Herbal Medicine Containing Aristolochic Acid and the Risk of Primary Liver Cancer in Patients with Hepatitis C Virus Infection

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Disclosure of Potential Conflicts of Interest

The authors declare no potential conflicts of interest.

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Abbreviations

AA, aristolochic acid; ACE, angiotensin-converting enzyme; CI, confidence interval; HCV, hepatitis C virus; HM, herbal medicine; HR, hazard ratio; ICD-9, International Classification of Diseases, 9th Edition; NHI, National Health Insurance; NHRI, National Health Research Institutes; NSAIDs, nonsteroidal anti-inflammatory drugs; PLC, primary liver cancer.

ABSTRACT

Background: To investigate the association between taking herbal medicine (HM) containing aristolochic acid (AA) and the risk of primary liver cancer (PLC) among patients with hepatitis C virus (HCV)-infection. **Methods:** This is a prospective study for the long-term follow up of a nationwide population-based cohort of patients aged 18 years or older diagnosed with HCV infection during 1997-2010. A total of 223,467 HCV-infected patients were identified using the National Health Insurance Research Database in Taiwan. The use of HM containing AA was evaluated among patients who had visited traditional Chinese medicine (TCM) clinics beginning from 1997 to one year prior to the diagnosis of PLC or dates censored (2003). We tracked each individual patient from 1997 to 2013 to identify incident cases of PLC since 1999. **Results:** During the follow-up period of 3,052,132 person-years, we identified 25,502 PLC cases; this corresponding to an overall incidence rate was 835.5 PLCs per 100,000 person-years. The adjusted hazard ratios (HRs) were 1.21 (95% confidence interval [CI], 1.18 to 1.24), 1.48 (95% CI, 1.37 to 1.59), 1.50 (95% CI, 1.34 to 1.68), and 1.88 (95% CI, 1.61 to 2.19) for estimated AA usage groups: 1 to 250, 251 to 500, 501 to 1,000, and more than 1,000 mg, respectively, relative to no AA exposure (reference group). **Conclusions:** The current findings suggest that among HCV positive patients, increasing exposure to AA poses an increased risk of acquiring PLC. **Impact:** AA may increase the risk of PLC in HCV positive populations.

Key words: herbal medicine, aristolochic acid, hepatitis C virus infection, primary liver cancer

Abbreviations: AA, aristolochic acid; ACE, angiotensin-converting enzyme; CI, confidence interval; HCCs, hepatocellular carcinomas; HCV, hepatitis C virus; HM, herbal medicine ; HR, hazard ratio; ICD-9, International Classification of Diseases, 9th Edition; NHI, National Health Insurance; NSAIDs, nonsteroidal anti-inflammatory drugs; PLC, primary liver cancer.

INTRODUCTION

Aristolochic acid (AA) is found in some Chinese herbs, and occurs naturally in plants in the genera Aristolochia and Asarum, which grow worldwide (1). Prior to stricter regulation in 2003, more than one hundred million people used Chinese herbs containing AA in China (2). AA was first identified as a factor in urothelial carcinoma (3,4). Plants containing AA and AA itself are listed as human carcinogens by the International Agency for Research on Cancer (5,6). AA increases DNA adduct formation and mutant frequency in experimental animals in both liver and kidney (7). A new study also demonstrated that AA-induced liver cancer in mice (8). In Taiwan, hepatocellular carcinomas (HCCs) are nearly always associated with AA mediated mutations and this AA signature was also identified throughout the Asian world and again among Asian patients treated elsewhere (9). Furthermore, there was a significant dose-response relationship between the consumption of AA and HCC in patients with HBV infection in Taiwan (10).

Persons infected with hepatitis C virus (HCV) had more than 15-fold higher HCC risk than uninfected persons (11). HCV infection is still a major public health burden in Taiwan, USA and worldwide, especially as relates to HCC and cancer-related mortality (12). Plants containing AA were prohibited in some countries and in Taiwan since November 2003 (13), however, more than thirty percent of the population in Taiwan was reportedly prescribed

Chinese herbs containing AA between 1997 and 2003 (14). Given the lack of a large population-based study assessing the effect of AA exposure on risk of PLC in HCV infected individuals, the objective of this research was to investigate whether the use of herbal medicine (HM) containing AA was associated with increased risk of PLC among HCV-infected patients.

MATERIALS AND METHODS

Data source

National Health Insurance (NHI) Research Database was used for the current analysis and the study design has been described in detail previously (15). In brief, the NHI program has provided compulsory universal health insurance since 1995 including both traditional Chinese and western medicine for most of the country's population (99%). Data were collected from both hospital admissions and outpatient visits and included patient characteristics: sex, birthdates, dates of visits, date of admission, date of discharge, and up to three outpatient visit diagnoses or five discharge diagnoses defined by International Classification of Diseases, Ninth Revision classification (ICD-9). Patient prescription information, duration and drug dosage were also collected. According to the study criteria mentioned above, a population-based cohort of patients, aged 18 years or older who had a diagnosis of HCV infection (ICD-9 codes 070.41, 070.44, 070.51, 070.54, 070.7, and V02.62) without HBV infection (ICD-9 codes 070.2, 070.3, and V02.61) between January 1, 1997 and December 31, 2010, was identified and included in the final analysis (Fig 1) (16,17). For ensuring comparability, only patients who had visited traditional Chinese medicine (TCM) clinics between January 1, 1997 and one year prior to the diagnosis of PLC or the censor dates were included. In accordance with personal electronic data protection regulations, strict confidentiality guideline was closely followed. The National

Health Research Institutes (NHRI) of Taiwan maintains and anonymizes the NHI reimbursement data as files suitable for study. This research was also approved by the Institutional Review Board of the National Taiwan University Hospital.

Exposure of HM containing AA

Following standard prescriptions recommended by the Committee on Chinese Medicine and Pharmacy in Taiwan, HM produced prior to promulgation of new regulations in November 2003 could include the following herbs containing AA: Guang Fang Ji (Aristolochia fangji), Guan Mu Tong (Aristolochia manshuriensis), Ma Dou Ling (fruits of Aristolochia contorta or Aristolochia debilis), Qing Mu Xiang (roots of Aristolochia debilis), Tian Xian Teng (stems and leaves of Aristolochia contorta or Aristolochia debilis), and Xi Xin (Asarum sieboldii or Asarum heterotropoides) (18). Upon the recommendation of ancient Chinese medicine books, these herbs were taken as either single products or components of mixed herbal formulas. The original amount of herbs was determined in the unit of grams for each mixture of HM and the overall dose for each AA-containing herb. The cumulative dose of AA was then calculated by summing the estimated average of AA from each herb for individuals who met the inclusion criteria: (1) one year prior to the diagnosis of PLC or the censor dates and (2) at least one year induction time. The AA dose associated with Guan Mu Tong, Guang Fang Ji, Ma Dou Ling, Xi Xin, Tian Xian Teng, and Qing Mu Xiang were

respectively estimated as 2.59, 2.04, 0.63, 0.042, 0.026, and 0.009 mg (19-23). Body mass was not consistently available, thus for the analysis, AA exposure was presented as total intake (mg).

Diagnosis of PLC

Patients with PLC were identified by diagnostic code, ICD-9 code 155.0, and the initial diagnosis used as the index date. The diagnosis of PLC was made following the recommendation of the American Association for the Study of Liver Diseases Practice Guidelines, NHI (24). To improve the validity of the diagnosis, only PLC patients who met the diagnosis criteria aforementioned were included in the analysis. Additional analyses of newly diagnosed patients from January 1, 1999 to December 31, 2013 were performed to ensure that all subjects had a minimum two years' exposure to HM prior to diagnosis with PLC.

Potential confounders

Comorbid diagnoses were identified as potential confounders recorded beginning January 1, 1997 to one year before the diagnosis of PLC or the censor dates. These included by diagnostic code, diabetes (250), hypertension (401), hyperlipidemia (272), nonalcoholic steatohepatitis (571.8 and 571.9), alcohol-related disease (291, 303.0, 303.9, 305.0, 571.0,

571.1, 571.2, and 571.3), liver cirrhosis (ICD-9 codes 571.2, 571.5, 571.6, 572.2, 572.3, 572.4, 572.8, and 573.0), cholelithiasis (574), and chronic obstructive pulmonary disease (491 and 492). Potential confounding prescription medications were evaluated as well including anti-HCV treatments (interferon or ribavirin), aspirin, non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs), statins (i.e., atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin), metformin, and angiotensin-converting enzyme (ACE) inhibitors (i.e., benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, perindopril, quinapril, and ramipril). In the model, sociodemographic characteristics (sex, age, income, and urbanization level) were also considered. Levels of urbanization in Taiwan are divided into four strata by the NRHI publications, with level I referring to the most urbanized areas and level IV referring to the least urbanized areas.

Statistical analyses

Kaplan-Meier method was used to the estimate PLC cumulative incidences. The log-rank test was further performed to examine differences in the risk of PLC in the cohort. The direct method to adjust the incidence rates was employing the sex and age distributions for the general population of Taiwan between 1999 and 2013. Finally, the analyses used Cox proportional hazards models that computed the hazard ratios (HRs) and accompanied 95% CIs before and after adjusting for variables including sex, age, urbanization level, monthly

income, diabetes, and liver cirrhosis (25,26). The analyses were censored for patients lost to follow-up and those death was recorded in the database. Tests for trend were also applied when appropriate. The categories for each HM containing AA or estimated AA were given ordinal scores (0, 1, 2, …) and then modeled as a continuous variable in the trend analysis.

Sensitivity analyses were performed to evaluate the difference and consistency between exposure to HM containing AA and the risk of PLC as well as to the examination of potential interaction in the subgroup effects. Cox proportional hazards regression models were applied coupled with propensity score matching for the analysis. Induction time, competing mortality, and time-dependent covariates were also accordingly evaluated for potential confounding or time-related biases when examining the associations of interest. A two-tailed P-value of 0.05 was set as being significant. SAS statistical software (version 9.4; SAS Institute, Cary, NC) was used for all statistical analyses.

RESULTS

A total of 223,467 HCV-infected patients were included in the study cohort, of whom 59.5% had recorded exposure to HM containing AA. Table 1 shows the demographic characteristics, medical conditions, and medication usage of patients.

There were 25,502 PLCs during the follow-up period of 3,052,132 person-years and the overall incidence rate was 835.5 PLCs per 100,000 person-years. There was a higher risk (adjusted HR, 1.23; 95% CI, 1.20 to 1.26) of PLC in patients with HCV infection who took HM containing AA. There were also significant dose-dependent trends ($p < 0.0001$) for those using Guan Mu Tong, Qing Mu Xiang, and Xi Xin (Table 2).

Regarding the cumulative dose of AA, the adjusted HRs were 1.21 (95% CI, 1.18 to 1.24), 1.48 (95% CI, 1.37 to 1.59), 1.50 (95% CI, 1.34 to 1.68), and 1.88 (95% CI, 1.61 to 2.19) for estimated AA of 1 to 250, 251 to 500, 501 to 1,000, and more than 1,000 mg, respectively, relative to no AA exposure. There was also a significant dose-dependent trend ($p < 0.0001$) (Table 2). The log-rank tests identified significant observed differences ($p < 0.001$) over the entire Kaplan-Meier curves (Fig 2).

A significant risk of PLC in patients with HCV was also identified using Cox proportional

hazards regression with propensity score matching, 2-year induction time, and time-varying changes. Additional covariates in the sensitivity analysis had little effect on the observed associations between AA exposure and the risk of PLC in different models. When the data were further stratified by age, sex, diabetes, nonalcoholic steatohepatitis, alcohol-related disease, liver cirrhosis, anti-HCV treatment, statin, aspirin, or metformin use, the association of interests remained significant in the subgroup analysis. In addition, significant interaction effects were observed for alcohol-related disease ($p < 0.0018$) and sex ($p < 0.0017$). This interaction may reflect the greater incidence of alcohol-related disease in men (Table 3).

DISCUSSION

Higher risk of PLC was observed among HCV infected patients who took HM containing AA. Furthermore, significant dose-dependent relationships were found between the specific herbal medicines, Guan Mu Tong and Qing Mu Xiang, or overall AA exposure and the risk of PLC in patients with HCV infection.

Despite its profound toxicity and carcinogenicity, the health effects of AA have not been well recognized. This changed in the early nineties when a cohort of Belgian women developed kidney disease (27) that was later accompanied by the upper urinary tract cancer (3,4). The unique course of the disease, now known as aristolochic acid nephropathy (AAN) (28), and its pathophysiology provided insights to solve the long-standing mystery of Balkan endemic nephropathy (BEN). Those diagnosed with BEN were exposed to AA from flour contaminated with Aristolochia clematatis seeds. BEN is now recognized as an example of AAN (29).

Aristolactam-DNA adducts (30) and their consequently AA-specific mutation signature (31,32) are now accepted biomarkers of AA exposure and effects. These biomarkers have aided in establishing links between AA exposure and upper urinary tract cancer (33,34), clear cell renal cell carcinoma (35), bladder cancer (36), and HBV-positive HCC (37) in

Taiwan. Thus, AA associated carcinogenesis is not only limited to the upper urinary tract. Ng et al. (9) just presented evidence that the majority of liver tumors sampled in Taiwan were positive for this AA mutational signature. Although they did not present evidence on AA exposure and viral hepatitis, their findings provide additional support for the association between AA exposure and HBV-positive HCC published by Chen et al. (10).

In the current analyses, AA exposure as well as Guan Mu Tong and Qing Mu Xiang may increase the risks for PLC in HCV-infected patients in a dose-dependent manner (Table 2). HCV induces mitogenic, angiogenic and metastatic pathways, blocks cell death, triggers persistent inflammation and ROS production, and dysregulates host lipid metabolism (38). AA may interact with HCV to increase the risk of PLC. Among three significant herbs containing AA, over 84% of Mu Tong herbal formulas contained Guan Mu Tong (Aristolochia manshuriensis), and the estimated average dose of AA was 2.59 mg per gram of Guan Mu Tong [21]. Of the top ten herbal formulas prescribed for chronic hepatitis in Taiwan, 2002 (39), two types of herbal formulas containing Mu Tong—Long Dan Xie Gan Tang and Gan Lou Xiao Du Dan—comprised 23.5% and 6.3% of all prescriptions, respectively. Furthermore, Qing Mu Xiang which is not among the popular chronic hepatitis formulas but contains significant AA, also increases the associated risk of PLC. Thus, herbal medicines intended to improve hepatic distress may actually contribute to an

unforeseen hepatic pathogenesis.

The current study has several strengths. First, a large size highly representative population-based computerized database was analyzed as the study population in Taiwan. The possibility of selection bias and losses to follow up was minimized due to the patients being recruited from an insured general population with approximately 99% population coverage. Second, because of the NHI reimbursement database identifies all prescription information prospectively, the possibility of patient recall bias for the intake doses of various HM and AA can be ruled out, though compliance is another issue (see below). Third, the included clinical endpoint, PLC is well defined with a well-established clinical importance. Fourth, a one-year minimum induction time was addressed by calculating cumulative AA exposures up to one year prior to diagnosis or the censor dates. Finally, sensitivity analyses including analytical designs, additional covariates, and subgroup effects were conducted and the results remained robust.

The increased risk observed in the current analysis might be associated with a subgroup of those with more severe liver hepatitis, who are more likely to develop liver cancer. If this subgroup were more likely to employ these herbal medicines a potential behavioral confound would result. Despite this possibility, the results presented indicate the AA

dose-dependency remained evident in both non-cirrhotic or in cirrhotic subgroups. Further, AA consumption was assessed in 1997-2003 during which AA was withdrawn from the market, where as cases of PLC were identified between 1999 and 2013 (ten years after market withdrawal). Therefore, the confounding effect by disease severity, if present, would be diluted.

Several potential limitations should be noted. Because of the data on herbal prescriptions are incomplete prior to 1997, information prior to that date were not included and the data may significantly underestimate AA intake. In addition, the AA estimates presumed that patients were compliant and took all prescribed medications. To the extent they did not, the AA consumption would thus be an overestimate. This may bias the current analysis to the null hypothesis. Furthermore, several unmeasured confounders, including body mass index, exposure to aflatoxin B1, alcohol intake, and smoking which are likewise associated with PLC (40-42) were not included in the database. Nevertheless, the sensitivity analyses on hypertension, hyperlipidemia, alcohol-related disease, nonalcoholic steatohepatitis, and chronic obstructive pulmonary disease as additional covariates were negative suggesting that these common concerns were not confounders (Table 3).

In summary, the current analyses suggest that AA may increase the efficacy of HCV in the

pathogenesis of PLC. The mechanism for this proposed interaction remains unclear and understanding the process could provide important insight into the oncogenic process and potential points of intervention. Although HM containing AA has largely been banned in Taiwan and other countries, HCV patients with current or past exposure to these herbal medicines should be followed closely.

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Table 1. Demographics and Clinical Characteristics of the Patients with HCV Infection

Demographics and Clinical Characteristics	Patients Who Took HM Containing AA (n = 132,951)	Patients Who Did Not Take HM Containing AA (n = 90,516)
Sex		
Female	57.8	47.1
Male	42.3	53.0
Age, year		
18-29	11.0	13.9
30-39	18.0	18.3
40-49	25.2	23.7
50-59	21.9	21.3
>=60	24.0	22.8
Mean	48.5	47.5
Standard deviation	14.2	14.7
Monthly income, NT$		
0	14.7	15.0
1-15,840	14.4	16.6
15,841-25,000	54.9	52.0
>25,000	16.0	16.4
Urbanization level		
I	22.1	21.7
II	44.9	44.3
III	21.6	22.4
IV (rural area)	11.4	11.6
Disease		
Liver cirrhosis	17.5	17.8
Alcohol-related disease	5.9	7.7
Nonalcoholic steatohepatitis	15.9	15.1
Cholelithiasis	14.0	13.5
Hypertension	51.7	51.4
Diabetes	34.8	35.1
Hyperlipidemia	35.1	33.4
Chronic obstructive pulmonary disease	20.0	17.1
Medication		
Anti-HCV treatment	13.5	13.9

Aspirin	31.8	30.1
Non-aspirin NSAIDs	95.1	91.9
ACE inhibitors	32.5	31.3
Metformin	19.4	20.6
Statins	17.9	17.9

AA, aristolochic acid; ACE, angiotensin-converting enzyme; HCV, hepatitis C virus; HM, herbal medicine; NSAIDs, nonsteroidal anti-inflammatory drugs; NT$, New Taiwan Dollar.

Table 2. Crude and Adjusted HRs of PLC Associated with HM Containing AA During the Follow-Up Period in the Patients with HCV Infection

HM Containing AA	No. of Patients	No. of Person-Years	No. of Patients with PLC	Incidence (per 10^5)	95% CI	Crude HR	95% CI	Adjusted HR[a]	95% CI	P for Trend
All patients	223467	3052132.0	25502	835.5	825.3 to 845.8					
No	90516	1246243.4	9239	741.3	726.2 to 756.5	1.00		1.00		
Yes	132951	1805888.6	16263	900.6	886.7 to 914.4	1.22	1.19 to 1.25	1.23	1.20 to 1.26	
Guan Mu Tong, g										<.0001
0	162286	2219248.1	17758	800.2	788.4 to 811.9	1.00		1.00		
1-30	41398	563737.6	4973	882.1	857.6 to 906.7	1.10	1.07 to 1.14	1.18	1.15 to 1.22	
31-60	9513	130135.3	1218	935.9	883.4 to 988.5	1.17	1.10 to 1.24	1.26	1.19 to 1.34	
61-100	4652	63651.1	638	1002.3	924.6 to 1080.1	1.25	1.16 to 1.36	1.36	1.26 to 1.47	
101-200	3617	48810.0	559	1145.3	1050.3 to 1240.2	1.44	1.32 to 1.56	1.57	1.44 to 1.70	
>200	2001	26549.9	356	1340.9	1201.6 to 1480.2	1.69	1.52 to 1.88	1.72	1.55 to 1.91	
Guang Fang Ji, g										0.0937
0	153866	2100515.8	17353	826.1	813.8 to 838.4	1.00		1.00		
1-30	60183	824280.9	6925	840.1	820.3 to 859.9	1.02	0.99 to 1.05	1.03	1.00 to 1.06	
31-60	5561	75180.9	704	936.4	867.2 to 1005.6	1.14	1.06 to 1.23	1.05	0.97 to 1.13	
61-100	1983	26876.6	279	1038.1	916.3 to 1159.9	1.26	1.12 to 1.42	1.10	0.97 to 1.23	
101-200	1304	17609.6	169	959.7	815.0 to 1104.4	1.17	1.00 to 1.36	1.00	0.86 to 1.16	
>200	570	7668.3	72	938.9	722.1 to 1155.8	1.14	0.91 to 1.44	0.88	0.70 to 1.11	
Ma Dou Ling, g										0.0952
0	222324	3036560.9	25352	834.9	824.6 to 845.2	1.00		1.00		
1-30	680	9305.2	85	913.5	719.3 to 1107.7	1.09	0.88 to 1.35	1.12	0.90 to 1.38	
31-60	254	3461.4	29	837.8	532.9 to 1142.8	1.00	0.70 to 1.45	1.00	0.70 to 1.44	

61-100	114	1510.3	20	1324.2	743.9 to 1904.6	1.61	1.04 to 2.49	1.59	1.03 to 2.47	
>100	95	1294.2	16	1236.3	630.5 to 1842.0	1.49	0.91 to 2.43	1.15	0.71 to 1.88	
Qing Mu Xiang, g										<.0001
0	164387	2246644.5	18462	821.8	809.9 to 833.6	1.00		1.00		
1-30	47168	644085.0	5452	846.5	824.0 to 868.9	1.03	1.00 to 1.06	1.11	1.07 to 1.14	
31-60	6533	88723.0	809	911.8	849.0 to 974.7	1.11	1.04 to 1.19	1.16	1.08 to 1.24	
61-100	2642	35946.9	348	968.1	866.4 to 1069.8	1.18	1.06 to 1.31	1.19	1.07 to 1.32	
101-200	1835	24673.4	281	1138.9	1005.7 to 1272.0	1.39	1.24 to 1.57	1.34	1.19 to 1.51	
>200	902	12059.2	150	1243.9	1044.8 to 1442.9	1.53	1.30 to 1.80	1.38	1.17 to 1.62	
Tian Xian Teng, g										0.1254
0	223158	3047930.1	25458	835.3	825.0 to 845.5	1.00		1.00		
1-30	242	3267.3	32	979.4	640.1 to 1318.7	1.18	0.83 to 1.67	1.12	0.79 to 1.58	
>30	67	934.6	12	1284.0	557.5 to 2010.5	1.54	0.88 to 2.71	1.55	0.88 to 2.73	
Xi Xin, g										<.0001
0	140495	1919817.2	15547	809.8	797.1 to 822.5	1.00		1.00		
1-30	59399	808728.7	7126	881.1	860.7 to 901.6	1.09	1.06 to 1.12	1.10	1.07 to 1.13	
31-60	11694	160498.2	1367	851.7	806.6 to 896.9	1.05	1.00 to 1.11	1.05	1.00 to 1.11	
61-100	5631	77389.3	654	845.1	780.3 to 909.8	1.04	0.97 to 1.13	1.03	0.96 to 1.12	
101-200	4180	57353.4	540	941.5	862.1 to 1020.9	1.16	1.07 to 1.27	1.12	1.03 to 1.22	
>200	2068	28345.3	268	945.5	832.3 to 1058.7	1.17	1.04 to 1.32	1.05	0.93 to 1.18	
Estimated AA, mg										<.0001
0	90516	1246243.4	9239	741.3	726.2 to 756.5	1.00		1.00		
1-250	124671	1694233.6	15009	885.9	871.7 to 900.1	1.20	1.17 to 1.23	1.21	1.18 to 1.24	
251-500	5332	72328.8	770	1064.6	989.4 to 1139.8	1.44	1.34 to 1.55	1.48	1.37 to 1.59	
501-1000	2127	28495.7	322	1130.0	1006.6 to 1253.4	1.54	1.37 to 1.72	1.50	1.34 to 1.68	

>1000	821	10830.6	162	1495.8	1265.4 to 1726.1	2.04	1.75 to 2.39	1.88	1.61 to 2.19

AA, aristolochic acid; CI, confidence interval; HCV, hepatitis C virus; HM, herbal medicine; HR, hazard ratio; PLC, primary liver cancer.

[a] Adjusted for sex, age, monthly income, urbanization, liver cirrhosis, and diabetes.

Table 3. Sensitivity Analysis of Adjusted HRs of Estimated AA in Risk Estimation for PLC During the Follow-Up Period in the Patients with HCV Infection

	Estimated AA						
	1-250 mg		251-500 mg		>500 mg		P for
Model	HR	95% CI	HR	95% CI	HR	95% CI	Interaction
Main model[a]	1.21	1.18 to 1.24	1.48	1.37 to 1.59	1.61	1.47 to 1.76	–
Analytical designs							
Model with 2-year induction time	1.21	1.18 to 1.24	1.48	1.37 to 1.60	1.57	1.43 to 1.73	–
Model with competing mortality	1.19	1.16 to 1.23	1.48	1.37 to 1.59	1.59	1.45 to 1.74	–
Model with propensity score matching[b]	1.24	1.20 to 1.28	1.45	1.25 to 1.68	1.67	1.39 to 2.02	–
Model with time-varying changes[c]	1.35	1.27 to 1.44	1.79	1.35 to 2.37	–		–
Additional covariates[d]							
Main model + alcohol-related disease	1.21	1.18 to 1.24	1.50	1.40 to 1.62	1.65	1.50 to 1.81	–
Main model + nonalcoholic steatohepatitis	1.21	1.18 to 1.24	1.48	1.37 to 1.59	1.62	1.48 to 1.77	–
Main model + hypertension	1.20	1.17 to 1.24	1.47	1.36 to 1.58	1.59	1.46 to 1.75	–
Main model + hyperlipidemia	1.21	1.18 to 1.25	1.51	1.40 to 1.62	1.64	1.49 to 1.79	–
Main model + chronic obstructive pulmonary disease	1.23	1.20 to 1.26	1.52	1.41 to 1.63	1.66	1.51 to 1.82	–
Main model + anti-HCV treatment	1.20	1.17 to 1.24	1.49	1.38 to 1.60	1.61	1.47 to 1.77	–
Main model + aspirin	1.22	1.19 to 1.25	1.50	1.40 to 1.62	1.61	1.47 to 1.76	–
Main model + non-aspirin NSAIDs	1.27	1.24 to 1.31	1.55	1.44 to 1.67	1.64	1.49 to 1.79	–
Main model + ACE inhibitors	1.21	1.18 to 1.25	1.48	1.37 to 1.59	1.62	1.48 to 1.77	–
Main model + metformin	1.21	1.18 to 1.24	1.48	1.38 to 1.59	1.61	1.47 to 1.77	–
Main model + statins	1.20	1.17 to 1.23	1.48	1.37 to 1.59	1.60	1.46 to 1.75	–
Subgroup effects							

Sex							0.0017
Female	1.17	1.13 to 1.22	1.40	1.26 to 1.56	1.40	1.20 to 1.62	
Male	1.24	1.20 to 1.29	1.56	1.41 to 1.72	1.80	1.60 to 2.02	
Age, years							0.4128
18-39	1.22	1.09 to 1.36	1.58	1.14 to 2.19	2.54	1.79 to 3.61	
≥40	1.22	1.18 to 1.25	1.45	1.35 to 1.57	1.54	1.40 to 1.69	
Liver cirrhosis							0.8872
No	1.21	1.17 to 1.25	1.47	1.34 to 1.62	1.73	1.54 to 1.94	
Yes	1.21	1.16 to 1.27	1.51	1.34 to 1.70	1.46	1.25 to 1.69	
Alcohol-related disease							0.0018
No	1.18	1.13 to 1.23	1.18	1.01 to 1.38	1.33	1.09 to 1.63	
Yes	1.23	1.19 to 1.27	1.64	1.51 to 1.79	1.77	1.60 to 1.96	
Nonalcoholic steatohepatitis							0.3289
No	1.20	1.17 to 1.24	1.45	1.33 to 1.57	1.61	1.46 to 1.78	
Yes	1.22	1.14 to 1.30	1.66	1.39 to 1.99	1.68	1.35 to 2.09	
Diabetes							0.3306
No	1.21	1.17 to 1.25	1.46	1.33 to 1.60	1.70	1.52 to 1.90	
Yes	1.20	1.15 to 1.25	1.51	1.33 to 1.70	1.45	1.24 to 1.71	
Anti-HCV treatment							0.0953
No	1.20	1.17 to 1.23	1.48	1.37 to 1.60	1.57	1.43 to 1.73	
Yes	1.26	1.12 to 1.41	1.56	1.15 to 2.12	2.32	1.67 to 3.22	
Aspirin							0.1483
No	1.24	1.20 to 1.28	1.56	1.43 to 1.70	1.57	1.41 to 1.75	
Yes	1.18	1.12 to 1.23	1.36	1.18 to 1.56	1.69	1.42 to 2.01	
Metformin							0.3124

No	1.21	1.17 to 1.24	1.48	1.36 to 1.60	1.68	1.52 to 1.86	
Yes	1.21	1.15 to 1.28	1.51	1.28 to 1.78	1.31	1.04 to 1.66	
Statins							0.4595
No	1.21	1.18 to 1.24	1.46	1.35 to 1.57	1.60	1.45 to 1.75	
Yes	1.12	1.01 to 1.24	1.69	1.29 to 2.21	1.62	1.12 to 2.33	

AA, aristolochic acid; ACE, angiotensin-converting enzyme; CI, confidence interval; HCV, hepatitis C virus; HR, hazard ratio; NSAIDs, nonsteroidal anti-inflammatory drugs; PLC, primary liver cancer.

[a] Adjusted for sex, age, monthly income, urbanization, liver cirrhosis, and diabetes.

[b] Subject number = 181,032.

[c] The estimated AA was divided into only two categories of use (1 to 250 and > 250 mg) in the model with time-varying changes.

[d] The models were adjusted for covariates in the main model as well as each additional listed covariate.

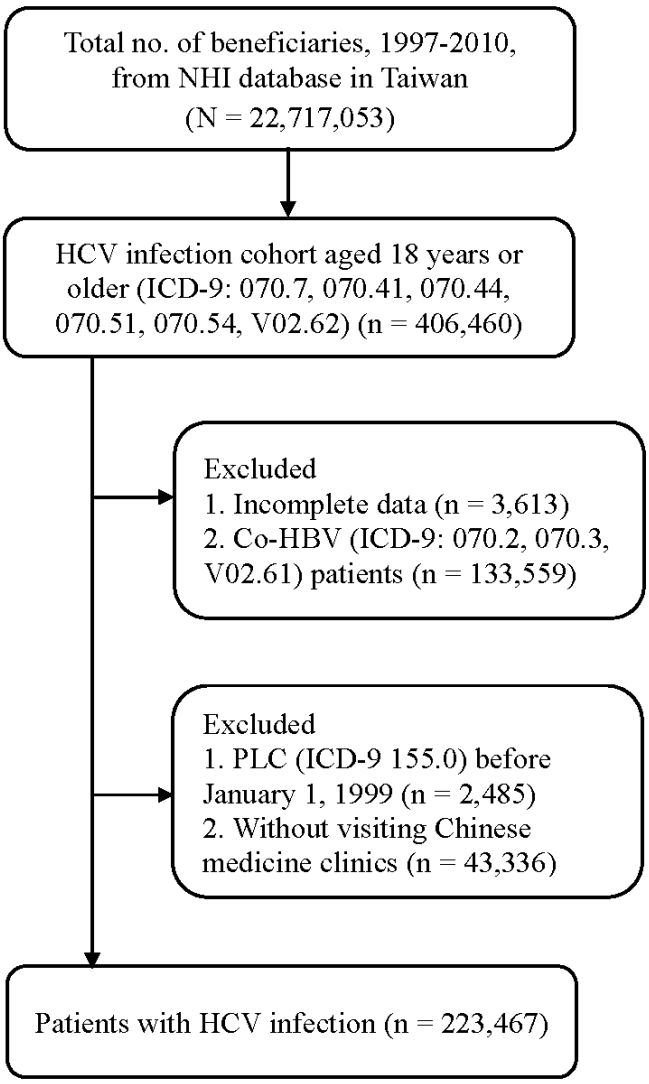
Figure Legends

Figure 1. Study design flowchart.

Abbreviations: HCV, hepatitis C virus; ICD-9, International Classification of Diseases, ninth revision; NHI, National Health Insurance; PLC, primary liver cancer.

Figure 2. Cumulative rates of PLCs according to estimated AA in the HCV-infected cohort.

Abbreviations: AA: aristolochic acid; and HCV, hepatitis C virus; PLC, primary liver cancer.



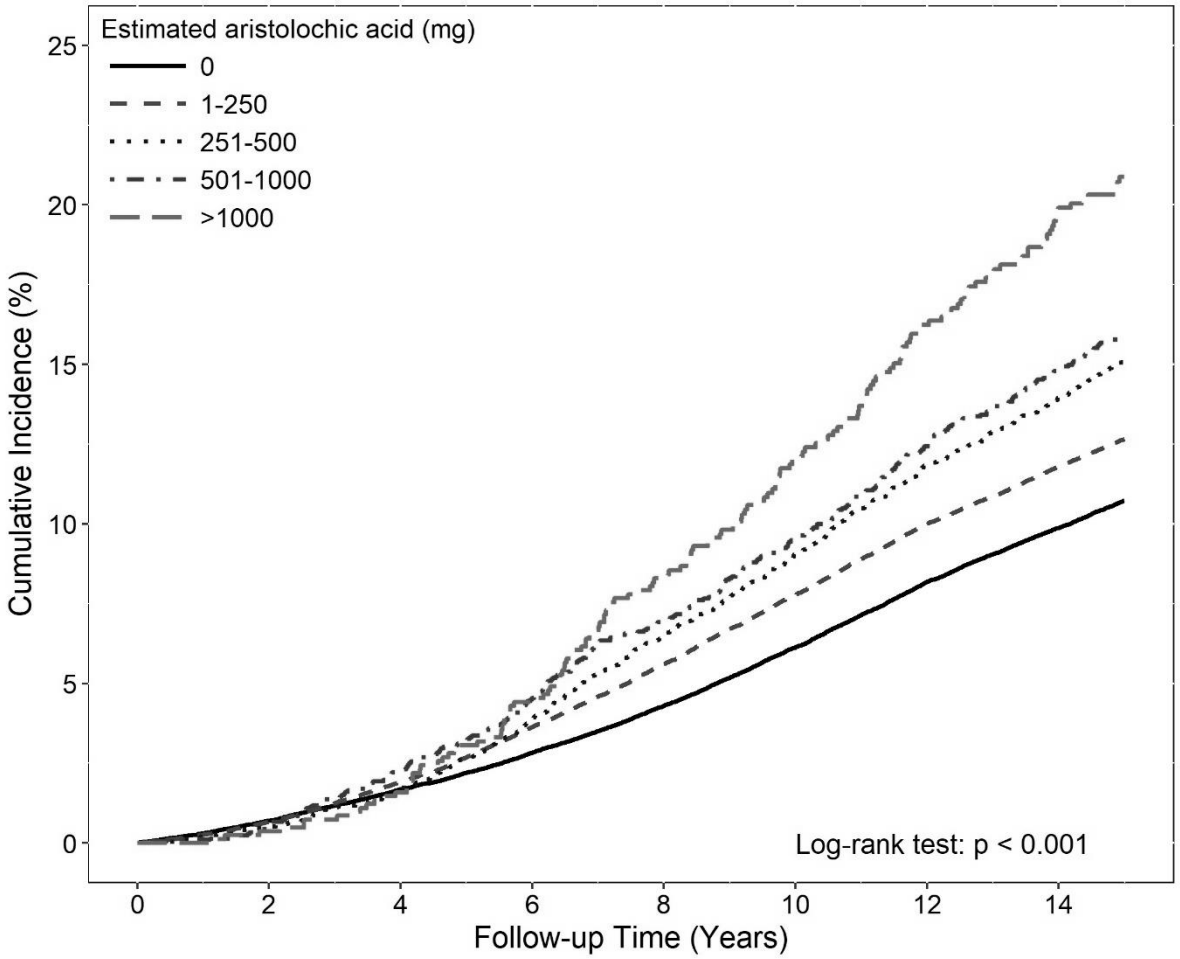


Figure 2

Herbal Medicine Containing Aristolochic Acid and the Risk of Primary Liver Cancer in Patients with Hepatitis C Virus Infection

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