

Alcohol intake, viral hepatitis B infection, viral hepatitis C infection, and the risk of primary liver cancer: systematic review and meta-analysis

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Abstract

Background

Liver cancer is a leading cause of morbidity and mortality globally. Accurate estimation of the contribution of major risk factors of liver cancer namely, alcohol intake and viral hepatitis infection, is essential for guiding health policy. We undertook a systematic literature review and meta-analysis to calculate unbiased relative risk estimates for each risk factor.

Methods

We systematically collected published data on relative risks of liver cancer related to intake of alcohol and viral hepatitis B and C infections. For each risk factor, we used meta-regression techniques to estimate the relative risk adjusted for the other two risk factors. In the case of alcohol intake, we used first degree fractional polynomials to choose the best model. In the case of viral hepatitis, we adjusted for alcohol using country-level alcohol data. We assessed for publication bias and study heterogeneity. We used mixed-effects logistic regression to explore sex and age interactions.

Results

The relative risk related to alcohol intake increased from 1.05 (95% confidence interval 1.04 – 1.06) at 20 grams of ethanol per day to 1.6 (1.4 – 1.7) and 6.1 (4.4 – 8.6) at 60 and 120 grams per day, respectively. This relation was not modified by sex or age. The relative risk related to hepatitis B infection was 11.6 (8.3 – 16.3) and did not change after adjusting for country-level alcohol. For hepatitis C infection, the relative risk was 7.4 (4.3 – 12.8) after adjusting for alcohol. Female sex reduced the relative risk of cancer related to hepatitis infection. This relation was significant for hepatitis C only.

Conclusions

Unbiased relative risk estimates of liver cancer adjusted for major risk factors are essential for quantification of the contribution of each risk factor to liver cancer burden. Our results will help cast public health attention to risk factors with the largest contribution to liver cancer burden.

Introduction

Primary liver cancer (liver cancer) is the third leading cause of cancer deaths globally resulting in approximately 740,806 deaths among both males and females in 2010 (Unpublished data from the Global Burden of Disease Study 2010). It is the second most common cause of cancer deaths after lung cancer in males and the seventh leading cause of cancer deaths in females. The most prevalent risk factors associated with primary liver cancer are viral hepatitis B infection, viral hepatitis C infection, and alcohol intake. The increased risk of liver cancer associated with these three factors has been demonstrated repeatedly in previously published literature¹⁻¹¹. Other risk factors are also associated with increased risk of liver cancer, and include tobacco^{7,12,13}, environmental toxins particularly aflatoxins¹⁴⁻¹⁶, and iron over-load syndromes¹⁷⁻¹⁹. There is also growing evidence that metabolic diseases including Diabetes Mellitus and non-alcoholic liver disease are also associated with increased risk of liver cancer²⁰⁻²⁵. Marked variation in prevalence of these risk factors exists across countries, and is responsible for variation in liver cancer incidence and mortality. Hence, there is a genuine public health interest in quantification of the attribution fraction of each of the most important risk factors on liver cancer in different countries. The currently ongoing Global Burden of Diseases, Injuries, and Risk Factors (GBD) 2010 Study, includes an estimation of the attributable fraction of risk factors on disease burden including liver cancer. The GBD study is an effort to

produce comprehensive and comparable global estimates of the burden of over 200 diseases. Quantification of population attributable fraction of a risk factor on a disease requires two inputs; prevalence of the risk factor and the relative risk of disease due to the risk factor. In the case of liver cancer, available data on relative risks related to alcohol intake and viral hepatitis is biased and inaccurate, since the relative risk related to one risk factor is not adjusted for the other two major risk factors. In this study, we undertook a systematic review of the literature on risk associations between liver cancer, alcohol, and viral hepatitis B and C. We used meta-regression methods to calculate adjusted relative risk estimates for each risk factor. We aimed to calculate unbiased relative risk estimates of liver cancer due to alcohol intake and viral hepatitis B and C infections.

Methods

Literature search

We performed a systematic review of the literature in Medline on the association between liver cancer and any of alcohol intake, hepatitis B virus infection, and/or hepatitis C virus infection.

We restricted our search to human studies, but did not limit it by language or time period of publication. We used the following search syntax: "liver neoplasms"[MeSH Terms] AND

("hepatitis b"[MeSH Terms] OR "hepatitis c"[MeSH Terms] OR ("ethanol"[MeSH Terms] OR "alcohols"[MeSH Terms])) AND "humans"[MeSH Terms]. We included studies if: (i) exposure

was evaluated and reported as follows: (a) alcohol intake reported in quantitative categories and at least two alcohol intake categories were reported, (b) hepatitis B virus infection

ascertained using hepatitis B surface antigen (HbsAg) serology, and/or (c) hepatitis C virus

infection ascertained using serum hepatitis C virus antibodies; (ii) liver cancer cases were

identified by lesion biopsy or imaging modalities; (iii) endpoint included liver cancer incidence or liver cancer as a cause of death; (iv) risk ratio estimates such as relative risk (RR), hazard ratio (HR), or odds ratio (OR), and confidence intervals were reported, or enough relevant information was included to calculate these measures; and (v) the study design was a case-control or a cohort design. We excluded studies if: (i) study participants were restricted to those with underlying liver cirrhosis; (ii) study was a clinical trial investigating therapy for chronic hepatitis B or C virus infection; (iii) control group encompassed AIDS patients or participants with immunosuppression, for example, liver transplant patients; (iv) liver cancer was a recurrence; (v) study design was cross-sectional; and (vi) data on the study population was reported in another study which included more comprehensive data.

Data extraction

We initially screened for “preliminary eligibility” the title and abstract of all studies retrieved from the literature search. Then, “preliminary eligible” studies were further assessed for definitive eligibility through review and evaluation of the full text. Consequently, a pool of studies usable for analysis was assembled. Since different methods of analysis were conducted for each risk factor, a different subset of the pooled studies was extracted and used for each analysis. On a standardized spreadsheet, we extracted data on the following variables: title, author, year of publication, country, start and end years for recruitment, parameters on participant age including range, mean, median, standard deviation, upper and lower confidence intervals, sex, percent of male participants, exposure (alcohol, hepatitis B, or hepatitis C), study design, liver cancer endpoint (incidence and/or death), diagnostic test for liver cancer, test for assessing exposure (questionnaire in the case of alcohol, enzyme-linked immunoassay or radio-

immunoassay in the cases of viral hepatitis infection), subgroups restrictions or exclusions (hepatitis B e antigen positive group, for example), alcohol type (ethanol, wine, etc...), alcohol unit (grams/day, drinks/weeks, etc...), alcohol conversion formula to grams per day of ethanol, alcohol intake duration, alcohol measurement (baseline versus lifetime), risk ratio type (RR, HR, or OR), risk ratio estimate, lower and upper confidence intervals, and standard error, variables adjusted for, comparative category and size, referent category and size, total sample size, language of the article, and catchment population (hospital versus community).

Data quality

We used several criteria to assess the quality of each study. We flagged studies with poor quality for the purpose of identification of outliers in the analysis. The quality score included the variables indicated in Table 1.

Standardization of alcohol consumption

We standardized alcohol consumption to permit comparability across all studies. All data on alcohol intake was converted to grams of ethanol per day. Ethanol content in beverages or drinks reported in the studies was calculated using conversion factors available in the studies themselves; otherwise we sought these formulae in the International Monitoring Guide for Monitoring Alcohol Consumption and Related Harm²⁶. We used the midpoint of reported alcohol intake ranges. In case no upper bound was reported, we assumed an upper bound value so that the range width was 75% of the lower alcohol intake category range width. This method of transforming alcohol intake ranges into a continuous variable has been adopted in previous studies^{27–29}.

Hepatitis infection markers

We used positive hepatitis B surface antigen (HbsAg) and hepatitis C virus antibody tests (anti-HCV) as markers of chronic hepatitis B and C infections, respectively.

Statistical analysis

We use the term relative risk (RR) when referring to risk ratio estimates including ORs, RRs, or HRs. To note, each method of analysis uses different subsets of the pool of eligible studies identified in the literature review. A more detailed explanation of these methods follows.

RR by alcohol intake

In this analysis, we included only studies that reported RR of liver cancer by alcohol intake level adjusted for both HbsAg and anti-HCV. We used random effects meta-regression proposed by Greenland et al.³⁰ to evaluate the association between liver cancer and alcohol intake. We modeled alcohol as a continuous covariate and included a sex effect. We used first degree fractional polynomial method to explore a possible non-linear relation between alcohol and the natural logarithm of RR. We used likelihood ratio test to compare linear to polynomial transformations of alcohol using powers -2, -1, 0.5, 0, 0.5, 2, and 3 where 0 implies natural logarithm³¹. We chose our final model based on the deviance difference and the likelihood ratio statistic. We simulated a 1000 draws of the model output to estimate uncertainty where the lower and upper bound reflect 2.5th and 97.5th percentiles output, respectively. We assessed for heterogeneity using the Q-statistic. We used unadjusted age-specific RR data from one study to evaluate for an interaction between age and alcohol intake on the RR of liver cancer.

RR by hepatitis infection

We analyzed the risk of liver cancer by viral hepatitis for each virus, B and C, separately. We employed the same analysis, however. Studies that reported RR adjusted for all risk factors, for

example, the RR by hepatitis B infection adjusted for hepatitis C and alcohol intake, was lacking. A subset of the pooled eligible studies, however, included data on the RR related to one virus adjusted for the second. We used random effects meta-regression on this subset of studies to evaluate the association between viral hepatitis and RR of liver cancer³². In the case that only the joint probabilities of HbsAg, anti-HCV, and liver cancer were reported, which is the case in most studies of this subset, we calculated RR related to one virus adjusted for the second using Mantel-Haenszel method. We calculated RR adjusted for the third risk factor, alcohol intake, in the meta-regression using country-level alcohol intake levels. We used the average global alcohol intake level for all years between 1980 and 2011 as a reference for which we predicted an adjusted RR. This covariate is a country- year-specific liters per capita alcohol consumption estimate derived from country-level alcohol production and trade levels. It was created using data from the Food and Agriculture Organization of the United Nations³³ and data in the World Drink Trends publication³⁴. We assessed for heterogeneity using the Cochran Q-test and the I^2 statistic. We assessed for publication bias using Begg and Mazumdar³⁵ and Egger et al.³⁶ tests. The pooled RR estimate by hepatitis infection in our analysis above was not age and sex specific. We evaluated for sex and age interactions with hepatitis infection on the RR of liver cancer using a different subset of studies. These studies, while not adjusted for any of the other risk factors, did include age and/or sex specific RR by virus infection. We used mixed effects logistic regression with fixed effect on either sex or age and random effect on study. We used three age groups; < 40 years, ≥ 40 and < 70 years, and ≥ 70 years.

We analyzed our data in STATA version 11.1³⁷. We used the GLST and METAREG commands for meta-regression analyses of RR by alcohol and hepatitis virus infections, respectively.

Results

We used a broad literature search strategy to retrieve 7,745 articles in Medline. Of these, 92 articles were eligible and constituted the pool of studies usable for our analysis. Depending on the method of analysis used for each risk factor, a different subset of pooled studies was abstracted. Not all 92 articles were included in our analysis. While all studies satisfied the inclusion criteria, only some satisfied the requirements of the different analyses. Figure 1 describes our literature search and review strategy, and indicates the number of studies in each analysis subset.

Alcohol intake

Table 2 describes studies used in the meta-regression analysis of RR by alcohol intake level. All studies reported RR adjusted for both HbsAg and anti-HCV. 3 studies provided female specific RRs, 4 included male specific RRs, and 5 reported RRs on both sexes collectively.

We tested a total of 7 transformations of the alcohol intake variable and compared those with the linear transformation. The meta-regression model which included the quadratic transformation produced the largest positive deviance difference, 69.48, and a statistically significantly likelihood ratio test (P-value (P) < 0.001). Table 3 shows the deviance difference and the likelihood ratio test p-values for all tested power transformations. The RR of liver cancer increased with higher intake of alcohol. Drinking any amount incurred a higher risk of liver cancer as compared to lifetime abstainers. The RR was 2.24 (95% confidence interval (CI) 1.94 – 2.61) at 80 g/d and increased to 6.12 (95% CI 4.64 – 8.66) at 120 g/d (Figure 2, Table 4). There was marked heterogeneity in the data as suggested by the statistically significant Q statistic (Q=69.8; P=0.0003), and evident, in Figure 2, particularly at high alcohol intake levels.

There was no statistically significant interaction between alcohol intake and sex or age where age is above or below 40 years.

Hepatitis B virus infection

We included 22 studies in the meta-regression analysis of RR of liver cancer by hepatitis B virus infection. All input RRs were adjusted for infection with hepatitis C. Table 5 presents a summary description of these studies. Included studies came from 20 countries that are markedly variable in prevalence of alcohol intake. They were primarily case-control studies of newly diagnosed liver cancer cases. The funnel plot in Figure 3 suggests no substantial publication bias. In addition, both Egger's ($P= 0.164$) and Begg's ($P= 0.205$) tests for funnel plot asymmetry were not significant indicating no evidence of important publication bias.

The pooled RR estimate adjusted for hepatitis C was 11.7 (95% CI 8.3 – 16.3) (Figure 4). The I^2 statistic was 81.1% suggesting significant heterogeneity in the true effect sizes. Adjusting for country-level alcohol intake levels did not explain any of the observed heterogeneity and the adjusted I^2 statistic was 81.7%. The effect of country-level alcohol on the hepatitis B incurred RR was not significant ($P= 0.95$). The RR adjusted for alcohol was 11.6 (95% CI 7.7 – 17.5) (Table 7). In patients with hepatitis B infection, females were at a slightly lower risk of developing liver cancer (RR= 0.97; 95% CI 0.87 – 1.07). The interaction term was not statistically significant with a $P=0.498$. Similarly, the association between hepatitis B infection and liver cancer was not modified by age. P-value for all interaction terms was > 0.05 .

Hepatitis C virus infection

Our analysis of RR of liver cancer by hepatitis C virus infection included 21 studies. RR estimates included in the meta-regression were adjusted for hepatitis B infection. Table 6 includes several characteristics of this subset of studies.

Similar to the hepatitis B infection analysis above, studies used in the hepatitis C meta-analysis came from several countries of variable prevalence of alcohol intake. They were also primarily case-control studies of newly diagnosed liver cancer cases. Test on publication bias were not significant indicating no evidence of substantial publication bias found. The p-values for Egger's and Begg's tests were 0.590 and 0.415, respectively. Figure 5 is a funnel plot of the RRs reported in this subset of studies. More RR estimates were clustered within the pseudo 95% CI suggesting non-substantial publication bias.

The pooled RR estimate adjusted for hepatitis B was 9.5 (95% CI 6.0 – 15.1) (Figure 6). The I^2 statistic was 86.1% suggesting significant heterogeneity in the true RRs. After adjusting for country-level alcohol intake levels, the I^2 statistic was reduced only minimally to 82.3% which suggest significant heterogeneity not explained by alcohol only. Increased country-level alcohol consumption was associated with increased RR of liver cancer in patients with hepatitis C infection (RR= 1.12; 95% CI 0.99 – 1.27; P=0.074) (Figure 7). The estimated RR of liver cancer in patient with hepatitis C infection adjusted for hepatitis B infection and at a global mean for alcohol intake was 7.4 (95% CI 4.3 - 12.8) (Table 7).

The effect of age on the relation of hepatitis C infection and liver cancer risk was not significant at all age categories. The p-value for all interaction terms was > 0.05. Females with hepatitis C

infection had a 11% lower risk of developing cancer as compared to infected males (RR=0.89; 95% CI 0.78 – 0.99; p-value= 0.046).

Discussion

Liver cancer is a major global burden of disease and a leading cause of cancer deaths worldwide. Its main risk factors, alcohol intake and chronic hepatitis infection, are largely amenable to prevention. Accurate quantification of the contribution of these risk factors to liver cancer burden is essential to guide targeted prevention health policies. Our study is a first attempt at assimilating known evidence for estimation of unbiased relative risk estimates of main risk factors used in calculation of population attributable fractions of liver cancer. Alcohol intake increased the risk of liver cancer with increasingly larger increments at higher intake levels. Heavy alcohol intake, defined as daily intake of more than 60g of ethanol^{38,39}, which is equivalent to 5-6 glasses of wine or 6 bottles of beer⁴⁰, incurred a 1.6 fold increase in risk of liver cancer as compared to abstainers. Intake of 120g/d increased the relative risk to 6 folds. The impact of this quadratic amplification in relative risk is substantial particularly in countries with heavy alcohol drinking such as Mexico, Russia, and several countries in Central and Eastern Europe. There is evidence that not only the amount of drinking but also the type of alcohol affect the risk of liver injury and, eventually, liver cancer. Studies primarily done on liver cirrhosis, an almost omnipresent intermediate stage in progression to liver cancer, showed that different alcohol types have varying hepatotoxic effects. Homemade hard fruit liquor poses a larger risk of liver injury as compared to other alcohol types^{41,42}. This theory has been implicated in the very high mortality rates of liver cirrhosis in countries such as Moldova and Hungary where hard fruit alcohol is common⁴³.

Chronic infection with viral hepatitis B and C was associated with a 7.4 and 11.6 fold increase in risk of liver cancer, respectively. Geographic variation in liver cancer mortality reflects prevalence of these two risk factors such as China and countries in western sub-Saharan Africa where high liver cancer mortality is related to endemic hepatitis B⁴⁴. There was large heterogeneity in the published RRs for both hepatitis virus infections. This heterogeneity was not explained by country-level alcohol consumption in the case of hepatitis B and only slightly explained in the case of hepatitis C infection. This may reflect a true minimal effect of alcohol on the RR of cancer in those with chronic hepatitis, or may actually relate to bias related to poor correlation between country-level and individual-level alcohol intake. A portion of this heterogeneity maybe explained by differences in access to care and advances in treatment of liver cancer across countries and over time which is reflected in the studies included in this analysis. Such data was lacking, however, and precluded quantification of the fraction of heterogeneity related to variation in healthcare. Other possible risk factors of liver cancer which are less prevalent yet markedly variable across countries may lead to variation in the RR estimates. Aflatoxins and non-alcoholic fatty liver disease which is related to the obesity endemic in several Western countries were not accounted for in our analysis.

Our study is a best attempt at quantifying unbiased RR estimates of liver cancer given the limitations in the available literature. Recall bias and ascertainment of alcohol intake levels using questionnaires was a significant limitation in all studies used in this meta-analysis exercise. While ascertainment of chronic hepatitis B or C infections utilized more objective tests, those tests are limited by imperfect accuracy measures so that serological evidence of disease may not perfectly correlate with chronic infection status. In chronic hepatitis B

infection, 0.5% of patients clear the HbsAg annually, a state known as occult infection⁴⁵. In the case of hepatitis C infection, some people may have cleared the disease, i.e. are cured, yet still have evidence of infection due to presence of anti-HCV antibodies in their blood⁴⁶. Another important limitation in using serological viral hepatitis markers in estimation of RR is the lack information on duration of the infection. It is well-known that longer duration of hepatitis infection is associated with a higher risk of developing liver cancer.

Several global and national programs and policies have been implemented to reduce prevalence of risk factors of liver cancer primarily those related to reduction in transmission of hepatitis viruses and alcohol consumption. In 1992, the World Health Organization mandated administration hepatitis B vaccination to newborns⁴⁷. In 2009, 177 (92%) WHO member states were routinely administering the vaccine⁴⁸. Several studies confirmed the protective effect of the vaccine against transmission of viral hepatitis B which translated to reduction in prevalence in chronic hepatitis B virus infection^{49,50}. Screening of transfused blood products is another example aimed at minimizing transmission hepatitis B and C viruses among other sexually transmitted diseases. National laws that restrict alcohol trade and production have been effective at reducing alcohol related burden including liver cirrhosis and liver cancer⁵¹⁻⁵³. As a matter of fact, liver cirrhosis mortality has been used historically to track country-level alcohol consumption^{54,55}. Implementing preventative national strategies aimed at reducing prevalence of major risk factors of liver cancer is the highly cost-effective means to halt increase and, eventually, reduce burden related to liver cancer disease.

In conclusion, the estimation of adjusted and unbiased relative risk estimates of liver cancer for main risk factors is essential for accurate quantification of the contribution of each risk factor to the burden of the disease. Given that both alcohol intake and viral hepatitis infection are amenable to prevention, quantification of their attribution to liver cancer burden is essential to guide national health policy and funnel health resources to where there is maximal benefit. In addition, determination of attribution will allow better appreciation of the impact of risk factors on liver cancer globally and promote evidence based discussion on global health policy aimed at minimizing risk factors with largest contribution to disease in the world.

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Table 1: Quality of study scoring system

Variable	Scoring criteria
Adjustment for risk factors (alcohol intake, HbsAg, anti-HCV)	0= no adjustment for other risk factors 1= only one risk factor adjusted for 2= both other risk factors adjusted for
Sex-specific	0= for a particular risk factor, no sex-specific risk ratios reported 1= for a particular risk factor, sex-specific risk ratios reported
Sex reported	0= fraction by sex of total study sample size not reported 1= fraction by sex of total study sample size reported
Age-specific	0= for a particular risk factor, no age-specific risk ratios reported 1= for a particular risk factor, age-specific risk ratios reported
Age reported	0= fraction by age categories of total study sample size not reported 1= fraction by age categories of total study sample size reported
Study design	0= case-control 1= cohort
Endpoint	0= liver cancer as a cause of death 1= incidence of liver cancer
Number of alcohol categories*	0= 2 alcohol categories reported 1= 3 alcohol categories reported 2= ≥ 4 alcohol categories reported
Alcohol period measurement*	0= baseline measurement 1= lifetime measurement

* Related to studies on alcohol only

Table 2: Studies used in RR of liver cancer by alcohol meta-regression

	Author	Recruitment date	Country	Study design	Endpoint	Sample size	Quality score
1	Donato ¹¹	1995 - 2000	Italy	cc	incidence	1,066	9
2	Franceschi ⁵⁶	1999 - 2002	Italy	cc	incidence	660	8
3	Hassan ⁵⁷	1994 - 1995	United States	cc	incidence	345	7
4	Hassan ⁵⁸	2000 - 2006	United States	cc	incidence	895	8
5	Pyong ⁵⁹	1989 - 1992	Korea	cc	incidence	177	8
6	Trichopoulos ⁷	1992 - 2000	Europe*	Nested cc	incidence	239	8
7	Yun ⁶⁰	2002 - 2006	Korea	cc	incidence	1,035	7

* European countries includes: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom; cc=Case-control

Table 3: First degree polynomial powers for RR by alcohol meta-regression

Power	Deviance difference	P-value for LR test
-2	-32.14	1
-1	-19.79	1
-0.5	-31.69	1
0*	-31.96	1
0.5	-6.96	1
1	0.00	1
2	69.48	7.72e ⁻¹⁷
3	37.97	7.18e ⁻¹⁰

LR=Likelihood ratio; *power 0= natural logarithm

Table 4: Adjusted RR of liver cancer by alcohol intake

Alcohol consumption (ethanol grams/day)	RR (95% CI)
0 (referent group)	1
20	1.05 (1.04 – 1.06)
40	1.22 (1.18 – 1.27)
60	1.6 (1.4 – 1.7)
80	2.2 (1.9 – 2.6)
100	3.5 (2.8 – 4.4)
120	6.1 (4.4 – 8.6)
140	11.8 (7.5 – 18.6)

Table 5: Studies used in RR of liver cancer by hepatitis B virus infection meta-regression

	Author	Recruitment date	Country	Study design	Endpoint	Sample size	Quality score	RR	95% CI
1	Chang ⁶¹	1984 - 1986	China	nested cc	incidence	190	5	81.8	9.0 - 740.7
2	Chuang ⁶²	1992 [†]	China	cc	incidence	512	4	6.9	3.2 - 15.1
3	Donato ³⁸	1995 - 2000	Italy	cc	incidence	1,066	9	6.9	4.4 - 10.7
4	Franceschi ⁵⁶	1999 - 2002	Italy	cc	incidence	660	6	12.4	4.4 - 34.7
5	Hassan ⁵⁸	2000 - 2006	United States	cc	incidence	895	5	6.7	2.2 - 20.3
6	Hassan ⁵⁷	1994 - 1995	United States	cc	incidence	345	5	23.8	3.9 - 141.6
7	Kaklamani ⁶³	1976 - 1984	Greece	cc	incidence	617	3	11.4	6.7 - 19.4
8	Kew ⁶⁴	1997 [†]	South Africa	cc	incidence	462	4	15.1	8.6 - 26.5
9	Kirk ⁶⁵	1997 - 2001	Gambia	cc	incidence	624	3	12.6	8.0 - 19.9
10	Kuper ⁶⁶	1995 - 1998	Greece	cc	incidence	693	4	45.5	23.4 - 88.6
11	Olubuyide ⁶⁷	1995 - 1995	Nigeria	cc	incidence	128	4	1.4	.72 - 2.93
12	Simonetti ⁶⁸	1982 - 1988	Italy	cc	incidence	424	4	8.2	2.7 - 24.3
13	Stroffoliini ⁶⁹	1990 - 1990	Italy	cc	incidence	164	4	7.2	2.3 - 22.3
14	Tanaka ⁷⁰	1985 - 1989	Japan	cc	incidence	501	4	22.2	5.5 - 89.5
15	Trichopoulos ⁷	1992 - 2000	Europe*	nested cc	incidence	344	7	9.1	2.1 - 39.5
16	Tsai ⁷¹	1990 - 1992	Taiwan	cc	incidence	306	3	5.5	3.7 - 8.2
17	Wang ⁷²	1999 [†]	China	cc	incidence	294	2	16.2	8.5 - 31
18	Wang ⁷³	1990 - 1992	Taiwan	cohort	death	11,837	5	13.1	8.6 - 20.8
19	Ye ⁷⁴	1989 - 1989	China	cc	incidence	330	2	5.7	3.13 - 10.5
20	Yu ⁷⁵	1997 [†]	China	cc	incidence	690	2	6.8	4.8 - 9.6
21	Yun ⁶⁰	2002 - 2006	Korea	cc	incidence	1,035	5	67.0	32.9 - 136.7
22	Zhang ⁷⁶	1994 - 1995	China	cc	incidence	267	4	29.0	10.9 - 76.6

[†] Year of publication

* European countries includes: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom;

cc=Case-control

Table 6: Studies used in RR of liver cancer by hepatitis C virus infection meta-regression

	Author	Recruitment dates	Country	Study design	Endpoint	Sample size	Quality score	RR	95% CI
1	Chang ⁶¹	1984 - 1986	China	nested cc	incidence	190	5	88.2	5.1 - 1509
2	Chuang ⁶²	1992 [†]	China	cc	incidence	512	4	9.8	5.8 - 16.5
3	Franceschi ⁵⁶	1999 - 2002	Italy	cc	incidence	660	6	16.3	10.4 - 25.5
4	Hassan ⁵⁷	1994 - 1995	United States	cc	incidence	345	5	14.1	4 - 49.7
5	Hassan ⁵⁸	2000 - 2006	United States	cc	incidence	895	6	41.1	18.9 - 89.7
6	Kaklamani ⁶³	1976 - 1984	Greece	cc	incidence	617	3	6.3	3.7 - 11
7	Kew ⁶⁴	1997 [†]	South Africa	cc	incidence	462	4	6.7	3.5 - 12.7
8	Kirk ⁶⁵	1997 - 2001	Gambia	cc	incidence	624	3	14.6	6.4 - 33.4
9	Kuper ⁶⁶	1995 - 1998	Greece	cc	incidence	693	4	28.5	7.4 - 109.4
10	Olubuyide ⁶⁷	1995 - 1995	Nigeria	cc	incidence	128	4	1.8	.68 - 5.0
11	Simonetti ⁶⁸	1982 - 1988	Italy	cc	incidence	424	4	40.8	20.7 - 80.4
12	Stroffoliini ⁶⁹	1990 - 1990	Italy	cc	incidence	164	4	15.2	6.4 - 36.1
13	Tanaka ⁷⁰	1985 - 1989	Japan	cc	incidence	501	4	68.6	20.5 - 229.1
14	Trichopoulos ⁷	1992 - 2000	Europe*	nested cc	incidence	344	7	13.3	4.1 - 43.4
15	Tsai ⁷¹	1990 - 1992	Taiwan	cc	incidence	306	3	8	4.3 - 14.6
16	Wang ⁷³	1990 - 1992	Taiwan	cohort	death	11,837	5	2.4	1.4 - 4.2
17	Wang ⁷²	1999 [†]	China	cc	incidence	294	2	3.3	.6 - 18.3
18	Ye ⁷⁴	1989 - 1989	China	cc	incidence	330	2	3.7	1.5 - 8.8
19	Yu ⁷⁵	1997 [†]	China	cc	incidence	690	2	4	2.2 - 7.2
20	Yun ⁶⁰	2002 - 2006	Korea	cc	incidence	1,035	5	24.7	6.7 - 90.7
21	Zhang ⁷⁶	1994 - 1995	China	cc	incidence	267	4	0.5	.21 - 1.3

[†] Year of publication

* European countries includes: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom;
cc=Case-control

Table 7: Adjusted RR of liver cancer in viral hepatitis infection

	RR* (95% CI)
Hepatitis B	11.6 (7.7 – 17.5)
Hepatitis C	7.4 (4.3 – 12.8)

*RR estimated at the mean global alcohol intake level

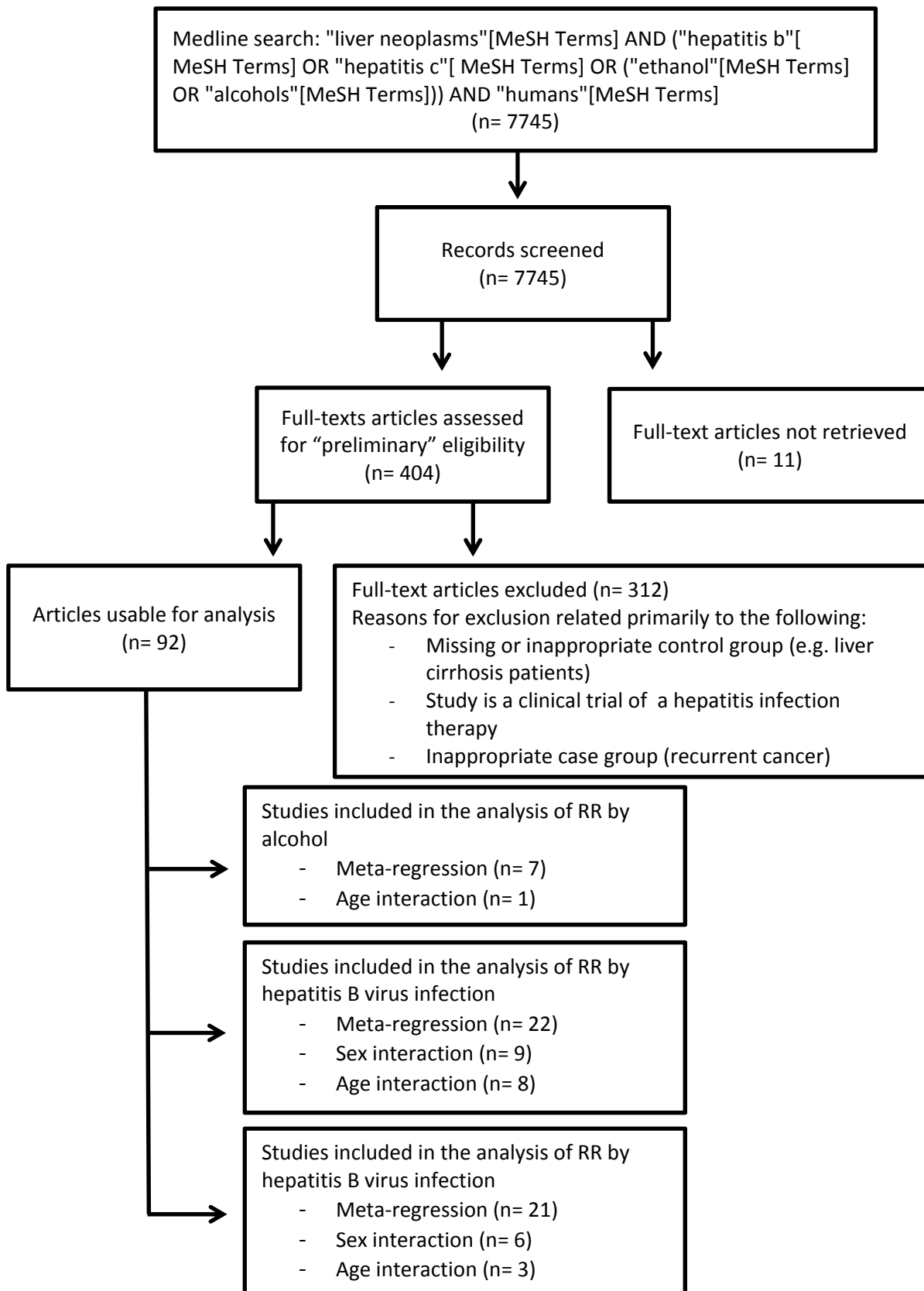


Figure 1: Flow diagram of the literature search

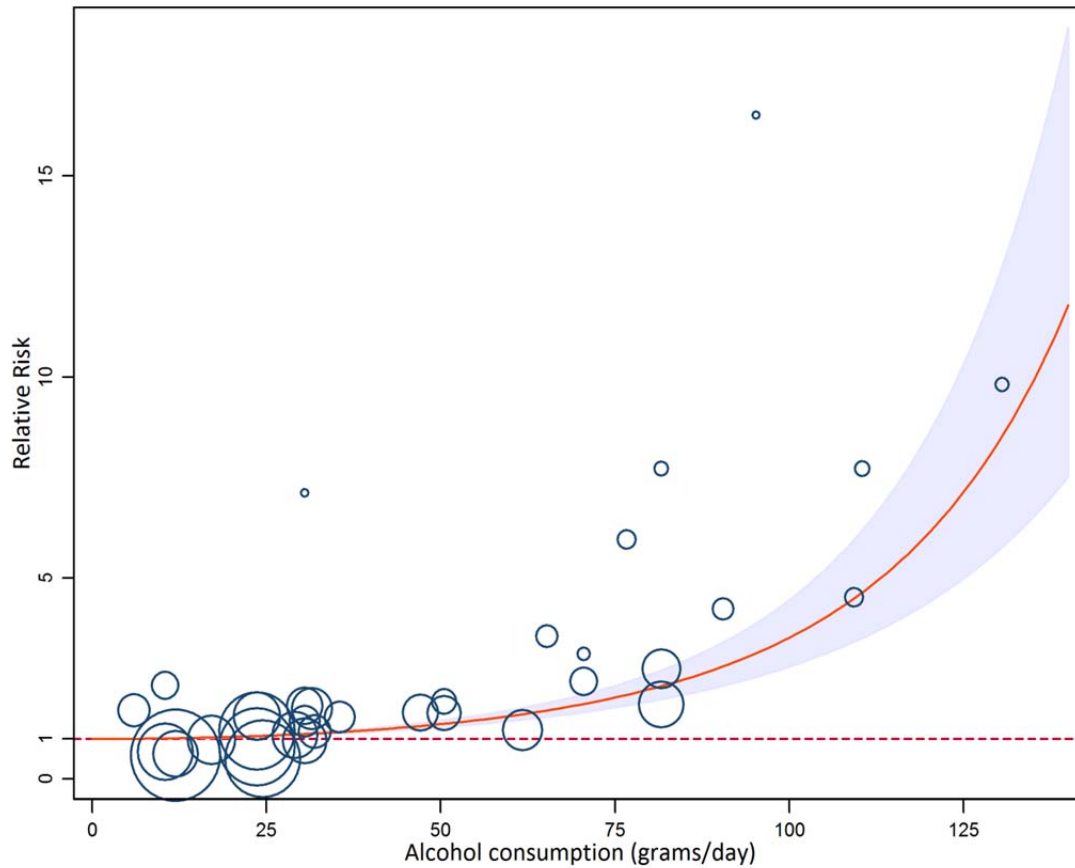


Figure 2: RR of liver cancer by daily ethanol intake. Raw data is represented with circles where the size is proportional to the inverse variance. Predicted RR estimates and 95% CI are indicated by the solid line and shaded area, respectively.

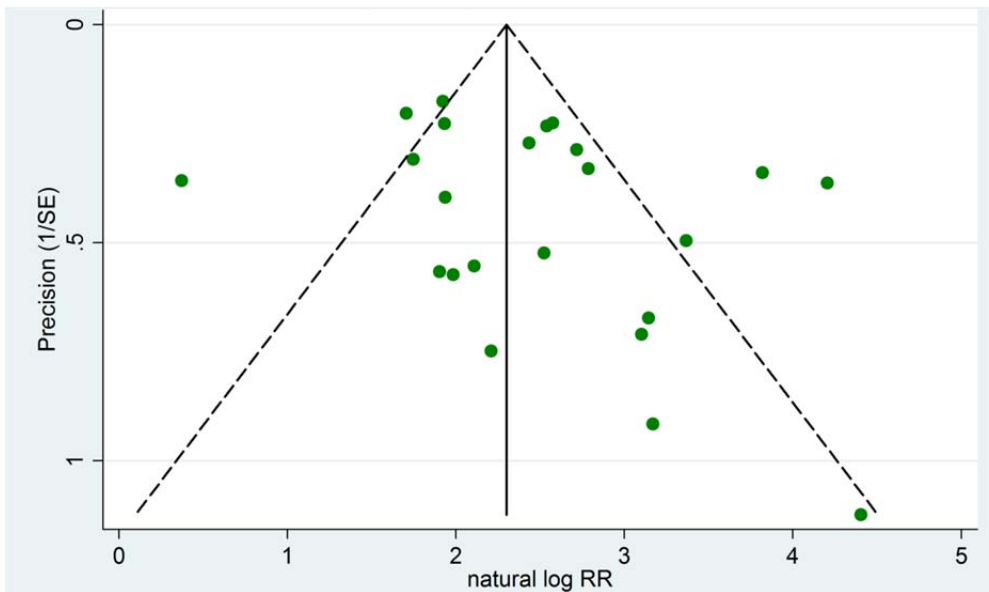


Figure 3: Funnel plot of hepatitis B analysis studies with pseudo 95% confidence limits. SE= standard error

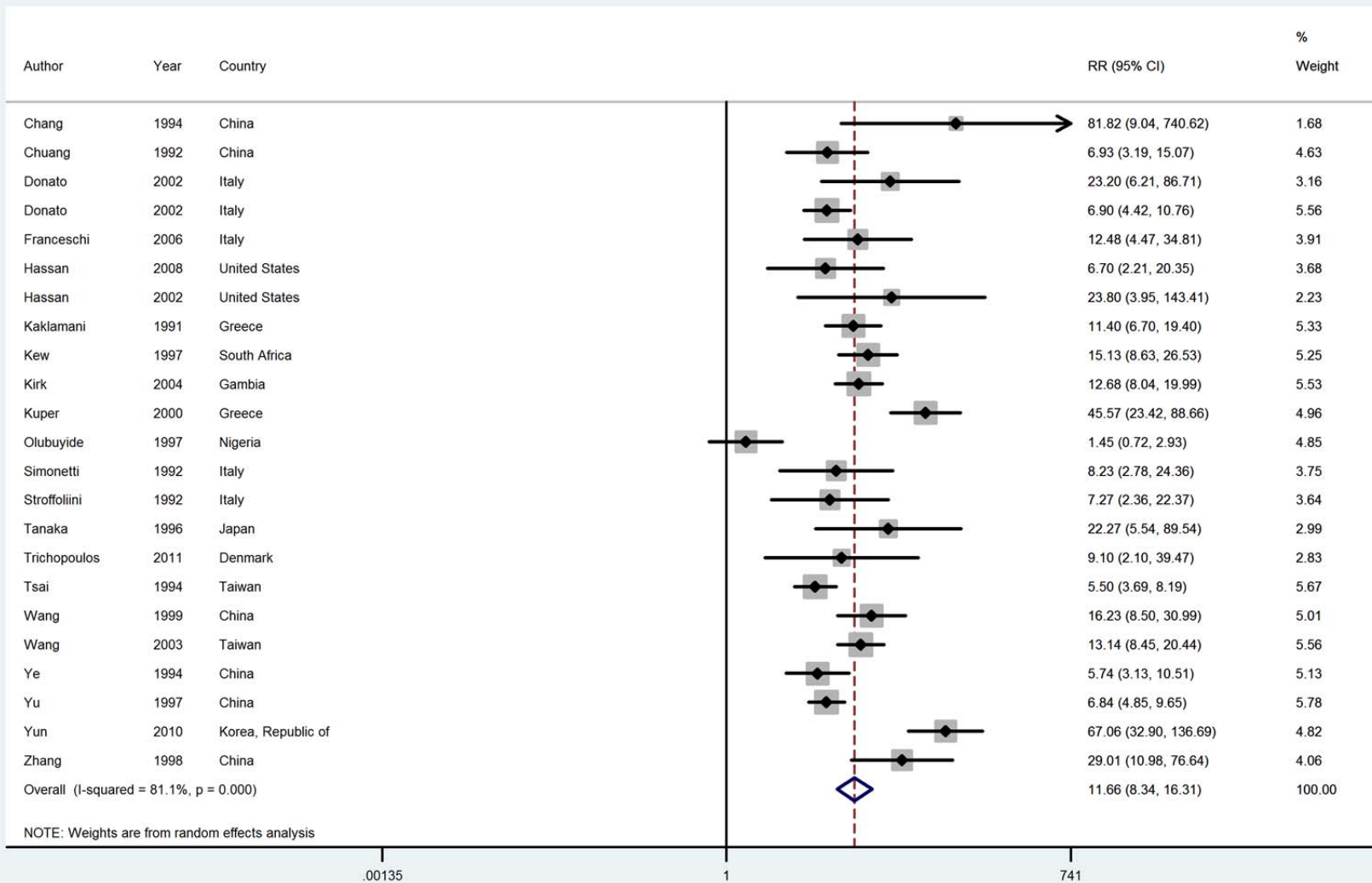


Figure 4: Pooled RR of liver cancer by hepatitis B using random effects meta-analysis. The pooled effect is adjusted for anti-HCV only.

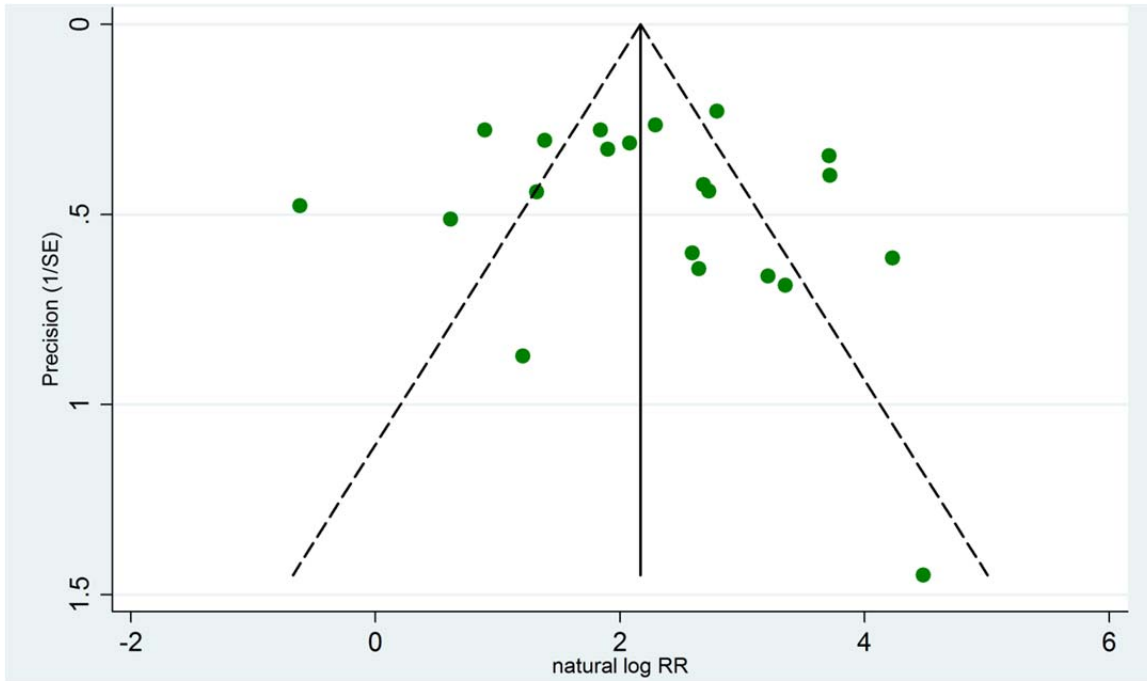


Figure 5: Funnel plot of hepatitis C analysis studies with pseudo 95% confidence limits.
SE= standard error

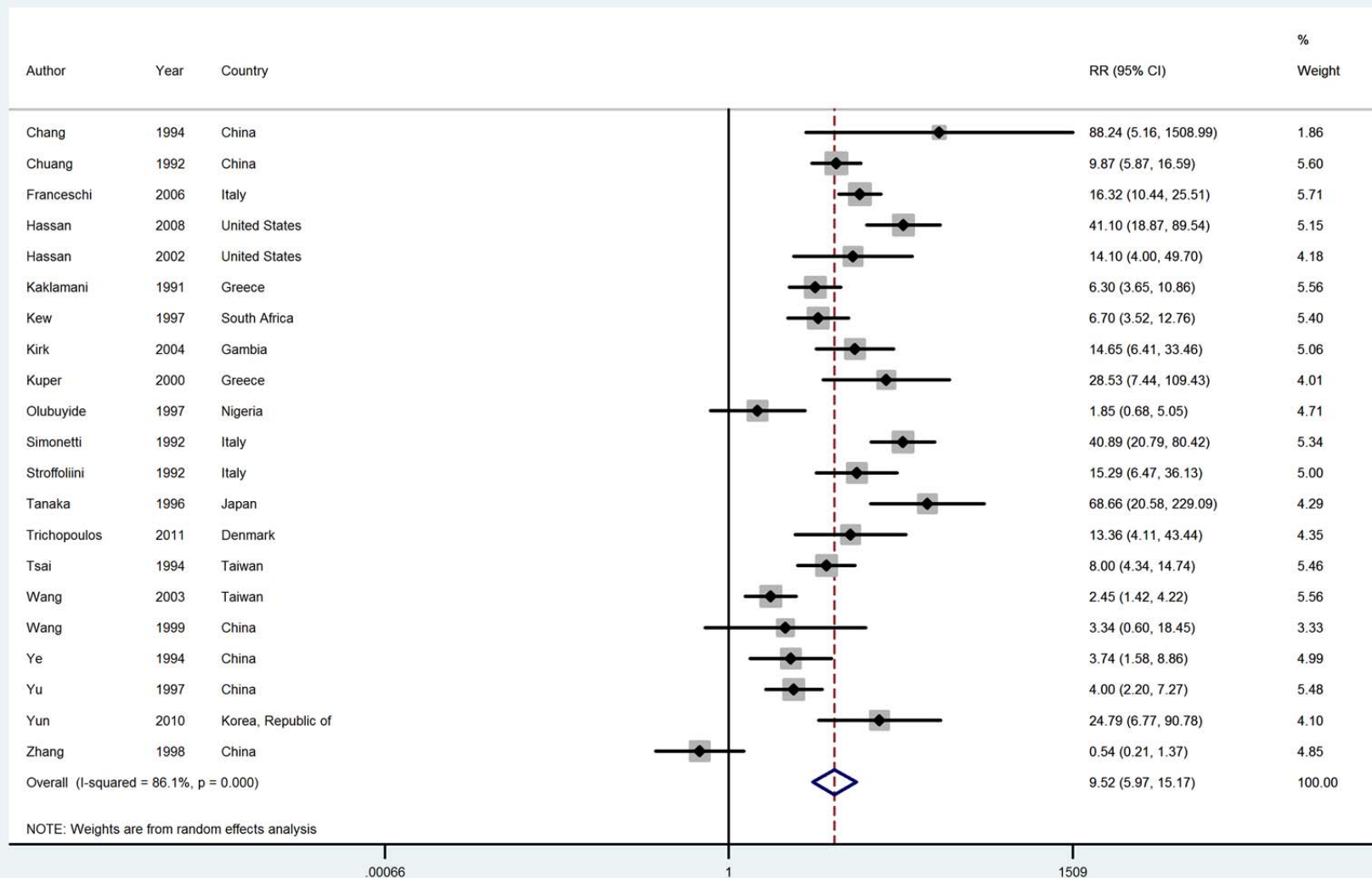


Figure 6: Pooled RR of liver cancer by hepatitis C using random effects meta-analysis. The pooled effect is adjusted for HbsAg only.

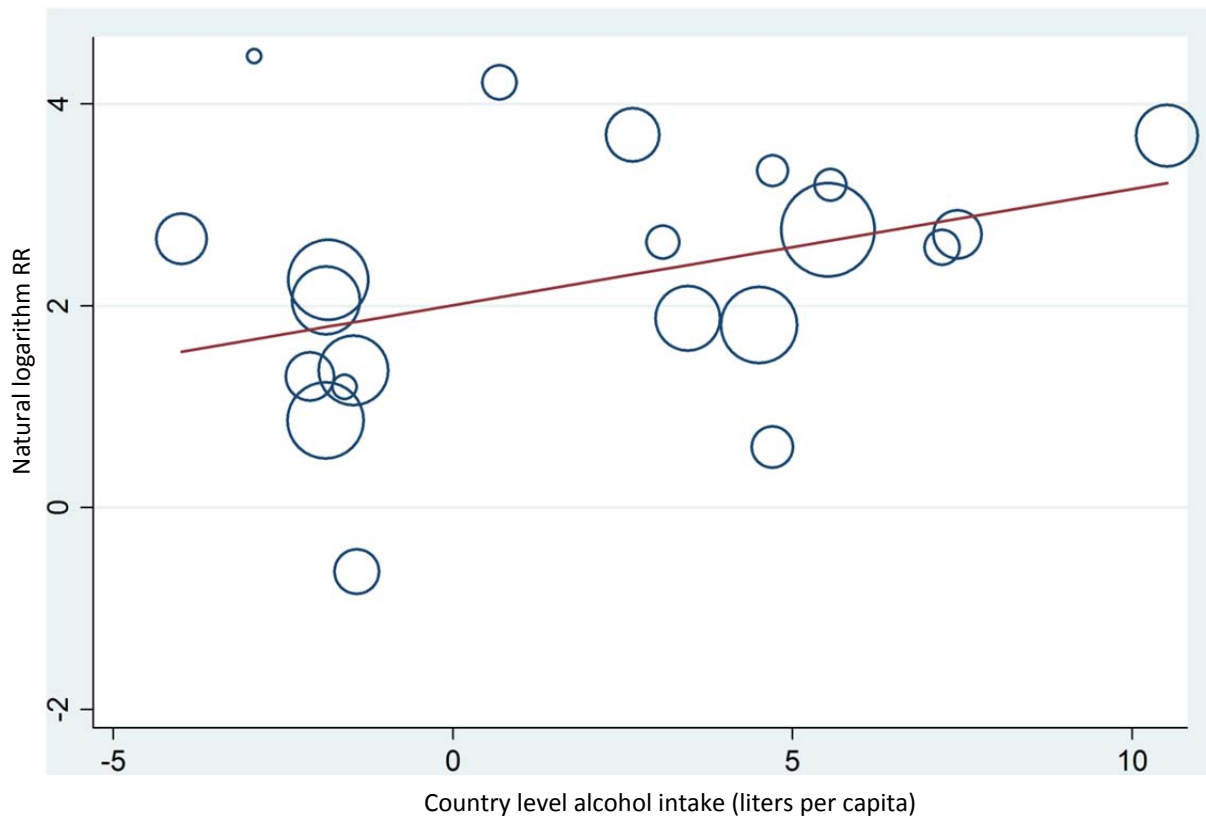


Figure 7: RR of liver cancer in patients with hepatitis C infection over country level alcohol intake levels. Alcohol consumption was centered at the global mean for all years available for analysis. Raw data are depicted in circles where the size of the circle is proportional to the inverse variance of the data point.