ISSN 1948-5182 (online)

World Journal of *Hepatology*

World Journal of Hepatology 2020 February 27; 12(2): 34-71

Published by Baishideng Publishing Group Inc

WJH World Journal of Hepatology

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Monthly Volume 12 Number 2 February 27, 2020

World Journal of Hepatology
Volume 12 Number 2 February 27, 2020

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The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Scopus, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Yan-Xia Xing*

Proofing Production Department Director: *Xiang Li*

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
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https://www.wjgnet.com/1948-5182/editorialboard.htm

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PUBLICATION DATE
February 27, 2020

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World Journal of Hepatology 2020 February 27; 12(2): 34-45

DOI: 10.4254/wjh.v12.i2.34

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Case Control Study

Clinical characteristics and treatment outcomes in patients with liver cirrhosis and lymphoma

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Author contributions: González-Regueiro JA and Ruiz-Margáin A contributed equally to this article; González-Regueiro JA, Demichelis-Gómez R, Ruiz-Margáin A and Macías-Rodríguez RU contributed to study conception and design; González-Regueiro JA, Cruz-Contreras M, Montaña-Duclaud AM and Cavazos-Gómez A contributed to acquisition of clinical data from medical records; Ruiz-Margáin A and Macías-Rodríguez RU performed the analysis of data and contributed with critical revisions for the content of the manuscript; González-Regueiro JA, Cruz-Contreras M, Montaña-Duclaud AM, Cavazos-Gómez A, Demichelis-Gómez R, Ruiz-Margáin A and Macías-Rodríguez RU contributed to interpretation of data and writing the final version of the article to be published.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of the Instituto Nacional de Ciencias Médicas y

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Abstract

BACKGROUND

A significant number of patients with liver cirrhosis concomitantly develop some type of solid or hematological cancer, including lymphoma. Treatment of patients with lymphoma and cirrhosis is challenging for physicians due to the clinical characteristics related to cirrhosis, including biochemical and functional abnormalities, as well as portal hypertension and lack of scientific evidence, limiting the use of chemotherapy. Currently, experts recommend only offering oncological treatment to patients with compensated cirrhosis.

AIM

To evaluate the clinical characteristics and treatment outcomes in patients with cirrhosis and lymphoma treated with chemotherapy.

METHODS

This was a case-control study conducted at a tertiary care center in Mexico. Data was recorded from medical files and from 8658 possible candidates with cirrhosis and/or lymphoma (2000 to 2018). Only 23 cases had both diseases concomitantly; 10 patients with cirrhosis and lymphoma (cases) met the selection criteria and were included, and 20 patients with lymphoma (controls) were included and matched according to age, sex, and date of diagnosis, type and clinical stage of lymphoma. All patients received treatment with chemotherapy. For statistical analysis, descriptive statistics, Shapiro-Wilk test, Mann-Whitney *U* test, chi-square test and Fisher's exact test were used. Survival was evaluated using

Nutrición Salvador Zubirán (INCMNSZ) (Ref. No. "2914").

Informed consent statement: Given the retrospective design of this study, patients were not required to provide informed consent as the data was obtained from medical records after the completion of treatment.

Conflict-of-interest statement: All authors declare no conflicts of interest related to this article.

Data sharing statement: No additional data are available.

STROBE statement: The guidelines of the STROBE statement have been adopted.

Manuscript source: Unsolicited manuscript

Received: September 10, 2019
Peer-review started: September 10, 2019
First decision: September 26, 2019
Revised: November 21, 2019
Accepted: December 19, 2019
Article in press: December 19, 2019
Published online: February 27, 2020

P-Reviewer: Dourakis SP, Takahashi T, Tarantino G, Zapater P
S-Editor: Ma RY
L-Editor: A
E-Editor: Xing YX

Kaplan-Meier curves and Log-rank test.

RESULTS

There were differences in biochemical variables inherent to liver disease and portal hypertension in patients with cirrhosis. The most frequent etiology of cirrhosis was hepatitis C virus (50%); 80% were decompensated, the median Child-Turcotte-Pugh score was 7.5 (6.75-9.25), and mean Model for End-stage Liver Disease was 11.5 ± 4.50. Regarding lymphomas, non-Hodgkin's were the most common (90%), and diffuse large B cell subtype was the most frequent, with a higher International Prognostic Index in the cases (3 *vs* 2, P = 0.049). The chemotherapy regimens had to be adjusted more frequently in the case group (50% *vs* 5%, P = 0.009). The complications derived from chemotherapy were similar between both groups (80% *vs* 90%, P = 0.407); however, non-hematological toxicities were more common in the case group (30% *vs* 0%, P = 0.030). There was no difference in the response to treatment between groups. Survival was higher in the control group (56 wk *vs* 30 wk, P = 0.269), although it was not statistically significant.

CONCLUSION

It may be possible to administer chemotherapy in selected cirrhotic patients, regardless of their severity, obtaining satisfactory clinical outcomes. Prospective clinical trials are needed to generate stronger recommendations.

Key words: Cirrhosis; Cancer; Lymphoma; Chemotherapy; Treatment; Survival; Toxicity; Adverse events

Core tip: Treatment in patients with liver cirrhosis and lymphoma represents a challenge for physicians given the lack of scientific evidence. Experts recommend offering oncological treatment only to patients with compensated cirrhosis. In this study, we included mainly decompensated patients with cirrhosis and lymphoma, and when compared to patients with lymphoma, we observed that clinical characteristics, response rate and complications derived from chemotherapy were similar in both groups. Chemotherapy was adjusted more in patients with liver dysfunction; however, this did not alter the response to treatment or prognosis. We propose that lymphoma treatment can be provided in patients with cirrhosis at any clinical state.

Citation: González-Regueiro JA, Ruiz-Margáin A, Cruz-Contreras M, Montaña-Duclaud AM, Cavazos-Gómez A, Demichelis-Gómez R, Macías-Rodríguez RU. Clinical characteristics and treatment outcomes in patients with liver cirrhosis and lymphoma. *World Journal of Hepatology* 2020; 12(2): 34-45
URL: https://www.wjgnet.com/1948-5182/full/v12/i2/34.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i2.34

INTRODUCTION

Liver cirrhosis and cancer are two major public health issues due to the high incidence and prevalence of each of these diseases. The fact that these diseases are relatively frequent in the general population increases the likelihood of having them simultaneously[1,2]. It is well known that liver cirrhosis is the main risk factor for developing hepatocellular carcinoma (HCC); however, it also increases the risk of having other extrahepatic malignancies[3,4]. Considering that both entities have common risk factors, such as alcohol consumption, smoking, obesity and metabolic syndrome, they may appear concomitantly[5-10].

Since HCC is the most common type of cancer in cirrhotic patients, most of the literature is centered on its management. Therefore, the management of other types of cancer, especially those less frequent in this population, may present a bigger challenge given that treatment-related hepatotoxicity can significantly affect the prescription and response, as well as the incidence of adverse events.

Lymphomas are malignant neoplasms of B, T and natural killer cells that represent approximately 4.7% of all malignant tumors[11]. There are two main types, Hodgkin lymphomas and non-Hodgkin lymphomas, which represent 10% and 90% of all of these, respectively. Lymphomas can be divided into indolent or aggressive according to their immunophenotype. With treatment, the five-year survival rates for non-Hodgkin and Hodgkin lymphomas are 72% and 86.6%, respectively[11]. Currently, with advances in chemotherapy, it is estimated that between 50% and 80% of all cases are likely to be cured when optimal therapy is provided[12,13]. However, treatment of lymphoma in patients with cirrhosis is a difficult task, and even the involvement of a multidisciplinary team may still not be enough, considering the lack of published literature about clinical outcomes, the narrow therapeutic index of the drugs and the complicated safety problems that are typical in these patients.

A review article by the European Society of Medical Oncology published in 2016 establishes expert recommendations on the treatment of this type of patient, indicating that it is always important to consider the severity of cirrhosis to establish the best therapeutic strategy[14]. However, there are no available studies evaluating survival and the different chemotherapy regimens used in patients who present concomitantly with lymphoma and cirrhosis.

The appropriate strategy for chemotherapy treatment in patients with lymphoma and liver cirrhosis has not yet been defined. Since most clinical trials for lymphoma exclude patients with cirrhosis and vice versa, the available knowledge about clinical outcomes and the use of chemotherapeutic agents in this context is based only on recommendations from pharmaceuticals and experts, case reports, case series and clinical trials investigating the usefulness of different drugs in the context of hepatocellular carcinoma.

Due to the lack of guidelines for the management of these patients, there are only small case series and case reports where the authors describe the therapy used in patients with lymphoma and cirrhosis, treated effectively and safely[15-17]. Therefore, the aim of this study was to evaluate clinical characteristics and treatment outcomes (survival, the type of chemotherapeutic regimen used, and the response rate and complications derived from it) in patients with liver cirrhosis and lymphoma compared with patients with lymphoma.

MATERIALS AND METHODS

This was a case-control study conducted at a tertiary care center in Mexico City (Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, INCMNSZ). The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by our Institutional Ethics Committee with Ref. No. 2914. All patients over 18 years of age with a diagnosis of liver cirrhosis and/or lymphoma from January 1, 2000, to December 31, 2018, were considered for inclusion. No written informed consent was obtained for the retrospective nature of this study.

Patients with liver cirrhosis and lymphoma (case group)

The cases included patients with an established diagnosis of liver cirrhosis and lymphoma. The diagnosis of cirrhosis was established by clinical tests (non-invasive markers, biochemical markers and evidence of portal hypertension in ultrasonography or the presence of esophageal varices in endoscopy) or liver biopsy. The severity of the liver disease was assessed according to Child-Turcotte-Pugh (CTP), Model for End-stage Liver Disease (MELD), and the MELD-Na (MELD sodium) scores. The criterion for decompensated cirrhosis was defined by a CTP class B or C or by the presence of an overt clinical decompensation (jaundice, variceal hemorrhage, ascites, hepatic encephalopathy). The diagnosis of lymphoma was established by histopathology. Patients who had a diagnosis of lymphoma prior to the diagnosis of liver cirrhosis, those who refused treatment with chemotherapy, those who died prior to administration of the chemotherapy regimen, and those with incomplete data in the medical records were excluded.

Patients with lymphoma (control group)

The control group included patients with lymphoma diagnosed by histopathology, with no evidence of liver disease. This group was electronically randomized for selection. Patients who were not treated with chemotherapy and those with incomplete data in the medical records were excluded. The ratio of controls and cases was 2:1. Controls were adjusted with cases according to age (older or younger than 60 years), sex, date of diagnosis of lymphoma, type of lymphoma, and early (I and II) or advanced (III and IV) clinical stage.

Medical care

The patients in both groups received the standard medical care from the tertiary care center. The patients in the case group were evaluated by the Departments of Internal Medicine, Gastroenterology and Hematology, and the patients in the control group were evaluated by the Departments of Internal Medicine and Hematology. The chemotherapeutic treatment was proposed by the interdisciplinary group, and the final decision was made by the Department of Hematology.

Statistical analysis

The distribution of continuous variables was evaluated using the Shapiro-Wilk test. For the baseline clinical characteristics, descriptive statistics were used, for quantitative variables, mean ± standard deviation or median and (p25-p75) were used according to the distribution, while for qualitative variables, absolute frequencies were used.

Student's *t* test was used whenever the data had a normal distribution, and Mann-Whitney *U* test was used when the data distribution was nonparametric. Chi-square or Fisher's exact tests were used for categorical data, and Kaplan-Meier curves and log-rank tests were used to evaluate survival. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS software package version 25 (International Business Machines, Armonk, NY, United States).

RESULTS

We reviewed 8658 medical records of patients with diagnosis of lymphoma and/or cirrhosis and found 23 patients with a concomitant diagnosis of liver cirrhosis and lymphoma, of whom only 10 patients underwent chemotherapy. These were paired with 20 patients with lymphoma without liver disease to perform the analysis. The flowchart for patient inclusion is shown in Figure 1.

Baseline characteristics of the study population

The demographic, clinical and biochemical characteristics of the study population are shown in Table 1. Differences were found, as expected, only in biochemical parameters with changes inherent to cirrhosis compared to the non-cirrhotic population.

Characteristics of the population with cirrhosis

The etiology and severity of liver disease in the case group are shown in Table 2. The etiologies of liver cirrhosis were hepatitis C virus (HCV) in 5 (50%) patients, non-alcoholic fatty liver disease (NAFLD) in 2 (20%) patients, alcohol in 1 (10%) patient, autoimmune hepatitis in 1 (10%) patient and cryptogenic in 1 (10%) patient. In relation to the five patients with viral cirrhosis (HCV), four of them were treatment naïve at the time of lymphoma diagnosis, and one of them had been treated previously and achieved sustained virologic response before the onset of lymphoma. Only one treatment naïve patient could receive antiviral treatment after chemotherapy. Regarding liver disease, eight (80%) patients were in a decompensated state of cirrhosis. The events of decompensation in the population included ascites in 6 (60%) patients, variceal bleeding in 5 (50%) patients, jaundice in 1 (10%) patient, hepatic encephalopathy in 1 (10%) patient and spontaneous bacterial peritonitis in 1 (10%) patient. The median score according to the CTP scale was 7.5 points, and the distribution for classes A/B/C of this scoring system was n = 2, 6, 2, respectively. The mean score according to the MELD prognostic index was 11.5 ± 4.50 points, and MELD-Na was 16.5 ± 5.12 points. The median time between the diagnosis of cirrhosis and lymphoma was 2.16 years, with a range between the same diagnostic time and 8.6 years after the diagnosis of cirrhosis.

Characteristics of lymphoma in the population

The characteristics of patients with lymphoma in both groups are shown in Table 3. Non-Hodgkin's lymphomas were the most common type (90%, n = 27), and within these, the predominant histologic subtype was diffuse large B cell (80%) in 24 patients (7 cases and 17 controls). In the case group, there was only one patient with Hodgkin's lymphoma with histological variety of lymphocytic depletion. In relation to the clinical stage, there were no significant differences between the two groups. The severity, according to the different prognostic scales validated for the different types of lymphoma, was greater in patients with cirrhosis [International Prognostic Index (IPI): 3 *vs* 2, P = 0.049]. The quality of life according to the Eastern Cooperative Oncology Group scale of performance status and the presence of B symptoms was similar between both groups. Finally, we observed that from the established risk

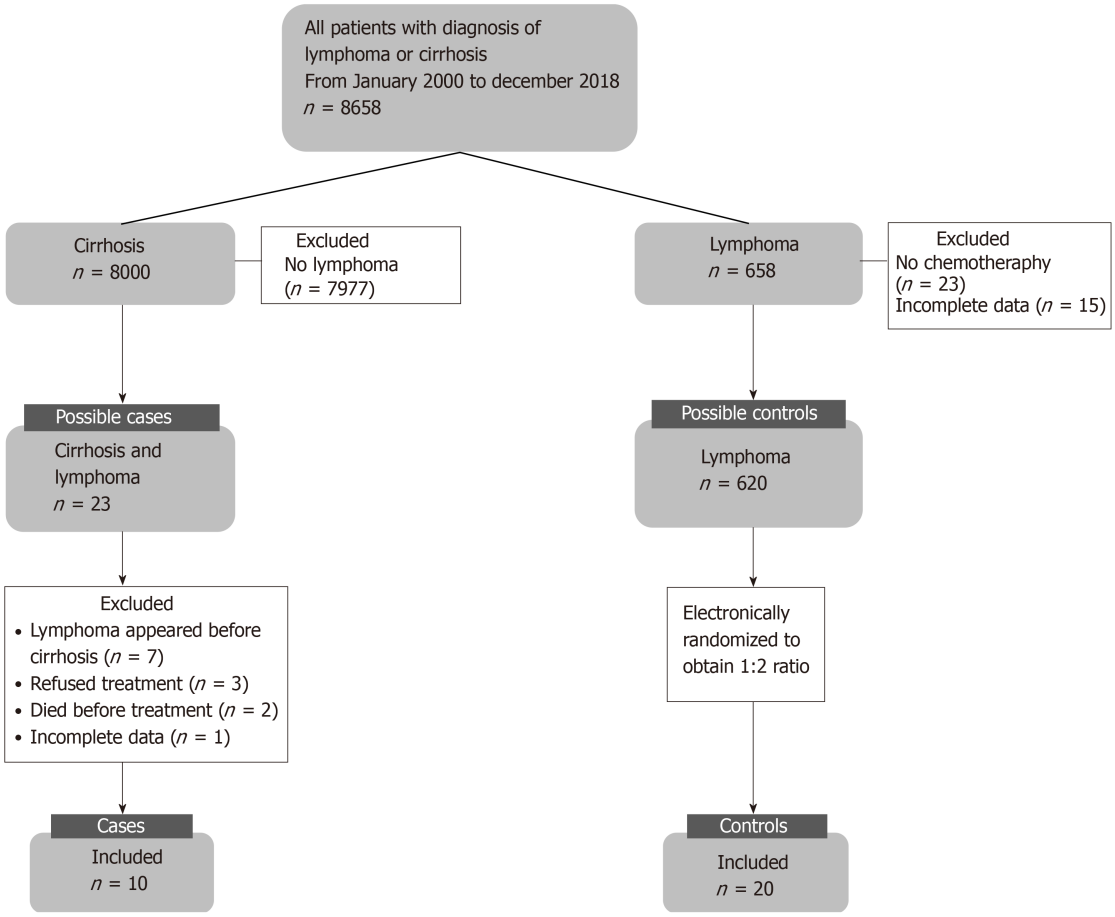

Figure 1 Flowchart for patient inclusion in the study. Flowchart showing the inclusion criteria of patients in the control group (n = 20) and the case group (n = 10). We reviewed 8000 medical records of patients with cirrhosis, 23 of whom had a concomitant diagnosis of lymphoma and only 10 met the inclusion criteria for the study. Additionally, we reviewed 658 medical records of patients with lymphoma, of which 620 were possible control candidates, and only 20 were electronically randomized to maintain a 1:2 ratio of cases and controls.

factors for the development of lymphoma, the case group had a higher prevalence of *Helicobacter pylori* infection (3 *vs* 0, P = 0.030).

Treatment and its outcomes

The type of chemotherapy and its outcomes in the study population are described in Table 4. The drugs used in the most relevant chemotherapy regimens were very similar between both groups. Chemotherapy was adjusted more frequently in patients with cirrhosis and lymphoma than in patients with lymphoma alone (50% and 5%, respectively, P = 0.009). In relation to complications derived from chemotherapy, there were similarities between both groups (80% *vs* 90%, P = 0.407); however, non-hematological toxicities were more frequent in the case group (30% *vs* 0%, P = 0.030). Regarding non-hematological toxicities, in the case group, 1 patient (10%) presented acute kidney injury AKIN II, 1 patient (10%) presented atypical pneumonia, and 1 patient (10%) had acute non-infectious diarrhea grade II. In the control group, 18 (90%) patients had hematological toxicity, and there was no other type of toxicity.

Among the complications derived from chemotherapy, we found that most were hematological toxicities, mainly anemia. On the other hand, it was remarkable that patients with lymphoma and cirrhosis had a trend to develop less hematological toxicity than the controls (cases 60% *vs* controls 90%, P = 0.141), which may be related to the adjustment in chemotherapy. Within hepatic toxicities, only one decompensating event (hepatic encephalopathy) occurred in one patient, which had implications in the continuation of chemotherapeutic treatment but did not directly correlate with the cause of death. Other important complications are infections, which were higher in patients with cirrhosis and lymphoma, probably because of the susceptibility of patients due to depletion of immunity, in addition to the state of decompensation of the patients.

Treatment response was evaluated according to international recommendations

Table 1 Baseline characteristics of the study population, *n* (%)

General characteristics	Cases (*n* = 10)	Controls (*n* = 20)
Female sex	6 (60)	12 (60)
Age (yr)	56 ± 14.2	57 ± 12.9
BMI (kg/m^2)	25.57 ± 3.44	27.30 ± 7.57
Lymphoma type		
Hodgkin lymphoma	1 (10)	2 (10)
Non-Hodgkin lymphoma	9 (90)	18 (90)
Laboratory data		
Hemoglobin (g/dL)	12.13 ± 2.39	11.97 ± 3.18
Platelets (K/μL)	166.60 ± 143.8[a]	293.9 ± 157.6
Leukocytes (K/μL)	5.4 ± 1.79	7.47 ± 3.79
INR	1.24 ± 0.19[b]	1.0 ± 1.0
Total bilirubin (mg/dL)	1.74 ± 1.31[a]	0.97 ± 0.75
Direct bilirubin (mg/dL)	0.76 ± 0.82[a]	0.27 ± 0.34
ALT (U/L)	31.82 ± 33.30	25.6 ± 13.35
AST (U/L)	48.50 ± 27.31[a]	32.1 ± 16.52
Alkaline phosphatase (U/L)	210.40 ± 261.2	136.40 ± 149.1
Albumin (g/dL)	2.78 ± 0.69	3.3 ± 0.82
Sodium (mmol/L)	132 (130-138)	137 (132-140)
Creatinine (mg/dL)	0.82 ± 0.21	1.63 ± 2.25
β-2 Microglobulin (g/dL)	3.17 ± 1.39	3.02 ± 3.08
LDH (U/L)	198.29 ± 119.81	356.5 ± 304.86
Comorbidities		
Obesity	2 (20)	6 (30)
Dyslipidemia	0 (0)	4 (20)
Diabetes mellitus	3 (30)	3 (15)
Arterial hypertension	2 (20)	8 (40)
Alcohol consumption	3 (30)	5 (25)
Tobacco consumption	5 (50)	7 (35)
Rheumatoid arthritis	0 (0)	2 (10)

mean ± SD, median (p25-p75), absolute frequencies (%);
[a]$P < 0.05$ *vs* controls,
[b]$P < 0.01$ *vs* controls. BMI: Body mass index; INR: International normalized ratio; ALT: Alanine transaminase; AST: Aspartate transaminase; β-2 Microglobulin: Beta-2 microglobulin; LDH: Lactic dehydrogenase.

and divided into complete response (4 cases *vs* 10 controls, $P = 0.488$), partial response (4 cases *vs* 9 controls, $P = 1.0$), stable disease (2 cases *vs* 1 controls, $P = 0.251$), disease progression (5 cases *vs* 5 controls, $P = 0.231$) and relapse (2 cases *vs* 3 controls, $P = 1.0$). The number of deaths in patients with cirrhosis was 7 (70%), and in patients without liver disease, there were 8 deaths (40%), although it was not significant ($P = 0.209$).

The survival of patients in both groups can be seen in Figure 2 in the Kaplan-Meier curve. Table 5 shows that the mean survival of patients with lymphoma without liver disease was 56 wk, while in patients with lymphoma and cirrhosis, it was 30 weeks on average, although no statistically significant difference ($P = 0.269$) was observed, most likely due to the sample size.

DISCUSSION

Liver cirrhosis is a frequent disease worldwide and is the 14th cause of mortality globally[18]. In addition, these patients have risk factors related both to the development of cirrhosis (such as HCV infection) and disease *per se* (alterations in immune surveillance), which confers an increased risk of developing neoplastic diseases, including lymphomas[19-21]. Due to the progressive increase in hepatic diseases such as NAFLD, it is expected that in the next years, there will be more cases of cirrhosis, and concomitantly, these patients may develop extrahepatic tumors such as lymphoma, making it necessary to know how to approach these patients with both

Table 2 Characteristics of the population with liver cirrhosis, *n* (%)

Characteristics of patients with cirrhosis	Cases (*n* = 10)
Etiologies of cirrhosis	
HCV	5 (50)
NAFLD	2 (20)
Alcohol	1 (10)
Autoimmune hepatitis	1 (10)
Cryptogenic	1 (10)
States of cirrhosis	
Compensated	2 (20)
Decompensated	8 (80)
Events of decompensation	
Jaundice	1 (10)
Variceal bleeding	5 (50)
Ascites	6 (60)
Spontaneous bacterial peritonitis	1 (10)
Encephalopathy	1 (10)
CTP score	7.5 (6.75-9.25)
CTP class A	2 (20)
CTP class B	6 (60)
CTP class C	2 (20)
MELD	11.5 ± 4.50
MELD-Na	16.5 ± 5.12

mean ± SD, median (p25-p75), absolute frequencies (%). HCV: Hepatitis C virus; NAFLD: Non-alcoholic fatty liver disease; CTP: Child-Turcotte-Pugh; MELD: Model for End-stage Liver Disease; MELD-Na: Model for End-stage Liver Disease sodium.

conditions.

Chronic liver disease (especially cirrhosis) poses multiple challenges when deciding the type of chemotherapy due to clinical characteristics of these patients, including hypersplenism, with a lower margin of safety for hematological complications such as thrombocytopenia, anemia, and leukopenia; the elevation in bilirubin levels is itself one of the few parameters recommended for chemotherapy adjustment, but which may be biased in interpretation in patients with cholestatic diseases such as primary biliary cholangitis; and finally, the patient's general condition, in which cachexia, deconditioning, low protein/albumin levels and decreased liver function are observed.

Currently, the recommendations issued by different experts are based solely on the evaluation of liver function as the main determinant of its prognosis. Patients with compensated cirrhosis, whose prognosis they consider to be defined primarily by cancer, are considered for chemotherapy. On the other hand, decompensated patients, whose prognosis is estimated to be determined by liver disease, are not considered for treatment because experts believe it may further worsen liver function and only provide supportive care. While these factors should be taken into account, there is no strong recommendation that more objectively delimits the optimal parameters for offering chemotherapy treatment, which gives the patient a greater survival rate without neglecting their safety.

In the present study, expected differences between cases and controls were observed in biochemical parameters, including liver function tests abnormalities, high INR and low platelet levels. These differences are secondary to liver disease itself and portal hypertension leading to hypersplenism.

It is noteworthy that most (80%) of the patients with cirrhosis in the study were in a decompensated phase of the disease. This is of utmost importance, since according to the current expert recommendations, these patients would be excluded from oncological treatment. However, despite decompensation, treatment with chemotherapy is feasible in this population without affecting safety or presenting greater toxicity or adverse events.

Regarding the general characteristics of lymphomas, the majority (66%) of patients in the study were diagnosed at advanced stages (clinical stage III and IV) of the

Table 3 Characteristics of the lymphoma cases, *n* (%)

Characteristics of lymphomas	Cases (*n* = 10)	Controls (*n* = 20)
Lymphoma type and subtypes		
Non-Hodgkin lymphoma	9 (90)	18 (90)
Diffuse large B cell	7 (70)	17 (85)
Follicular	1 (10)	0 (0)
Others	1 (10)	1 (5)
Hodgkin lymphoma	1 (10)	2 (10)
Nodular sclerosis	0 (0)	1 (5)
Lymphocyte predominant	0 (0)	1 (5)
Lymphocyte depleted	1 (10)	0 (0)
Clinical stages		
I	1 (10)	4 (20)
II	2 (20)	3 (15)
III	1 (10)	6 (30)
IV	6 (60)	7 (35)
Extra nodal involvement	6 (60)	9 (45)
Prognostic scale		
IPI score	3 (2.5-3)[a]	2 (1-3)
FLIPI score	3	-
IPS score	3	2.5
ECOG Performance Status	2 (1-2.25)	2 (1-2)
B symptoms		
Fever	3 (30)	6 (30)
Weight loss	9 (90)	11 (55)
Nocturnal diaphoresis	4 (40)	6 (30)
Helicobacter pylori	3 (30)[a]	0 (0)

Median (p25-p75), absolute frequencies (%);
[a]$P < 0.05$ *vs* controls; [b]$P < 0.01$ *vs* controls. IPI: International Prognostic Index; FLIPI: Follicular Lymphoma International Prognostic Index; IPS: International Prognostic Score; ECOG: Eastern Cooperative Oncology Group scale of Performance Status.

disease, most patients had B symptoms, with weight loss being the most common (66%) and one of the main reasons for seeking medical care. In the case of diffuse large B cell non-Hodgkin's lymphomas, the IPI was significantly higher in cases than in controls (cases 3 *vs* 2 controls, $P = 0.049$), maybe due to increased extra nodal involvement.

Finally, it should be noted that in relation to the risk factors associated with the development of lymphoma, the group of patients with liver disease had a higher incidence of a history of *H. pylori* infection, which has been reported in previous studies. These studies have shown an increased risk of *H. pylori* infection in patients with liver cirrhosis, especially in those with viral etiology (HCV and hepatitis B virus), most likely due to impaired immune function[22,23].

In relation to survival, as seen in the Kaplan-Meier curves, patients with cirrhosis had a lower survival rate (almost half compared to those without liver disease), although it failed to reach statistical significance, probably due to the sample size; however, despite the extensive search of nearly 9000 patients (including those with cirrhosis and those with lymphoma), only the cases described in this study were found.

Even though the presence of portal hypertension-related complications contributes to the increased mortality in patients with cirrhosis and lymphoma, this should not preclude the evaluation and treatment of hematological disease with curative intentions. Regarding this, in the group of cases, we found a CTP class C patient who received non-adjusted chemotherapy and had an overall survival of 82 week to finally receive a liver transplant. This is important because it shows that even in decompensated patients, curative treatment is feasible and not confining the patient to palliative treatment only.

It is important to note that chemotherapy is usually adjusted with the value of bilirubin; therefore, cirrhotic patients, having high bilirubin levels from the liver

Table 4 Type of chemotherapy and treatment outcomes, *n* (%)

Chemotherapy	Cases (*n* = 10)	Controls (*n* = 20)
Drugs		
Rituximab	5 (50)	9 (45)
Cyclophosphamide	8 (80)	17 (85)
Doxorubicin	5 (50)	15 (75)
Vincristine	8 (80)	17 (85)
Prednisone	6 (60)	17 (85)
Bleomycin	2 (20)	3 (15)
Dacarbazine	1 (10)	2 (10)
Etoposide	4 (40)	3 (15)
Vinblastine	0 (0)	2 (10)
Methotrexate	1 (10)	4 (20)
Ifosfamide	2 (20)	2 (10)
Carboplatin	2 (20)	2 (10)
Dexamethasone	2 (20)	1 (5)
Others	2 (20)	4 (20)
Chemotherapy adjustment	5 (50)[b]	1 (5)
Radiotherapy	1 (10)	2 (10)
Treatment response		
Complete response	4 (40)	10 (50)
Partial response	4 (40)	9 (45)
Stable disease	2 (20)	1 (5)
Disease progression	5 (50)	5 (25)
Relapse	2 (20)	3 (15)
Complications	8 (80)	18 (90)
Hematologic toxicity	6 (60)	18 (90)
Gastrointestinal toxicity	1 (10)	0 (0)
Renal toxicity	1 (10)	0 (0)
Infectious toxicity	1 (10)	0 (0)
Decompensating events	1 (10)	-
Death	7 (70)	8 (40)

Absolute frequencies (%). [a]$P < 0.05$ *vs* controls;
[b]$P < 0.01$ *vs* controls.

disease itself, an adjustment is often made that may even contraindicate certain drugs and are not given a complete regimen that they could tolerate.

Although there are clinical characteristics that make patients with cirrhosis not the best candidates to receive chemotherapy, in this study it was observed that this treatment is feasible even in decompensated patients, observing greater renal, gastrointestinal and infectious toxicity. None of these complications caused death, which will help to more closely monitor this type of complications in patients with cirrhosis and lymphoma treated with chemotherapy, as well as to develop more studies to establish the safety margin of these drugs and to adequately define the adjustment and effectiveness of chemotherapy.

It is important to emphasize that currently, there are no international guidelines on the management of these patients, and in the field of clinical research, there is very little work to develop scientific evidence in this regard. Although several positions have been proposed by different experts in the field, they do not have satisfactory clinical validation. The absence of randomized clinical trials with this patient group means that the choice of treatment is not evidence-based. Therefore, it is important that in the future, clinical trials on chemotherapy treatments that include patients with cirrhosis are designed to generate recommendations based on the evidence and to know their outcomes.

This is the first study evaluating the clinical characteristics, the type of chemotherapy, the response and complications arising from the treatment, and survival in patients with cirrhosis and lymphoma.

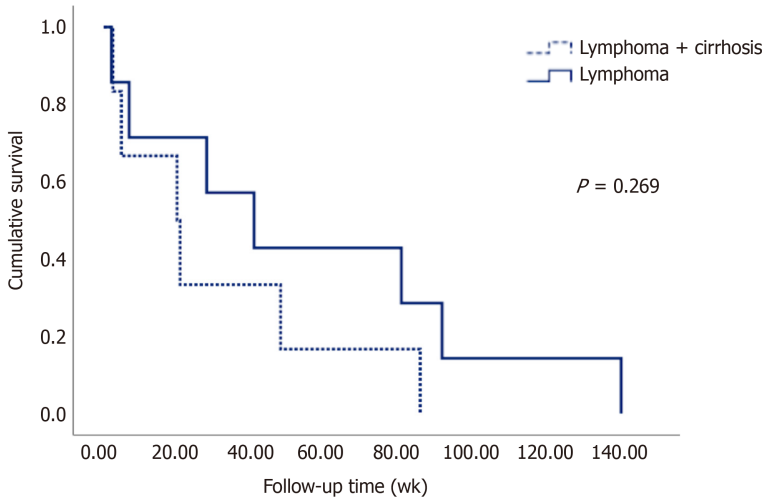

Figure 2 Kaplan-Meier survival curve. Kaplan-Meier curve showing survival in both groups during 140 wk of follow-up.

The strengths of the study include an adequate methodology and the fact that all patients had a thorough evaluation of the characteristics of cirrhosis and its complications, sometimes in other studies overlooked when evaluated only by one type of specialist. In this study, the type and adjustment of chemotherapy was decided by a multidisciplinary team (Internal Medicine, Hematology, Gastroenterology and Hepatology) according to the available guidelines in a non-cirrhotic population.

This study has some limitations; first, being a retrospective study, it has an inherent risk of information and selection bias. Some of the bias that may be present in this study may be due to the fact that we analyzed patients from a large time period, which could cause differences in the available treatments. Additionally, we excluded patients who refused chemotherapy or died before receiving it, which could be considered as bias; however, since we wanted to evaluate the response to chemotherapy, it was considered necessary to exclude them for the purpose of the study. On the other hand, the number of cases (lymphoma and cirrhosis) is relatively small, but despite that, the present study has the largest number of patients with both diseases than any other, since previously conducted studies included only case reports or case series with a maximum of two patients. Furthermore, the fact that all patients from our center with concomitant diseases in an 18-year period were included is to be noted, as the small sample size is due to the low frequency of the cases, which clearly supports the case-control methodology.

In conclusion, this study demonstrates that the clinical characteristics of patients with lymphoma and cirrhosis are similar to those with lymphoma, except for some changes inherent to cirrhosis and portal hypertension. Additionally, it may be possible to administer chemotherapy regimens in selected cirrhotic patients, regardless of their severity or even in decompensated patients, obtaining acceptable treatment outcomes (response to treatment and complications), always closely monitoring their toxicity.

Table 5 Numerical data on the survival of the study population

Population	Mean
Case group (wk)	30.66 ± 30.05
Control group (wk)	56.46 ± 51.15
Global (wk)	44.56 ± 43.76

mean ± SD.

ARTICLE HIGHLIGHTS

Research background

A significant number of patients with liver cirrhosis concomitantly develop some type of solid or hematological cancer, including lymphoma. Treatment of patients with lymphoma and cirrhosis is challenging for physicians due to the clinical characteristics related to cirrhosis and lack of scientific evidence, limiting the use of chemotherapy. Currently, experts recommend only offering oncological treatment to patients with compensated cirrhosis and the best supportive care to those in a decompensated state.

Research motivation

The treatment of lymphomas in patients with cirrhosis is a difficult task, and even the involvement of a multidisciplinary team may still not be enough, considering the lack of published literature about clinical outcomes, the narrow therapeutic index of the drugs and the safety issues that are typical in these patients. A study that evaluates treatment with chemotherapy in patients with cirrhosis and lymphoma is necessary to address this knowledge gap.

Research objectives

To evaluate the clinical characteristics and treatment outcomes (type of chemotherapy regimen, response rate and complications derived from it, and survival) in patients with cirrhosis and lymphoma treated with chemotherapy to generate scientific evidence in this regard.

Research methods

This was a case-control study conducted at a tertiary care center in Mexico. Data was recorded from medical files from 2000 through 2018, and from 8658 possible candidates with cirrhosis and/or lymphoma, only 23 cases had both diseases concomitantly; 10 patients with cirrhosis and lymphoma (cases) met the selection criteria and were included, and 20 patients with lymphoma (controls) were included and matched according to age, sex, and date of diagnosis, type and clinical stage of lymphoma. All patients received treatment with chemotherapy. For statistical analysis, descriptive statistics, Shapiro-Wilk test, Mann-Whitney *U* test, chi-square test and Fisher's exact test were used. Survival was evaluated using Kaplan-Meier curves and the log-rank test.

Research results

There were differences in biochemical variables inherent to liver disease and portal hypertension in patients with cirrhosis. The most frequent etiology of cirrhosis was hepatitis C virus (50%); 80% were decompensated, the median Child-Turcotte-Pugh score was 7.5 (6.75-9.25), and mean Model for End-stage Liver Disease was 11.5 ± 4.50. Regarding lymphomas, non-Hodgkin's were the most common (90%), and diffuse large B cell subtype was the most frequent, with a higher International Prognostic Index in the cases (3 *vs* 2, $P = 0.049$). The chemotherapy regimens had to be adjusted more frequently in the case group (50% *vs* 5%, $P = 0.009$). The complications derived from chemotherapy were similar between both groups (80% *vs* 90%, $P = 0.407$); however, non-hematological toxicities were more common in the case group (30% *vs* 0%, $P = 0.030$). There was no difference in the response to treatment between groups. Survival was higher in the control group (56 wk *vs* 30 wk, $P = 0.269$), although it did not show statistical significance. This study included mainly decompensated patients with cirrhosis and lymphoma with acceptable treatment outcomes.

Research conclusions

The clinical characteristics of patients with lymphoma and cirrhosis are similar to those with lymphoma, except for some changes inherent to cirrhosis. It may be possible to administer chemotherapy in selected cirrhotic patients, regardless of their severity, obtaining satisfactory clinical outcomes.

Research perspectives

We propose that lymphoma treatment can be provided in patients with cirrhosis at any clinical state without neglecting their safety, although more prospective clinical trials are needed to generate stronger recommendations and better establish safety margins.

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World Journal of Hepatology 2020 February 27; 12(2): 46-63

DOI: 10.4254/wjh.v12.i2.46

ISSN 1948-5182 (online)

SYSTEMATIC REVIEWS

Early treatment efficacy of S-adenosylmethionine in patients with intrahepatic cholestasis: A systematic review

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Author contributions: All authors contributed to the conception and development of the paper, approved the final version, and agree to be accountable for all aspects of the work.

Conflict-of-interest statement: Noureddin M has been on the advisory board or a speaker for Allergan, Gilead, Intercept, Pfizer, Novartis, Blade, EchoSens North America, OWL, Simply Speaking, and Abbott. He has also received research support from Allergan, BMS, Gilead, Galmed, Galectin, Genfit, Conatus, Enanta, Novartis, Shire, and Zydus. He is a minor shareholder in Anaetos and Viking. Sander-Struckmeier S is an employee of Abbott. Mato JM has served on advisory boards for Abbott, Galmed, and OWL Metabolomics, and is a shareholder of OWL Metabolomics.

PRISMA 2009 Checklist statement: The manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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Abstract

BACKGROUND

S-adenosylmethionine (AdoMet) is a metabolically pleiotropic molecule used to treat intrahepatic cholestasis (IHC) and chronic liver diseases. While the efficacy of AdoMet has been demonstrated previously, it has not been systematically investigated within the early weeks of treatment.

AIM

To systematically review the early treatment efficacy of AdoMet in adult patients with IHC.

METHODS

Studies reporting the efficacy of intravenous, intramuscular, or oral forms of AdoMet within 8 wk of treatment initiation were considered; three randomized and six non-randomized studies were eligible for inclusion (PROSPERO registration number CRD42018090936). Of the three randomized studies, two were double-blind and placebo-controlled, and one was comparator-controlled with unclear blinding and a relatively high risk of bias. Mean serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (γGT) following AdoMet treatment *vs* placebo, comparator, or baseline were summarized to determine differences in liver enzymes. Changes in patient-reported clinical symptoms of cholestasis were also summarized.

RESULTS

Both placebo-controlled randomized studies reported significant reductions in serum ALT levels with AdoMet *vs* placebo within 2 wk. One of these also reported significant ALP reductions, and the other reported significant AST and

Manuscript source: Unsolicited manuscript

Received: September 11, 2019
Peer-review started: September 11, 2019
First decision: November 2, 2019
Revised: December 4, 2019
Accepted: December 13, 2019
Article in press: December 13, 2019
Published online: February 27, 2020

P-Reviewer: Bailey SM, El-Shabrawi MHF, Mousa N, Pellicano R
S-Editor: Ma RY
L-Editor: A
E-Editor: Xing YX

γGT reductions within 2 wk. The comparator-controlled randomized study, which had a number of notable limitations, reported significant reductions in serum ALT and AST levels with AdoMet *vs* potassium magnesium aspartate within 4 wk, but not within2 wk. All of the non-randomized studies (4/4) that investigated ALT, AST, ALP and/or γGT reported significant reductions in at least two of these parameters within 2 wk. Of the five studies that evaluated fatigue, reductions were observed within 2 wk in one randomized and two non-randomized studies. The remaining two non-randomized studies reported improvements in fatigue within 6 and 8 wk. Of the four studies reporting symptoms of depression, two non-randomized studies observed improvements within 2 wk and the other two observed improvements within 17 d and 8 wk.

CONCLUSION

Data from both randomized and non-randomized studies suggest that AdoMet improves some biochemical liver parameters and symptoms of cholestasis within 2 wk, with further improvements observed in some studies after 4 and 8 wk of treatment.

Key words: S-adenosylmethionine; Intrahepatic cholestasis; Chronic liver disease; Liver enzymes; Symptoms of cholestasis

Core tip: Chronic liver diseases are associated with substantial mortality and morbidity, and are a significant healthcare burden. Therapies that rapidly reverse or inhibit the deterioration of liver function in patients with intrahepatic cholestasis would therefore be beneficial. In this study, we provide new insight into the efficacy of S-adenosylmethionine in treating these patients, demonstrating that S-adenosylmethionine improves some biochemical liver parameters and symptoms of cholestasis within 2 wk, with further improvements observed in some studies after 4 and 8 wk of treatment.

Citation: Noureddin M, Sander-Struckmeier S, Mato JM. Early treatment efficacy of S-adenosylmethionine in patients with intrahepatic cholestasis: A systematic review. *World J Hepatol* 2020; 12(2): 46-63
URL: https://www.wjgnet.com/1948-5182/full/v12/i2/46.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i2.46

INTRODUCTION

Intrahepatic cholestasis (IHC) is the impairment of bile formation or bile flow resulting from hepatocellular functional defects or obstructive lesions of the intrahepatic biliary tract[1]. IHC is a feature of several chronic liver diseases including alcoholic liver disease (ALD), later stages of non-alcoholic fatty liver disease (NAFLD), drug-induced liver injury (DILI) and others[2,3]. Chronic liver diseases are associated with substantial mortality and morbidity, and are a significant healthcare burden[1,4,5].

The clinical signs and symptoms of IHC include pruritus, jaundice, and fatigue, which may subsequently be associated with depression, autonomic dysfunction, and sleep disturbances[1]. Liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (γGT) may be elevated in patients with IHC, and changes in their levels are important for guiding diagnosis and assessing response to treatment[1,6]. For example, ALP is an early marker that is often increased in asymptomatic patients, and levels 1.5 times the upper limit of normal have been proposed as a threshold for further diagnostic analysis[1]. Indeed, improvement in ALP is considered a biomarker of disease response in cholestatic liver diseases[7-9]. In addition, improvement in ALT has recently been proposed to predict treatment response in patients with NAFLD[10]. Furthermore, a sustained > 10-fold rise in liver transaminases is indicative of a higher risk of mortality in patients with liver disease[6].

S-adenosylmethionine (AdoMet, also abbreviated as SAMe and SAM) is a molecule that participates in multiple cellular reactions (transmethylation, transsulfuration, and

aminopropylation; Figure 1), as the precursor for the synthesis of glutathione; it is the principal methyl donor in methyltransferase reactions that modify DNA, RNA, histones, and other proteins[11-14]. The synthesis of AdoMet is known to be reduced in chronic liver diseases, and depletion of AdoMet promotes increased cellular proliferation and growth, which may be deleterious in chronic conditions[11,13-15].

Preclinical studies support a potential role for AdoMet in the treatment of chronic liver diseases[16-21]. In mouse models of NAFLD, progression to the more severe nonalcoholic steatohepatitis (NASH) is associated with AdoMet depletion[18], while AdoMet supplementation reduces the severity of NASH and improves liver function[17,20]. In a rat model of cholestasis *via* bile duct ligation, animals treated with AdoMet before ligation showed less oxidative stress and a reduced ratio of oxidized to total glutathione, as well as improvements in biochemical liver parameters[16]. The molecular mechanisms by which AdoMet attenuates downregulation of glutathione synthetic enzymes and increases glutathione levels during bile duct ligation have recently been delineated, and they appear to involve the induction of nuclear factor-erythroid 2related factor 2 and suppression of Maf proteins[19,21].

Pharmacokinetic studies in healthy volunteers have shown that AdoMet has a short terminal half-life [81 min following a 100 mg intravenous (iv) dose; 101 min following a 500 mg iv dose] and is rapidly cleared (3.7 mL/min/kg and 3.1 mL/min/kg for 100 mg and 500 mg doses, respectively)[22]. Time to maximum concentration (T_{max}) is 3-5 h after single oral doses of 400-1000 mg, with concentrations declining to baseline levels within 24 h[13]. This is in agreement with previous measurements of labile methyl balance in healthy volunteers, which indicated that the synthesis and catabolism of AdoMet was very rapid[23]. Due to a significant first-pass effect[24], AdoMet is readily bioavailable in the liver, *i.e.*, the target organ. The rapid hepatic metabolism associated with oral administration means that the bioavailability of AdoMet is increased with parenteral administration[13].

A systematic review and meta-analysis of clinical studies assessing the efficacy and safety of AdoMet for the treatment of chronic liver diseases demonstrated that AdoMet treatment was associated with significant improvements in some biochemical liver parameters (total bilirubin and AST); however, the efficacy of AdoMet in the early weeks of treatment was not specifically evaluated[25]. While sustained treatment efficacy is crucial in patients with IHC, early onset of efficacy may also be a key consideration to facilitate a rapid improvement in liver function and, subsequently, a reduction in the debilitating symptoms of cholestasis. The primary objective of this systematic review was to evaluate the efficacy of AdoMet in improving biochemical liver parameters (ALT, AST, ALP, and γGT) within 8 wk of initiating treatment in adult patients with IHC. The secondary objective was to analyze the efficacy of AdoMet in improving clinical symptoms of cholestasis in these patients during this timeframe.

MATERIALS AND METHODS

Protocol registration

The protocol for this systematic review is registered with the international prospective register of systematic reviews (PROSPERO; registration number CRD42018090936) and can be viewed at https://www.crd.york.ac.uk/PROSPERO/. This study followed the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[26].

Eligibility criteria

Published clinical trials reporting the efficacy of AdoMet within 8 wk of treatment initiation were considered for inclusion in this systematic review.

The inclusion criteria were: (1) Full-text articles published between January 1, 1990, and June 10, 2019 reporting prospective, randomized, open-label, and observational studies; (2) Studies including male and female adults with IHC; (3) Studies reporting the efficacy of iv, intramuscular (im), or oral forms of AdoMet in terms of changes in biochemical liver parameters (ALT, AST, ALP, and γGT); and (4) Studies reporting the efficacy of AdoMet within the first 8 wk of treatment compared with control (comparator or placebo) or baseline values.

Studies of IHC in pregnancy were not eligible for inclusion, due to differences in underlying pathology between patients with IHC of pregnancy compared with those with nonpregnancyrelated IHC[27]. Review articles, retrospective studies, and animal studies were also excluded. Shortlisted full-text articles published in non-English languages were translated to English.

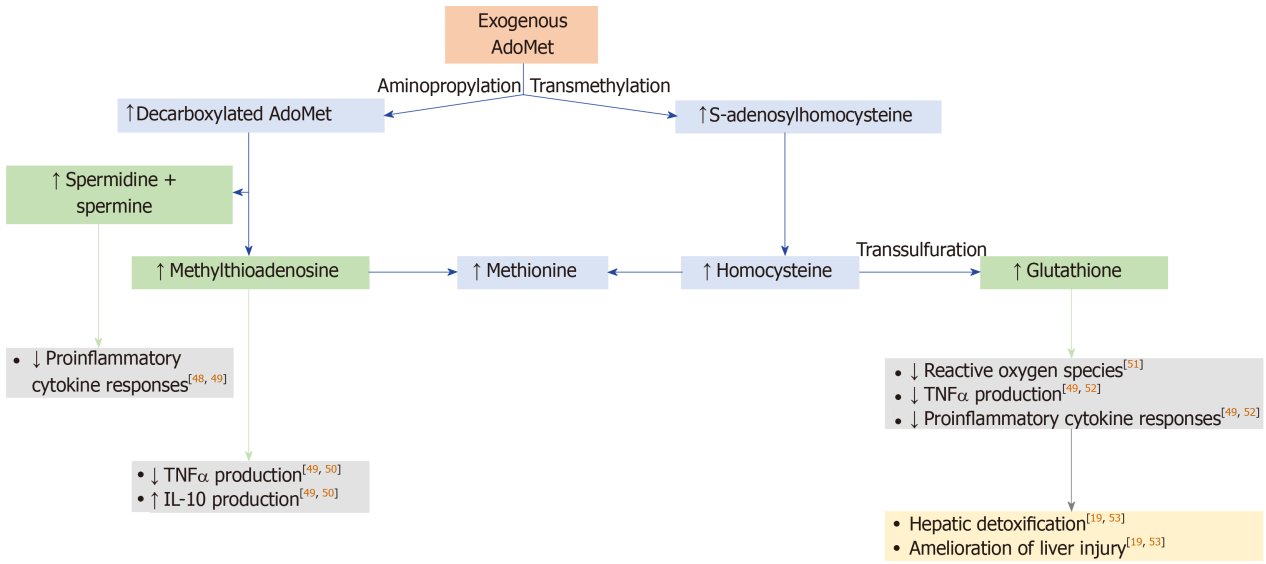

Figure 1 Mechanism of action of S-adenosylmethionine[11,13,47]**.** S-adenosylmethionine (AdoMet)-dependent methylation reactions yield S-adenosylhomocysteine as a by-product, and S-adenosylhomocysteine is cleaved into adenosine and homocysteine by S-adenosylhomocysteine hydrolase. Remethylation of homocysteine to form methionine occurs by methionine synthase and betaine homocysteine methyltransferase. In the transsulfuration pathway, homocysteine is converted to cysteine, which is the rate-limiting precursor for glutathione, *via* an enzymatic process catalyzed by cystathionine β-synthase and cystathionase. To synthesize polyamines, AdoMet is decarboxylated in a reaction catalyzed by AdoMet decarboxylase. The predominant polyamines in mammalian cells are spermidine and spermine, which are made by sequential addition of aminopropyl groups from AdoMet decarboxylase; methylthioadenosine is a by-product of these reactions. These metabolites of AdoMet have biological effects[48-52], which may improve hepatic detoxification and amelioration of liver injury[19,53]. AdoMet: S-adenosylmethionine; IL: Interleukin; TNF: Tumor necrosis factor.

Information sources

PubMed and Embase databases were searched for relevant articles that met the inclusion criteria summarized above. Additional articles that were not available through PubMed or Embase were identified via searches of reference lists and contact with authors from this study.

The following terms were used to search PubMed for articles published between January 1, 1990 and June 10, 2019: AdoMet OR samyr OR transmetil OR heptral OR ademetionine OR Sadenosyl-L-methionine OR S-adenosyl-L-methionine 1,4-butanedisulfonate OR toluenesulfonate OR tosylate OR tosylate disulfate OR disulfate monooleate AND (intrahepatic cholestasis OR alcoholic liver disease OR non-alcoholic liver disease OR non-alcoholic steatohepatitis OR drug-induced liver injury OR chronic liver disease) NOT pregnancy. These terms were adapted for Embase searches.

Study selection

The initial results from the literature searches were screened against the pre-established criteria for inclusion to remove articles, using their titles and abstracts. Excluded articles included those reporting studies in children, studies that did not report the prespecified outcome measures of interest, and conference abstracts. At this initial screening stage, all studies reporting outcomes in chronic liver diseases were included to ensure that any studies involving patients with IHC were not incorrectly excluded. The full texts of the remaining articles were then examined in detail to determine their suitability for inclusion in the review; studies reporting outcomes in chronic liver diseases that did not include patients with IHC were removed at this point. At least two review team members assessed studies for eligibility and disagreements were resolved by discussion.

Data extraction

Data were extracted from the included articles and findings were discussed between authors. Queries regarding data reported in the articles were resolved *via* consultation with statisticians and correspondence with study investigators.

Information extracted from the selected articles included reference citation, country in which the research was carried out, study design, patient population, details of study interventions, number of patients in each treatment group, AdoMet treatment protocol, duration of study treatment, and data relating to the prespecified outcome measures (biochemical parameters and symptoms of cholestasis).

Summary measures

Mean serum levels of ALT, AST, ALP, and γGT following AdoMet treatment *vs* placebo, comparator, or baseline were summarized to assess the efficacy of AdoMet within the first 8 wk of treatment. Additionally, mean scores of patient-reported fatigue and symptoms of depression *vs* placebo, comparator, or baseline were summarized to determine differences in the clinical symptoms of cholestasis following treatment with AdoMet. The results of this research were described using subjective evaluations.

Risk of bias

The risk of bias across studies was limited by ensuring comprehensive searches for all eligible published studies, and by independent assessment of studies identified from these searches by review team members.

Both randomized and non-randomized studies were eligible for inclusion in this review. As the domains that may be subject to bias vary between randomized and non-randomized studies, separate risk-of-bias analyses were performed for the two types of study. The risk of bias in individual randomized studies was determined by assessment of the following domains in accordance with the Cochrane tool for assessing risk of bias in randomized studies[28]: (1) The adequacy of sequence generation; (2) The adequacy of allocation concealment; (3) The adequacy of blinding; (4) The handling of incomplete outcome data; (5) Selective reporting of outcomes; and (6) Any other source of bias. The risk of bias in individual non-randomized studies was determined by assessment of the following domains in accordance with the risk-of-bias assessment tool for non-randomized studies (RoBANS)[29]: (1) The adequacy of the selection of participants; (2) The adequacy of consideration of confounding variables; (3) The adequacy of measurement of exposure; (4) The adequacy of blinding outcome assessments; (5) The handling of incomplete outcome data; and (6) Selective reporting of outcomes.

RESULTS

Study selection

The study selection process is summarized in Figure 2. In total, 115 abstracts were retrieved from the database searches (28 from PubMed, 87 from Embase). Ten further references were identified by searching reference lists and through contact with the authors of this study. After 12 duplicate records were removed, 113 abstracts remained and were screened against the eligibility criteria; of these, 87 were excluded. Full-text articles were assessed for the remaining 26 studies, and a further 17 were excluded. In total, 9 studies were determined to be eligible for inclusion in the systematic review.

Study characteristics

The characteristics of the included studies are provided in Table 1.

Three randomized studies and six non-randomized (observational) studies of patients with IHC were included. In terms of liver conditions, two studies included patients with IHC and DILI[30,31], three reported IHC and chronic liver diseases from various etiologies[32-34], one reported IHC with ALD[35], and one reported IHC with NAFLD[36]. The two remaining studies included patients with IHC and viral hepatitis[37] and IHC due to acute hepatitis or chronic liver disease[38].

In two of the three randomized studies, the intervention was AdoMet *vs* placebo[33,38]. The remaining randomized study evaluated AdoMet *vs* potassium magnesium aspartate[39-41]. No comparators were used in the observational studies.

AdoMet was administered intravenously, intramuscularly, or orally; iv doses were mostly in the range of 400-1000 mg/d; im doses were 400-800 mg/d; and oral doses were 800-1600 mg/d. Several studies initiated AdoMet treatment with iv or im dosing, then switched to oral dosing after 2-4 wk. The total duration of AdoMet treatment varied from 2 to 8 wk (8 wk was the predefined upper limit of inclusion).

All of the included studies reported biochemical liver parameters and six also provided data on the prespecified symptoms of cholestasis. Overall, data from 1791 patients were collected for this review, of whom, 1503 received AdoMet, 273 received placebo, and 15 received a comparator (potassium magnesium aspartate).

Risk of bias

Randomized studies: A summary of the risk of bias within each of the three randomized studies is provided in Table 2. The included domains adhere to the Cochrane tool for assessing risk of bias in randomized studies[28]. None of the studies

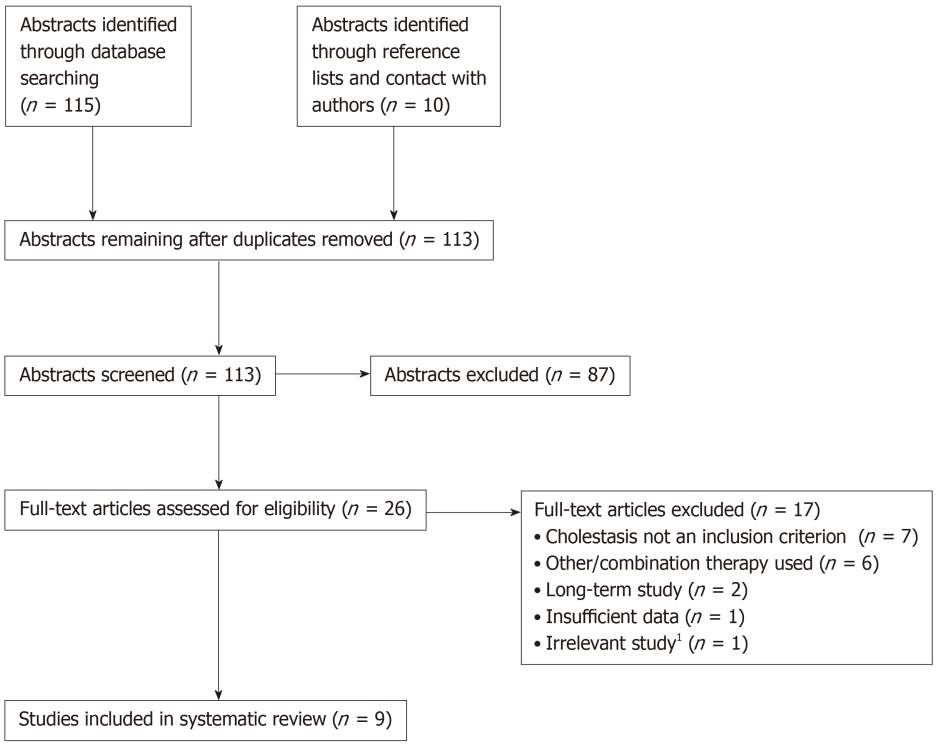

Figure 2 Flow diagram of the study selection process. [1]This study evaluated the use of AdoMet in psoriasis and included no data on chronic liver disease.

reported information on allocation concealment. The blinding scheme was unclear in one study, and the same study used an active comparator in its control group, giving rise to possible bias[37]. This study was also very small, with only 15 patients in each treatment group, and the study endpoints were not clearly defined in the methodology, potentially introducing additional bias. Therefore, greater weighting was assigned to the two placebo-controlled studies throughout the systematic review.

Non-randomized studies: A summary of the risk of bias within each of the six non-randomized studies is provided in Table 3. The included domains adhere to the RoBANS tool for assessing risk of bias in non-randomized studies[29]. Confounding variables were not reported in most of the studies, but one did highlight differences in baseline clinical characteristics between treatment groups[32]. Five studies included patient-reported outcomes and, therefore, were judged to be at a high risk of measurement bias[30,31,34-36]. Two studies appeared to have incomplete outcome data: Virukalpattigopalratnam *et al*[36] reported data for 244 patients at baseline and 243 patients at Visit 1, with no explanation for the missing patient; and Larionova *et al*[30] reported data for 99, 95, and 73 patients at Baseline, Day 14, and Day 42, respectively, with no explanation for the missing data.

Early treatment efficacy of AdoMet: Liver parameters

Efficacy within 2 wk: The two randomized, double-blind, placebo-controlled studies reported significant reductions in two or more of the four liver parameters studied within 2 wk of starting AdoMet treatment (Table 4)[33,38]. Frezza *et al*[33] demonstrated significant reductions in ALT at Week 2 ($P < 0.05$), and ALP at Week 1 ($P < 0.05$) and Week 2 ($P < 0.01$), but did not observe significant changes in AST or γGT at either of these timepoints. Manzillo *et al*[38] demonstrated significant reductions in ALT, AST, and γGT ($P < 0.01$ to $P < 0.05$) at Week 2, but did not observe significant changes in ALP.

In contrast, the small comparator-controlled study did not report significant reductions in ALT, AST, or ALP *vs* potassium magnesium aspartate at Week 2[37].

All of the four non-randomized studies that investigated changes in ALT, AST, ALP, and/or γGT within 2 wk of AdoMet treatment initiation reported significant reductions in at least two of these parameters (Table 5)[30-32,35]. Fiorelli[32] and Perlamutrov *et al*[31] reported significant reductions in ALT, AST, ALP, and γGT within this timeframe ($P < 0.01$ to $P < 0.05$). Larionova *et al*[30] reported significant reductions in ALT and AST at Week 2 (both $P < 0.001$) and Ivashkin *et al*[35] reported significant reductions in ALP and γGT at Week 2 ($P < 0.0001$ for both parameters).

Table 1 Overview of the included studies

Study	Country	Study design	Disease	Intervention and dose		Duration of treatment	Available parameters
Randomized studies							
Frezza *et al*[33], 1990	Italy	Multicenter, randomized, double-blind, placebo-controlled study	Chronic liver disease with IHC (*N* = 220)	AdoMet 1600 mg oral daily (*n* = 110)	Placebo (*n* = 110)	2 wk	ALT, AST, ALP, γGT, fatigue
Manzillo *et al*[38], 1992	Italy	Multicenter, randomized, double-blind, placebo-controlled study	IHC (*N* = 343)	AdoMet 800 mg iv daily (*n* = 180)	Placebo (*n* = 163)	2 wk	ALT, AST, ALP, γGT
Qin *et al*[37], 2000[1]	China	Randomized, parallel-group, comparator-controlled study	Intrahepatic cholestatic viral hepatitis (*N* = 30)	AdoMet 1000 mg iv daily (*n* = 15)	Potassium magnesium aspartate 20 mL daily (*n* = 15)	4 wk	ALT, AST, ALP
Non-randomized studies							
Podymova *et al*[34], 1998[1]	Russia	Observational, baseline-controlled study	Chronic liver disease with IHC (*N* = 32)	AdoMet 800 mg iv daily for 16 d (first phase), then 1600 mg oral daily for 16 d (second phase)		32 d	ALT, AST, ALP, γGT, asthenic syndrome[2]
Fiorelli[32], 1999	Italy	Multicenter, baseline-controlled, open-label study	IHC complicating chronic liver disease (*N* = 640)	AdoMet 500 mg im (*n* = 338) or 800 mg iv (*n* = 302) daily		15 d	ALT, AST, ALP, γGT
Virukalpattigo-palratnam *et al*[36], 2013	India	Multicenter, observational, baseline-controlled study	IHC due to chronic NAFLD (*N* = 250)	AdoMet 800-1200 mg daily for 239/243 (98.4%) patients[3]		6 wk	ALT, AST, ALP, γGT, fatigue
Perlamutrov *et al*[31], 2014	Russia	Multicenter, observational, baseline-controlled study	DILI with IHC (*N* = 105)	AdoMet 400-800 mg iv or im daily for 2 wk (first phase), then 800-1600 mg oral daily for 4 wk (second phase)		6 wk	ALT, AST, ALP, γGT, fatigue, depressed mood
Larionova *et al*[30], 2015[1]	Russia	Multicenter, observational, baseline-controlled study	DILI due to CT and evidence of IHC (*N* = 99)	AdoMet 400-800 mg iv or im daily for 2 wk (first phase), then 800-1600 mg oral daily for 4 wk (second phase)		6 wk	ALT, AST, fatigue, low mood
Ivashkin *et al*[35], 2018	Russia	Multicenter, baseline-controlled, open-label study	IHC due to ALD (*N* = 72)	AdoMet 1500 mg oral daily or 500/800 mg iv daily for 2 wk, then 1500 mg oral daily for 6 wk		8 wk	ALP, γGT, fatigue, depressed mood

[1]Published studies translated to English from original language;
[2]Typically involving irritability, weakness, fatigue, and unstable mood;
[3]4/243 patients (1.6%) received doses < 800 mg/d. AdoMet: S-adenosylmethionine; ALD: Alcoholic liver disease; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CT: Chemotherapy; DILI: Drug-induced liver injury; γGT: Gamma-glutamyl transferase; IHC: Intrahepatic cholestasis; im: Intramuscular; iv: Intravenous; NAFLD: Non-alcoholic fatty liver disease.

Efficacy in 2-4 wk: Neither of the two placebo-controlled randomized studies investigated changes in liver parameters between 2 and 4 wk of AdoMet treatment.

The comparator-controlled study reported significant reductions in ALT and AST, but not ALP, *vs* potassium magnesium aspartate at Week 4 ($P < 0.05$)[37].

Two non-randomized studies reported outcomes for liver parameters following 2-4 wk of AdoMet treatment (Table 5)[32,34]. Fiorelli[32] showed significant reductions in ALT, AST, ALP, and γGT at Day 15 (all $P < 0.01$). In contrast, Podymova *et al*[34] reported no significant changes in ALT, AST, ALP, or γGT at Day 17.

Efficacy in 4-8 wk: Neither of the two placebo-controlled randomized studies, nor the comparator-controlled study, investigated changes in liver parameters in 4-8 wk of AdoMet treatment.

Four out of five non-randomized studies demonstrated significant reductions in two or more of the four liver parameters in 4-8 wk of AdoMet treatment (Table 5)[30,31,35,36]. Virukalpattigopalratnam *et al*[36] and Perlamutrov *et al*[31] reported significant reductions in ALT, AST, ALP, and γGT at Week 6 (all $P < 0.05$). Larionova *et al*[30] observed significant reductions in ALT and AST at Week 6 (both $P < 0.001$), while Ivashkin *et al*[35] reported significant reductions in ALP and γGT at Week 8 ($P < 0.0001$

Table 2 Risk-of-bias assessments for included randomized studies

Study	Risk of bias (domains)					
	Inadequate sequence generation	Inadequate allocation concealment	Inadequate blinding	Inadequate handling of incomplete outcome data	Selective reporting of outcomes	Other bias
Frezza *et al*[33], 1990	Low	Unclear	Low	Low	Low	Low
Manzillo *et al*[38], 1992	Low	Unclear	Low	Low	Low	Low
Qin *et al*[37], 2000	Low	Unclear	Unclear	Low	Low	High

Risk of bias within each domain was judged as low, unclear, or high, using the Cochrane risk-of-bias tool for randomized studies[28].

for both parameters). In contrast, Podymova *et al*[34] observed no significant changes in ALT, AST, ALP, or γGT at Day 33.

Early treatment efficacy of AdoMet: Symptoms and consequences of cholestasis

One of the randomized studies reported the effects of AdoMet treatment on fatigue (Table 6)[33]; Frezza *et al*[33] demonstrated significant reductions *vs* placebo at Weeks 1 and 2 (both $P < 0.01$). Of the four non-randomized studies that reported changes in fatigue from baseline, statistically significant reductions were demonstrated by Virukalpattigopalratnam *et al*[36] at Week 6 ($P < 0.0001$). Both Perlamutrov *et al*[31] and Larionova *et al*[30] observed reductions in fatigue at Weeks 2 and 6, but statistical analyses were not reported. Similarly, Ivashkin *et al*[35] reported a reduction in fatigue at Week 8, but statistical analyses were not provided.

None of the randomized studies reported changes in symptoms of depression (Table 6). Of the non-randomized studies, Perlamutrov *et al*[31] reported a significant reduction in the number of patients with depressed mood *vs* baseline at Weeks 2 and 6 ($P < 0.001$ for both) and Larionova *et al*[30] reported a similar trend for improvement in low mood at Weeks 2 and 6, but did not report any statistical analyses. Similarly, Podymova *et al*[34] observed improvements in asthenic syndrome (typically involving irritability, weakness, fatigue, and unstable mood) at Days 17 and 33, but no statistical analyses were reported. Ivashkin *et al*[35] reported a reduction in depressed mood at Week 8, but statistical analyses were not provided.

Improvements in pruritus and jaundice were also reported in some of the included studies[30,31,33-36]. Six studies demonstrated a reduction in pruritus (Table 6)[30,31,33-36], with statistically significant reductions reported by Frezza *et al*[33] at Weeks 1 and 2 (both $P < 0.01$ *vs* placebo) and Virukalpattigopalratnam *et al*[36] at Week 6 ($P < 0.0001$ *vs* baseline). Reductions in jaundice were observed in five studies (data not shown)[30,31,34-36], although statistical significance was only reported in a non-randomized study, by Virukalpattigopalratnam *et al*[36] at Week 6 ($P < 0.0001$ *vs* baseline).

DISCUSSION

When treating patients with IHC, the speed of onset of AdoMet efficacy could be an important consideration for clinicians as it may determine the rate of improvement in liver function, as well as help with the debilitating symptoms of cholestasis (such as fatigue and depressed mood). It should be noted, though, that the speed of improvement in liver enzymes may depend on the subtype and severity of underlying chronic liver disease, and that each enzyme may react slightly differently to AdoMet therapy in this context.

The efficacy of AdoMet in the early weeks of treatment in patients with chronic liver diseases has been described in several clinical studies[30-33,35-44]. These data have been supported by findings suggesting that its pharmacokinetic parameters may positively affect its speed of clinical efficacy[22]. Furthermore, a recent systematic review and meta-analysis demonstrated that AdoMet treatment was associated with significant improvements in some biochemical liver parameters[25]; however, the authors did not systematically investigate the early effects of AdoMet within 8 wk of treatment initiation, and did not investigate important symptoms of cholestasis such as fatigue and depression.

The placebo-controlled randomized studies included in this review reported

Table 3 Risk-of-bias assessments for included non-randomized studies

Study	Risk of bias (domains)					
	Inadequate selection of participants	Inadequate consideration of confounding variables	Inadequate measurement of exposure	Inadequate blinding of outcome assessments	Inadequate handling of incomplete outcome data	Selective reporting of outcomes
Podymova *et al*[34], 1998[1]	Unclear	Unclear	High	Unclear	Unclear	Low
Fiorelli[32], 1999	High	High	Low	Unclear	Low	Low
Virukalpattigo-palratnam *et al*[36], 2013	Unclear	Unclear	High	Unclear	High	Low
Perlamutrov *et al*[31], 2014	Unclear	Unclear	High	Unclear	Low	Low
Larionova *et al*[30], 2015[1]	Unclear	Unclear	High	Unclear	High	Low
Ivashkin *et al*[35], 2018	Unclear	Unclear	High	Unclear	Low	Low

[1]Published studies translated to English from original language. Risk of bias within each domain was judged as low, unclear, or high, using the risk-of-bias assessment tool for non-randomized studies[29].

significant reductions in plasma ALT levels in patients treated with AdoMet *vs* placebo within 2 wk[33,38]. These data are in contrast to the previous meta-analysis, which identified no significant differences in the change in ALT levels between AdoMet and controls[25]. The conflicting results may reflect the different study populations: Of the six studies included in the previous meta-analysis, four evaluated pregnant women and one evaluated children, whereas our review only included studies in nonpregnant adults. The current review also included a third randomized study by Qin *et al*[37], which reported significant reductions in ALT at Week 4, but not at Week 2. However, this study was not placebo-controlled (the comparator was potassium magnesium aspartate) and the study was small, with only 15 patients in each treatment group giving limited power to detect treatment effects. Therefore, results from this study should be interpreted with caution, and as such, greater weighting was given throughout the review to the two placebo-controlled studies.

The previous meta-analysis showed that AST levels were significantly reduced with AdoMet *vs* control[25], and this is partially supported by our findings; one of the two double-blind placebo-controlled randomized studies in the current systematic review reported significant AST reductions *vs* placebo or comparator within 2 wk[38]. Of three non-randomized studies that investigated AST levels within 2 wk, two reported significant reductions at Week 2[30,31] and one reported significant reductions at Week 1[32].

The previous meta-analysis did not include ALP or γGT as outcome measures[25]. However, we found that one of the two placebo-controlled randomized studies reported a significant reduction in ALP levels at Week 1 and Week 2[33]. Furthermore, one of the two placebo-controlled randomized studies reported significant reductions in γGT within 2 wk[38].

Overall, it is notable that both of the double-blind placebo-controlled randomized studies[33,38] and all of the non-randomized studies (4/4)[30-32,35] that investigated changes in ALT, AST, ALP, and/or γGT within 2 wk of AdoMet treatment initiation reported significant reductions in at least two of these parameters. However, significant reductions were not always observed for all four parameters within each study within this short timeframe, possibly because the specific underlying liver diseases may influence the effect and speed of efficacy onset of AdoMet on liver enzymes.

We also identified improvements in the symptoms of cholestasis. Fatigue was improved in five studies, with two studies reporting significant reductions within 6 wk, one of which reported significant reductions within 1 wk[33,36]. Symptoms of depression were improved in four studies[30,31,34,35], although statistical significance was only reported in one (non-randomized) study at Week 2 and Week 6[31]. Pruritus improved in six studies[30,31,33-36], with statistical significance reported in two of these studies within 6 wk, one of which reported significant reductions within 1 wk[33,36].

Although we did not perform a systematic literature review for changes in bilirubin

Table 4 Summary of outcome data relating to prespecified biochemical liver parameters (randomized studies)

Parameter	Study	Disease	Intervention	Baseline		After treatment		Statistical method	*P* value
				AdoMet	Placebo / comparator	AdoMet	Placebo / comparator		
ALT									
ALT (μkat/L)	Frezza *et al*[33], 1990	Chronic liver disease with IHC (*N* = 220)	AdoMet (*n* = 110) *vs* placebo (*n* = 110)	3.3 ± 0.4 (mean ± SE)	2.8 ± 0.3	2.3 ± 0.2 (Week 1), 1.5 ± 0.1 (Week 2)	2.4 ± 0.2 (Week 1), 2.2 ± 0.2 (Week 2)	Split-plot analysis	Not reported at Week 1, *P* < 0.05 *vs* placebo at Week 2
ALT (μkat/L)	Manzillo *et al*[38], 1992	IHC (*N* = 343)	AdoMet (*n* = 180) *vs* placebo (*n* = 163)	1.2 (1.1, 1.4) (mean, 95% CI)	1.2 (1.1, 1.4)	0.8 (0.7, 0.9)	1.0 (0.9, 1.2)	Split-plot analysis (MANOVA)	*P* < 0.01 treatment-to-time interaction *vs* placebo at Week 2
ALT (U/L)	Qin *et al*[37], 2000[1]	Intrahepatic cholestatic viral hepatitis (*N* = 30)	AdoMet (*n* = 15) *vs* potassium magnesium aspartate (*n* = 15)	198.5 ± 75.2 (mean ± SD)	190.6 ± 71.2	127.0 ± 47.5 (Week 2), 48.2 ± 43.5 (Week 4)	130.2 ± 47.2 (Week 2), 67.7 ± 27.2 (Week 4)	x^2 test for comparison of rates; *t* test for comparison of means	NS at Week 2 *P* < 0.05 *vs* comparator at Week 4
AST									
AST (μkat/L)	Frezza *et al*[33], 1990	Chronic liver disease with IHC (*N* = 220)	AdoMet (*n* = 110) *vs* placebo (*n* = 110)	2.4 ± 0.2 (mean ± SE)	2.1 ± 0.2	1.8 ± 0.1 (Week 1), 1.3 ± 0.1 (Week 2)	1.8 ± 0.2 (Week 1), 1.7 ± 0.2 (Week 2)	Split-plot analysis	NS *vs* placebo at Weeks 1 and 2
AST (μkat/L)	Manzillo *et al*[38], 1992	IHC (*N* = 343)	AdoMet (*n* = 180) *vs* placebo (*n* = 163)	1.4 (1.2, 1.6) (mean, 95% CI)	1.3 (1.2, 1.5)	0.9 (0.9, 1.1)	1.0 (0.9, 1.2)	Split-plot analysis (MANOVA)	*P* < 0.05 treatment-to-time interaction *vs* placebo at Week 2
AST (U/L)	Qin *et al*[37], 2000[1]	Intrahepatic cholestatic viral hepatitis (*N* = 30)	AdoMet (*n* = 15) *vs* potassium magnesium aspartate (*n* = 15)	127.0 ± 60.7 (mean ± SD)	118.2 ± 58.7	84.6 ± 33.8 (Week 2), 45.6 ± 28.2 (Week 4)	78.3 ± 38.5 (Week 2), 52.7 ± 25.3 (Week 4)	x^2 test for comparison of rates; *t* test for comparison of means	NS at Week 2 *P* < 0.05 *vs* comparator at Week 4
ALP									
ALP (μkat/L)	Frezza *et al*[33], 1990	Chronic liver disease with IHC (*N* = 220)	AdoMet (*n* = 110) *vs* placebo (*n* = 110)	4.5 ± 0.3 (mean ± SE)	4.7 ± 0.3	3.7 ± 0.3 (Week 1), 3.2 ± 0.2 (Week 2)	4.6 ± 0.3 (Week 1), 4.4 ± 0.3 (Week 2)	Split-plot analysis	*P* < 0.05 *vs* placebo at Week 1, *P* < 0.01 *vs* placebo at Week 2
ALP (μkat/L)	Manzillo *et al*[38], 1992	IHC (*N* = 343)	AdoMet (*n* = 180) *vs* placebo (*n* = 163)	4.8 (4.2, 5.5) (mean, 95% CI)	4.9 (4.3, 5.7)	3.9 (3.4, 4.4)	4.0 (3.4, 4.7)	Split-plot analysis (MANOVA)	NS *vs* placebo at Week 2
ALP (U/L)	Qin *et al*[37], 2000[1]	Intrahepatic cholestatic viral hepatitis (*N* = 30)	AdoMet (*n* = 15) *vs* potassium magnesium aspartate (*n* = 15)	203.2 ± 39.5 (mean ± SD)	202.8 ± 39.4	93.5 ± 33.7 (Week 2), 85.6 ± 20.6 (Week 4)	97.5 ± 33.0 (Week 2), 89.1 ± 27.8 (Week 4)	*x2* test for comparison of rates; *t* test for comparison of means	NS *vs* comparator at Weeks 2 and 4
γGT									
γGT (μkat/L)	Frezza *et al*[33], 1990	Chronic liver disease with IHC (*N* = 220)	AdoMet (*n* = 110) *vs* placebo (*n* = 110)	2.5 ± 0.3 (mean ± SE)	2.2 ± 0.2	1.9 ± 0.3 (Week 1), 1.5 ± 0.2 (Week 2)	1.9 ± 0.1 (Week 1), 1.7 ± 0.1 (Week 2)	Split-plot analysis	NS at Week 1 or Week 2
γGT (μkat/L)	Manzillo *et al*[38], 1992	IHC (*N* = 343)	AdoMet (*n* = 180) *vs* placebo (*n* = 163)	1.9 (1.6, 2.2) (mean, 95% CI)	1.8 (1.6, 2.2)	1.2 (1.0, 1.3)	1.3 (1.1, 1.6)	Split-plot analysis (MANOVA)	*P* < 0.05 treatment-to-time interaction *vs* placebo at Week 2

[1]Published studies translated to English from original language. AdoMet: S-adenosylmethionine; ALP: Alkaline phosphatase; ALT: Alanine

aminotransferase; AST: Aspartate aminotransferase; CI: Confidence interval; γGT: Gamma-glutamyl transferase; IHC: Intrahepatic cholestasis; MANOVA: Multivariate analysis of variance; NS: Not significant; SD: Standard deviation; SE: Standard error.

levels, both of the double-blind placebo-controlled randomized studies reported significant reductions in serum combined and/or total bilirubin levels with AdoMet *vs* placebo or comparator within 2 wk of treatment initiation[33,38]. Furthermore, four of the five non-randomized studies that investigated this parameter found that AdoMet significantly reduced serum combined and/or total bilirubin levels *vs* baseline within 8 wk of treatment initiation[30-32,36], and three of these demonstrated significant reductions within 2 wk[30-32]. Since bilirubin levels are a read out of the functional capacity of the liver[6], the improvement in bilirubin levels in patients who received AdoMet treatment may therefore reflect a corresponding improvement in liver function; however, further investigations are required to validate this observation.

AdoMet is a natural compound, synthesized and metabolized mainly by the liver *via* multiple pathways[24]; it is likely that exogenous AdoMet is rapidly metabolized, improving overall liver metabolic homeostasis[14]. AdoMet can be formulated for iv, im, or oral administration, with the three routes appearing to have comparable efficacy within 8 wk of treatment initiation, although it should be noted that most of the studies analyzed here did not directly compare different formulations. However, the trial reported by Fiorelli *et al*[32], which used both iv and im delivery, showed similar improvements in liver parameters with the two formulations. In several studies, initial iv administration was followed by maintenance oral administration[30,31,34,35]; while a comparison of efficacy between the two formulations is difficult, it might be expected that iv administration would result in a faster onset of efficacy compared with the oral route due to increased bioavailability. Ivashkin *et al*[35] reported that improvements in ALP and γGT after 8 wk of treatment were greater in patients initially treated with iv AdoMet *vs* oral AdoMet, however the authors highlighted that patients receiving initial iv treatment had higher baseline values as the initial treatment route was based on baseline disease severity. Finally, all the studies included in this review used pharmaceutical grade AdoMet; it is uncertain whether similar findings would be observed from AdoMet formulations of different quality or from alternative manufacturing techniques.

Although this systematic review did not include an assessment of adverse events, AdoMet has a favorable tolerability profile that has been established in several clinical studies and by long-term marketing experience[45]. The rapid efficacy of AdoMet, combined with its favorable tolerability profile, makes it an attractive therapeutic option for a wide range of patients.

We acknowledge that this review has several limitations. Firstly, this study retrospectively evaluated data from prospective clinical studies that were not designed to evaluate the speed of onset of the treatment effect of AdoMet. Secondly, we included non-randomized studies, which increased bias. It is noticeable that, in general, the non-randomized studies reported more substantial and rapid improvements in the measured outcomes than the randomized studies. The lack of a control arm in the non-randomized studies is a significant confounding factor; therefore, while the results of these studies support the rapid efficacy of AdoMet, more weighting must be given to the data arising from the two double-blind placebo-controlled randomized studies. Thirdly, to ensure that all eligible studies were included in the review, we did not restrict our database searches to English-language abstracts. As a result, several of the included studies were translated to English from other languages. Fourthly, we included studies of patients with a broad variety of underlying chronic liver diseases, and we recognize that the heterogeneity of the studied populations was a confounding factor that may mask the true treatment effects in specific underlying diseases. For example, it is possible that variations in the severity and duration of IHC across patients could have had an impact on clinical outcomes, and it is possible that not all patients with IHC had a deficiency in AdoMet synthesis. Fifthly, we recognize that a relatively limited number of studies were eligible for inclusion (three randomized and six non-randomized studies), and that sample sizes in some of the included studies were small. Finally, we acknowledge that a meta-analysis would have provided a more robust assessment of the early effects of AdoMet on liver enzymes, but this was not possible due to the heterogeneous nature of the available studies.

To our knowledge, this is the first systematic review investigating the efficacy of AdoMet within the early weeks of treatment in adults with IHC. Clinical data from randomized and non-randomized studies suggest that AdoMet significantly reduces plasma ALT, AST, ALP, and γGT levels, as well as clinical symptoms of cholestasis within the first 8 wk of treatment. Furthermore, AdoMet has clinical efficacy within

Table 5 Summary of outcome data relating to prespecified biochemical liver parameters (non-randomized studies)

Parameter	Study	Disease	Intervention	Baseline	After treatment	Statistical method	*P* value
ALT							
ALT (IU)	Podymova *et al*[34], 1998[1]	Chronic liver disease with IHC (*N* = 32)	AdoMet	109.3 ± 26.4 (mean ± SD)	74.4 ± 167.0 (Day 17), 40.5 ± 14.5 (Day 33)	NR	*P* > 0.1 *vs* baseline at Day 17, *P* > 0.05 *vs* baseline at Day 33
ALT (U/L)	Fiorelli[32], 1999	IHC complicating chronic liver disease (*N* = 640)	AdoMet im or iv	im: 96 ± 6.1, iv: 92 ± 5.6 (mean ± SE)	im: 84 ± 5.3 (Day 7), 76 ± 5.6 (Day 15), iv: 84 ± 4.3 (Day 7), 76 ± 3.6 (Day 15)	Friedman non-parametric test	*P* < 0.01 *vs* baseline at Days 7 and 15 for both im and iv
ALT (U/L)	Virukalpattigo-palratnam *et al*[36], 2013	IHC due to chronic NAFLD (*N* = 250)	AdoMet	124.4 (mean)	62.1	Wilcoxon signed-rank test	*P* < 0.05 *vs* baseline at Week 6
ALT (U/L)	Perlamutrov *et al*[31], 2014	DILI with IHC (*N* = 105)	AdoMet	NR	NR	Spearman's correlation coefficient	*P* < 0.05 *vs* baseline at Weeks 2 and 6
ALT (U/L)	Larionova *et al*[30], 2015[1]	DILI due to CT and evidence of IHC (*N* = 99)	AdoMet	137.3 (median)	68.5 (Day 14), 55.2 (Day 42)	NR	*P* < 0.001 *vs* baseline at Days 14 and 42
AST							
AST (IU)	Podymova *et al*[34], 1998[1]	Chronic liver disease with IHC (*N* = 32)	AdoMet	105.9 ± 21.5 (mean ± SD)	66.4 ± 13.9 (Day 17), 32.5 ± 10.0 (Day 33)	NR	*P* > 0.1 *vs* baseline at Day 17 *P* > 0.01 *vs* baseline at Day 33
AST (U/L)	Fiorelli[32], 1999	IHC complicating chronic liver disease (*N* = 640)	AdoMet im or iv	im: 104 ± 4.2, iv: 116 ± 5.7 (mean ± SE)	im: 96 ± 4.4 (Day 7), 88.0 ± 4.8 (Day 15), iv: 100 ± 4.1 (Day 7), 92.0 ± 4.1 (Day 15)	Friedman non-parametric test	*P* < 0.01 *vs* baseline at Days 7 and 15 for both im and iv
AST (U/L)	Virukalpattigo-palratnam *et al*[36], 2013	IHC due to chronic NAFLD (*N* = 250)	AdoMet	130.8 (mean)	61.6	Wilcoxon signed-rank test	*P* < 0.05 *vs* baseline at Week 6
AST (U/L)	Perlamutrov *et al*[31], 2014	DILI with IHC (*N* = 105)	AdoMet	NR	NR	Spearman's correlation coefficient	*P* < 0.05 *vs* baseline at Weeks 2 and 6
AST (U/L)	Larionova *et al*[30], 2015[1]	DILI due to CT and evidence of IHC (*N* = 99)	AdoMet	103.3 (median)	49.7 (Day 14), 41.0 (Day 42)	NR	*P* < 0.001 *vs* baseline at Days 14 and 42
ALP							
ALP (U/L)	Podymova *et al*[34], 1998[1]	Chronic liver disease with IHC (*N* = 32)	AdoMet	676.9 ± 154.8 (mean ± SD)	596.5 ± 144.2 (Day 17), 446.2 ± 175.1 (Day 33)	NR	*P* > 0.1 *vs* baseline at Day 17, *P* > 0.1 *vs* baseline at Day 33
ALP (U/L)	Fiorelli[32], 1999	IHC complicating chronic liver disease (*N* = 640)	AdoMet im or iv	im: 259.5 ± 10.8, iv: 276.5 ± 13.7 (mean ± SE)	im: 217.0 ± 10.5 (Day 7), 191.5 ± 10.2 (Day 15), iv: 234.0 ± 14.3 (Day 7), 208.5 ± 13.6 (Day 15)	Friedman non-parametric test	*P* < 0.01 *vs* baseline at Days 7 and 15 for both im and iv
ALP (U/L)	Virukalpattigo-palratnam *et al*[36], 2013	IHC due to chronic NAFLD (*N* = 250)	AdoMet	230.6 (mean)	165.3	Wilcoxon signed-rank test	*P* < 0.05 *vs* baseline at Week 6
ALP (U/L)	Perlamutrov *et al*[31], 2014	DILI with IHC (*N* = 105)	AdoMet	NR	NR	Spearman's correlation coefficient	*P* < 0.05 *vs* baseline at Week 2, *P* < 0.05 *vs* baseline at Week 6
ALP (U/L)	Ivashkin *et al*[35], 2018	IHC due to ALD (*N* = 72)	AdoMet	241.2	167.9 (Week 2), 152.2 (Week 8)	ANCOVA	*P* < 0.0001 *vs* baseline at Weeks 2 and 8
γGT							
γGT (IU)	Podymova *et al*[34], 1998[1]	Chronic liver disease with IHC (*N* = 32)	AdoMet	153.5 ± 36.1 (mean ± SD)	145.1 ± 50.1 (Day 17), 51.8 ± 16.1 (Day 33)	NR	*P* > 0.1 *vs* baseline at Day 17, *P* > 0.05 *vs* baseline at Day 33

γGT (U/L)	Fiorelli[32], 1999	IHC complicating chronic liver disease (*N* = 640)	AdoMet im or iv	im: 240.7 ± 20.0, iv: 303.4 ± 36.1	im: 195.1 ± 16.0 (Day 7), 160.9 ± 10.0 (Day 15), iv: 218.0 ± 17.7 (Day 7), 189.4 ± 17.3 (Day 15)	Friedman non-parametric test	$P < 0.01$ *vs* baseline at Days 7 and 15 for both im and iv
γGT (U/L)	Virukalpattigo-palratnam *et al*[36], 2013	IHC due to chronic NAFLD (*N* = 250)	AdoMet	151.5 (mean)	90.8 (mean)	Wilcoxon signed-rank test	$P < 0.05$ *vs* baseline at Week 6
γGT (U/L)	Perlamutrov *et al*[31], 2014	DILI with IHC (*N* = 105)	AdoMet	NR	NR	Spearman's correlation coefficient	$P < 0.05$ *vs* baseline at Week 2, $P < 0.05$ *vs* baseline at Week 6
γGT (U/L)	Ivashkin *et al*[35], 2018	IHC due to ALD (*N* = 72)	AdoMet	NR	Mean change ± SD: -197.0 ± 403.5 (Week 2), -233.8 ± 407.1 (Week 8)	ANCOVA	$P < 0.0001$ *vs* baseline at Weeks 2 and 8

[1]Published studies translated to English from original language. AdoMet: S-adenosylmethionine; ALD: Alcoholic liver disease; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; ANOVA: Analysis of variance; ANCOVA: Analysis of covariance; AST: Aspartate aminotransferase; CI: Confidence interval; CT: Chemotherapy; DILI: Drug-induced liver injury; γGT: Gamma-glutamyl transferase; IHC: Intrahepatic cholestasis; im: Intramuscular; iv: Intravenous; NAFLD: Non-alcoholic fatty liver disease; NR: Not reported; SD: Standard deviation; SE: Standard error.

the first 2 wk of treatment as some studies indicated that it reduced the levels of liver enzymes and improved clinical symptoms of cholestasis within this short timeframe. AdoMet has also been shown to have long-term efficacy in liver diseases[46], further supporting the use of AdoMet in this patient population. However, prospective, randomized, placebo-controlled clinical studies are needed to establish the speed of onset of AdoMet efficacy, and the subsequent clinical impact on patient outcomes, in the treatment of specific liver diseases.

In summary, this systematic review provides new insight into the efficacy of AdoMet in treating IHC, and demonstrates that most studies that investigated the efficacy of AdoMet within 2 wk of treatment initiation, showed improvements in some biochemical liver parameters and symptoms of cholestasis (such as fatigue and symptoms of depression) within this short timeframe; further improvements were observed in some studies after 4 and 8 wk of treatment.

Table 6 Summary of outcome data relating to prespecified symptoms and consequences of cholestasis

Parameter	Study	Scale / scoring system	Disease	Inter-vention	Baseline		After treatment		Statistical method	*P* value
					AdoMet	Control	AdoMet	Control		
Fatigue										
Fatigue (cm)	Frezza *et al*[33], 1990[1]	10 cm visual analog scale: 0 = lack of symptom to 10 = maximal severity	Chronic liver disease with IHC (*N* = 220)	AdoMet (*n* = 110) *vs* placebo (*n* = 110)	5.5 ± 0.3 (mean ± SE)	5.3 ± 0.3	3.5 ± 0.2 (Week 1), 2.6 ± 0.2 (Week 2)	5.0 ± 0.3 (Week 1), 4.8 ± 0.3 (Week 2)	Fisher's exact test	*P* < 0.01 *vs* placebo at Weeks 1 and 2
Fatigue (% patients)	Virukalpatti gopalratnam *et al*[36], 2013[2]	NR	IHC due to chronic NAFLD (*N* = 250)	AdoMet	75.8	-	32.5	-	McNemar's test	*P* < 0.0001 *vs* baseline at Week 6
Fatigue (% patients)	Perlamutrov *et al*[31], 2014[2]	Two-degree scale: 0 = absent; 1 = present	DILI with IHC (*N* = 105)	AdoMet	81.0	-	29.5 (Day 14), 11.4 (Day 42)	-	NR	NR
Fatigue (% patients)	Larionova *et al*[30], 2015[2,3]	NR	DILI due to CT and evidence of IHC (*N* = 99)	AdoMet	42.4	-	25.3 (Day 14), 17.2 (Day 42)	-	NR	NR
Fatigue (% patients without)	Ivashkin *et al*[35], 2018	Six-point scale: 0 = no symptoms to 5 = maximal symptoms	IHC due to ALD (*N* = 72)	AdoMet	18.1	-	49.2	-	NR	NR
Depressed mood										
Asthenic syndrome[4] (% patients)	Podymova *et al*[34], 1998[2,3]	Four-degree scale: 0 = absent to 3 = severe	Chronic liver disease with IHC (*N* = 32)	AdoMet	100%	-	50 (Day 17), 46 (Day 33)	-	NR	NR
Depressed mood (% patients without)	Perlamutrov *et al*[31], 2014[2]	Four-degree scale: 0 = absent to 3 = severe	DILI with IHC (*N* = 105)	AdoMet	12.4%	-	50.5 (Day 14), 74.3 (Day 42)	-	x^2 test	*P* < 0.001 *vs* baseline at Days 14 and 42
Low mood (No. patients)	Larionova *et al*[30], 2015[2,3]	NR	DILI due to CT and evidence of IHC (*N* = 99)	AdoMet	NR	-	No. of patients without low mood increased on Days 14 and 42	-	NR	NR
Depressed mood (% patients without)	Ivashkin *et al*[35], 2018[2]	Six-point scale: 0 = no symptoms to 5 = maximal symptoms	IHC due to ALD (*N* = 72)	AdoMet	16.7	-	73.0	-	NR	NR
Pruritus										
Pruritus (cm)	Frezza *et al*[33], 1990[1]	10 cm visual analog scale: 0 = lack of symptom to 10 = maximal severity	Chronic liver disease with IHC (*N* = 220)	AdoMet (*n* = 110) *vs* placebo (*n* = 110)	5.3 ± 0.3 (mean ± SE)	5.3 ± 0.3	3.5 ± 0.3 (Week 1), 2.7 ± 0.2 (Week 2)	4.8 ± 0.2 (Week 1), 4.1 ± 0.2 (Week 2)	Fisher's exact test	*P* < 0.01 *vs* placebo at Weeks 1 and 2
Pruritus (% patients)	Podymova *et al*[34], 1998[2,3]	Four-degree scale: 0 = absent to 3 = severe	Chronic liver disease with IHC (*N* = 32)	AdoMet	63	-	53 (Day 17), 41 (Day 33)	-	NR	NR
Pruritus (% patients)	Virukalpatti gopalratnam *et al*[36], 2013[2]	NR	IHC due to chronic NAFLD (*N* = 250)	AdoMet	38.9	-	17.3	-	McNemar's test	*P* < 0.0001 *vs* baseline at Week 6

Pruritus (% patients)	Perlamutrov *et al*[31], 2014[2]	Two-degree scale: 0 = absent; 1 = present	DILI with IHC (*N* = 105)	AdoMet	81.0	-	42.9 (Day 14), 6.7 (Day 42)	-	NR	NR
Pruritus (% patients)	Larionova *et al*[30], 2015[2,3]	NR	DILI due to CT and evidence of IHC (*N* = 99)	AdoMet	24.2	-	7.1 (Day 14), 6.1 (Day 42)	-	NR	NR
Pruritus (% patients without)	Ivashkin *et al*[35], 2018[2]	Six-point scale: 0 = no symptoms to 5 = maximal symptoms	IHC due to ALD (*N* = 72)	AdoMet	45.8	-	88.9	-	NR	NR

[1]Randomized studies;
[2]Observational studies;
[3]Published studies translated to English from original language;
[4]Typically involving irritability, weakness, fatigue, and unstable mood. AdoMet: S-adenosylmethionine; ALD: Alcoholic liver disease; CT: Chemotherapy; DILI: Drug-induced liver injury; IHC: Intrahepatic cholestasis; NAFLD: Non-alcoholic fatty liver disease; NR: Not reported; SE: Standard error.

ARTICLE HIGHLIGHTS

Research background

Intrahepatic cholestasis (IHC) is a key feature of several chronic liver diseases and is associated with clinical signs and symptoms such as pruritus, jaundice, and fatigue, which may subsequently be associated with depression, autonomic dysfunction, and sleep disturbances. S-adenosylmethionine (AdoMet) is a metabolically pleiotropic molecule that is used to treat IHC.

Research motivation

The efficacy of AdoMet has been demonstrated by several clinical studies and a previous systematic review and meta-analysis; however, the efficacy of AdoMet in the early weeks of treatment has not been systematically investigated.

Research objectives

The primary objective of this systematic review was to evaluate the efficacy of AdoMet in improving biochemical liver parameters [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (γGT)] in adult patients with IHC within 8 wk of initiating treatment. The secondary objective was to assess the efficacy of AdoMet in improving the clinical symptoms of cholestasis within this timeframe.

Research methods

Published clinical trials reporting the efficacy of AdoMet (intravenous, intramuscular, or oral forms) within 8 wk of treatment initiation in adult patients with IHC were considered for inclusion in the review. Mean serum levels of ALT, AST, ALP, and γGT following AdoMet treatment *vs* placebo, comparator, or baseline were summarized. Changes in patient-reported clinical symptoms of cholestasis (such as fatigue and depression) were also reviewed.

Research results

In total, three randomized and six non-randomized (observational) studies of patients with IHC were included in the systematic review. Of the three randomized studies, two were double-blind and placebo-controlled, and one was comparator-controlled with unclear blinding and a relatively high risk of bias. Both of the double-blind placebo-controlled randomized studies and all of the non-randomized studies (4/4) that investigated changes in ALT, AST, ALP, and/or γGT within 2 wk of AdoMet treatment initiation reported significant reductions in at least two of these parameters. Reductions in patient-reported fatigue and depression were also reported within 2 wk in some studies.

Research conclusions

Clinical data from the randomized and non-randomized studies included in this systematic review suggest that AdoMet shows clinical efficacy within the first 2 wk of treatment, as some studies reported reductions in liver enzymes and improvements in clinical symptoms of cholestasis within this short timeframe.

Research perspectives

Sustained treatment efficacy is crucial in patients with IHC, but the early onset of efficacy may also be a key consideration to facilitate rapid improvements in liver function, leading to a prompt reduction in the distressing symptoms of cholestasis. In terms of future research, further targeted clinical studies are desired to determine the speed of onset of the clinical impact of AdoMet in patients with specific liver diseases.

ACKNOWLEDGEMENTS

The authors thank Dr Shelly Lu for her critical review of the manuscript. Editorial support was provided by Josh Lilly of Alpharmaxim Healthcare Communications and funded by Abbott.

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World Journal of Hepatology 2020 February 27; 12(2): 64-71

DOI: 10.4254/wjh.v12.i2.64

ISSN 1948-5182 (online)

CASE REPORT

Nonsense variant of *ATP8B1* gene in heterozygosis and benign recurrent intrahepatic cholestasis: A case report and review of literature

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Author contributions: Di Leo A, Barone M and Ierardi E designed the research; Piazzolla M and Castellaneta N followed up the patient both during hospitalization and outpatient visits; Novelli A, Agolini E and Cocciadiferro D performed genetic analysis; Resta L and Duda L performed histological examination; Piazzolla M and Ierardi E wrote the paper; all authors critically revised the manuscript.

Informed consent statement: The patient gave written informed consent before the submission of the manuscript.

Conflict-of-interest statement: No conflict of interest.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

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Abstract

BACKGROUND

Benign recurrent intrahepatic cholestasis is a genetic disorder with recurrent cholestatic jaundice due to *ATP8B1* and *ABCB11* gene mutations encoding for hepato-canalicular transporters. Herein, we firstly provide the evidence that a nonsense variant of *ATP8B1* gene (c.1558A>T) in heterozygous form is involved in BRIC pathogenesis.

CASE SUMMARY

A 29-year-old male showed severe jaundice and laboratory tests consistent with intrahepatic cholestasis despite normal gamma-glutamyltranspeptidase. Acute and chronic liver diseases with viral, metabolic and autoimmune etiology were excluded. Normal intra/extra-hepatic bile ducts were demonstrated by magnetic resonance. Liver biopsy showed: Cholestasis in the centrilobular and intermediate zones with bile plugs and intra-hepatocyte pigment, Kupffer's cell activation/hyperplasia and preserved biliary ducts. Being satisfied benign recurrent intrahepatic cholestasis diagnostic criteria, *ATP8B1* and *ABCB11* gene analysis was performed. Surprisingly, we found a novel nonsense variant of *ATP8B1* gene (c.1558A>T) in heterozygosis. The variant was confirmed by Sanger sequencing following a standard protocol and tested for familial segregation, showing a maternal inheritance. Immunohistochemistry confirmed a significant reduction of mutated gene related protein (familial intrahepatic cholestasis 1). The patient was treated with ursodeoxycholic acid 15 mg/kg per day and

Manuscript source: Invited manuscript

Received: November 14, 2019
Peer-review started: November 14, 2019
First decision: December 12, 2019
Revised: December 19, 2019
Accepted: January 1, 2020
Article in press: January 1, 2020
Published online: February 27, 2020

P-Reviewer: Deneau M, Li XH
S-Editor: Ma RY
L-Editor: A
E-Editor: Xing YX

colestyramine 8 g daily with total bilirubin decrease and normalization at the 6th and 12th mo.

CONCLUSION
A genetic abnormality, different from those already known, could be involved in familial intrahepatic cholestatic disorders and/or pro-cholestatic genetic predisposition, thus encouraging further mutation detection in this field.

Key words: Benign recurrent intrahepatic cholestasis; *ATP8B1/ABCB11* genes; Jaundice; Heterozygous variant of *ATP8B1* gene (c.1558A>T); Familial inheritance; Case report

Core tip: Benign recurrent intrahepatic cholestasis is a rare genetic disorder characterized by recurrent jaundice due to the mutation of the *ATP8B1/ABCB11* genes encoding for hepato-canalicular transporters. The original finding, which characterizes the case we observed, is the association of a novel nonsense variant of *ATP8B1* gene (c.1558A>T) in a heterozygous condition with hepato-canalicular transporter protein deficiency. Indeed, the disorder has been described until now as an autosomal recessive one, whereas, in this case, the patient expressed the disease despite having only one mutated allele of *ATP8B1* gene.

Citation: Piazzolla M, Castellaneta N, Novelli A, Agolini E, Cocciadiferro D, Resta L, Duda L, Barone M, Ierardi E, Di Leo A. Nonsense variant of *ATP8B1* gene in heterozygosis and benign recurrent intrahepatic cholestasis: A case report and review of literature. *World J Hepatol* 2020; 12(2): 64-71
URL: https://www.wjgnet.com/1948-5182/full/v12/i2/64.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i2.64

INTRODUCTION

Benign recurrent intrahepatic cholestasis (BRIC) is a rare genetic disorder characterized by recurrent episodes of cholestatic jaundice that undergo spontaneous resolution within a period lasting from few weeks to some months without an evolution towards chronic liver disease[1]. The first case of BRIC was described by Summerskill *et al*[2] in 1956. Its exact prevalence remains unknown, but estimated incidence is approximately 1 in 50000 to 100000 people worldwide. BRIC is an autosomal recessive disorder. Two subtypes have been described according to associated gene mutations. BRIC-1 is associated with a mutation in the *ATP8B1* gene (chromosome 18q21-22) that encodes a protein called familial intrahepatic cholestasis 1 (FIC1)[3]. BRIC-2 is caused by a mutation in the *ABCB11* gene (chromosome 2q24) encoding bile salt export pump protein[4]. Consequent impaired function of these hepato-canalicular transporters, responsible for the secretion of bile components, induces an intrahepatic cholestasis. Mutations in *ATP8B1* and *ABCB11* genes are also present in progressive familial intrahepatic cholestasis (PFIC - type 1 and 2, respectively). Despite both are due to mutations in the same gene, phenotypes of BRIC and PFIC differ, since PFIC is characterized by a progressive liver damage up to end-stage liver disease[5].

BRIC diagnostic criteria have been proposed by Luketic *et al*[1]; at the moment, they represent the basics in the diagnosis of the disorder. They include: (1) At least two episodes of jaundice separated by a symptom-free interval lasting several months to years; (2) Laboratory values consistent with intrahepatic cholestasis; (3) Gamma-glutamyltranspeptidase (GGT) either normal or only minimally elevated; (4) Severe pruritus secondary to cholestasis; (5) Liver histology demonstrating centrilobular cholestasis; (6) Normal intra- and extra-hepatic bile ducts by cholangiography (endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiography); and (7) Absence of factors known to be associated with cholestasis (*i.e.*, drugs, pregnancy)[1].

On these bases, we believe of interest to report the case of a young adult patient with clinical, biochemical and histological features of BRIC, where we have, at the best of our knowledge, firstly provided the evidence of a heterozygous pathogenic

nonsense variant in the *ATP8B1* gene (c.1558A>T).

CASE PRESENTATION

Chief complaints

A 29-year-old male was admitted into our Gastroenterology Unit for jaundice, severe pruritus, dark colored urine, clay colored stool and weight loss.

History of present illness

Patient symptoms started one month before the admission in our Unit. He had undergone two previous hospitalizations without clinical benefit. No infections or fever were reported.

History of past illness

The patient had a free previous medical history. In the past, he had never suffered from jaundice.

Personal and family history

There was no history of drug exposure, blood transfusions or alcohol intake. Family history was negative for similar complaints. Mother had never reported intrahepatic cholestasis of pregnancy during his two pregnancies.

Physical examination upon admission

On physical examination, the patient showed an evident jaundice, widespread scratch marks over the whole body, hepatomegaly (1 cm below the right costal margin) without clinical signs of liver failure (ascites, edema, encephalopathy).

Laboratory examinations

Laboratory investigations showed low hemoglobin (10 g/dL) and mean corpuscular volume (78.1 fl) with normal white blood cell (6330/microL) and platelet (295000/microL) count. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were normal. Markers of cholestasis showed normal GGT, alkaline phosphatase (ALP) 2.9 times upper normal limit and total bilirubin value of 32.70 mg/dL (conjugated fraction: 28.59 mg/dL). Markers of hepatic parenchymal activity showed hypoalbuminemia (1.9 g/dL) with normal values of international normalized ratio of prothrombin time (0.94), Partial thromboplastin time (ratio: 1.03; normal: < 1.20), coagulation factor V, ammonium, cholinesterase, blood sugar and cholesterol levels. Other altered biochemical tests were: low vitamin D level (14 ng/mL; normal: 30-100 ng/mL), mild elevation of amylase and lipase (1.1 times and 1.4 times upper normal limit, respectively), reduced renal function test (estimated Glomerular filtration rate - eGFR 67 mL/min), reduced serum potassium (3.1 mmol/L).

Serological investigations excluded viral, metabolic and autoimmune causes of acute and chronic liver diseases. Negative results were found for the following parameters: IgM antibodies to hepatitis A virus, hepatitis B surface antigen, hepatitis B virus DNA, hepatitis C virus RNA, antibodies to hepatitis C virus, serum levels of iron, transferrin, and ferritin, serum copper and ceruloplasmin levels, alpha$_1$-antitrypsin, anti-nuclear, anti-smooth muscle, anti-liver kidney microsomal anti-mitochondrial antibodies, serum protein electrophoresis and immunoglobulins (IgG, IgA, IgM).

Imaging examinations

Ultrasonographic abdominal investigation revealed mild hepatomegaly with normal echogenicity. Magnetic resonance cholangiopancreatography showed normal intra- and extra-hepatic biliary tree and pancreatic ductal system.

Histological examination

Liver biopsy showed: severe cholestasis in the centrilobular hepatocytes, involvement of intermediate zone with evidence of bile plugs as well as intra-hepatocyte bile pigment (Figure 1A), activation and hyperplasia of Kupffer's cells (Figure 1B) and preserved biliary ducts in portal areas (Figure 1C).

Genetic analyses

Being satisfied BRIC diagnostic criteria, *ATP8B1* and *ABCB11* gene analysis was performed. After obtaining the informed consent for the genetic analyses, targeted enrichment and panel parallel sequencing were performed on genomic DNA extracted from circulating leukocytes of the affected subject. Patient library

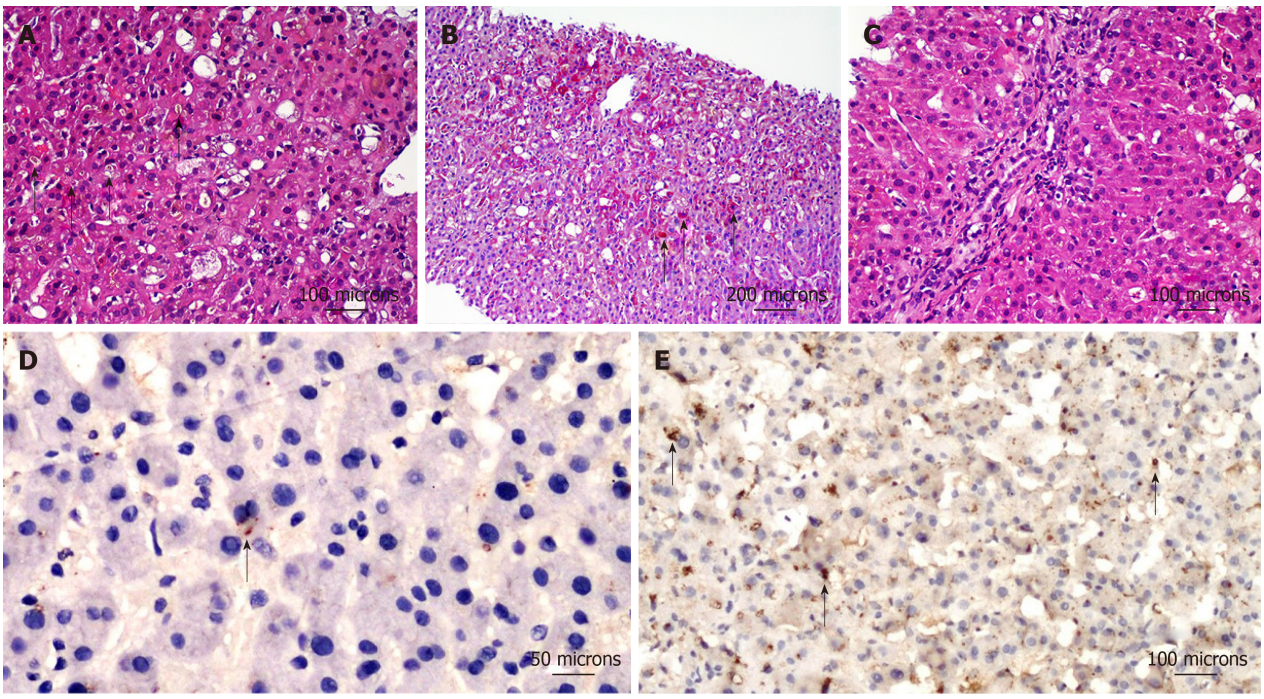

Figure 1 Histological pictures of liver biopsy and immunohistochemical analysis of *ATP8B1* gene encoded protein in liver biopsy. A: Severe cholestasis in zone 3 (centrilobular area) with evidence of bile plugs (black arrow) as well as intra-hepatocyte bile pigment [hematoxylin and eosin (HE), 200 ×]; B: Activation and hyperplasia of Kupffer's cells, which are stained red by periodic acid schiff (PAS) reaction (black arrow) and counterstained blue by haematoxylin (PAS, 100 ×); C: Portal area showing preserved biliary duct and few inflammatory cells (HE, 200 ×); D: Canalicular immunoreactivity (diaminobenzidine chromogen and hematoxylin counterstain) for *ATP8B1* gene encoded protein showing mild (black arrow) or absent staining in our benign recurrent intrahepatic cholestasis patient (400 ×); E: Normal immunohistochemical *ATP8B1* gene encoded protein (black arrow) staining in a healthy control (200 ×). Scale bars with length expressed as microns are reported.

preparation and targeted re-sequencing were performed by using the NimbleGenSeqCap Target Enrichment kit (Roche, Pleasanton, CA, United States) on a NextSeq550 (Illumina Inc, San Diego, CA, United States) platform, according to the manufacture's protocol. The BaseSpace pipeline (Illumina, https://basespace.illumina.com/) and the TGex software LifeMap Sciences (http://tgex.genecards.org/) were used for the variant calling and annotating variants, respectively. Sequencing data have been aligned to the hg19 human reference genome. The variants were analyzed in silico by using Scale-Invariant Feature Transform and Polymorphism Phenotyping v2 (http://genetics.bwh.harvard.edu/pph2) for the prediction of deleterious non-synonymous SNVs for human diseases. Based on the guidelines of the American College of Medical Genetics and Genomics[6], a minimum mean depth coverage of 30 × was considered suitable for analysis. Variants were examined for coverage and Qscore (minimum threshold of 30), and visualized by the Integrative Genome Viewer. Clinical investigations and genetic analyses were approved by the institutional scientific board of the institutes involved, and were conducted in accordance with the Helsinki Declaration.

FINAL DIAGNOSIS

Sequencing analysis identified a heterozygous nonsense variant, c.1558A>T, in exon14 of *ATP8B1* gene (NM_005603). This variant has never been described before, and is not present in dbSNP, Exome Variant Server, or ExAC databases. The finding was confirmed by Sanger sequencing following a standard protocol (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems by Life Technologies) and tested for familial segregation, showing a maternal inheritance. According to the American College of Medical Genetics and Genomics criteria, the c.1558A>T (p.Lys520Ter) variant can be classified as likely pathogenic. Figure 2 reports pedigrees and electropherograms of *ATP8B1* c.1558A>T variant, which were identified in the patient family.

To confirm the pathogenetic role of observed mutation, immunohistochemical study was performed with anti-ATP8B1 Antibody (rabbit polyclonal antibody - HPA018674, 1:20 working dilution, Atlas Antibodies, Stockholm, Sweden). This

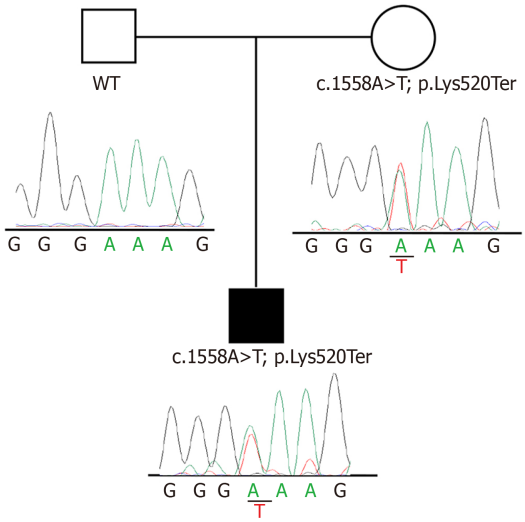

Figure 2 Pedigree and electropherograms of *ATP8B1* c.1558A>T variant identified in the family of the BRIC patient.

antibody has a specific affinity for the protein codified by *ATP8B1* gene (https://www.atlasantibodies.com/products/antibodies/primary-antibodies/triple-a-polyclonals/atp8b1-antibody-hpa018674/). The protein expression in the liver of the patient with c.1558A>T mutation was significantly reduced (Figure 1D) as compared to that of a healthy control (Figure 1E).

TREATMENT

The patient was treated with ursodeoxycholic acid 300 mg thrice daily (15 mg/kg per day) and colestyramine 4 g twice daily with a clinical improvement. After 15 d, total bilirubin decreased to 12.38 mg/dL which was near to normal values after 2 mo of therapy (1.99 mg/dL) with a complete normalization at the 6th and 12th mon.

OUTCOME AND FOLLOW-UP

A 24-mo follow up did not develop any further episode of jaundice; laboratory investigations showed normal values of hemoglobin (13 g/dL), AST, ALT, GGT, ALP and total bilirubin (1.32 mg/dL) at this time. Figure 3 summarizes the course of most significant laboratory tests and a trend chart with the therapeutic process.

DISCUSSION

BRIC is a self-limiting disorder. There is no progression to cirrhosis: liver histology remains normal during remission. Therefore, it does not cause chronic liver disease. The knowledge of this entity is crucial as an early recognition can suggest the optimal prognostic and therapeutic approach.

Review of the literature: general overview

The first report of BRIC dates back to 1956. Its estimated incidence is approximately 1 in 50000 to 100000 people worldwide[1]. It is due to an autosomal recessive disorder even if two subtypes are known according to respective associated gene mutations, *i.e.*, BRIC-1 (mutation in the *ATP8B1* gene)[3] and BRIC-2 (mutation in the *ABCB11* gene)[4]. These mutations induce an impaired function of hepato-canalicular transporters for the secretion of bile components with a consequent intrahepatic cholestasis. Mutations in *ATP8B1* and *ABCB11* genes are also involved in a PFIC - type 1 and 2 characterized by a liver damage up to end-stage liver disease[5]. BRIC diagnostic criteria proposed by Luketic and Shiffman represent the basics in the diagnosis of the disorder[1]. Indeed, rare cases of BRIC that subsequently transitioned to a persistent progressive form of the disease (PFIC) have been described, thus suggesting the possibility of a clinical continuum among the different phenotypic

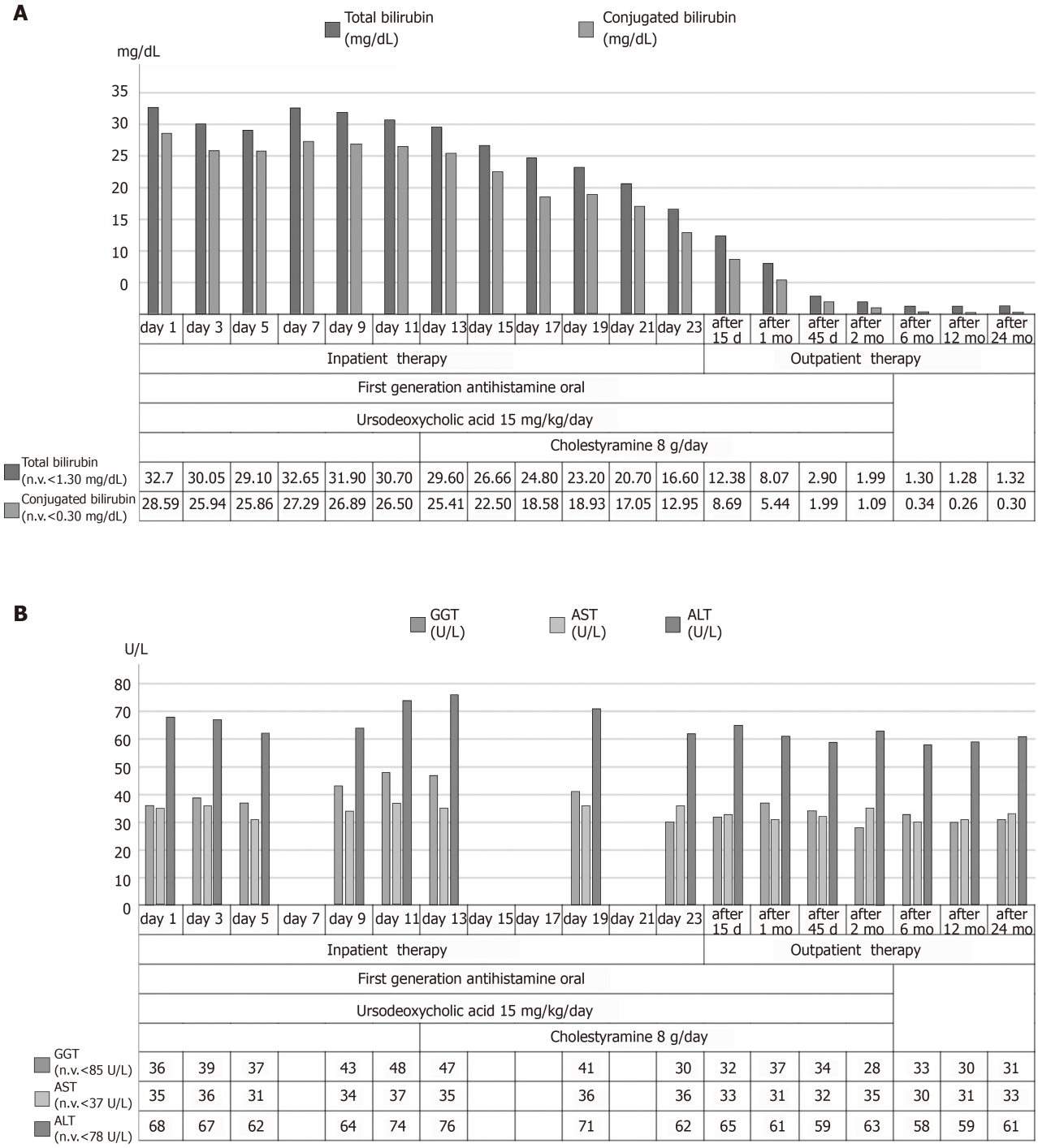

Figure 3 Course of most significant laboratory tests and trend chart with the therapeutic process. A: Total and conjugated bilirubin values, expressed as mg/dL; B: Aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltranspeptidase, expressed as U/L. n.v.: Normal values; GGT: Gamma-glutamyltranspeptidase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

expressions due to gene defect[7]. Since both diseases (BRIC and PFIC) are referred to *ATP8B1* and *ABCB11* deficiency, as described by van der Woerd *et al*[5], the central role of genetic abnormalities is evident and molecular analysis may be useful both to predict the disease phenotype and determine a tailored treatment strategy.

Our case in the context of recent advances about genetic scenario

BRIC clinical, biochemical and histological pattern of our patient fully agreed with the criteria of Luketic and Shiffman[1]. The finding of the nonsense variant (c.1558A>T), in exon14 of *ATP8B1* gene, associated with immunohistochemical demonstration of FIC1 deficiency, confirmed our diagnostic hypothesis. At the best of our knowledge, the mutation we found (c.1558A>T) has not been described in the scientific literature and international databases (ClinVar, Human Gene Mutation Database, Leiden Open

Variation Database). Indeed, even if BRIC is an autosomal recessive disease, a single heterozygous variant in the *ATP8B1* gene has been found in a single case of this disorder[8]. This unexpected finding has induced to hypothesize that a counter allele could be present but not detected, since some mutations (such as large deletions) may be undetectable by common methods or involve not analyzed regions (untranslated regions, upstream regulatory sequences, introns)[9]. On the other hand, the state of art demonstrates that the most common *ATP8B1* mutation identified in European BRIC patients (I661T) shows an incomplete penetrance in homozygous form, while its compound heterozygous form has been even associated to a case of PFIC-1[9]. In addition, mutation type or location correlate with disease severity[9].

In this multifaceted setting of hepato-canalicular transporter genetic disorders, an apparently autosomal dominant transmission in a family with BRIC has been recently reported[10]. These data suggest the likehood of an additional unmapped locus for autosomal recessive BRIC and PFIC[11]. To support this possibility, biallelic *MYO5B* mutations has been recently reported in patients with isolated low-GGT cholestasis[12]. This finding suggests that patients lacking mutations in both *ATP8B1* and *ABCB11* genes should be screened for additional gene mutations involved in bile secretion and transport pathways[13]. Indeed, at this regard, a recent systematic review highlighted the "expanded PFIC disorders" thus suggesting the possible involvement of genes other than already known ones, such as those related to *TJP2*, *NR1H4* (FXR protein), *MYO5B*, *USP53* and *LSR* defects, being these genes are all involved in bile transport physiology[14].

Differently from the other sequence variants of *ATP8B1* gene found in heterozygous form, we observed a "nonsense" mutation able to induce the insertion of a premature stop codon (p.Lys520Ter) and impair the production of FIC1. Additionally, this variant has never been described at the best of our knowledge, being not present in dbSNP, Exome Variant Server, or ExAC databases. Finally, a mechanistic study of its biological effect was performed by immunohistochemistry for gene codified protein deficiency and unquestionably confirmed that FIC1 level was severely lacking[15].

In this context, the mutation found in our patient perfectly fits with what known about the complex genetic scenario. Additionally, the nonsense variant was confirmed by Sanger sequencing and tested for familial segregation, showing a maternal inheritance, thus supporting the known familiarity of the disorder.

CONCLUSION

FIC refers to a heterogeneous group of autosomal recessive liver disorders, which occur worldwide. In all cases of FIC, a wide range of variations in clinical phenotypes has been observed, possibly due to the variability of the mutations and type of segregation, which have even been sporadically found as compound heterozygosity. Molecular analysis is mandatory for a correct and complete diagnosis and our case provides the novelty that the finding of a single nonsense heterozygous mutation does not exclude an inherited disorder. In this case, however, immunostaining for the corresponding encoded protein is recommended to confirm a cause-effect relationship. Therefore, the genetic heterogeneity and the complexity of the mechanisms related to the biliary flow suggest that inherited abnormalities other than those already known, could be not only at the basis of the spectrum of familial intrahepatic cholestatic disorders, but also of specific pro-cholestatic genetic predisposition, thus encouraging further investigations in this field.

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