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Complete List of Authors:	Jing, Jing; Medical School of Chinese PLA, Department of Integrative Medicine; Beijing 302 Hospital, Division of Integrative Medicine Wang, Rui-lin; Beijing 302 Hospital, Division of Integrative Medicine Zhu, Yun; Beijing 302 Hospital, Division of Integrative Medicine Niu, Ming; Beijing 302 Hospital, Institute of Chinese Herbal Medicine Wang, Li-fu; Beijing 302 Hospital, Division of Integrative Medicine Song, Xue-ai; Beijing 302 Hospital, Division of Integrative Medicine He, Ting-ting; Beijing 302 Hospital, Division of Integrative Medicine Sun, Yong-qiang; Beijing 302 Hospital, Division of Integrative Medicine Zhang, Fan; Beijing 302 Hospital, Division of Integrative Medicine Xu, Wen-tao; Beijing 302 Hospital, Division of Integrative Medicine Yu, Si-miao; Beijing 302 Hospital, Division of Integrative Medicine Guo, Yu-ming; Beijing 302 Hospital, Institute of Chinese Herbal Medicine Bai, Zhao-fang; Beijing 302 Hospital, Institute of Chinese Herbal Medicine Xiao, Xiao-he; Medical School of Chinese PLA, Department of Integrative Medicine; Beijing 302 Hospital, Institute of Chinese Herbal Medicine Wang, Jia-bo; Beijing 302 Hospital, Institute of Chinese Herbal Medicine
Keywords:	drug-induced liver injury, Herbal medicine < THERAPEUTICS, chronic liver disease, prognosis

Association between the Concurrence of Pre-existing Chronic Liver Diseases and Malignant Prognosis in Patients with Herb-induced Liver Injury: A Retrospective Observational Cohort Study

Jing Jing[1, 2†], Rui-lin Wang[2†], Yun Zhu[2], Ming Niu[3], Li-fu Wang[2], Xue-ai Song[2], Ting-ting He[2], Yong-qiang Sun[2], Fan Zhang[2], Wen-tao Xu[2], Si-miao Yu[2], Yu-ming Guo[3], Zhao-fang Bai[3], Xiao-he Xiao[1, 2*], Jia-bo Wang[3*]

[1] Department of Integrative Medicine, Medical School of Chinese PLA, Beijing 100853, China;
[2] Division of Integrative Medicine, Beijing 302 Hospital, Beijing 100039, China;
[3] Institute of Chinese Herbal Medicine, Beijing 302 Hospital, Beijing 100039, China.

[†] Jing Jing and Rui-lin Wang contributed equally to this paper.

[*] To whom correspondence should be addressed: Jia-bo Wang, China Military Institute of Chinese Medicine, 302 Military Hospital. Email: pharm_sci@126.com; Xiao-he Xiao, Medical School of Chinese PLA; China Military Institute of Chinese Medicine, 302 Military Hospital, Beijing 100039, China. Email: pharmacy302xxh@126.com. Jia-bo Wang (pharm_sci@126.com) as show in Scholar One. Jia-bo Wang and Xiao-he Xiao contributed equally to this paper.

Abstract

Objectives: Herb-induced liver injury (HILI) is a frequent concern in patients with pre-existing chronic liver diseases (CLDs). The present study aimed to evaluate the association between the concurrence of pre-existing CLDs and malignant prognosis in patients with HILI.

Design: A retrospective observational cohort study, using data from the electronic medical records; Adjusted analysis using logistic regression.

Setting: Tertiary hospital specializing in liver diseases in China.

Participants: 145 hospitalized HILI patients and 200 matched CLDs cases were assessed with respect to prognosis by comparing HILI with or without pre-existing CLDs from February 2007 to February 2017.

Primary outcome measures: Non-recoverable outcomes, including chronicity and fatality, in HILI patients with or without pre-existing CLDs, and patients with matched CLDs.

Results: Of the 7001 hospitalized patients with temporal association between liver injury and drug exposure, 5703 patients met the diagnostic criteria for drug-induced liver injury (DILI), which was attributed to *Polygonum multiflorum* Thunb. (PMT) in 145 patients. Among these HILI patients, 22.8% (33 of 145) had pre-existing CLDs, including 17 (51.5%) with alcoholic liver disease (ALD), 8 (24.2%) with non-alcoholic fatty liver disease (NAFLD), 5 (15.2%) with chronic viral hepatitis and 3 (9.1%) with autoimmune liver disease. Compared with HILI patients without pre-existing CLDs, HILI patients with pre-existing CLDs showed higher mortality (0.9% vs 9.1%, p=0.037) and higher chronicity (12.5% vs 30.3%, p=0.016). Compared with matched ALD (136 patients) or NAFLD (64 patients) patients, HILI patients with pre-existing ALD showed higher chronicity (35.3% vs 11.8%, p=0.019). Multivariate logistic regression analysis found that

concurrence of pre-existing CLDs was an independent risk factor for chronicity (OR 3.035, 95%CI: 1.115-8.259, *p*=0.030) and non-recovery (including chronicity and fatality) (OR 3.966, 95% CI: 1.501–10.477, *p*=0.005).

Conclusions: Concurrence of pre-existing CLDs could be an independent risk factor for malignant prognosis, especially chronicity, in HILI.

Key words: drug-induced liver injury; herbal medicine; chronic liver disease; prognosis

An article summary

- In the present study, the evidence for the better understanding on the association between concurrence of pre-existing chronic liver diseases and malignant prognosis of HILI was provided.
- As different drugs might have differential effects on prognosis, the HILI cases attributed to the same herb were found in order to avoid the confounding effects of different drugs.
- To investigate the effects of different pre-existing chronic liver diseases (CLDs) on the prognosis of HILI, we also enrolled patients with matched CLDs as the control group, who compared with HILI patients with pre-existing CLDs, according to the matching conditions.
- The enrolled cases involved in the study were from a racially and medically homogeneous background in China.
- Our study was limited by the single-centre nature of the study (ie, a tertiary hospital).

Introduction

Concurrence of pre-existing chronic liver diseases (CLDs) with drug-induced liver injury (DILI) is a special challenge in clinical settings, which might render the liver sensitive to drug toxicity and cause higher fatality rates[1]. For instance, a case report showed that long-term alcohol intake could potentiate the hepatotoxicity of low doses of acetaminophen[2]. In addition, it was noted that non-alcoholic fatty liver disease (NAFLD) and obesity might increase the risk for acute liver injury caused by several synthetic agents, such as methotrexate and tamoxifen, resulting in more severe liver injury[3-5]. According to published data from the Drug-Induced Liver Injury Network (DILIN), a higher total fatality rate (19.0%) occurred in patients with known pre-existing liver diseases 6 months after the onset of DILI than in those without CLDs (8.1%)[6]. However, these results in DILIN registry could be different from outcomes of DILI patients with pre-existing CLDs in China due to different spectra of CLDs and medication systems. Furthermore, no studies have tested whether the concurrence of pre-existing CLDs is a major risk factor for malignant prognosis in DILI[3].

In particular, herbal medications are frequently used as alternative or supplementary agents to conventional synthetic drugs to treat chronic diseases in low- and middle-income countries (LMICs). In previous population surveys, herbal and diet supplements (HDS) were used by one-third to one-half of the adult population in developed countries[7]. In a previous population survey of LMICs, the widespread use of traditional Chinese medicines (TCMs) was reported among 24.5% of middle-aged and older patients with chronic diseases in China[8]. However, the risk of herbal hepatotoxicity has not been fully addressed, especially in patients with pre-existing CLDs. It was reported that the herbal formula, Xiao Chai Hu Tang, caused jaundice and abnormal

liver function in a middle-aged woman with known pre-existing liver disease[9,10]. In addition, there has been also a rising trend in the use of HDS in developed countries, although they are not prescribed by physicians. Therefore, HILI coupled with pre-existing CLDs is a critical and expanding issue in most of these countries. However, knowledge about the intersection between herb-induced liver injury (HILI) and pre-existing CLDs has been largely limited.

In this study, we analyzed the clinical characteristics and prognosis of HILI, especially in patients with pre-existing CLDs from a single center in China, and we tested whether the concurrence of pre-existing CLDs was an independent risk factor for malignant prognosis in HILI.

Methods

Study design

This study aimed to investigate the clinical characteristics and prognosis of HILI in patients with pre-existing CLDs. Since different drugs might have differential effects on prognosis, we screened all hospitalized patients suspected of having DILI and found the HILI cases attributed to the same herb in order to avoid the confounding effects of different drugs. Finally, *Polygonum multiflorum* Thunb. (PMT) was found to be the most frequent herb attributed to HILI, and this herb has been widely considered to cause hepatotoxicity over the past three decades[11,12]. Then, to determine the effects of different pre-existing CLDs on the prognosis of HILI, we also enrolled patients with matched CLDs (1:8) as the control group. The matching conditions included sex, age, body mass index (BMI), type of pre-existing CLD, amount of alcohol ingested and the presence or absence of cirrhosis.

Patient and public involvement

This retrospective cohort study was performed in Beijing 302 Hospital, a tertiary hospital specializing in liver diseases in the Capital Region of China. We examined inpatients who met the diagnostic criteria for DILI or HILI from February 2007 to February 2017. Patients were excluded if they ingested synthetic agents, biological products or Chinese herbal medicines without PMT, or if they lack data from suspected agents. In this study, we also divided patients with PMT-related HILI into patients with pre-existing CLDs and those without pre-existing CLDs. Meanwhile, patients with CLDs were selected and matched with HILI patients with pre-existing CLDs by some matching conditions. The follow-up visits in eligible cases were scheduled at 6 or 12 months through telephone dictation or uploaded clinical data from EMR. The patient was defined as lost to follow-up if we were unable to contact with him or her at follow-up visit for any reason. Detailed data about demographics, medical history, clinical symptoms and clinical serological tests in all eligible patients was extracted from the electronic medical record (EMR). The study protocol was approved by the ethics committee of the 302 Military Hospital, and written informed consent was obtained from each enrolled patient, guardian or next of kin. The study flowchart is depicted in Figure 1.

Diagnostic criteria

DILI or HILI diagnosis was performed according to the ACG clinical guideline for DILI[3], which consists of three parts: (i) any recent abnormal liver biochemistry indices; and (ii) chronological use of all drugs and HDS within 6 months prior to the onset of abnormalities in liver testing; and (iii) exclusion of recent acute liver injury indicating alternative causes. Abnormal liver biochemistries should meet any of the three following conditions: (i) only a recent rise in alanine or aspartate aminotransferase (ALT or AST) ≥5 times the upper limit of normal (ULN); (ii) alkaline

phosphatase (AKP) ≥2 times ULN; (iii) jaundice [serum total bilirubin (TB) ≥2 mg/dl] and elevations of liver enzymes (ALT ≥3 ULN). For HILI patients with pre-existing CLDs, the ULN was replaced with the previously obtained baseline value prior to exposure to the suspected drugs. When assessing alternative causes of HILI, anti-hepatitis A virus IgM, hepatitis B surface antigen, anti-hepatitis B core IgM, hepatitis B virus DNA, anti-hepatitis C virus, hepatitis C virus RNA, anti-hepatitis E virus IgM and anti-hepatitis E virus IgG testing, non-hepatotropic virus infection and acute alcoholism within 3 months prior to onset were considered [3,13-16]. ALD, NAFLD, HBV and AIH were diagnosed according to clinical practice guidelines[17-22].

Procedures

In this study, we assessed clinical patterns of liver injury, causality and severity in all eligible patients. According to the Council for International Organizations of Medical Sciences scale[23], the clinical pattern of DILI, based on identified liver biochemistry abnormalities at onset after intake of suspected drugs, was defined using R values, where R=(ALT/ULN)/(ALP/ULN). Hepatocellular DILI was defined as an R value ≥5, cholestatic as R ≤2 and mixed as R>2 to R<5. Using the Roussel Uclaf Causality Assessment Method (RUCAM), a typical method for the judgment of a causal relationship between liver injury and implicated agents[24], the causality of eligible patients with HILI was classified into highly probable (≥9), probable (6-8), possible (3-5), unlikely (1-2), or excluded (≤0). According to national and international practice guidelines, the severity assessments of HILI were categorized into five grades, including mild, moderate, severe, liver failure and fatal[25,26]. Additionally, the Model for End-Stage Liver Disease (MELD) score was calculated as follows: 9.6*ln [creatinine (mg/dl)] + 3.8*ln [bilirubin (mg/dl)] + 11.2*ln (INR) + 6.4.

The discontinuance of the causal agent(s) and alcohol intake was performed in every eligible

patient at HILI or CLDs recognition, and at least 6 months of follow-up is available for those. Chronicity was considered as the elevations of ALT, AST, TB or ALP >1 ULN or hepatic imaging or histological data in line with chronicity after 6 months from the recognition of HILI or CLDs. According to detailed descriptions of the follow-up, all of the eligible patients were categorized with three current outcomes: (i) the recovery group, consisting of cases who had obtained persistent normalization of liver biochemistry after the withdrawal of implicated agent(s) over the 6-month follow-up; (ii) the chronic group, including cases with chronicity beyond 6-month follow-up; and (iii) the fatal group, including patients who underwent liver transplantation or died.

Statistics.

The data are characterized by the means ± SDs for normal distribution, the median (Q1, Q3) for abnormal distribution and the frequency distributions for categorical variables. Differences between groups in continuous variables were assessed using Student's t-test and one-way analysis of variance (ANOVA) or Wilcoxon's rank-sum test and the Kruskal-Wallis test based on test of normality and homogeneity of variance, respectively. Differences between groups in categorical variables were analyzed by the chi-squared test or Fisher's exact test, while results of multiple comparisons were corrected by the Bonferroni's correction. The identification of factors with p values less than 0.1 in univariate analysis was explored through multivariable logistic regression analysis. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated from the model coefficients and standard errors. $p<0.05$ was considered statistically significant. All of the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software, version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographics.

Of the 7001 hospitalized patients with temporal association between liver injury and drug exposure among the 193,714 inpatients with liver diseases in the liver unit at Beijing Military 302 Hospital between February 2007 and January 2017, 5703 patients met the diagnostic criteria for DILI, of whom 145 cases were attributed to PMT-related HILI (Figure S1, Table S1 and S2). Among these cases, 33 (22.76%) with HILI had pre-existing CLDs, while 112 cases (77.24%) did not have pre-existing CLDs (Figure 1). Liver biopsies were performed in 10 cases (30.30%) with pre-existing CLDs and in 60 patients (53.57%) without pre-existing CLDs to confirm the diagnosis of HILI. There was no difference in the mean ages between the HILI patients with (45.60 years old, range 21.67-86.74) or without (42.61 years old, range 8.47-70.79) pre-existing CLDs. However, HILI patients with pre-existing CLDs were more likely to be male than those without pre-existing CLDs (67.7% vs 40.2%, p=0.007) (Table 1).

Clinical characteristics

The clinical features of HILI patients with or without pre-existing CLDs are showed in Table 1. Among the 145 enrolled cases with HILI, 22.76% (33 of 145) had pre-existing CLDs, including 17 with alcoholic liver disease (ALD), 8 with non-alcoholic fatty liver disease (NAFLD), 5 with chronic viral hepatitis and 3 with autoimmune liver disease (Figure 1 and Table S3). In particular, the clinical patterns of liver biochemistry in the HILI cases with pre-existing CLDs were similar to those in HILI without pre-existing CLDs, but they were different from those in the matched CLDs patients. Nevertheless, compared to the levels in HILI patients without pre-existing CLDs, higher

levels of serum TB (at peak, median, 10.38 vs 18.75 mg/dl, p=0.008) and lower levels of serum albumin (at lowest, median, 35 vs 33 g/l, p=0.036) and cholinesterase (at lowest, 5138.79±1659.09 vs 4197.70±1969.99 U/l, p=0.007) were found in HILI patients with pre-existing CLDs. In addition, MELD scores in HILI patients with pre-existing CLDs were significantly higher than in those without pre-existing CLDs (median, 15 vs 17, p=0.038). The main presenting symptoms, including jaundice (93.9% vs 93.8%), anorexia (72.7% vs 75%), generalized weakness (72.7% vs 68.8%), nausea (51.5% vs 42.9%), abdominal discomfort (27.3% vs 31.3%) and vomiting (9.1% vs 16.1%), were all profiled and showed fewer differences in HILI patients with or without pre-existing CLDs. Further, there were no differences in comorbidities among HILI cases with or without pre-existing CLDs, except for cardiovascular disease (12.1% vs 1.8%, p=0.024) (Table S4).

To investigate the impacts of different pre-existing CLDs on HILI, we selected and analyzed two major types of pre-existing CLDs (ALD and NAFLD) in HILI patients and matched ALD or NAFLD patients with HILI patients (1:8) that who had corresponding pre-existing CLDs (Table 2 and 3). The compared results indicated that HILI patients with pre-existing ALD or NAFLD had more severe abnormalities in liver biochemistry, including ALT, AST, ALP, TB, INR, serum albumin and cholinesterase, than matched ALD or NAFLD patients (p for all <0.05). In all the enrolled HILI patients with pre-existing CLDs and in those without pre-existing CLDs, male patients accounted for a larger proportion of HILI patients with pre-existing CLDs than those without pre-existing CLD (p<0.01) (Table 1). However, HILI patients with pre-existing ALD comprised well over 50% of male patients compared with those without pre-existing CLDs (p<0.001), whereas HILI patients with pre-existing NAFLD showed no sex differences from those without pre-existing CLDs (Table 2 and 3). In contrast, BMI values were significantly higher in HILI cases with pre-existing NAFLD than in

HILI cases without pre-existing NAFLD, but there was no difference in HILI cases with or without pre-existing ALD groups (Tables 2 and 3).

Outcomes

Recorded data on clinical outcomes during follow-up visits are shown in Table 1 and Table S5. All enrolled patients with HILI or CLDs were followed up until the end of the study. Compared with HILI patients without pre-existing CLDs, HILI patients with pre-existing CLDs had more severe non-recovery outcomes, including a higher mortality rate (0.9% vs 9.1%, p=0.037) and a greater rate of chronicity (12.5% vs 27.3%, p=0.041) (Table 1). Moreover, HILI patients with pre-existing ALD had higher chronicity (11.8% vs 35.3%, p=0.038) and a lower recovery rate (88.2% vs 58.8%, p=0.011) than the matched ALD patients (Table 2). Of patients with fatal outcomes, 3 HILI patients with pre-existing CLDs and 1 HILI patient without a pre-existing CLD died because of hemorrhagic diseases, whereas all of the matched CLD patients survived. It was noted that the 3 HILI patients with pre-existing CLDs who died had accompanying by pre-existing alcohol-induced liver cirrhosis (n=1), inactive chronic virus hepatitis (n=1) and autoimmune liver disease (n=1). In the univariate logistic regression analysis, the concurrence of pre-existing CLDs was considered a significant risk factor for malignant outcomes, including non-recovery (OR 4.203, 95% CI: 1.735-10.185, p=0.001), chronicity (OR 3.043, 95% CI: 1.201-7.713, p=0.019) and fatality (OR 11.100, 95% CI: 1.114-110.584, p=0.040) (Table 4).

In the multivariate logistic regression analysis, clinically relevant variables (age and sex) and those with statistical significance (p<0.1) in the univariate analysis (pre-existing CLD, liver biochemistries and MELD score) were introduced as covariates (Table 4). As variables with known co-linearity or high correlations, the selection of one predictor for modeling was judged by

clinical practice. Multivariate logistic regression analysis showed that the concurrences of pre-existing CLDs (OR 3.966, 95% CI: 1.501-10.477, *p*=0.005) and peak ALT (OR 0.999, 95% CI: 0.998-1.000, *p*=0.022) were independently associated with non-recovery outcomes, including chronicity and fatality outcomes. In multivariate logistic regression analysis for different non-recovery outcomes, the concurrence of pre-existing CLDs was likely to be an independent risk factor for chronic outcomes of HILI (OR 3.035, 95%CI: 1.115-8.259, *p*=0.030) as well as MELD scores for fatal outcomes (OR 1.222, 95%CI: 1.052-1.421, *p*=0.009). In addition, the concurrence of pre-existing CLDs might be a potentially relevant factor with a trend close to significance for fatal outcomes of HILI (*p*=0.078).

Discussion

In LMICs, herbal medications, rather than synthetic drugs, are frequently used as alternative or supplementary agents to replace conventional synthetic drugs to treat chronic diseases due to the lower cost of TCM and limited access to conventional medicines in remote areas of LMICs[27,28]. In China, many patients are treated for chronic conditions using herbal medications. Thus, this study might partly explain why the proportion of HILI patients with pre-existing CLDs among all enrolled HILI patients from China (a LMIC) was markedly higher than the proportion of DILI patients with pre-existing CLDs among all DILI patients from the United States (a developed country) [13] (22.8% vs 9.9%, respectively). In addition, the use of HDS and the constituent ratio of HILI in DILI cohorts appeared to show increasing trends[29]. Self-medication among patients with CLDs often accounts for a proportion of herbal medication use[27]. Therefore, HILI coupled with pre-existing CLDs is a critical and expanding issue in most of these countries. However, knowledge

about the intersection between HILI and pre-existing CLDs has been limited.

In this study, we found that HILI patients with pre-existing CLDs showed higher mortality (0.9% vs 9.1%, *p*=0.037) and higher chronicity (12.5% vs 30.3%, *p*=0.016) than HILI patients without pre-existing CLDs. Multivariate logistic regression analysis illustrated that concurrence of pre-existing CLDs was an independent risk factor for chronicity (OR 3.035, 95%CI: 1.115-8.259, p=0.030) and non-recovery (including chronicity and fatality) (OR 3.966, 95% CI: 1.501–10.477, p=0.005). Thus, the concurrence of pre-existing CLDs is likely to be an independent risk factor for malignant prognosis, especially chronicity, in HILI. These results provide new insights into the clinical management of alternative treatment with herbal medications, especially in patients with pre-existing CLDs.

In addition, we noted that ALD was the primary type of pre-existing CLD involved in HILI, followed by non-alcoholic fatty liver disease (NAFLD), chronic viral hepatitis and autoimmune liver disease. In contrast, pre-existing hepatitis C or NAFLD often underlie DILI in the DILIN registry[13]. The difference between this study and the DILIN registry might be associated with the different spectra of liver diseases, medication systems and socioeconomic backgrounds. According to a retrospective nationwide analysis, the risk of acute liver injury caused by suspected agents could increase with pre-existing ALD (aOR 6.46; 95% CI: 4.53-9.21) and NAFLD (aOR 7.43; 95% CI: 3.30-16.7)[30]. Thus, herbal TCM and its products should be prudently administered to patients with pre-existing ALD or NAFLD.

Interestingly, the biochemistry patterns of HILI patients with pre-existing CLDs were similar to those of HILI patients, rather than to those of patients with corresponding CLDs. These results showed that abnormal liver biochemistries were dominated by herbal medications with potential

hepatotoxicity in HILI patients, although these HILI patients had pre-existing CLDs. For instance, HILI patients with pre-existing CLDs and those without pre-existing CLDs could have patterns of sharply increasing levels of ALT, AST, ALP and TB, while CLD patients could have trends of slightly elevated levels of these factors. Thus, the diagnosis of HILI is likely to depend on the pattern of increasing levels of liver biochemistries, especially in patients with pre-existing CLDs. However, compared with those in HILI patients without pre-existing CLDs, the peak value of serum TB and the lowest values of serum albumin and cholinesterase were more severe in HILI patients with pre-existing CLDs, most of whom were diagnosed as having a hepatocellular type of liver injury. Previous studies and Zimmerman's observations have confirmed that increased bilirubin levels and hepatocellular liver injury caused by drugs were associated with 10%-50% mortality and liver transplantation rates from liver failure[31]. More severe hypoalbuminemia and lower choline esterase activity could be explained by underlying impaired liver function due to reduced synthesis[32,33]. In a previous study of hepatotoxicity caused by active antiretroviral agents, patients with acute liver injury owing to these implicated drugs appeared to be more severe in those with chronic viral hepatitis[34]. Consequently, care should be taken to monitor and manage patients with pre-existing CLDs who digest herbal medications by either physician prescription or self-medication.

Although HILI patients with pre-existing CLDs (9.10%) in this study showed similar liver-related mortality rates as DILI patients with pre-existing CLDs (9.12%) in the DILIN registry, patients with both pre-existing CLDs and HILI were more likely to develop chronic outcomes (30.3%) than DILI patients with pre-existing CLDs (13.7%) in the DILIN study[13]. Furthermore, HILI patients with pre-existing ALD were more likely to have chronic liver diseases than matched ALD

patients. In a study of pulmonary TB patients treated with various antituberculosis drugs, multivariate analysis revealed prior alcohol consumption to be a risk factor for recurrent DILI[35]. Acute liver injury in individuals with pre-existing CLDs was hypothesized to result in severe liver injury or slower to recovery due to impaired liver regeneration[3]. Further, it might be inferred that the interaction of immunopathogenesis between emerging HILI and pre-existing CLDs promoted the exacerbation of HILI patients with pre-existing CLDs, leading to poor outcomes. It is reasonable to hypothesize that some herbs with potential hepatotoxicity, such as PMT, perhaps cause idiosyncratic DILI due to immunopathogenesis and that the active ingredients of herbal TCM induce immunological idiosyncratic hepatotoxicity by enhancing immune function in patients[36,37]. Simultaneously, advanced CLDs or even cirrhosis could lead to systematic immune dysfunction[38,39]. Therefore, patients with pre-existing CLDs following ingestion of herbal TCM should be considered, with a focus on the increased risk of HILI and its malignant prognosis.

In conclusion, HILI patients with pre-existing CLDs should receive heightened attention from healthcare professionals, pharmaceutical companies, academic institutions and the public owing to an increased risk of malignant prognosis. Although patients with pre-existing CLDs might benefit from the use of complementary and alternative medicines (CAM), especially herbal remedies, they are most likely to experience fatality or chronicity after suffering from HILI caused by herbal TCM. Consequently, providing strict monitoring and supervision of CAM, including herbal TCM, in the treatment of patients with pre-existing CLDs is crucial in LMICs. This study revealed the likelihood of a malignant prognosis in PMT-related HILI in patients with pre-existing CLDs, but it was limited by potential selection bias because of its small-sample, single-center and retrospective design. Therefore, further investigation based on multi-center and prospective

studies with big data are needed to find the distinctive characteristics, risk factors, predictors, and mechanisms underlying alternative causality and the pathogenesis of all-cause DILI with pre-existing CLDs.

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Footnotes

Jing Jing and Rui-lin Wang contributed equally.

Contributors: Jia-bo Wang, Xiao-he Xiao and Jing Jing designed the report. Jing Jing, Rui-lin Wang, Yun Zhu, Ming Niu, Li-fu Wang, Xue-ai Song, Ting-ting He, Yong-qiang Sun, Fan Zhang, Wen-tao Xu and Si-miao Yu collected the patient's clinical data; Jing Jing, Yu-ming Guo, Zhao-fang Bai analyzed the data. Jing Jing and Rui-lin Wang translated and wrote the paper. Xiao-he Xiao and Jia-bo Wang took charge of the project and amended the paper. All authors read and approved the final version of manuscript.

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Table 1. The characteristics among HILI patients with or without pre-existing CLDs.

Characteristic	Entire cohort of HILI (n=145, 100%)	HILI with pre-existing CLDs (n=33, 22.76%)	HILI without CLD (n=112, 77.24%)	*p* value
Males (%)	67 (46.2%)	22 (66.7%)	45 (40.2%)	0.007
Age (years, mean±SD)	43.29±13.68	45.60±13.04	42.61±13.85	0.272
BMI (kg/m^2, mean±SD)	23.37±3.40	24.64±3.57	23.00±3.27	0.015
Prior drug allergies (%)	11(7.6%)	1(3.0%)	10(8.9%)	0.236
Latency (days, median [IQR])	50.0(31.0,91.0)	45.0(29.5,105.0)	51.0(31.0,88.5)	0.777
Re-challenge (%)	7(4.8%)	1(3.0%)	6(5.4%)	0.499
Alcohol use† (%)	28(19.3%)	17(51.5%)	11(9.8%)	<0.001
Laboratory index in DILI recognition				
WBC (×10^9/L, median [IQR])	5.34(4.30, 6.56)	4.89(4.39,6.50)	5.37(4.23,6.56)	0.899
HGB (g/L, mean±SD)	135.59±18.20	137.82±18.72	134.94±18.08	0.426

PLT (×10^9/L, mean±SD)	213.47±71.00	196.79±78.72	218.37±68.16	0.125
Peripheral eosinophilia (×10^9/L, median [IQR])	0.16(0.10,0.28)	0.21(0.14,0.28)	0.15(0.09,0.28)	0.114
Peak values of laboratory index				
ALT (U/L, median [IQR])	1208.70(826.05,1537.00)	1276.00(806.00,1671.00)	1173.00(833.00,1472.05)	0.551
AST (U/L, median [IQR])	739.00(494.00,1051.00)	873.00(445.00,1292.50)	716.90(493.50,1041.60)	0.341
ALP (U/L, median [IQR])	179.0(141.5,215.0)	177.00(145.50,230.55)	180.00(139.50,213.50)	0.786
TB (mg/dL, median [IQR])	10.76(6.15,18.96)	18.75(7.69,25.18)	10.38(5.59,16.77)	0.008
Albumin (g/L, median [IQR])	34(31,38)	33.00(27.50,37.00)	35.00(32.00,38.00)	0.036
Cholinesterase (U/L, mean±SD)	4924.61±1772.28	4197.70±1969.99	5138.79±1659.09	0.007
INR (median [IQR])	1.07(0.98,1.02)	1.09(0.97,1.40)	1.07(0.99,1.15)	0.488
Pattern of liver injury				
HC/Chol/Mixed (%)	137/4/4	30/2/1	107/2/3	0.399

RUCAM score (median [IQR])	8(7,8)	7(6,8)	7(7,8)	0.003
Possible/probable/highly probable	9/120/16	2/30/1	7/90/15	0.275
Severity of Liver Injury‡ (% of column total)				0.120
Mild	8(5.5%)	1(3.0%)	7(6.3%)	
Moderate	18(12.4%)	2(6.1%)	16(14.3%)	
Severe	105(72.4%)	25(75.8%)	80(71.4%)	
Liver failure	10(6.9%)	2(6.1%)	8(7.1%)	
Fatal	4(2.8%)	3(9.1%)	1(0.9%)	
MELD score (median [IQR])	15(12,18)	17(13,20)	15(11,17)	0.038
Prognosis (% of column total)				
Recovery	120(82.8%)	20(60.6%)	97(86.6%)	0.001
Chronic	20(13.8%)	10(30.3%)	14(12.5%)	0.016

Fatal	5(3.4%)	3(9.1%)	1(0.9%)	0.037

† The patients with histories of alcoholism (alcohol intake of >2 drinks per day in women and >3 drinks per day in men) did not drink during the month prior to the onset of liver injury.

‡ The severity assessments of HILI were graded as follows[31,32]: mild, reversible elevations of serum ALT and/or ALP levels, TB <2.5 mg/dl and international normalized ratio (INR) <1.5; moderate elevations of serum ALT and/or ALP levels with associated TB ≥2.5 mg/dl or INR ≥1.5; severe, elevations of serum ALT and/or ALP levels and TB ≥5 mg/dl, with or without INR ≥1.5; liver failure, elevation of serum ALT and/or ALP level with TB ≥10 mg/dl or a sharp increase of 1 mg/dl per day, INR ≥1.5, with relevant ascites, hepatic encephalopathy or other organ failure related to DILI; death or liver transplantation because of DILI.

Abbreviations: ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; Chol, cholestatic; CLD, chronic liver diseases; DILI, drug-induced liver injury; HC, hepatocellular; HGB, hemoglobin; HILI, herb induced liver injury; INR, international normalized ratio; IQR, interquartile range (25-75%); MELD, Model for End-Stage Liver Disease; PLT, platelet; RUCAM, the Roussel Uclaf Causality Assessment Method; SD, standard deviation; TB, serum total bilirubin; WBC, white blood cell

Table 2 The characteristics of HILI patients with pre-existing ALD compared to those of HILI and ALD patients.

Characteristic	**HILI group (n=112)**	**HILI with pre-existing ALD group (n=17)**	**Matched ALD group (n=136)**	***p* value***	***p* value****	***p* value*****	***p* value******
Males (%)	45(40.2%)	15(88.2%)	120(88.2%)	<0.001	<0.001	<0.001	1.00
Age (years, median [IQR])	43.79(33.80, 53.41)	45.15(38.68, 56.00)	45.17(38.34, 51.65)	0.567			
BMI (kg/m^2, median [IQR])	22.48(20.45, 24.96)	24.06(20.67, 26.76)	23.51(22.10, 24.73)	0.133			
Liver cirrhosis (%)	8(7.1%)	3(17.6%)	32(23.5%)	0.001	0.480	<0.001	1.00
Peak of serum ALT (U/L, median [IQR])	1173.00(833.00,1472.05)	1389.00(911.85,1842.50)	54.50(30.00, 90.75)	<0.001	0.801	<0.001	<0.001
Peak of serum AST (U/L, median [IQR])	716.90(493.50,1041.60)	878.00(423.50,1359.00)	46.50(26.00, 91.00)	<0.001	1.00	<0.001	<0.001

Characteristic	HILI group (n=112)	HILI with pre-existing ALD group (n=17)	Matched ALD group (n=136)	*p* value*	*p* value**	*p* value***	*p* value****
Peak of serum ALP (U/L, median [IQR])	180.00(139.50,213.50)	179.00(149.50,230.55)	103.00(80.00, 165.50)	<0.001	1.00	<0.001	0.003
Peak of serum TB (mg/dL, median [IQR])	10.38(5.59, 16.77)	18.75(8.15,29.51)	1.11(0.74, 2.12)	<0.001	0.057	<0.001	<0.001
Peak of serum GGT (U/L, median [IQR])	164.00(96.25, 260.00)	160.00(113.00, 187.00)	125.00(46.75, 336.00)	0.508			
Peak of serum INR (median [IQR])	1.07(0.99, 1.15)	1.11(1.02, 1.40)	0.99(0.93, 1.10)	<0.001	0.744	<0.001	0.009
Peak of serum TC (mmol/L, median [IQR])	3.79(2.96, 4.42)	3.78(2.45, 4.44)	4.75(3.90, 5.43)	<0.001	0.957	<0.001	0.009

Characteristic	HILI group (n=112)	HILI with pre-existing ALD group (n=17)	Matched ALD group (n=136)	*p* value*	*p* value**	*p* value***	*p* value****
Peak of serum TG (mmol/L, median [IQR])	2.32(1.61, 3.25)	2.34(1.77, 3.56)	1.70(1.10, 3.01)	0.004	1.00	0.009	0.156
Laboratory index in the recognition							
Serum albumin (g/L, median [IQR])	37.00(35.00, 40.00)	35.00(30.50, 39.00)	39.00(35.00, 43.00)	0.001	0.303	0.003	0.021
Serum cholinesterase (U/L, median [IQR])	5754.50(4715.25,6615.75)	4506.00(3196.50,5561.00)	6702.00(4861.50,8223.75)	<0.001	0.018	<0.001	<0.001
Recovery (%)	97(86.6%)	10(58.8%)	120(88.2%)	0.011	0.030	1.000	0.015
Chronic (%)	14(12.5%)	6(35.3%)	16(11.8%)	0.038	0.027	0.860	0.019
Fatal (%)	1(0.9%)	1(5.9%)	0(0.0%)	0.058			

[*] The comparisons were analyzed among the 3 groups, including the DILI group, DILI with pre-existing ALD group and matched ALD group.

[**] The comparisons were analyzed between the DILI group and the DILI with pre-existing ALD group.

[***] The comparisons were analyzed between the DILI group and the matched ALD group.

[****] The comparisons were analyzed between the DILI with pre-existing ALD group and the matched ALD group.

Abbreviations: ALD, alcoholic liver disease; ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transpeptidase; HILI, herb-induced liver injury; Ig, immunoglobulin; INR, international normalized ratio; IQR, interquartile range (25-75%); SD, standard deviation; TB, serum total bilirubin; TC: total cholesterol; TG: total glyceride

Table 3 The characteristics of HILI patients with pre-existing NAFLD compared with those of HILI patients and NAFLD patients.

Characteristic	**HILI group (n=112)**	**HILI with pre-existing NAFLD group (n=8)**	**Matched NAFLD group (n=64)**	***p* value***	***p* value****	***p* value*****	***p* value******
Males (%)	45(40.2%)	4(50.0%)	24(37.5%)	0.805			
Age (years, median [IQR])	43.79(33.80,53.41)	40.27(33.81,48.39)	39.17(34.08,48.69)	0.337			
BMI (kg/m^2, median [IQR])	22.48(20.45,24.96)	27.16(25.48,28.53)	26.15(23.69,28.53)	<0.001	0.003	<0.001	1.000
Diabetes mellitus (%)	5(4.5%)	0(0.0%)	4(6.3%)	0.817			
Liver cirrhosis (%)	8(6.90%)	0(0.00%)	0(0.00%)	0.104			
Complications (%)	17(14.3%)	2(25.0%)	0(0.0%)	0.001	1.000	<0.001	0.009
Peak of serum ALT (U/L, median [IQR])	1173.00(833.00,1472.05)	1490.50(861.75,1681.50)	88.00(58.50,152.25)	<0.001	1.000	<0.001	<0.001

Characteristic	HILI group (n=112)	HILI with pre-existing NAFLD group (n=8)	Matched NAFLD group (n=64)	*p* value*	*p* value**	*p* value***	*p* value****
Peak of serum AST (U/L, median [IQR])	716.90(493.50,1041.60)	945.00(645.18,1377.75)	50.00(34.00, 75.25)	<0.001	0.468	<0.001	<0.001
Peak of serum ALP (U/L, median [IQR])	180.00(139.50,213.50)	149.00(105.25,192.75)	98.50(77.50,121.75)	<0.001	0.864	<0.001	0.045
Peak of serum TB (mg/dL, median [IQR])	10.38(5.59,16.77)	21.08(7.65,21.93)	0.74(0.57,0.90)	<0.001	0.252	<0.001	<0.001
Peak of serum GGT (U/L, median [IQR])	164.00(96.25,260.00)	209.00(196.25,257.25)	80.00(40.50,141.75)	<0.001	0.396	<0.001	<0.001
Peak of serum INR (median [IQR])	1.07(0.99,1.15)	1.21(0.97,1.40)	0.94(0.88, 0.96)	<0.001	1.000	<0.001	0.003

Characteristic	HILI group (n=112)	HILI with pre-existing NAFLD group (n=8)	Matched NAFLD group (n=64)	*p* value*	*p* value**	*p* value***	*p* value****
Peak of serum TC (mmol/L, median [IQR])	3.79(2.96,4.42)	4.18(3.76, 4.63)	5.04(4.39, 5.58)	<0.001	0.780	<0.001	0.084
Peak of serum TG (mmol/L, median [IQR])	2.32(1.61,3.25)	3.11(1.71,4.37)	2.24(1.58,3.37)	0.530	0.711	1.000	1.000
Laboratory index in the recognition							
Serum albumin (g/L, median [IQR])	37.00(35.00,40.00)	39.50(33.25,40.00)	42.00(40.00,44.00)	<0.001	1.000	<0.001	0.003
Serum cholinesterase (U/L, mean±SD)	5664.79±1613.11	5856.38±1941.11	8589.23±1254.07	<0.001	1.000	<0.001	<0.001

Characteristic	HILI group (n=112)	HILI with pre-existing NAFLD group (n=8)	Matched NAFLD group (n=64)	*p* value*	*p* value**	*p* value***	*p* value****
Recovery (%)	97(86.6%)	5(62.5%)	55(85.9%)	0.187			
Chronic (%)	14(12.5%)	3(37.5%)	9(14.1%)	0.137			
Fatal (%)	1(0.9%)	0(0.00%)	0(0.00%)	1.000			

* The comparisons were analyzed among the 3 groups, including the DILI group, DILI with pre-existing NAFLD group and matched NAFLD group.

** The comparisons were analyzed between the DILI group and the DILI with pre-existing NAFLD group.

*** The comparisons were analyzed between the DILI group and the matched NAFLD group.

**** The comparisons were analyzed between the DILI with pre-existing NAFLD group and the matched NAFLD group.

Abbreviations: ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transpeptidase; HILI, herb-induced liver injury; Ig, immunoglobulin; INR, international normalized ratio; IQR, interquartile range (25-75%); NAFLD, non-alcoholic fatty liver disease; SD, standard deviation; TB, serum total bilirubin; TC: total cholesterol; TG:

total glyceride

Table 4 Logistic regression for the prognosis of HILI with/without pre-existing CLDs caused by herbal TCM.

Parameters[‡]	Univariable				Multivariate[†]			
	OR	95%CI		*p* value	OR	95%CI		*p* value
Non-recovery[§]								
Age	1.028	0.996	1.061	0.089	1.021	0.985	1.058	0.265
Sex	0.828	0.363	1.890	0.654	0.819	0.300	2.236	0.696
BMI	1.143	1.010	1.294	0.034				
Pre-existing CLDs	4.203	1.735	10.185	0.001	3.966	1.501	10.477	0.005
Peak value of ALT	0.999	0.998	1.000	0.031	0.999	0.998	1.000	0.022
Peak value of total bilirubin	1.052	1.005	1.101	0.028				
Peak value of INR	7.708	1.986	29.923	0.003				
Lowest albumin	0.804	0.726	0.890	<0.001				
Lowest Cholinesterase	0.999	0.999	1.000	<0.001				

MELD score	1.068	0.988	1.154	0.096	1.077	0.989	1.172	0.087
Chronic								
Age	1.023	0.990	1.058	0.175	1.014	0.977	1.052	0.465
Sex	1.018	0.423	2.452	0.968	0.970	0.348	2.707	0.954
BMI	1.146	1.006	1.306	0.040				
Pre-existing CLDs	3.043	1.201	7.713	0.019	3.035	1.115	8.259	0.030
Peak value of ALT	0.999	0.998	1.000	0.078	0.999	0.998	1.000	0.102
Peak value of total bilirubin	1.025	0.976	1.075	0.323				
Peak value of INR	2.596	0.858	7.855	0.091				
Lowest albumin	0.879	0.802	0.964	0.006				
Lowest Cholinesterase	1.000	0.999	1.000	0.010				
MELD score	1.015	0.936	1.100	0.727	1.017	0.933	1.109	0.703
Fatality								
Age	1.042	0.965	1.124	0.293	1.028	0.960	1.101	0.433
Sex	0.277	0.028	2.729	0.271	0.512	0.085	3.076	0.465

BMI	1.071	0.804	1.426	0.640				
Pre-existing CLDs	11.100	1.114	110.584	0.040	4.385	0.846	22.714	0.078
Peak value of ALT	0.999	0.997	1.001	0.212	0.999	0.997	1.000	0.169
Peak value of total bilirubin	1.169	1.039	1.316	0.010				
Peak value of INR	12.448	2.429	63.779	0.002				
Lowest albumin	0.545	0.328	0.904	0.019				
Lowest Cholinesterase	0.997	0.996	0.999	0.008				
MELD score	1.326	1.088	1.616	0.005	1.222	1.052	1.421	0.009

† Peak value of total bilirubin, INR and lowest serum albumin and cholinesterase were excluded for multivariate analysis.

‡ Choosing clinically relevant variables (age and sex) and those with $p<0.1$ on univariate analysis. For variables with known co-linearity or high correlations, clinical judgment was used to select one predictor for additional modeling.

§ Non-recovery outcomes involving chronic and fatal outcomes

Abbreviations: ALT, serum alanine transaminase; BMI, body mass index; CLD, chronic liver

diseases; HILI, herb-induced liver injury; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; OR, odds ratio; CI, confidence interval; TCM, traditional Chinese medicine

Figure 1 Flowchart depicting the process for case enrollment.

Abbreviations: ALD, alcoholic liver disease; CMV, cytomegalovirus; DILI, drug-induced liver injury; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HILI, herb-induced liver injury; NAFLD, non-alcoholic fatty liver disease; PMT, *Polygonum multiflorum* Thunb.

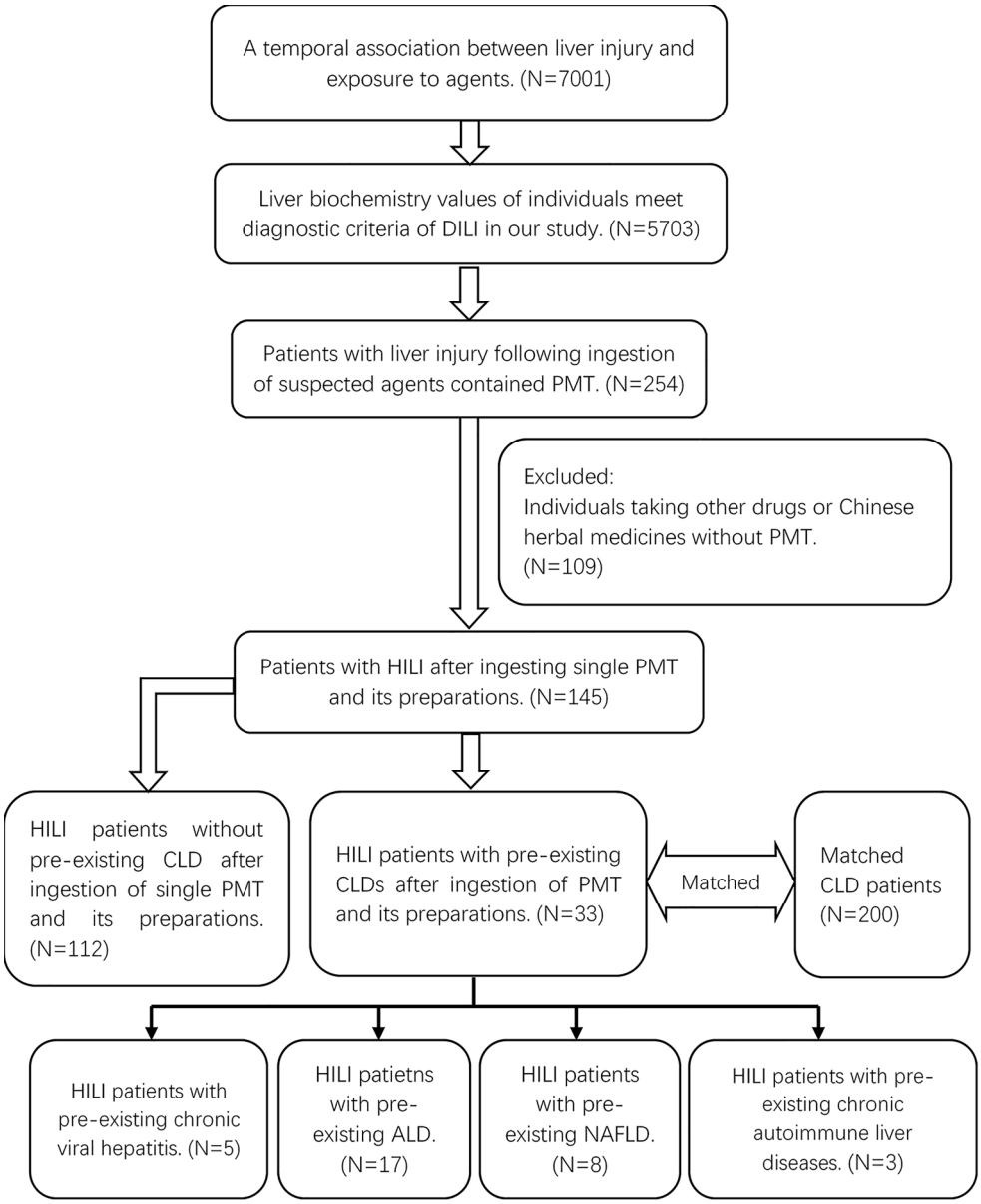

Figure 1 Flowchart depicting the process for case enrollment.

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 5-6
Objectives	3	State specific objectives, including any pre-specified hypotheses	Page 5-6
Methods			
Study design	4	Present key elements of study design early in the paper	Page 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 7-8
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Page 6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 8-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*Page 6-8*
Bias	9	Describe any efforts to address potential sources of bias	Page 6-7
Study size	10	Explain how the study size was arrived at	Page 7-8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 9
		(*b*) Describe any methods used to examine subgroups and interactions	Page 9
		(*c*) Explain how missing data were addressed	We had no missing data in this study.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	No patient loss to

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	follow-up.
		(*e*) Describe any sensitivity analyses	We did not make sensitivity analyses.
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 10, 12
		(b) Give reasons for non-participation at each stage	Page 9
		(c) Consider use of a flow diagram	Page 7, 40
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 10-12
		(b) Indicate number of participants with missing data for each variable of interest	We had no missing data.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Page 7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Page 12-13
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 12-13
		(*b*) Report category boundaries when continuous variables were categorized	Page 12-13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 10-13
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 16-17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 14-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 14-17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between the Concurrence of Pre-existing Chronic Liver Disease and Worse Prognosis in Patients with an Herb- Polygonum multiflorum Thunb. induced Liver Injury: A Case-control Study from a Specialized Liver Disease Center in China

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Complete List of Authors:	Jing, Jing; Medical School of Chinese PLA, Department of Integrative Medicine; Beijing 302 Hospital, Division of Integrative Medicine Wang, Rui-lin; Beijing 302 Hospital, Division of Integrative Medicine Zhao, Xin-yan; Capital Medical University Affiliated Beijing Friendship Hospital, Liver Research Center Zhu, Yun; Beijing 302 Hospital, Division of Integrative Medicine Niu, Ming; Beijing 302 Hospital, Institute of Chinese Herbal Medicine Wang, Li-fu; Beijing 302 Hospital, Division of Integrative Medicine Song, Xue-ai; Beijing 302 Hospital, Division of Integrative Medicine He, Ting-ting; Beijing 302 Hospital, Division of Integrative Medicine Sun, Yong-qiang; Beijing 302 Hospital, Division of Integrative Medicine Xu, Wen-tao; Beijing 302 Hospital, Division of Integrative Medicine Yu, Si-miao; Beijing 302 Hospital, Division of Integrative Medicine Wang, Li-ping; Beijing 302 Hospital, Division of Integrative Medicine Guo, Yu-ming; Beijing 302 Hospital, Institute of Chinese Herbal Medicine Bai, Zhao-fang; Beijing 302 Hospital, Institute of Chinese Herbal Medicine Xiao, Xiao-he; Medical School of Chinese PLA, Department of Integrative Medicine; Beijing 302 Hospital, Institute of Chinese Herbal Medicine Wang, Jia-bo; Beijing 302 Hospital, Institute of Chinese Herbal Medicine
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Keywords:	Herbal medicine < THERAPEUTICS, chronic liver disease, prognosis, drug induced liver injury[MeSH Terms]

Association between the Concurrence of Pre-existing Chronic Liver Disease and Worse Prognosis in Patients with an Herb- *Polygonum multiflorum* Thunb. induced Liver Injury: A Case-control Study from a Specialized Liver Disease Center in China

Jing Jing[1, 2†], Rui-lin Wang[2†], Xin-yan Zhao[3], Yun Zhu[2], Ming Niu[4], Li-fu Wang[2], Xue-ai Song[2], Ting-ting He[2], Yong-qiang Sun[2], Wen-tao Xu[2], Si-miao Yu[2], Li-ping Wang[2], Yu-ming Guo[4], Zhao-fang Bai[4], Xiao-he Xiao[1, 4*], Jia-bo Wang[4*]

[1] Department of Integrative Medicine, Medical School of Chinese PLA, Beijing 100853, China;
[2] Division of Integrative Medicine, Beijing 302 Hospital, Beijing 100039, China;
[3] Liver Research Center, Beijing Friendship Hospital, Capital Medial University, Beijing, 100069, China.
[4] Institute of Chinese Herbal Medicine, Beijing 302 Hospital, Beijing 100039, China.

[†] Jing Jing and Rui-lin Wang contributed equally to this paper.

[*] To whom correspondence should be addressed: Jia-bo Wang, China Military Institute of Chinese Medicine, 302 Military Hospital. Email: pharm_sci@126.com; Xiao-he Xiao, Medical School of Chinese PLA; China Military Institute of Chinese Medicine, 302 Military Hospital, Beijing 100039, China. Email: pharmacy302xxh@126.com. Jia-bo Wang (pharm_sci@126.com) as show in Scholar One. Jia-bo Wang and Xiao-he Xiao contributed equally to this paper.

Abstract

Objectives: The present study aimed to evaluate the association between the concurrence of pre-existing chronic liver diseases (CLD) and worse prognosis in patients with HILI.

Design: A case-control study.

Setting: Tertiary hospital specializing in liver diseases in China.

Participants: 145 hospitalized HILI patients were assessed with respect to prognosis by comparing HILI with or without pre-existing CLD from February 2007 to February 2017. 25 HILI cases with pre-existing alcoholic liver disease (ALD) or non-alcoholic fatty liver disease (NAFLD) and 200 ALD or NAFLD controls matched 1:8 for sex, age (± 4 years old), body mass index (± 2 kg/m^2), the type of CLD, alcohol intake (± 5 g/d) and the presence or absence of cirrhosis.

Primary outcome measures: Mortality and chronicity in HILI patients with or without pre-existing CLD, and matched CLD patients.

Results: Of the 193714 hospitalized patients with liver diseases, 5703 patients met the diagnostic criteria for drug-induced liver injury (DILI), which was attributed to *Polygonum multiflorum* Thunb. (PMT) in 145 patients. Among these HILI patients, 22.8% (33 of 145) had pre-existing CLD, including 17 (51.5%) with ALD, 8 (24.2%) with NAFLD, 5 (15.2%) with chronic viral hepatitis and 3 (9.1%) with autoimmune liver disease. Compared with HILI patients without CLD, HILI patients with pre-existing CLD showed higher mortality (0.9% vs 9.1%, p=0.037) and higher chronicity (12.5% vs 30.3%, p=0.016). Compared with matched ALD (136 patients) or NAFLD (64 patients) patients, HILI patients with pre-existing ALD showed higher chronicity (35.3% vs 11.8%, p=0.019). Multivariate logistic regression analysis found that concurrence of pre-existing CLD was an independent risk factor for both of chronicity and mortality (OR 3.966, 95% CI: 1.501–10.477,

p=0.005), especially the chronicity (OR 3.035, 95%CI: 1.115-8.259, *p*=0.030).

Conclusions: Concurrence of pre-existing CLD could be an independent risk factor for worse prognosis, especially chronicity, in PMT-related HILI.

Key words: drug-induced liver injury; herbal medicine; chronic liver disease; prognosis

Strengths and limitations of this study

- This was a matched (1:8) case-control study in a large clinical database (n=193714) from a specialized liver disease center.
- This study focused on patients with one herb- *Polygonum multiflorum* Thunb. induced liver injury in order to avoid the confounding effects of different drugs on prognosis.
- We had simultaneously made comparisons among the three groups (HILI with CLD group, HILI without CLD group and matched CLD without HILI group) for distinguishing between HILI and CLD interactions.
- The present study was limited by the sample size, single-centre and retrospective nature of the study (ie, a tertiary hospital).

Introduction

Concurrence of pre-existing chronic liver disease (CLD) with drug-induced liver injury (DILI) is a special challenge in clinical settings, which might render the liver sensitive to drug toxicity and cause higher fatality rates[1]. For instance, a case report showed that long-term alcohol intake could potentiate the hepatotoxicity of low doses of acetaminophen[2]. In addition, non-alcoholic fatty liver disease (NAFLD) and obesity might increase the risk for acute DILI caused by several synthetic agents, such as methotrexate and tamoxifen, resulting in more severe liver injury[3-5]. According to published data from the Drug-Induced Liver Injury Network (DILIN), a higher total fatality rate (19.0%) occurred in patients with known pre-existing liver diseases 6 months after the onset of DILI than in those without CLD (8.1%)[6]. However, these results in DILIN registry could be different from outcomes of DILI patients with pre-existing CLD in China due to different spectra of CLD and medication systems. Furthermore, no studies have tested whether the concurrence of pre-existing CLD is a major risk factor for worse prognosis in DILI[3].

In particular, herbal medications are frequently used as alternative or supplementary agents to conventional synthetic drugs to treat chronic diseases in low- and middle-income countries (LMICs). In previous population surveys, herbal and dietary supplements (HDS) were used by one-third to one-half of the adult population in developed countries[7]. In a previous population survey of LMICs, the widespread use of traditional Chinese medicines (TCMs) was reported among 24.5% of middle-aged and older patients with chronic diseases in China[8]. However, the risk of herbal hepatotoxicity has not been fully addressed, especially in patients with pre-existing CLD. It was reported that the herbal formula, Xiao Chai Hu Tang, caused jaundice and abnormal liver function in a middle-aged woman with known pre-existing liver disease[9,10]. In addition, there

has been also a rising trend in the use of HDS in developed countries, although they are not prescribed by physicians. Therefore, HILI coupled with pre-existing CLD is a critical and expanding issue in most of these countries. However, knowledge about the intersection between herb-induced liver injury (HILI) and pre-existing CLD has been largely limited.

In this study, we analyzed the clinical characteristics and prognosis of HILI, especially in patients with pre-existing CLD from a single center in China, and tested whether the concurrence of pre-existing CLD was an independent risk factor for worse prognosis in HILI patients.

Methods

Study design

The case-control study included inpatients in Beijing 302 Hospital, a tertiary hospital specializing in liver diseases in the Capital Region of China, from February 2007 to February 2017. Since different drugs might have differential effects on prognosis, we found the HILI cases attributed to the same herb in order to avoid the confounding effects of different drugs. Finally, *Polygonum multiflorum* Thunb. (PMT) was found to be the most frequent herb attributed to HILI, and this herb has been widely considered to cause hepatotoxicity over the past three decades[11,12]. Then, we also divided enrolled patients with PMT-related HILI into patients with pre-existing CLD and those without CLD. To determine the effects of different pre-existing CLD on the prognosis of HILI, we selected PMT-related HILI patients with pre-existing CLD as the case group, and also identified matched CLD patients without HILI as the control group. For each case, we selected eight controls matched by sex, age (± 4 years old), body mass index (BMI) (± 2 kg/m^2), the type of CLD, the daily amount of alcohol intake (± 5 g/d) and the presence or absence of cirrhosis.

Detailed data about demographics, medical history, clinical features, laboratory tests, and histological findings in all eligible patients was extracted from the electronic medical record (EMR). The study protocol was approved by the ethics committee of the 302 Military Hospital, and written informed consent was obtained from each enrolled patient, guardian or next of kin. The study flowchart is depicted in Figure 1.

Diagnostic criteria

DILI or HILI diagnosis was performed according to the ACG clinical guideline for DILI[3], which consists of three parts: (i) any recent abnormal liver biochemistry indices; and (ii) chronological use of all drugs and HDS within 6 months prior to the onset of abnormalities in liver testing; and (iii) exclusion of recent acute liver injury indicating alternative causes. Abnormal liver biochemistries should meet any of the three following conditions: (i) only a recent rise in alanine or aspartate aminotransferase (ALT or AST) ≥5 times the upper limit of normal (ULN); (ii) alkaline phosphatase (AKP) ≥2 times ULN; (iii) jaundice [serum total bilirubin (TB) ≥2 mg/dl] and elevations of liver enzymes (ALT ≥3 ULN). For HILI patients with pre-existing CLD, the ULN was replaced with the previously obtained baseline value prior to exposure to the suspected drugs. When assessing alternative causes of HILI, cases with positive anti-hepatitis A virus IgM, anti-hepatitis B core IgM, hepatitis B virus DNA, anti-hepatitis E virus IgM and anti-hepatitis E virus IgG testing, or with non-hepatotropic virus infection, or with alcoholism within 3 months prior to onset were excluded [3,13-16].

In the case and control groups, CLD were defined as persistent liver diseases over 6 months, including ALD, NAFLD, chronic viral hepatitis (CVH) and autoimmune liver diseases (AILD). ALD was diagnosed in CLD patients with a history of excessive alcohol consumption over 5 years, ⩾

40 g/d for men and ⩾20 g/d for women, and other causes of CLDs were excluded [17]. NAFLD was diagnosed in patients with the radiographic imaging or histological findings compatible with hepatic steatosis in the absence of excessive alcohol intake and other alternative causes such as viral hepatitis, use of agents associated with hepatotoxicity, and iron overload [18]. CVH was diagnosed based on positive serologic parameters, and in this study CVH involved chronic HBV infection and chronic HCV infection [19, 20] AILD consisted of autoimmune hepatitis, primary biliary cholangitis and the overlap syndrome between both of these conditions, and it was diagnosed according to the antibody profiles and liver biopsy findings [21, 22].

Procedures

In this study, we assessed clinical patterns of liver injury, causality and severity in all eligible patients. By using R value, the ratio of ALT (as a multiple of its ULN) to ALP (as a multiple of its ULN) at onset after intake of suspected drugs [23], the clinical pattern of DILI was classified into hepatocellular (R⩾5), cholestatic (R⩽2) and mixed liver injury (2<R<5). Based on the Roussel Uclaf Causality Assessment Method (RUCAM)[24], a causal relationship between liver injury and implicated agents among eligible patients was judged as highly probable (≥9), probable (6-8), possible (3-5), unlikely (1-2), or excluded (≤0). . According to national and international practice guidelines, the severity assessments of HILI were categorized into five grades, including mild, moderate, severe, liver failure and fatal[25,26]. Additionally, the Model for End-Stage Liver Disease (MELD) score was calculated as follows: 9.6*ln [creatinine (mg/dl)] + 3.8*ln [bilirubin (mg/dl)] + 11.2*ln (INR) + 6.4.

Liver biopsies were reviewed by two hepatic pathologists, who were blinded to clinical information including patients and suspected agents. And the pathological pattern of liver injury

was classified into acute hepatitis and chronic hepatitis, acute and chronic cholestasis, and cholestatic hepatitis[27].

The discontinuance of the causal agent(s) and alcohol intake was performed in every eligible patient at HILI or CLD recognition. The follow-up visits in eligible cases were scheduled at 6 or 12 months through telephone dictation or uploaded clinical data from EMR. The patient was defined as lost to follow-up if we were unable to contact with him or her at follow-up visit for any reason. Chronicity was considered as the elevations of ALT, AST, TB or ALP >1 ULN or hepatic imaging or histological data in line with chronicity after 6 months from the recognition of HILI or CLD. According to detailed descriptions of the follow-up, all of the eligible patients were categorized with three current outcomes: (i) the recovery group, consisting of cases who had obtained persistent normalization of liver biochemistry after the withdrawal of implicated agent(s) over the 6-month follow-up; (ii) the chronic group, including cases with chronicity beyond 6-month follow-up; and (iii) the fatal group, including patients who underwent liver transplantation or died due to liver diseases.

Patient involvement

No patient was involved in setting the research question, the design of the study, or their outcome measures. Regular contact with enrolled patients was to improve the implementation of the study. Finally, no patient had advised on dissemination including describing the research and its results.

Statistics.

The data are characterized by the means ± SDs for normal distribution, the median (Q1, Q3) for

abnormal distribution and the frequency distributions for categorical variables. Differences between groups in continuous variables were assessed using Student's t-test and one-way analysis of variance (ANOVA) or Wilcoxon's rank-sum test and the Kruskal-Wallis test based on test of normality and homogeneity of variance, respectively. Differences between groups in categorical variables were analyzed by the chi-squared test or Fisher's exact test, while results of multiple comparisons were corrected by the Bonferroni's correction. The identification of factors with *p* values less than 0.1 in univariate analysis was explored through multivariable logistic regression analysis. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated from the model coefficients and standard errors. $p<0.05$ was considered statistically significant. All of the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software, version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographics.

Of the 7001 hospitalized patients with temporal association between liver injury and drug exposure among the 193,714 inpatients with liver diseases in the liver unit at Beijing Military 302 Hospital between February 2007 and January 2017, 5703 patients met the diagnostic criteria for DILI, of whom 145 cases were attributed to PMT-related HILI (Figure S1, Table S1 and S2). Among these cases, 33 (22.76%) with HILI had pre-existing CLD, while 112 cases (77.24%) did not have pre-existing CLD (Figure 1). There was no difference in the mean ages between the HILI patients with (45.60 years old, range 21.67-86.74) or without (42.61 years old, range 8.47-70.79) pre-existing CLD. However, male patients accounted for a larger proportion of HILI patients with

pre-existing CLD than those without pre-existing CLD (67.7% vs 40.2%, *p*=0.007) (Table 1).

Among enrolled patients with PMT-related HILI, 22.76% (33 of 145) had pre-existing CLD, including 17 with ALD, 8 with NAFLD, 5 with CVH and 3 with AILD (Figure 1 and Table S3). HILI patients with pre-existing ALD comprised well over 50% of male patients compared with those without pre-existing CLD (*p*<0.001), whereas HILI patients with pre-existing NAFLD implied no sex differences from those without pre-existing CLD (Table 2 and 3). In contrast, BMI values might be significantly higher in HILI cases with pre-existing NAFLD than in HILI cases without pre-existing CLD, but there was no difference in HILI cases with pre-existing ALD and those without CLD (Tables 2 and 3).

Clinical characteristics

The clinical features of HILI patients with or without pre-existing CLD are showed in Table 1. By the use of R values, 145 eligible cases were classified into hepatocellular (n=137, 94.6%), cholestatic (n=4, 2.7%) and mixed liver injury (n=4, 2.7%). Of HILI cases with and without pre-existing CLD, based on the RUCAM scale, 11.0% were considered highly probable, 82.8% were probable, 6.2% were possible, and no one was unlikely or excluded. The clinical patterns and RUCAM scales in the HILI cases with pre-existing CLD were similar to those in HILI cases without CLD.

The main presenting symptoms, including jaundice (93.9% vs 93.8%), anorexia (72.7% vs 75%), generalized weakness (72.7% vs 68.8%), nausea (51.5% vs 42.9%), abdominal discomfort (27.3% vs 31.3%) and vomiting (9.1% vs 16.1%), were all profiled and showed fewer differences in HILI patients with or without pre-existing CLD. Further, there were no differences in comorbidities among HILI cases with or without pre-existing CLD, except for cardiovascular disease (12.1% vs

1.8%, *p*=0.024) (Table S4).

Nevertheless, there were more differences in clinical and laboratory findings among HILI patients with pre-existing CLD, those without CLD, and matched CLD patients. Compared to the levels in HILI patients without CLD, higher levels of serum TB (at peak, median, 10.38 vs 18.75 mg/dl, *p*=0.008) and lower levels of serum albumin (at lowest, median, 35 vs 33 g/l, *p*=0.036) and cholinesterase (at lowest, 5138.79±1659.09 vs 4197.70±1969.99 U/l, *p*=0.007) were found in HILI patients with pre-existing CLD. In addition, MELD scores in HILI patients with pre-existing CLD were significantly higher than in those without CLD (median, 15 vs 17, *p*=0.038). To investigate the impacts of different pre-existing CLD on HILI, we selected and analyzed two major types of pre-existing CLDs (ALD and NAFLD) in HILI patients and matched ALD or NAFLD patients with HILI patients (1:8) that who had corresponding pre-existing CLD (Table 2 and 3). The compared results indicated that HILI patients with pre-existing ALD or NAFLD had more severe abnormalities in liver biochemistry, including ALT, AST, ALP, TB, INR, serum albumin and cholinesterase, than matched ALD or NAFLD patients (*p* for all <0.05).

Histological findings

In 145 enrolled PMT-related HILI patients, liver biopsies were performed in 70 cases with and without pre-existing CLD to confirm the diagnosis of HILI. The most common histological patterns were acute (58.5%) hepatitis and acute cholestasis (24.6%), followed by chronic hepatitis (15.4%), and cholestatic hepatitis (1.5%). Lobular inflammation, portal inflammation, Interface hepatitis, with typical confluent necrosis, apoptosis and neutrophils, were frequently found in over 50% of HILI cases with histologic information. Additionally, hepatocellular and/or canalicular cholestasis in 26 HILI case, all of whose clinical patterns were hepatocellular liver injury. Histological patterns

between HILI patients with and without pre-existing CLD were similar (p>0.05).

Outcomes

Recorded data on clinical outcomes during follow-up visits are shown in Table 1 and Table S5. All enrolled patients with HILI or CLD were followed up until the end of the study. In 145 patients with PMT-related HILI, 4 patients with hepatocellular (n=2) or cholestatic liver injury (n=2) died because of hemorrhagic disease, one complication of liver diseases. Four patients progressed to acute and chronic liver failure (ACLF) in 33 HILI patients with pre-existing CLD, while no one developed ACLF in 112 HILI cases without CLD. Among 33 patients with HILI and CLD, 2 patients died in 4 ACLF patients, whereas only one died in 29 patients without ACLF.

Of HILI patients with fatal outcomes, 3 HILI patients with pre-existing CLD and 1 HILI patient without a pre-existing CLD died, whereas all of the matched CLD patients survived. Compared with HILI patients without pre-existing CLD, HILI patients with pre-existing CLD had a higher mortality rate (0.9% vs 9.1%, p=0.037) and a greater rate of chronicity (12.5% vs 27.3%, p=0.041) (Table 1). Moreover, HILI patients with pre-existing ALD had higher chronicity (11.8% vs 35.3%, p=0.038) and a lower recovery rate (88.2% vs 58.8%, p=0.011) than the matched ALD patients (Table 2). In the univariate logistic regression analysis, the concurrence of pre-existing CLD was considered a significant risk factor for worse outcomes (OR 4.203, 95% CI: 1.735-10.185, p=0.001), including chronicity (OR 3.043, 95% CI: 1.201-7.713, p=0.019) and mortality (OR 11.100, 95% CI: 1.114-110.584, p=0.040) (Table 4).

In the multivariate logistic regression analysis, clinically relevant variables (age and sex) and those with statistical significance (p<0.1) in the univariate analysis (pre-existing CLD, liver biochemistries and MELD score) were introduced as covariates (Table 4). As variables with known

co-linearity or high correlations, the selection of one predictor for modeling was judged by clinical practice. Multivariate logistic regression analysis showed that the concurrences of pre-existing CLD (OR 3.966, 95% CI: 1.501-10.477, *p*=0.005) and peak ALT (OR 0.999, 95% CI: 0.998-1.000, *p*=0.022) were independently associated with worse outcomes, including chronicity and mortality . In multivariate logistic regression analysis for different worse outcomes, the concurrence of pre-existing CLD was likely to be an independent risk factor for chronic outcomes of HILI (OR 3.035, 95%CI: 1.115-8.259, *p*=0.030) as well as MELD scores for liver-related death (OR 1.222, 95%CI: 1.052-1.421, *p*=0.009). In addition, the concurrence of pre-existing CLD might be a potentially relevant factor with a trend close to significance for fatal outcomes of HILI (*p*=0.078).

Discussion

In this study, PMT-related HILI patients with pre-existing CLD showed a higher mortality rate (0.9% vs 9.1%, *p*=0.037) and a greater rate of chronicity (12.5% vs 30.3%, *p*=0.016) than those without CLD. Multivariate logistic regression analysis illustrated that concurrence of pre-existing CLD was an independent risk factor for chronic and fatal outcomes (OR 3.966, 95% CI: 1.501–10.477, *p*=0.005), especially the former (OR 3.035, 95%CI: 1.115-8.259, *p*=0.030). Thus, the concurrence of pre-existing CLD is likely to be an independent risk factor for worse prognosis, especially chronicity, in PMT-related HILI. These results provide new insights into the clinical study and management of alternative treatment with herbal medications, especially in patients with pre-existing CLD.

In LMICs, herbal medications, rather than synthetic drugs, are frequently used as alternative or supplementary agents to replace conventional synthetic drugs to treat chronic diseases due to

the lower cost of TCM and limited access to conventional medicines in remote areas of LMICs[26,28]. Thus, this study might partly explain why the proportion of PMT-related HILI patients with pre-existing CLD among all enrolled HILI patients from China (a LMIC) seemed to be markedly higher than the proportion of DILI patients with pre-existing CLD among all DILI patients from the United States (a developed country)[13] (22.8% vs 9.9%, respectively). In addition, the use of HDS and the constituent ratio of HILI in DILI cohorts appeared to show increasing trends[29]. Self-medication among patients with CLD often accounts for a proportion of herbal medication use[28]. Therefore, HILI coupled with pre-existing CLD is a critical and expanding issue in most of these countries.

In this study, we noted that ALD was the primary type of pre-existing CLD involved in PMT-related HILI, followed by NAFLD, CVH and AILD. In contrast, pre-existing hepatitis C or NAFLD often underlie DILI in the DILIN registry[13]. The difference between this study and the DILIN registry might be associated with the different spectra of liver diseases, medication systems and socioeconomic backgrounds. According to a retrospective nationwide analysis, the risk of acute DILI could increase with pre-existing ALD (aOR 6.46; 95% CI: 4.53-9.21) and NAFLD (aOR 7.43; 95% CI: 3.30-16.7)[30]. Thus, PMT and its products should be prudently administered to patients with pre-existing ALD or NAFLD.

Interestingly, the laboratory findings of HILI patients with pre-existing CLD were similar to those of HILI patients, rather than to those of patients with corresponding CLD. Additionally, histological patterns had no difference between PMT-related HILI patients with and without pre-existing CLD in this study. These results showed that abnormal liver biochemistries and histological findings were dominated by PMT and its products, although these HILI patients had

pre-existing CLD. For instance, HILI patients with pre-existing CLD and those without CLD could have patterns of sharply increasing levels of ALT, AST, ALP and TB, while CLD patients could have trends of slightly elevated levels of these factors. Thus, the diagnosis of HILI is likely to depend on the pattern of increasing levels of liver biochemistries and histological findings, especially in patients with pre-existing CLD. However, compared with those in HILI patients without CLD, the peak value of serum TB and the lowest values of serum albumin and cholinesterase were more severe in HILI patients with pre-existing CLD, most of whom were diagnosed with hepatocellular liver injury. Previous studies and Zimmerman's observations have confirmed that increased bilirubin levels and hepatocellular liver injury caused by drugs were associated with 10%-50% mortality and liver transplantation rates from liver failure[31]. More severe hypoalbuminemia and lower choline esterase activity could be explained by underlying impaired liver function due to reduced synthesis[32,33]. In a previous study of hepatotoxicity caused by active antiretroviral agents, patients with acute DILI appeared to be more severe in those with CVH[34]. Consequently, care should be taken to monitor and manage patients with pre-existing CLD who digest herbal medications by either physician prescription or self-medication.

Notably, this study showed that PMT-related HILI patients with pre-existing CLD had higher mortality and greater chronicity. Furthermore, concurrence of pre-existing CLD could be an independent risk factor for worse prognosis, especially chronicity, in PMT-related HILI. Although PMT-related HILI patients with pre-existing CLD (9.10%) in this study showed similar liver-related mortality rates as DILI patients with pre-existing CLD (9.12%) in the DILIN registry, PMT-related HILI patients with pre-existing CLD were more likely to develop chronic outcomes (30.3%) than DILI patients with pre-existing CLD (13.7%) in the DILIN study[13]. PMT-related HILI patients with

pre-existing ALD were more likely to have chronic liver diseases than matched ALD patients. In a study of pulmonary TB patients treated with various antituberculosis drugs, multivariate analysis revealed prior alcohol consumption to be a risk factor for recurrent DILI[35]. Additionally, ACLF might be increase the risk for liver related mortality in HILI patients with pre-existing CLD. In a retrospective cohort study, hepatic necrosis and hepatic encephalopathy could be significantly associated with liver related deaths in HILI caused by Ayurvedic and herbal medicines[36]. Acute DILI in individuals with pre-existing CLD was hypothesized to result in severe liver injury or slower to recovery due to impaired liver regeneration[3]. Therefore, patients with pre-existing CLD following ingestion of herbal TCM should be considered, with a focus on the increased risk of HILI and its worse prognosis.

The present study is noteworthy for several reasons. First, this was a matched (1:8) case-control study on HILI combined with CLD in a large clinical database (n=193714) from a specialized liver disease center in China. Second, the HILI cases in the present study attributed to the same herb were found in order to avoid differential effects of confounding variables (different drugs) on prognosis. Third, the comparisons were simultaneously analyzed among the three groups (HILI with CLD group, HILI without CLD group and matched CLD without HILI group) for the sake of distinguishing between HILI and CLD interactions. These methods of clinical study was rarely published in previous researches on DILI or HILI[3, 13]. Additionally, the association between concurrence of pre-existing CLD and worse prognosis of HILI was discovered in this study. In previous studies, knowledge about intersection between HILI and pre-existing CLD has been limited.

However, our study has some limitations. There were potential selection bias and recall bias

in this study because of its single-center and retrospective design. Furthermore, the present study investigated clinical characteristics and prognosis of an herb-PMT related HILI in patients with CLD, so this affected the sample size of enrolled patients and the power of our study.

In conclusion, HILI patients with pre-existing CLD should receive heightened attention owing to an increased risk of worse prognosis. Although patients with pre-existing CLD might benefit from the use of complementary and alternative medicines (CAM), especially herbal remedies, they are most likely to experience fatality or chronicity after suffering from HILI caused by herbal TCM. Therefore, providing strict monitoring and supervision of CAM, including herbal TCM, in the treatment of patients with pre-existing CLD is crucial in LMICs. Based on the present research design, further large samples, multi-center and prospective studies are needed to find the distinctive characteristics, risk factors, and predictors of prognosis in all-cause DILI patients with pre-existing CLD.

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Footnotes

Jing Jing and Rui-lin Wang contributed equally.

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Table 1. The characteristics among PMT-related HILI patients with or without pre-existing CLD.

Characteristic	Entire cohort of HILI (n=145, 100%)	HILI with pre-existing CLD (n=33, 22.76%)	HILI without CLD (n=112, 77.24%)	*p* value
Males (%)	67 (46.2%)	22 (66.7%)	45 (40.2%)	0.007
Age (years, mean±SD)	43.29±13.68	45.60±13.04	42.61±13.85	0.272
BMI (kg/m^2, mean±SD)	23.37±3.40	24.64±3.57	23.00±3.27	0.015
Prior drug allergies (%)	11(7.6%)	1(3.0%)	10(8.9%)	0.236
Latency (days, median [IQR])	50.0(31.0,91.0)	45.0(29.5,105.0)	51.0(31.0,88.5)	0.777
Re-challenge (%)	7(4.8%)	1(3.0%)	6(5.4%)	0.499
Alcohol use† (%)	28(19.3%)	17(51.5%)	11(9.8%)	<0.001
Laboratory index in DILI recognition				
WBC (×10^9/L, median [IQR])	5.34(4.30, 6.56)	4.89(4.39,6.50)	5.37(4.23,6.56)	0.899
HGB (g/L, mean±SD)	135.59±18.20	137.82±18.72	134.94±18.08	0.426

PLT (×10^9/L, mean±SD)	213.47±71.00	196.79±78.72	218.37±68.16	0.125
Peripheral eosinophilia (×10^9/L, median [IQR])	0.16(0.10,0.28)	0.21(0.14,0.28)	0.15(0.09,0.28)	0.114
Peak values of laboratory index				
ALT (U/L, median [IQR])	1208.70(826.05,1537.00)	1276.00(806.00,1671.00)	1173.00(833.00,1472.05)	0.551
AST (U/L, median [IQR])	739.00(494.00,1051.00)	873.00(445.00,1292.50)	716.90(493.50,1041.60)	0.341
ALP (U/L, median [IQR])	179.0(141.5,215.0)	177.00(145.50,230.55)	180.00(139.50,213.50)	0.786
TB (mg/dL, median [IQR])	10.76(6.15,18.96)	18.75(7.69,25.18)	10.38(5.59,16.77)	0.008
Albumin (g/L, median [IQR])	34(31,38)	33.00(27.50,37.00)	35.00(32.00,38.00)	0.036
Cholinesterase (U/L, mean±SD)	4924.61±1772.28	4197.70±1969.99	5138.79±1659.09	0.007
INR (median [IQR])	1.07(0.98,1.02)	1.09(0.97,1.40)	1.07(0.99,1.15)	0.488
Pattern of liver injury				
HC/Chol/Mixed (%)	137/4/4	30/2/1	107/2/3	0.399

RUCAM score (median [IQR])	8(7,8)	7(6,8)	8(7,8)	0.003
Possible/probable/highly probable	9/120/16	2/30/1	7/90/15	0.275
Severity of Liver Injury‡ (% of column total)				0.120
Mild	8(5.5%)	1(3.0%)	7(6.3%)	
Moderate	18(12.4%)	2(6.1%)	16(14.3%)	
Severe	105(72.4%)	25(75.8%)	80(71.4%)	
Liver failure	10(6.9%)	2(6.1%)	8(7.1%)	
Fatal	4(2.8%)	3(9.1%)	1(0.9%)	
MELD score (median [IQR])	15(12,18)	17(13,20)	15(11,17)	0.038
Prognosis (% of column total)				
Recovery	120(82.8%)	20(60.6%)	97(86.6%)	0.001
Chronic	20(13.8%)	10(30.3%)	14(12.5%)	0.016

Fatal	5(3.4%)	3(9.1%)	1(0.9%)	0.037

† The patients with histories of excessive alcohol use (alcohol intake of ≥40 g/d for men and ≥20 g/d for women) did not drink during three months prior to the onset of liver injury.

‡ The severity assessments of HILI were graded as follows[31,32]: mild, reversible elevations of serum ALT and/or ALP levels, TB <2.5 mg/dl and international normalized ratio (INR) <1.5; moderate elevations of serum ALT and/or ALP levels with associated TB ≥2.5 mg/dl or INR ≥1.5; severe, elevations of serum ALT and/or ALP levels and TB ≥5 mg/dl, with or without INR ≥1.5; liver failure, elevation of serum ALT and/or ALP level with TB ≥10 mg/dl or a sharp increase of 1 mg/dl per day, INR ≥1.5, with relevant ascites, hepatic encephalopathy or other organ failure related to DILI; death or liver transplantation because of DILI.

Abbreviations: ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; Chol, cholestatic; CLD, chronic liver diseases; DILI, drug-induced liver injury; HC, hepatocellular; HGB, hemoglobin; HILI, herb induced liver injury; INR, international normalized ratio; IQR, interquartile range (25-75%); MELD, Model for End-Stage Liver Disease; PMT, *Polygonum multiflorum* Thunb.; PLT, platelet; RUCAM, the Roussel Uclaf Causality Assessment Method; SD, standard deviation; TB, serum total bilirubin; WBC, white blood cell

Table 2 The characteristics of PMT-related HILI patients with pre-existing ALD compared to those of PMT-related HILI patients without CLD and matched ALD patients.

Characteristic	**HILI without CLD group (n=112)**	**HILI with pre-existing ALD group (n=17)**	**Matched ALD group (n=136)**	***p* value[A]**	***p* value[B]**	***p* value[C]**	***p* value[D]**
Males (%)	45(40.2%)	15(88.2%)	120(88.2%)	<0.001	<0.001	<0.001	1.00
Age (years, median [IQR])	43.79(33.80, 53.41)	45.15(38.68, 56.00)	45.17(38.34, 51.65)	0.567			
BMI (kg/m^2, median [IQR])	22.48(20.45, 24.96)	24.06(20.67, 26.76)	23.51(22.10, 24.73)	0.133			
Liver cirrhosis (%)	8(7.1%)	3(17.6%)	32(23.5%)	0.001	0.480	<0.001	1.00
Peak of serum ALT (U/L, median [IQR])	1173.00(833.00,1472.05)	1389.00(911.85,1842.50)	54.50(30.00, 90.75)	<0.001	0.801	<0.001	<0.001
Peak of serum AST (U/L, median [IQR])	716.90(493.50,1041.60)	878.00(423.50,1359.00)	46.50(26.00, 91.00)	<0.001	1.00	<0.001	<0.001

Characteristic	HILI without CLD group (n=112)	HILI with pre-existing ALD group (n=17)	Matched ALD group (n=136)	*p* value[A]	*p* value[B]	*p* value[C]	*p* value[D]
Peak of serum ALP (U/L, median [IQR])	180.00(139.50,213.50)	179.00(149.50,230.55)	103.00(80.00, 165.50)	<0.001	1.00	<0.001	0.003
Peak of serum TB (mg/dL, median [IQR])	10.38(5.59, 16.77)	18.75(8.15,29.51)	1.11(0.74, 2.12)	<0.001	0.057	<0.001	<0.001
Peak of serum GGT (U/L, median [IQR])	164.00(96.25, 260.00)	160.00(113.00, 187.00)	125.00(46.75, 336.00)	0.508			
Peak of serum INR (median [IQR])	1.07(0.99, 1.15)	1.11(1.02, 1.40)	0.99(0.93, 1.10)	<0.001	0.744	<0.001	0.009
Peak of serum TC (mmol/L, median [IQR])	3.79(2.96, 4.42)	3.78(2.45, 4.44)	4.75(3.90, 5.43)	<0.001	0.957	<0.001	0.009

Characteristic	HILI without CLD group (n=112)	HILI with pre-existing ALD group (n=17)	Matched ALD group (n=136)	*p* value[A]	*p* value[B]	*p* value[C]	*p* value[D]
Peak of serum TG (mmol/L, median [IQR])	2.32(1.61, 3.25)	2.34(1.77, 3.56)	1.70(1.10, 3.01)	0.004	1.00	0.009	0.156
Laboratory index in the recognition							
Serum albumin (g/L, median [IQR])	37.00(35.00, 40.00)	35.00(30.50, 39.00)	39.00(35.00, 43.00)	0.001	0.303	0.003	0.021
Serum cholinesterase (U/L, median [IQR])	5754.50(4715.25,6615.75)	4506.00(3196.50,5561.00)	6702.00(4861.50,8223.75)	<0.001	0.018	<0.001	<0.001
Recovery (%)	97(86.6%)	10(58.8%)	120(88.2%)	0.011	0.030	1.000	0.015
Chronic (%)	14(12.5%)	6(35.3%)	16(11.8%)	0.038	0.027	0.860	0.019
Fatal (%)	1(0.9%)	1(5.9%)	0(0.0%)	0.058			

[A] The comparisons were analyzed among the three groups.

[B] The pairwise comparison between the PMT-related HILI group without CLD and the PMT-related HILI with pre-existing ALD group.

[C] The pairwise comparison between the PMT-related HILI group without CLD and the matched ALD group.

[D] The pairwise comparison between the PMT-related HILI with pre-existing ALD group and the matched ALD group.

[B,C,D] Differences between groups in categorical variables were analyzed by the chi-squared test or Fisher's exact test, while results of multiple comparisons were corrected by the Bonferroni's correction.

Abbreviations: ALD, alcoholic liver disease; ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; CLD, chronic liver disease; GGT, gamma-glutamyl transpeptidase; HILI, herb-induced liver injury; Ig, immunoglobulin; INR, international normalized ratio; IQR, interquartile range (25-75%); PMT, *Polygonum multiflorum* Thunb.; SD, standard deviation; TB, serum total bilirubin; TC: total cholesterol; TG: total glyceride

Table 3 The characteristics of PMT-related HILI patients with pre-existing NAFLD compared with those of PMT-related HILI patients without CLD and matched NAFLD patients.

Characteristic	**HILI without CLD group (n=112)**	**HILI with pre-existing NAFLD group (n=8)**	**Matched NAFLD group (n=64)**	***p* value[A]**	***p* value[B]**	***p* value[C]**	***p* value[D]**
Males (%)	45(40.2%)	4(50.0%)	24(37.5%)	0.805			
Age (years, median [IQR])	43.79(33.80,53.41)	40.27(33.81,48.39)	39.17(34.08,48.69)	0.337			
BMI (kg/m^2, median [IQR])	22.48(20.45,24.96)	27.16(25.48,28.53)	26.15(23.69,28.53)	<0.001	0.003	<0.001	1.000
Diabetes mellitus (%)	5(4.5%)	0(0.0%)	4(6.3%)	0.817			
Liver cirrhosis (%)	8(6.90%)	0(0.00%)	0(0.00%)	0.104			
Complications (%)	17(14.3%)	2(25.0%)	0(0.0%)	0.001	1.000	<0.001	0.009
Peak of serum ALT (U/L, median [IQR])	1173.00(833.00,1472.05)	1490.50(861.75,1681.50)	88.00(58.50,152.25)	<0.001	1.000	<0.001	<0.001

Characteristic	HILI without CLD group (n=112)	HILI with pre-existing NAFLD group (n=8)	Matched NAFLD group (n=64)	*p* value[A]	*p* value[B]	*p* value[C]	*p* value[D]
Peak of serum AST (U/L, median [IQR])	716.90(493.50,1041.60)	945.00(645.18,1377.75)	50.00(34.00, 75.25)	<0.001	0.468	<0.001	<0.001
Peak of serum ALP (U/L, median [IQR])	180.00(139.50,213.50)	149.00(105.25,192.75)	98.50(77.50,121.75)	<0.001	0.864	<0.001	0.045
Peak of serum TB (mg/dL, median [IQR])	10.38(5.59,16.77)	21.08(7.65,21.93)	0.74(0.57,0.90)	<0.001	0.252	<0.001	<0.001
Peak of serum GGT (U/L, median [IQR])	164.00(96.25,260.00)	209.00(196.25,257.25)	80.00(40.50,141.75)	<0.001	0.396	<0.001	<0.001
Peak of serum INR (median [IQR])	1.07(0.99,1.15)	1.21(0.97,1.40)	0.94(0.88, 0.96)	<0.001	1.000	<0.001	0.003

Characteristic	HILI without CLD group (n=112)	HILI with pre-existing NAFLD group (n=8)	Matched NAFLD group (n=64)	*p* value[A]	*p* value[B]	*p* value[C]	*p* value[D]
Peak of serum TC (mmol/L, median [IQR])	3.79(2.96,4.42)	4.18(3.76, 4.63)	5.04(4.39, 5.58)	<0.001	0.780	<0.001	0.084
Peak of serum TG (mmol/L, median [IQR])	2.32(1.61,3.25)	3.11(1.71,4.37)	2.24(1.58,3.37)	0.530	0.711	1.000	1.000
Laboratory index in the recognition							
Serum albumin (g/L, median [IQR])	37.00(35.00,40.00)	39.50(33.25,40.00)	42.00(40.00,44.00)	<0.001	1.000	<0.001	0.003
Serum cholinesterase (U/L, mean±SD)	5664.79±1613.11	5856.38±1941.11	8589.23±1254.07	<0.001	1.000	<0.001	<0.001

Characteristic	HILI without CLD group (n=112)	HILI with pre-existing NAFLD group (n=8)	Matched NAFLD group (n=64)	*p* value[A]	*p* value[B]	*p* value[C]	*p* value[D]
Recovery (%)	97(86.6%)	5(62.5%)	55(85.9%)	0.187			
Chronic (%)	14(12.5%)	3(37.5%)	9(14.1%)	0.137			
Fatal (%)	1(0.9%)	0(0.00%)	0(0.00%)	1.000			

[A] The comparisons were analyzed among the three groups.

[B] The pairwise comparison between the PMT-related HILI group without CLD and the PMT-related HILI with pre-existing NAFLD group.

[C] The pairwise comparison between the PMT-related HILI group without CLD and the matched NAFLD group.

[D] The pairwise comparison between the PMT-related HILI with pre-existing NAFLD group and the matched NAFLD group.

[B,C,D] Differences between groups in categorical variables were analyzed by the chi-squared test or Fisher's exact test, while results of multiple comparisons were corrected by the Bonferroni's correction.

Abbreviations: ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass

index; CLD, chronic liver disease; GGT, gamma-glutamyl transpeptidase; HILI, herb-induced liver injury; Ig, immunoglobulin; INR, international normalized ratio; IQR, interquartile range (25-75%); NAFLD, non-alcoholic fatty liver disease; PMT, *Polygonum multiflorum* Thunb.; SD, standard deviation; TB, serum total bilirubin; TC: total cholesterol; TG: total glyceride

Table 4 Logistic regression for the prognosis of PMT-related HILI with and without pre-existing CLD.

Parameters[‡]	Univariable				Multivariate[†]			
	OR	95%CI		*p* value	OR	95%CI		*p* value
Chronic								
Age	1.023	0.990	1.058	0.175	1.014	0.977	1.052	0.465
Sex	1.018	0.423	2.452	0.968	0.970	0.348	2.707	0.954
BMI	1.146	1.006	1.306	0.040				
Pre-existing CLD	3.043	1.201	7.713	0.019	3.035	1.115	8.259	0.030
Peak value of ALT	0.999	0.998	1.000	0.078	0.999	0.998	1.000	0.102
Peak value of total bilirubin	1.025	0.976	1.075	0.323				
Peak value of INR	2.596	0.858	7.855	0.091				
Lowest albumin	0.879	0.802	0.964	0.006				
Lowest Cholinesterase	1.000	0.999	1.000	0.010				

MELD score	1.015	0.936	1.100	0.727	1.017	0.933	1.109	0.703
Mortality								
Age	1.042	0.965	1.124	0.293	1.028	0.960	1.101	0.433
Sex	0.277	0.028	2.729	0.271	0.512	0.085	3.076	0.465
BMI	1.071	0.804	1.426	0.640				
Pre-existing CLD	11.10	1.114	110.584	0.040	4.385	0.846	22.714	0.078
Peak value of ALT	0.999	0.997	1.001	0.212	0.999	0.997	1.000	0.169
Peak value of total bilirubin	1.169	1.039	1.316	0.010				
Peak value of INR	12.44	2.429	63.779	0.002				
Lowest albumin	0.545	0.328	0.904	0.019				
Lowest Cholinesterase	0.997	0.996	0.999	0.008				
MELD score	1.326	1.088	1.616	0.005	1.222	1.052	1.421	0.009
Mortality and chronicity								
Age	1.028	0.996	1.061	0.089	1.021	0.985	1.058	0.265

Sex	0.828	0.363	1.890	0.654	0.819	0.300	2.236	0.696
BMI	1.143	1.010	1.294	0.034				
Pre-existing CLD	4.203	1.735	10.185	0.001	3.966	1.501	10.477	0.005
Peak value of ALT	0.999	0.998	1.000	0.031	0.999	0.998	1.000	0.022
Peak value of total bilirubin	1.052	1.005	1.101	0.028				
Peak value of INR	7.708	1.986	29.923	0.003				
Lowest albumin	0.804	0.726	0.890	<0.001				
Lowest Cholinesterase	0.999	0.999	1.000	<0.001				
MELD score	1.068	0.988	1.154	0.096	1.077	0.989	1.172	0.087

† Peak value of total bilirubin, INR and lowest serum albumin and cholinesterase were excluded for multivariate analysis.

‡ Choosing clinically relevant variables (age and sex) and those with $p<0.1$ on univariate analysis. For variables with known co-linearity or high correlations, clinical judgment was used to select one predictor for additional modeling.

Abbreviations: ALT, serum alanine transaminase; BMI, body mass index; CI, confidence interval;

CLD, chronic liver disease; HILI, herb-induced liver injury; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; OR, odds ratio; PMT, *Polygonum multiflorum* Thunb.; TCM, traditional Chinese medicine

Figure 1 Flowchart depicting the process for case enrollment.

Abbreviations: ALD, alcoholic liver disease; CMV, cytomegalovirus; DILI, drug-induced liver injury; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HILI, herb-induced liver injury; NAFLD, non-alcoholic fatty liver disease; PMT, *Polygonum multiflorum* Thunb.

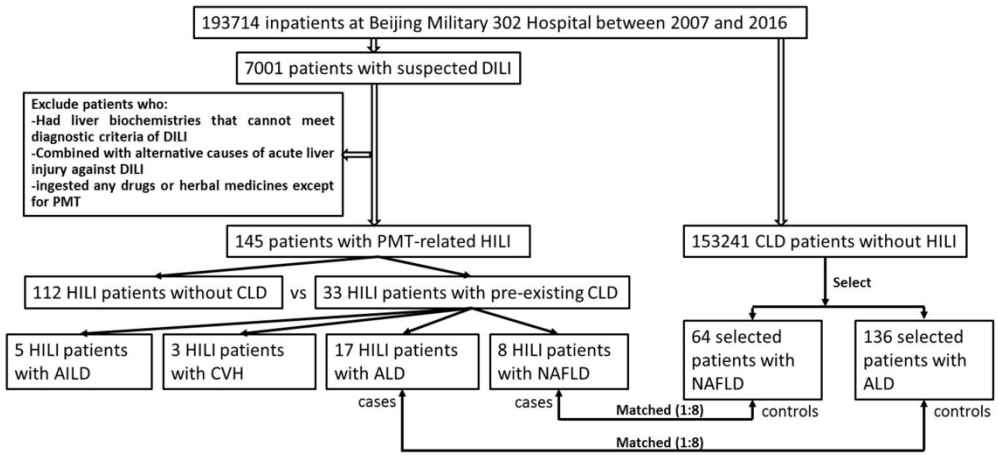

Figure 1 Flowchart depicting the process for case enrollment.
Abbreviations: ALD, alcoholic liver disease; CMV, cytomegalovirus; DILI, drug-induced liver injury; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HILI, herb-induced liver injury; NAFLD, non-alcoholic fatty liver disease; PMT, Polygonum multiflorum Thunb.

325x148mm (300 x 300 DPI)

Title: Association between the Concurrence of Pre-existing Chronic Liver Disease and Worse Prognosis in Patients with an Herb- *Polygonum multiflorum* Thunb. induced Liver Injury: A Case-control Study from a Specialized Liver Disease Center in China

Jing Jing[1, 2†], Rui-lin Wang[2†], Xin-yan Zhao[3], Yun Zhu[2], Ming Niu[4], Li-fu Wang[2], Xue-ai Song[2], Ting-ting He[2], Yong-qiang Sun[2], Wen-tao Xu[2], Si-miao Yu[2], Li-ping Wang[2], Yu-ming Guo[4], Zhao-fang Bai[4], Xiao-he Xiao[1, 2*], Jia-bo Wang[4*]

[1] Department of Integrative Medicine, Medical School of Chinese PLA, Beijing 100853, China;

[2] Division of Integrative Medicine, Beijing 302 Hospital, Beijing 100039, China;

[3] Liver Research Center, Beijing Friendship Hospital, Capital Medial University, Beijing, 100069, China.

[4] Institute of Chinese Herbal Medicine, Beijing 302 Hospital, Beijing 100039, China.

[†] Jing Jing and Rui-lin Wang contributed equally to this paper.

[*] To whom correspondence should be addressed: Jia-bo Wang, Institute of Chinese Herbal Medicine, Beijing 302 Hospital. Email: pharm_sci@126.com; Xiao-he Xiao, Department of Integrative Medicine, Medical School of Chinese PLA, Beijing 100039, China; Institute of Chinese Herbal Medicine, Beijing 302 Hospital, Beijing 100039,

China. Email: pharmacy302xxh@126.com. Jia-bo Wang (pharm_sci@126.com) as show in Scholar One. Jia-bo Wang and Xiao-he Xiao contributed equally to this paper.

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Supplementary materials:

Table S1 Twelve types of Chinese patent medicines with PMT associated with 2 or more patients with HILI

Name of Chinese patent medicines with PMT	**Number of cases (HILI with pre-existing CLD/entire HILI)**	**Constituents**	**Indications**	**Suggestion for patients with pre-existing CLD in the label**	**Hepatotoxic information**
Yang-xue-sheng-fa capsule	2/14	*Radix rehmanniae preparata, Radix angelica sinensis, Rhizoma et radix notopterygii, Fructus chaenomelis, Rhizoma ligustici chuanxiong, Radix paeoniae alba, Semen cuscutae, Rhizoma gastrodiae, Radix polygoni*	Hair loss (n=13); Alopecia areata (n=1)	Following doctor's advice	Reaction labelled in the product instruction

		5ultiflora preparata			
Jing-wu capsule/tablet	1/7	*Radix polygoni 5ultiflora preparata, Rhizoma 5 ultiflora, Fructus ligustri lucidi, Herba ecliptae*	Hair loss (n=4); Alopecia areata (n=1); Amnesia (n=2)	None	Reaction published but unlabeled
Xin-yuan capsule	1/5	*Radix polygoni 5ultiflora preparata, Salviae miltiorrhizae*; *Rehmanniae radix*	Coronary heart disease (n=4); Hyperlipoidemia (n=1)	None	Reaction published but unlabeled
Yan-shou tablet	1/4	*Polygonum multiflorum* Thunb., *Cuscutae semen, Eucommiae cortex, Ecliptae herba, Ligustri lucidi fructus, Rehmanniae radix, Achyranthis bidentatae radix,* mulberry, *Sesami semen nigrum*, *Sojae semen nigrum*	Health improvement (n=3); Hyperlipoidemia (n=1)	None	Reaction published but unlabeled

Qi-bao-mei-ran pill/granule	0/4	*Radix polygoni 6ultiflora preparata, Cuscutae semen, Poria, Radix angelica sinensis, Lycii fructus, Achyranthis bidentatae radix, Psoraleae fructus*	Hair loss (n=4)	Following doctor's advice	Reaction published but unlabeled
Jian-yang capsule	1/4	Extracts from *Rhizoma Polygonati*, *Morindae officinalis radix*, *Lycii fructus, Ganoderma, Radix polygoni 6ultiflora preparata, Radix Notoginseng*	Health improvement (n=4)	None	Reaction unknown
Gu-shen-sheng-fa pill	1/3	*Polygonum multiflorum* Thunb., *Lycii fructus, Notoptergii 6 ultifl et radix, Radix Polygoni 6 hizome 6 ra praeparata, Chuanxiong 6hizome, Chaenomelis fructus, Ligustri lucidi fructus, Radix angelica sinensis,* mulberry, *Salviae miltiorrhizae*; *Codonopsis radix*,	Hair loss (n=3)	Following doctor's advice	Reaction unknown

		Sesami semen nigrum			
An-shen-bu-nao liquid	1/2	*Cervi cornu pantotrichum*, *Radix polygoni 7 ultiflora preparata, Epimedii folium, Rhizoma Zingiberis, Radix Glycyrrhizae, Jujubae fructus, Vitamin* B_1	Insomnia (n=2)	Following doctor's advice	Reaction unknown
Kun-bao pill	1/2	*Ligustri lucidi fructus, Fructus Rubi, Semen Cuscutae, Fructus Lycii, Polygonum multijiorum* Thunb., *Carapax et Plastrum Testudinis*, *Cortex Lycii, Radix Adenophorae, Radix Ophiopogonis, Semen Ziziphi Spinosae, Radix Rehmanniae, Radix Paeoniae Alba, Radix Paeoniae Rubra, Radix Angelica sinensis, Caulis Spatholobi, Concha Margaritifera, Herba Dendrobii, Flos*	Gynecologic diseases (n=2)	None	Reaction published but unlabeled

		Chrysanthemi, Herba Ecliptae, Folium Mori, Radix Cynanchi Atrati, Rhizoma Anemarrhenae, Radix Scutellariae			
Run-zhao-zhi-yang capsule	2/2	*Polygonum multijiorum* Thunb., *Polygoni Multiflori Radix Praeparata, Radix Rehmanniae, Folium Mori, Radix Sophorae Flavescentis, Honghuoma*	Skin diseases (n=2)	Inappropriate use	Reaction published but unlabeled
Shou-wu-yan-shou tablet	0/2	*Polygonum multiflorum* Thunb.	Hair loss (n=1); Insomnia (n=1)	None	Reaction labelled in the product instruction

Abbreviations used: CLD, chronic liver disease; HILI, herb-induced liver injury;.PMT, *Polygonum multiflorum* Thunb.

Table S2. Herbs combined with PMT in 6 or more patients with HILI after ingestion of single PMT and its preparations.

Names of herbs	Numbers of patients with HILI (n)	Numbers of HILI patients with pre-existing CLD (n)	Clinical report of liver reaction induced by the single herb	Effect on liver in animal/cellular experiments		Hepatotoxic information by Chinese Pharmacopeia
				Hepatotoxicity	Hepatoprotective effect	
Rehmanniae radix	40	13	No	No	Yes	No
Radix angelica sinensis	31	7	No	No	Yes	No
Cuscutae semen	26	4	No	No	No	No
Ligustri lucidi fructus	21	5	No	No	Yes	No
Paeoniae Radix	20	5	No	No	Yes	No

Alba						
Notoptergii	19	3	No	No	Yes	No
rhizoma et radix						
Chaenomelis	19	3	No	No	No	No
fructus						
Chuanxiong	19	3	No	No	No	No
rhizoma						
Gastrodiae	17	2	No	No	No	No
Rhizoma						
Ecliptae Herba	17	4	No	No	No	No
Lycii fructus	17	5	No	No	No	No
Salviae	15	5	Yes	Yes	No	No
miltiorrhizae						
Polygonati	12	3	No	No	No	No

rhizoma						
Sesami semen nigrum	12	4	No	No	No	No
Achyranthis bidentatae radix	10	1	No	No	Yes	No
Poria	9	2	Yes	No	Yes	No
Mulberry	8	2	No	No	No	No
Sojae Semen Nigrum	7	1	No	No	No	No
Glycyrrhizae radix et rhizoma	7	2	No	No	Yes	No
Morindae officinalis radix	6	2	No	No	No	No

Abbreviations: CLD, chronic liver disease; HILI, herb-induced liver injury; PMT, *Polygonum multiflorum* Thunb.

Table S3. The clinical characteristics of all HILI patients with pre-existing CLD.

Characteristics	**HILI patients with pre-existing ALD (n=17)**	**HILI patients with pre-existing NAFLD (n=8)**	**HILI patients with pre-existing chronic viral hepatitis (n=5)**	**HILI patients with pre-existing autoimmune liver disease (n=3)**
Males (%)	15(88.2%)	4(50.0%)	3(60.0%)	0(0.0%)
Age (years, mean±SD)	45.10±11.51	41.12±8.55	48.94±22.47	54.81±12.52
BMI (kg/m^2, mean±SD)	24.13±3.60	26.71±2.19	24.66±2.49	21.91±6.33
Latency (day, median [IQR])	45.00(29.50,69.50)	29.00(21.00,156.00)	72.00(36.00,601.00)	133.00(45.00,133.00)
Peripheral eosinophilia (×10^9/L, mean±SD)	0.23±0.09	0.25±1.45	0.19±0.16	0.10±0.06
Positive autoantibody	2(11.8%)	5(62.5%)	1(20.0%)	3(100.0%)
Peak values of laboratory index				
ALT (U/L, mean±SD)	1469.36±810.41	1336.90±494.26	1328.60±515.81	264.67±220.76

AST (U/L, mean±SD)	924.75±586.88	978.36±481.11	855.40±493.82	331.67±243.55
ALP (U/L, mean±SD)	185.63±62.21	169.64±98.69	202.00±68.02	235.67±88.12
GGT (U/L, median [IQR])	160.00(113.00,187.00)	209.00(196.25,257.25)	141.00(76.00,240.00)	231(175.00,231.00)
TB (mg/dL, mean±SD)	18.96±11.54	17.63±9.83	16.00±13.56	13.27±8.46
INR (median, [IQR])	1.11(1.02,1.40)	1.21(0.97,1.40)	0.91(0.90,2.13)	1.20(0.91,1.20)
TC (mmol/L, mean±SD)	3.62±1.52	4.39±1.27	3.50±0.95	4.17±2.15
TG (mmol/L, mean±SD)	2.61±1.12	3.32±1.99	2.68±1.10	2.20±0.67
Pattern of liver injury				
HC/Chol/Mixed	17/0/0	8/0/0	4/1/0	1/1/1
RUCAM score (mean±SD)	6.65±0.86	7.88±0.99	7.00±1.00	5.67±0.58
Possible/probable/highly probable	1/16/0	0/7/1	0/5/0	1/2/0
Severity of Liver Injury[†]				

Mild/Moderate/Severe/Liver failure/Fatal	0/2/12/2/1	0/0/8/0/0	1/0/3/0/1	0/0/2/0/1
MELD score (mean±SD)	16.88±5.97	17.13±5.94	13.80±4.38	17.00±4.36
Liver cirrhosis	3/17	0/8	1/5	1/3
Prognosis				
Recovery (n)	10	5	4	1
Chronic (n)	6	3	0	1
Fatal (n)	1	0	1	1

† The severity assessments of HILI were graded as follows[31,32]: mild, reversible elevations of serum ALT and/or ALP levels, TB <2.5 mg/dl and international normalized ratio (INR) <1.5; moderate elevations of serum ALT and/or ALP levels with associated TB ≥2.5 mg/dl or INR ≥1.5; severe, elevations of serum ALT and/or ALP levels and TB ≥5 mg/dl, with or without INR≥1.5; liver failure, elevation of serum ALT and/or ALP level with TB ≥10 mg/dl or a sharp increase of 1 mg/dl per day, INR ≥1.5, with relevant ascites, hepatic encephalopathy, or other organ failure related to DILI; death or liver transplantation because of DILI.

Abbreviations: ALD, alcoholic liver disease; ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; Chol, cholestatic; DILI, drug-induced liver injury; GGT, gamma-glutamyl transpeptidase; HC, hepatocellular; HILI, herb-induced liver injury; Ig, immunoglobulin; INR, international normalized ratio; IQR, interquartile range (25-75%); MELD, Model for End-Stage Liver Disease; NAFLD, non-alcoholic fatty liver disease; RUCAM, the Roussel Uclaf Causality Assessment Method; SD, standard deviation; TB, serum total bilirubin; TC, total cholesterol; TG, total glyceride

Table S4. The comorbidities of patients with HILI.

Names of the comorbidities	Total (n=145)	HILI with pre-existing CLD (n=33)	HILI without pre-existing CLD (n=112)	*p* value
Gastritis	21 (14.5%)	6 (18.2%)	15 (13.4%)	0.332
Hypertension	17 (11.7%)	4 (12.1%)	13 (11.6%)	0.573
Cholecystic diseases	9 (6.2%)	3 (9.1%)	6 (5.4%)	0.335
Infectious diseases	8 (5.5%)	4 (12.1%)	4 (3.6%)	0.079
Cardiovascular disease	6 (4.1%)	4 (12.1%)	2 (1.8%)	0.024
Diabetes mellitus	5 (3.4%)	0 (0.00%)	5 (4.5%)	0.269
Connective tissue diseases	4 (2.8%)	2 (6.1%)	2(1.8%)	0.223
Kidney diseases	3 (2.1%)	1 (3.0%)	2 (1.8%)	0.542
Thyroid diseases	3 (2.1%)	1 (3.0%)	2 (1.8%)	0.542
Osteonosus	2 (1.4%)	2 (6.1%)	0 (0.0%)	0.051

Andrology	1 (0.7%)	0 (0.0%)	1 (0.9%)	0.772
Otopathy	1 (0.7%)	0 (0.0%)	1 (0.9%)	0.772

Abbreviations: CLD, chronic liver disease; HILI, herb-induced liver injury

Table S5. Comparison of the clinical characteristics in the overall PMT-related HILI patients between the recovery group and the chronic group.

Characteristics	**Recovery group (n=119)**	**Chronic group (n=23)**	***p* value**
Males (%)	55(46.2%)	10(43.5%)	0.809
Age (years, mean±SD)	42.45±14.06	47.25±10.08	0.121
BMI (kg/m^2, mean±SD)	22.46(20.51,25.39)	24.03(22.22,28.01)	0.021
Latency (day, median [IQR])	49(31,78)	57(30,121)	0.803
Duration of drug use (day, median [IQR])	36(30,72)	31(19,122)	0.708
Alcohol use^{+} (%)	21(17.6%)	6(26.1%)	0.249
Pre-existing CLD	22(18.5%)	9(39.1%)	0.028
Peak value of ALT (U/L, median [IQR])	1247.00(874.00,1583.00)	1000.00(739.10,1477.40)	0.075
Peak value of AST (U/L, median [IQR])	739.00(493.00,1050.00)	800.00(547.00,1294.00)	0.585
Peak value of ALP (U/L, median [IQR])	176.00(141.00,214.00)	193.00(141.00,221.10)	0.958
Peak value of GGT (U/L, median [IQR])	165.00(105.00,242.00)	174.00(71.00,257.00)	0.539
Peak value of TB (mg/dL, median [IQR])	10.67(6.89,17.74)	14.57(5.18,24.86)	0.448
Peak value of INR (median	1.05(0.98,1.13)	1.15(1.01,1.46)	0.022

[IQR])			
Lowest serum albumin (g/L, median [IQR])	35.00(32.00,38.00)	30.00(25.00,37.00)	0.003
Lowest cholinesterase (U/L, mean±SD)	5199.61±1579.26	3938.26±2055.55	0.010
TC (mmol/L, median [IQR])	3.79(2.94,4.35)	3.78(2.57,4.86)	0.771
TG (mmol/L, median [IQR])	2.51(1.71,3.45)	2.03(1.22,3.17)	0.080
Laboratory index in DILI recognition			
WBC (×10^9/L, median [IQR])	5.35(4.40,6.42)	5.26(4.12,6.79)	0.998
HGB (g/L, mean±SD)	136.67±18.10	129.04±16.40	0.063
PLT (×10^9/L, mean±SD)	222.11±66.04	176.96±78.20	0.004
peripheral eosinophilia (×10^9/L, median [IQR])	0.16(0.10,0.28)	0.18(0.11,0.27)	0.831
IgA (g/L, median [IQR])	2.41(1.64,2.57)	2.57(2.01,3.25)	0.197
IgG (g/L, median [IQR])	12.70(10.00,13.30)	13.18(11.56,18.48)	0.011
IgM (g/L, median [IQR])	0.89(0.52,1.01)	0.84(0.63,1.11)	0.538
Pattern of liver injury			
HC/Chol/Mixed (%)	115/1/3	21/1/1	0.250
RUCAM score (median [IQR])	8(7,8)	7(6,8)	0.113
Possible/probable/highly	7/99/13	2/18/3	0.729

probable			
Severity of Liver Injury‡ (% of column total)			
Mild/Moderate/Sever/Liver failure/Fatal	7/14/91/7/0	1/4/14/3/1	0.119
MELD score (mean±SD)	14.41±4.77	15.61±7.20	0.451

† Patients with a history of alcoholism (alcohol intake of >2 drinks per day in women and >3 drinks per day in men) did not drink during 3 months prior to the onset of liver injury.

‡ The severity assessments of HILI were graded as follows[31,32]: Mild, reversible elevations of serum ALT and/or ALP levels, TB <2.5 mg/dl, and international normalized ratio (INR) <1.5; moderate, elevations of serum ALT and/or ALP levels with associated TB ≥2.5 mg/dl or INR ≥1.5; severe, elevations of serum ALT and (or) ALP levels and TB ≥5 mg/dl, with or without INR ≥1.5; liver failure, elevation of serum ALT and/or ALP level with TB ≥10 mg/dl or a sharp increase of 1mg/dl per day, INR ≥1.5, with relevant ascites, hepatic encephalopathy, or other organ failure related to DILI; fatal, death or liver transplantation because of DILI.

Abbreviations: ALP, serum alkaline phosphatase; ALT, serum alanine transaminase; AST, serum aspartate aminotransferase; BMI, body mass index; Chol, cholestatic; CLD, chronic liver diseases; DILI, drug-induced liver injury; HC, hepatocellular; HGB, hemoglobin; HILI, herb-induced liver injury; INR, international normalized ratio; IQR,

interquartile range (25-75%); MELD, Model for End-Stage Liver Disease; PLT, platelets; RUCAM, the Roussel Uclaf Causality Assessment Method; SD, standard deviation; TB, serum total bilirubin; TC, total cholesterol; TG, total glyceride; WBC, white blood cell

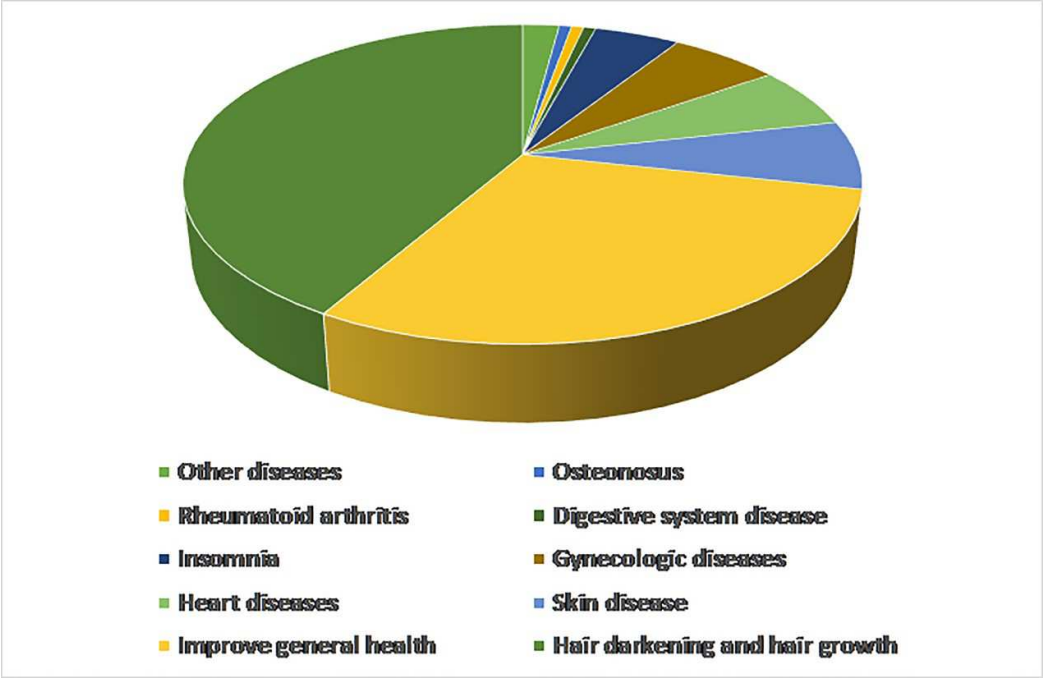

Figure S1. The indications for single PMT and its herbal products in total patients with HILI

Abbreviations: HILI, herb-induced liver injury; PMT, *Polygonum multiflorum* Thunb.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*
Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 5-6
Objectives	3	State specific objectives, including any pre-specified hypotheses	Page 5-6
Methods			
Study design	4	Present key elements of study design early in the paper	Page 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 6-7, 9
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 6-8
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Page 6-8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*Page 6-9*
Bias	9	Describe any efforts to address potential sources of bias	Page 6-7
Study size	10	Explain how the study size was arrived at	Page 7-8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 9
		(*b*) Describe any methods used to examine subgroups and interactions	Page 9
		(*c*) Explain how missing data were addressed	We had no missing data in this study.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	Page 6.

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	We did not make sensitivity analyses.
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 10, 12
		(b) Give reasons for non-participation at each stage	Page 9
		(c) Consider use of a flow diagram	Page 7, 42
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 10-13
		(b) Indicate number of participants with missing data for each variable of interest	We had no missing data.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Page 13-14
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 13-14
		(*b*) Report category boundaries when continuous variables were categorized	Page 13-14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 10-14
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 14-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 14-18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.