detailed comprehension of its natural history are important, since they would allow to identify risk factors for its transmission and to choose the correct timing for antiviral treatment (it has been suggested that an early start can lead to higher sustained virological response rates)[11]. Therefore, the aim of this study was to identify clinical characteristics and risk factors for aHCin a cohort of South American patients, to evaluate factors associated with its evolution towards chronicity (in an untreated cohort) and response to antiviral treatment (in a treated cohort).

Patients and Methods

Study population:A retrospective clinical record analysis of all patients ≥16 years old registered with diagnosis of aHCwas performed in thirteen hepatology units (eleven centers from Argentina, one center from Uruguay and one center from Paraguay) from January 2002 until December 2015. The diagnosis of aHCwas based on seroconversion to anti-HCV antibodies (preferred criteria) and/or the presence of an acute hepatitis syndrome (increases of alanine aminotransferase [ALT] at least 10 times the upper limit of normal) in individuals without preexisting liver disease after the exclusion of other infectious (acute hepatitis A and B), metabolic or toxic etiologies, accompanied by the presence of HCV RNA in the first serum sample (alternative criteria).The study protocol was evaluated and approved by a Clinical Research Committee; it was conducted in accordance with the Declaration of Helsinki.

Exclusion criteria: Patients with alternative diagnosis for acute hepatitis, patients with previously altered liver test/suspicion of chronic hepatitis, patients with concomitant severe illness that precluded exclusiveaHC diagnosis (systemic cytomegalovirus infection, severe sepsis, etc).

Demographic, possible transmission routes and clinical data: Data was collected in an *ad hoc* standardized form, including:

1. Demographic data: gender, age at diagnosis.
2. History of exposure to possible risk factors: if available in medical records, previous exposures within 6 months before the diagnosis of aHC were recorded. The considered variables were nosocomial (medical invasive procedures, surgical interventions, transfusions and hemodialysis), occupational (needle stick injuries or other type of exposure in health-care workers), exposure related to alternative medical procedures (i.e., ozone therapy), sexual-related (these were divided accordingly if the patient had a known sero-discordant anti-HCV positive sexual partner or if the patient had unsafe sex without knowledge of their couple´s HCV status) or intravenous drug use. If the patient did not recognized a possible risk factor or mentioned more than one, they were catalogued as unknown risk exposure.
3. Date ofonset (symptomatic patients) or first consult when referred for abnormal liver test (asymptomatic patients).

Serological and Molecular HCV RNA assays: Anti-HCV serology was performed by commercially available third generation enzyme immunoassays. When available before the diagnosis of aHC, its result and date of testing were recorded. During the episode of aHC, several features regarding anti-HCV were documented: date of testing in relation to the onset of symptoms/first consultation, number of serological test performed and their results. It was also noted whether seroconversion was confirmed. Regarding molecular HCV RNA testing, both qualitative and quantitative polymerase chain reaction (PCR) testing were performed according to available technology at different times and centers; when available, HCV genotype and viral load were recorded.

Biochemical features: Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TB) values were registered at aHC onset, and if available,

monthly during the following three months. The worst prothrombin time during each aHCepisode was also recorded.

Follow-up and evolution: If patients did not completed at least five-months of medical assessment, they were accounted as lost to follow-up; in the remaining cases, patients were sorted according to the decision of antiviral treatment. In those patients that were not treated, rate and timing of spontaneous resolution or evolution towards chronicity was registered. In the cohort of patients that were treated, treatment duration, selected drugs and sustained virological response (SVR) were registered.

Statistical analysis: Results are presented in percentages, mean and standard deviation for normally distributed data and in median and interquartile range for non-normal distributed data. Bivariate analysis was performed with: chi-square or fisher´s test according to the number of patients analyzed for nominal data, t student test or ANOVA for parametric data; and Mann-Whitney or Kruskal Wallis test for non-parametric data, depending on the number of groups evaluated.Multivariate analysis was performed by logistic regression. Statistical analysis was performed with STATA 11^{th} version. In all analysis the significance levels were set at $p \leq 0.05$.

Results

Demographic and clinical features: During the study period, 64 patients fulfilled inclusion criteria. Baseline demographic and clinical characteristics are described in *Table 1,* whereas possible sources of infection/risk factors and their distribution according to age and gender are detailed in *Table 2, 3 and 4.* The majority of patients were middle-aged (median age 46 years) females; most of them were symptomatic at diagnosis (79.6%). The most frequent complaint was jaundice (33 patients; 51.5%), followed by abdominal pain (9 patients; 14%), flu-like symptoms (8 patients; 12.5%) and in one case glomerulonephritis (1.6%). The remaining thirteen asymptomatic patients were diagnosed due to altered liver function test detected during hospitalization or regular check-ups. In 57 (89%) patients, ALT and/or AST were elevated ≥10 times the upper limit normal value (ULN); in the remaining 7 patients, AST values were 6±2.5 times ULN and ALT values reached 6±2 times ULN. Regarding bilirubin levels, 44(70.9%) patients developed jaundice during the aHC episode.

- HIV co-infected patients:A total of 8 patients were HIV positive. The median CD4 count, which was available in 6 patients, was 492 (IQR 180-849) cell/L. Two patients were receiving highly active antiretroviral treatment at the time of aHC diagnosis. Only one HIV positive patient was asymptomatic at diagnosis (CD4 count 112 cell/L).

- Disease severity: there were five cases with liver failure due to aHC: three patients presented with severe acute hepatitis (prothrombin time ≤50%); only one occurred in an immunosuppressed patient (HIV positive, CD4 count 41 cell/L). One patient had a fulminant evolution, requiring liver transplantation one week after aHC onset (with latter severe cholestatic HCV recurrence). Finally, one patient with NASH-related cirrhosis developed acute-on-chronic liver failure, requiring liver transplantation 96 hours after aHC episode onset.

Most of the aHC episodes were detected during or after the year 2010 (n=46). When the likely source of HCV transmission was analyzed according to the time period of aHC onset, nosocomial and sexual-related transmission were the most prevalent risk factors before the year 2010 (33.3% for each risk factor), followed by transfusions (16.7%), unknown (11.1%) and occupational related transmission(5.6%). During the 2010-2015 period, the nosocomial transmission accounted for 50% of the declared risk factors, followed by unknown (19.6%), sexual (13%), intravenous druguse (6.5%), occupational and alternative medical procedures (4.35% for each risk factor) and blood-transfusion related exposure (2.2%).

Serologic diagnosis

Prior HCV serology and seroconversion: Prior negative anti-HCV serology was available in 25(39%) patients; 18 of whom belonged to high risk groups for HCV infection (health care workers, sexual workers, HIV positive patients, patients with multiple hospital admissions, hemodialysis and/or known HCV positive sexual partner).

Seroconversion provided aHC diagnosis in 27 patients (42.1%). It occurred with a median 20 (IQR3-36) days from the onset of symptoms in the whole group. In 9 patients the first anti-HCV obtained after consultation was negative, with a median time of testing of 7 days (IQR3.5-23) since the onset of symptoms or altered liver tests date; these patients presented a positive anti-HCV on second testing, at a median of 59 days (IQR32-134).

Virologic diagnosis: In 37 patients, aHC diagnosis was performed by HCV RNA detection in the setting of an acute hepatitis syndrome without an alternative etiology. The median time for HCV RNA detection was 17 (IQR10-29) days from symptom´s onset.

HCV genotypes and viral kinetics: Genotype distribution (available in 52 patients) was as follows: genotype 1 (no subtype available) n=3 (4.7%), 1a n=18 (28.1%), 1b n=15

(23.4%); genotype 2a n=2 (3.1%), 2c n=3 (4.7%) 2a/2c n=7 (10.9%) and genotype 3a n=4 (6.3%). There were 12 (18.8%) patients without available genotype information. Intermittent HCV RNA negativity (at least one negative HCV RNA assay followed by a positive determination) occurred in 8(12.5%) patients; with a median time of 3 months (IQR1.3-5) after aHC onset. Viral load were available in 52(81.2%) patients; wide viremic fluctuations of >1 log were detected in 12(18.8%) patients.

Follow-up and evolution: After aHC diagnosis, median follow-up was 12.2 months (IQR5.1-22.2). Evolution was analyzed in 46 patients, since 16 patients did not completed the minimum required follow-up time of 5 months and 2 patients were transplanted due to fulminant hepatitis/ acute-on-chronic liver failure (as described above). The clinical outcome of patients who completed ≥5 months of follow-up is summarized in *Figure 1*.

Untreated cohort

- **Natural course of aHC:** The first described group comprised 24 patients who were not treated due to heterogeneous reasons (patient refusal and contraindications mainly); thus allowing analyzing the natural course of aHC. Their median follow-up time was 19 months; 12 patients evolved to chronic infection, whereas the remaining 12 patients presented spontaneous resolution. This latter group was divided according to their time to viral clearance. In 9 patients, early resolution (≤6 months from aHC onset) was recorded. In three patients, late resolution (>6 months from aHC onset) was confirmed. All of these patients had at least one positive HCV RNA assay 6 months after disease onset, with latter spontaneous viral clearance. Noteworthy, one of these patients was co-infected with HIV without HAART at time of aHC and the remaining two patients were initially treated as autoimmune hepatitis with high-dose steroid therapy, resolving aHC after steroid suspension.
- **Factors associated with spontaneous resolution of aHC.** Demographic, clinical and virological characteristics were analyzed regarding its association with

spontaneous resolution or chronic evolution. The results of the bivariate are detailed in Table 5. Spontaneous resolution was associated with higher values of AST /ALT during aHC course and with the absence of intermittent HCV RNA viremia in univariate analysis; whereas in multivariate analysis only AST levels were significant, reaching an OR: 1.13 (CI 95% 1-1.27; p=0.04) for each time above the ULN.

Treated cohort

The second group included patients who received antiviral treatment.

- **Antiviral treatment:**Only one patient was treated with pegylated interferon (Peg-IFN) monotherapy, the remaining 20 received both Peg-IFN and ribavirin (information regarding fixed or weight-adjusted dose of ribavirin was not obtained). In all patients the median time of treatment was 24 weeks. Median follow-up time was 14 months. Ten patients received early treatment (≤4 months since aHC onset); 9 of them achieved SVR (the remaining case refers to a patient that achieved rapid virological response, complete early virological response and end of treatment virological response to date, with SVR results not available at the time this manuscript was written. The remaining 11 patients initiated antiviral treatment >4 months since aHC onset; in this cohort SVR was achieved in 63.6% of patients.
- **Factors associated with sustained virological response.** Demographic, clinical and virological characteristics were analyzed regarding its association with viral clearance in patients that received antiviral treatment and had SVR information; the results are detailed in Table 5. Viral clearance was associated with possible nosocomial source of infection and an earlier onset of antiviral treatment (multivariate analysis could not be performed due to the small sample size).

Discussion

The paucity of data regarding aHC incidence, affected population and associated risk factors difficult designing strategies to prevent its transmission, or even when detected, to optimize treatment results. The lack of epidemiological information is quite remarkable in South America, thus inspiring the development of this study. In the above described aHC cohort, the majority of patients (65.6%) were females and their median age was 46 years old. Regarding the possible transmission sources (when known), the most prevalent were nosocomial, accounting for 45.3% of the cases, followed by sexual-related in 18.8%. The remaining risk factors reported (occupational, blood product transfusion, drug-use and alternative medical procedures exposures) accounted each for less than 7%. In 11 cases the possible cause of infection could not be determined, since the patient could not identify any risky event in the prior 6 months, or acknowledged more than one. Although the median age of our patients is similar to those reported in other aHC series [12-14], 44% (28 patients) were 50 years or older at the time of infection. This rather high proportion of older patients could be related to the predominance of a nosocomial transmission source; since it has been previously suggested that this risk factor prevails in older subjects (>50 years of age)[12], whereas patients reporting sexual or drug use related exposure were much younger (see *table 3*).

Another discrepancy found when comparing with other published series refers to gender distribution, since males are usually the predominant affected population [15, 16]. However, when sex distribution was considered according to the suspected source of infection, females were only significantly more frequent in the nosocomial transmission group (our largest cohort); whereas intravenous drug users are the predominant group analyzed in the majority of previously published aHC series, and in this form of acquisition, young males prevail [12, 13, 15]. The small percentage of patients who declared drug use as a risk factor is concordant with previous reports, since intravenous drug addiction related to HCV acquisition has been traditionally considered infrequent in our region[17]. Furthermore, in a prospectively design aHC study conducted in Rio de

Janeiro, age and gender distribution were very similar to our study population; their main suspected source of transmission was also associated with medical procedures[18].

Both in developed and developing countries, nosocomial transmission of aHC has been extensively reported, especially since the incidence of HCV infection due to blood product transfusions and intravenous drug use have fallen[5]. In a recent study conducted in Germany, medical procedures were the main suspected source of infection, accounting for one-third of all patients[6]; similarly, in a Spanish retrospective analysis of 131 patients, 40% of them reported a non-transfusion related nosocomial source of infection[12]. In Egypt, a country that stands out for having the highest prevalence of HCV worldwide, hospital admission, admission in a surgical unit, sutures, therapeutic intravenous injections and infusions were all independently associated with an increase in HCV risk in non-drug users[19].

In our study, the most frequently reported risk factor were programmed surgical procedure/interventions, followed by upper and/or lower endoscopy; multiple hospital admissions or prolonged hospitalizations (see *Table 2*). These findings are perhaps the most important data obtained in our study, since they highlight the impact that poor adherence to standard infection control measures has on HCV transmission in our region. Furthermore, these reports were not constrained to a single center, but stated by most of them; thus stressing the need for optimizing rules and universal precautions involved in HCV transmission, especially when considering the majority of these reports were obtained in recent years (2010-2015 period), thus underlying the fact that nosocomial aHC is a current issue, and these are not just colorful statistics from the past. It should be stressed out that due to the retrospective design of our protocol and data acquisition from clinical records, it is impossible to determine beyond "reasonable doubt" the source of aHC infection; however, all patients were thoroughly interrogated discarding other risk factors, and a likely transmission route was only considered if it occurred in the prior six months from disease onset.

Though HCV transmission associated with heterosexual intercourse in sero-discordant partners is somewhat controversial (and has even been suggested to be insignificant) [20], several studies report a sexual contact with a known HCV carrier as the most likely source of transmission, especially when moderate-high risk sexual practices were declared (anal sex, sex in presence of other sexually transmitted diseases, sex without a condom, sexual worker)[16, 21, 22]. To note, the relationship between sexual transmission and specific sexual practices could not be analyzed in our study, due to its retrospective design.

Seroconversion is the current gold standard for aHC diagnosis, but due to the unlikely chance that patients without risk factors were tested previously for HCV, most scientific societies and experts endorse alternative diagnostic approaches, such as HCV RNA detection in a patient with an acute hepatitis syndrome without an alternative diagnosis[4, 8, 9]. We established aHC diagnosis using the preferred seroconversion criteria in less than half of the studied patients (42%), while in the remaining subjects, HCV RNA detection in the above described clinical scenario was the diagnostic criteria used. It seems important to underline that in nine cases, first anti-HCV testing was negative despite the presence of symptoms or altered liver test, and seroconversion was only detected in a subsequent analysis performed with a median of 59 days after disease onset. Regarding HCV RNA detection, in 8 patients, intermittent HCV RNA negativity was observed, whereas in 12 patients wide viremic fluctuations were noted. These serological and virological dynamics have been extensively described[5, 23-25], reflecting the importance of repeating determinations of both anti-HCV and HCV ARN, not only in a diagnostic algorithm (to avoid false negative testing) but also during follow-up, since a patient with a single negative HCV ARN may be categorized as recovered, when in fact he/she might have evolved to a chronic infection[7].

Regarding clinical presentation, although aHC is usually described as an asymptomatic infection[7], in the majority of aHC series patients are symptomatic - hence motivating medical consult and allowing their timely identification-. In this cohort, 80% of patients

were symptomatic at diagnosis, and 71% developed jaundice during the episode. All patients presented with aminotransferase elevations>5 times per ULN and almost 90% of them reached >10 times per ULN of both ASL and ALT, as described in other series of symptomatic aHCpatients [10]. Paradoxically, the presence of symptoms has been traditionally welcomed by physicians, since features such as jaundice and flu-like symptoms have been reported to be associated with spontaneous resolution[6, 15]. Código de campo cambiado Although the presence of symptoms at diagnosis did not reached statistical significance for viral clearance in this study cohort, it showed a trend towards this outcome (p=0.09). Probably, our small sample size influenced these results. Nevertheless, the presence of necro-inflammatory activity did correlate with viral clearance: AST levels were significantly associated (both in bivariate and multivariate analysis performed by logistic regression) with this favorable evolution. To note, the association of aminotransferase levels and spontaneous resolution had been observed previously in an HIV-infected cohort[25].

Despite the fact that a severe/fulminant evolution associated with aHC is considered rare (except when occurring as a superinfection in HBV carriers[4]), we have detected five cases with associated liver failure; three of them with severe aHC (two occurring in previously healthy immunocompetent patients) and two cases that required liver transplantation (one of them due to fulminant hepatitis and the other due to acute-on-chronic liver failure). Due to the small sample size, we could not identify factors associated with disease severity; however, it should be mentioned that this evolution is perhaps not as infrequent in our region and should be considered in the severe acute hepatitis diagnostic algorithm.

Deciding who and when to treat has always been subject of concern in patients with aHC[5, 7]. Spontaneous resolution was observed in 50% of our untreated patients, and in the majority of them, it occurred during the first four months since disease onset. Despite the fact that only three patients presented with spontaneous viral clearance six months after diagnosis (in all three cases associated with some degree of immune

system depression), this late resolution is worth mentioning, since it portrays the difficulty in assessing aHC evolution despite close monitoring[26]. Prevalent early spontaneous resolution is in accordance to major guidelines recommendations, which establish that most patients with detectable HCV RNA six months after diagnosis will develop a chronic infection[27].

Regarding evolution in the treated cohort, those patients who received antiviral treatment in the first four months had a SVR rate of 100%. For those patients treated after this period, SVR only accounted for 64%. In the bivariate analysis, both time of treatment onset and a nosocomial transmission source were significantly associated with SVR. There has been some controversy regarding the utility of early treatment initiation, since a large prospective study that treated patients after 12 weeks of aHC diagnosis did not had lower SVR rates that those reported previously with earlier treatment onset (4 weeks since diagnosis)[6, 28]. In a recent revision published by Hullegie et al, where the rationale for the timing of treatment initiation in aHC is discussed, the authors mention that some major scientific societies advocate for treatment in the chronic phase of the disease- in order to avoid Peg-IFN regimens and instead use direct acting antivirals- .However, in regions where insurance coverage may only pay for these drugs in those patients with an urgent medical need for HCV treatment (such as advanced fibrosis/cirrhosis), Peg-IFN may still be the most effective and only available option for aHC treatment in the short-term future[3].

In conclusion, this is the largest aHC cohort evaluated in our region. Most of the patients had a symptomatic presentation, and the most prevalent suspected transmission source was of nosocomial origin, thus stressing the importance of revising universal precautions to prevent HCV transmission. A severe evolution, though infrequent, was observed in five patients (two of them required liver transplantation). Spontaneous resolution was associated with necro-inflammatory activity, represented by AST levels, whereas SVR was associated with a nosocomial transmission and early

treatment onset. In a setting of scarce availability of direct acting antiviral therapy, early Peg-IFN based treatment may still have a role in aHC treatment.

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Con formato: Izquierda

Table 1. **Baseline demographic and clinical characteristics of patients with acute hepatitis C (n=64)**

Variables	**Total n=64**
Age at onset (years) -median and IQR.	46 (40-57)
Gender (female) - no. (%)	42 (65.6%)
HIV positive - no. (%)	8 (12.5%)
Symptomatic at diagnosis - no. (%)	51 (79.6%)
AST* (times per ULN) -median and IQR	23 (12.5-36.5)
ALT* (times per ULN) -median and IQR	29 (17-48)
Total bilirubin (mg/dL) -median and IQR	7 (1.1-11)
Severe acute hepatitis- no. (%)	3 (4.7%)
Fulminant hepatitis - no. (%)	1 (1.6%)
Acute-on-chronic liver failure- no. (%)	1 (1.6%)

* the highest registered value

Table 2.**Prevalence of reported risk factors for HCV transmission**

Likely source of HCV infection	**Number of patients (n=64)**
Nosocomial- no. (%)	**29 (45.3%)**
Scheduled surgical procedures/ interventions*- no.	8
Upper and/or lower endoscopy - no.	8
Multiple hospital admissions/ prolonged hospital stay - no.	6
Short hospital admission with parenteral drug administration - no.	2
Hospital admission due to respiratory infection and bronchoalveolar lavage - no.	4
Hemodialysis - no.	1
Blood product transfusion- no. (%)	**4 (6.2%)**
Alternative medical procedures- no. (%)	**2 (3.1%)**
Ozone therapy- no.	2
Occupational exposure- no. (%)	**3 (4.7%)**
Needles stick injury among health-care workers- no.	3
Sexual related exposure- no. (%)	**n=12 (18.8%)**
Known HCV sexual partner- no.	9
Unsafe sexual practices/sexual worker- no.	3

Drug use related exposure- no. (%)	**3 (4.7%)**
Intravenous/Inhalation drug users- no.	3
More than one possible source of infection- no. (%)	**3 (4.7%)**
Unknown exposure- no. (%)	**8 (12.5%)**

*surgical procedures such as renal biopsy, tracheal stent placing, cataract surgery, coronary angioplasty, myomectomy and other obstetric surgery.

*Table 3.***Age distribution according to likely source of HCV infection (n=53)**

Likely source of HCV infection	**Age (years)**	*p value*
Alternative medical procedures	59 (57-61)	
Nosocomial	53 (41-60)	
Occupational	44 (31-47)	
Blood product transfusion	41 (27-49)	***0.02 ****
Sexual	41 (38-46)	
Drug use	34 (31-36)	

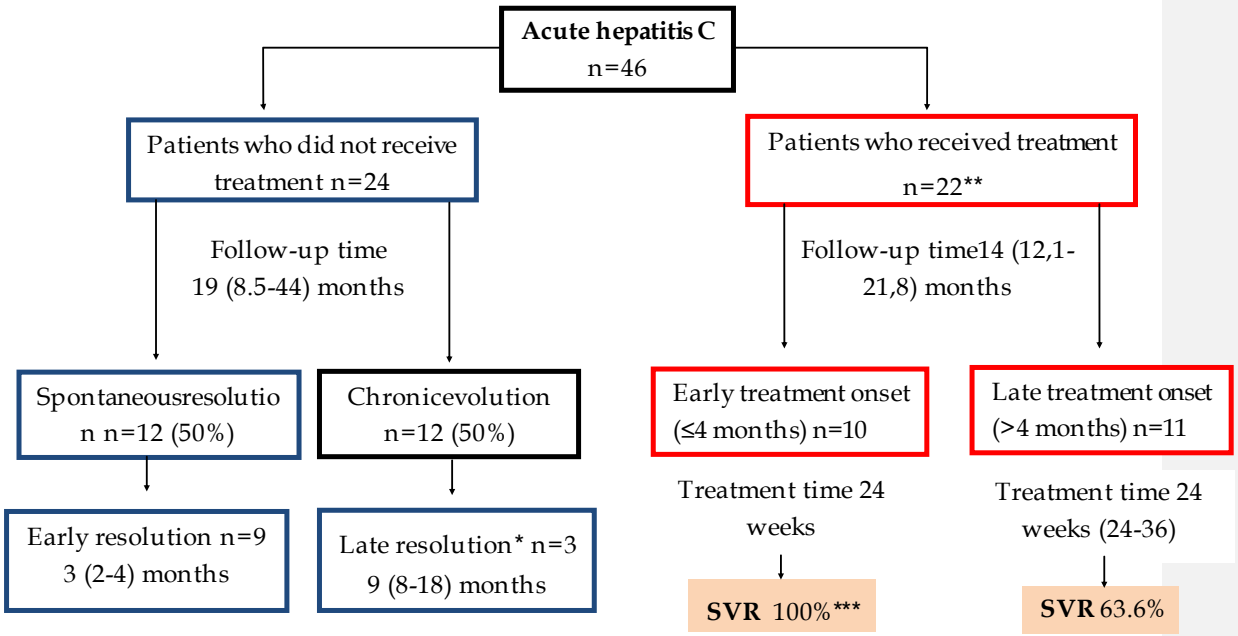
Note: All values are expressed in median and interquartile range. *Fisher test shows statistical difference between median ages when divided according to declared risk factors. 11 patients with unknown/more than one likely source of infection were excluded from this analysis.

*Table 4.***Sex distribution according to likely source of HCV infection (n=53)**

Likely source of HCV infection (n=53)	**Females (n=37)**	**Males (n=16)**	*p value*
Alternative medical procedures- no. (%)	2 (100%)	0	*0.54*
Nosocomial - no. (%)	19 (65.5%)	10 (34.4%)	***0.02***
Occupational - no. (%)	2 (66.7%)	1 (33.3%)	*0.71*
Blood product transfusion - no. (%)	3 (75%)	1 (25%)	*0.55*
Sexual - no. (%)	10 (83.3%)	2 (16.7%)	*0.18*
Drug use - no. (%)	1 (33.3%)	2 (66.7%)	*0.28*

Note: 11 patients with unknown/more than one likely source of infection were excluded from this analysis.

Figure 1. **Clinical outcome of aHC patients who completed ≥5 months of follow-up**



Note: All results are presented as median and interquartile range. * Refers to patients who had HCV RNA positive 6 months after disease onset and had latter spontaneous resolution. **22 patients received antiviral treatment for aHC; in one case, information regarding time of treatment onset is missing. ***One patient in the early treatment group has achieved rapid, early and virological response at the end of treatment; sustained virological response (SVR) results are pendant.

Table 5. **Factors associated with spontaneous resolution of aHC in patients without antiviral treatment (n=24)**

Variables	**Spontaneous resolution (n=12)**	**Chronic evolution (n=12)**	***p value***
Age at onset (years)-median and IQR.	45 (41-51)	41 (39-58)	*0.56*
Gender (female)- no. (%)	9 (75%)	9 (75%)	*1*
HIV status (positive)- no. (%)	3 (25%)	0	*0.21*
Symptomatic at diagnosis - no. (%)	12 (100%)	8 (66.6%)	*0.09*
Source of infection (nosocomial)- no. (%)	4 (33.3%)	5 (41.6%)	*1*
AST level (times per ULN)-median and IQR.	42.5 (27-68)	16 (8-23)	***0.002***
ALT level (times per ULN)-median and IQR.	41 (30-62)	20 (12.5-28)	***0.02***
Total bilirubin (mg/dL)-median and IQR.	8.8 (3.5-14)	6 (1.6-11)	*0.43*
Genotype (1,1a,1b)*- no. (%)	6 (50%)	9 (75%)	*0.40*
First available viral load (log^{10} UI/ml)**-median and IQR.	6.5 (3.4-7.3)	5.5 (3.9-6.2)	*0.54*
Intermittent HCV RNA viremia- no. (%)	0	5 (41.67%)	***0.03***

Note: * Genotype determination was available in 17 patients, 7 in the spontaneous resolution group and 10 in the chronic evolution group. ** Available in 16 patients, 6 in the spontaneous resolution group and 10 in the chronic evolution group.

Table 6. **Factors associated with viral clearance in aHC patients that received antiviral treatment and had SVR results at the time of enrollment**

Variables	**No SVR (n=5)**	**SVR (n=16)**	*p value*
Age at onset (years)-median and IQR.	48 (35-43)	43 (38-52)	*0.06*
Gender (female)- no. (%)	2 (40%)	9 (56.2%)	*0.63*
HIV status (positive)- no. (%)	1 (20%)	0	*0.23*
Symptomatic at diagnosis - no. (%)	5 (100%)	12 (75%)	*0.53*
Source of infection (nosocomial)- no. (%)	0	10 (62.5%)	***0.03***
AST level (times per ULN)-median and IQR.	22 (19-25)	22 (15-54)	*0.86*
ALT level (times per ULN)-median and IQR.	29 (27-32)	32 (18-62)	*0.71*
Total bilirubin (mg/dL)-median and IQR.	10.7 (7-11)	3.7 (1-8)	*0.11*
Genotype (1,1a,1b)*- no. (%)	4* (80%)	8* (57.1%)	*0.60*
First available viral load (log^{10} UI/ml)**-median and IQR.	5.85 (4.3-7.2)	4.7 (3.7-5.8)	*0.30*
Intermittent HCV RNA (-) assay- no. (%)	1 (20%)	2 (12.5%)	*1*
Treatment duration (weeks)-median and IQR.	24 (20-24)	24 (24-36)	*0.43*
Onset of antiviral treatment***-median and IQR.	6.5 (6-7.5)	4 (3-6)	***0.05***

Note: Values are presented in percentage, median and 1^{st}-3^{rd} interquartile range.*Genotype determination was available in 19 patients, 5 included in the non-sustained virological response group (SVR) and the remaining 14 in the SVR

group.**Data regarding viral load was available in 19 patients, 5 included in the non-SVR group and the remaining 14 in the SVR group. *** Data regarding time of treatment onset was available in 20 patients.