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Thrombocytosis is associated with worse survival in patients with hepatocellular carcinoma

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Abstract

Background & Aims: Thrombocytosis is associated with more aggressive tumour biology in many malignancies. There are limited data in patients with hepatocellular carcinoma (HCC), which often occurs in patients with cirrhosis and portal hypertension. We aimed to explore the prognostic value of thrombocytosis in two cohorts of patients with HCC.

Methods: We included 3561 patients from Taiwan and 1145 patients from the USA. Thrombocytopenia was defined as platelet count < 150×10^9/L and thrombocytosis as ≥ 300×10^9/L at HCC diagnosis. We used multivariable Cox proportional hazard models to identify independent predictors of survival.

Results: Thrombocytosis was present in 9.0% and 6.9% of Taiwan and USA patients respectively. Compared to patients with normal platelet counts and those with thrombocytopenia, patients with thrombocytosis had larger tumours, increased vascular invasion and a higher proportion had extrahepatic metastases in both cohorts. In multivariable analysis, thrombocytosis (aHR 1.40, 95% CI 1.23-1.60) and thrombocytopenia (aHR 1.13, 95% CI 1.04-1.23) were both associated with worse survival after adjusting for age, gender, liver disease aetiology, Child-Pugh score, maximal

Abbreviations: AA, African American; AFP, alpha-fetoprotein;; ALBI, albumin-bilirubin; BCLC, Barcelona Clínic Liver Cancer; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; INR, international normalized ratio; IQR, interquartile range; MELD, Model for End-stage Liver Disease; NHW, non-Hispanics White; PT, prothrombin time; SD, standard deviation.

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tumour size, tumour nodularity, vascular invasion, lymph node or distant metastasis, performance status and alpha-fetoprotein level. Patients with thrombocytosis had a median survival of 6 and 4 months in the Taiwan and USA cohorts, compared to 32 and 14 months for those with normal platelet counts and 38 and 16 months for thrombocytopenic patients.

Conclusion: Thrombocytosis is independently associated with increased tumour burden and worse overall survival among HCC patients.

KEYWORDS

liver cancer, platelet count, prognosis

1 | INTRODUCTION

With nearly 800 000 deaths annually, hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related mortality worldwide.[1] HCC is highly lethal and has a poor prognosis as a result of the lack of chemoprophylactic agents among high-risk individuals, delays in detection, frequent presentation at late stages and high rates of recurrence with currently available therapies.[2-5] While the highest incidence rates of HCC are still reported in Southeast Asia and sub-Saharan Africa, the incidence of HCC in the USA has tripled over the past 20 years.[6,7] While the mortality for most cancers in the USA is decreasing, HCC-related mortality continues to climb.[6]

Thrombocytosis is often considered as a paraneoplastic syndrome and is associated with poor prognosis in many types of malignancy such as ovarian cancer and gastric cancer.[8,9] However, few data evaluating the prognostic value of platelet counts in patients with HCC, which typically arises in a background of cirrhosis, where thrombocytopenia is prevalent as a result of portal hypertension and splenic sequestration.[10-14] The data that do exist have yielded conflicting information regarding the prognostic value of platelet counts. Thrombocytopenia has both been associated with increased HCC risk among patients with cirrhosis[15] as well as worse survival among HCC patients.[16,17] However, others have reported that thrombocytosis is associated with increased risk of vascular invasion and extrahepatic metastasis.[18,19] Furthermore, antiplatelet therapy, such as aspirin, may be associated with a reduction in HCC risk[20,21] and lower recurrence rate and improved survival after surgical resection.[22] Although the exact mechanism of this potential association is unknown, some have speculated that it may relate to aspirin's antiplatelet properties. Finally, the data that do exist have largely been derived from HBV-predominant populations in Asia and there are less data including Western patient populations with other aetiologies of liver disease such as HCV infection and alcohol-related liver disease.

To address this area of uncertainty, we performed a cohort study utilizing two large cohorts from Taiwan and the USA to examine the prognostic implications of thrombocytosis in patients with HCC.

Layman Summary

Thrombocytosis is independently associated with more advanced tumour stage and worse overall survival among patients with hepatocellular carcinoma. Our findings add to the growing literature about the complex interactions between platelet and hepatocellular carcinoma in patients with cirrhosis. Further translational studies are warranted in exploring the chemoprophylactic, prognostic and therapeutic roles of platelets and antiplatelet therapy among patients with hepatocellular carcinoma and patients at risk of hepatocellular carcinoma.

2 | PATIENTS AND METHODS

2.1 | Patients

This study consisted of two cohorts—one from Taiwan and the other from the USA. The Taiwan cohort included 3561 consecutive patients with newly diagnosed HCC admitted to Taipei Veterans General Hospital between 2002 and 2017. The USA cohort was comprised of 1145 consecutive newly diagnosed, treatment-naïve HCC patients seen at the University of Texas Southwestern Medical Center or Parkland Health & Hospital System between 2008 and 2017. Detailed information regarding the two cohorts has been reported previously.[23,24] For both cohorts, HCC diagnosis was based on the American Association for the Study of Liver Diseases (AASLD) and the Asian Pacific Association for the Study of the Liver (APASL) guidelines at the time of HCC diagnosis.[10,25] We excluded patients who did not meet AASLD or APASL criteria for HCC diagnosis or who were missing platelet count from the time of diagnosis. Patients were included independent of cirrhosis status, although prior data suggest over 90% of all HCC patients have cirrhosis at time of diagnosis.[26] The study protocols were approved by the Institutional Review Board of both institutions and complied with current ethical guidelines.

TABLE 1 Demographic, clinical and staging information among hepatocellular carcinoma patients stratified by blood platelet count in the Taiwan cohort

Variables	All patients	Stratified by platelet count (10^9/L)			
(Taiwan cohort)	(n = 3561)	<150 (n = 1727)	150-299 (n = 1513)	≥300 (n = 321)	*P* value
Age (years), median (IQR)	65 (55-75)	66 (57-76)	65 (54-75)	60 (48-73)	<.001
Male, %	77%	71%	82%	85%	<.001
[1]Aetiologies of chronic liver diseases (HBV/ HCV/alcohol/mixed/cryptogenic), %	40/22/5/18/15	37/29/5/19/10	43/16/5/16/20	42/12/8/17/21	<.001
Performance status (0/1/2-4), %	59/21/20	63/20/17	59/21/20	38/28/34	<.001
Mean/Median survival (months)	52/32	53/38	56/32	22/6	<.001
Tumour characteristics					
Maximal size 0-2/2-5/5-10/≥10 cm, %	18/39/25/18	26/48/19/7	12/33/31/24	3/11/31/55	<.001
Maximal size, median (IQR), cm	4.3 (2.5-8.8)	3.2 (2.0-5.2)	6.0 (3.0-10.0)	11.0 (7.5-15.0)	<.001
Multiple tumour, %	36%	37%	34%	41%	0.038
Any vascular invasion, %	24%	15%	29%	54%	<.001
Distant/lymph node metastasis, %	11%	6%	12%	28%	<.001
Laboratory values					
Platelet (10^9/L), mean ± SD	170 ± 96	97 ± 33	206 ± 40	389 ± 80	<.001
Albumin (g/dL), mean ± SD	3.7 ± 0.6	3.6 ± 0.6	3.8 ± 0.6	3.5 ± 0.6	<.001
Bilirubin (mg/dL), mean ± SD	1.5 ± 2.8	1.6 ± 2.6	1.4 ± 2.8	2.0 ± 4.1	<.001
INR of PT, mean ± SD	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	<.001
AFP (ng/mL), median (IQR)	43 (8-769)	32 (9-247)	49 (7-1467)	720 (15-28815)	<.001
Tumour staging					
BCLC staging (0/A/B/C/D), %	8/25/16/40/11	11/31/15/34/9	6/22/19/41/12	1/4/14/60/21	<.001
Liver functional reserve					
Child-Turcotte-Pugh class (A/B/C), %	73/22/5	71/24/5	78/18/4	61/33/6	<.001
Child-Turcotte-Pugh score, mean ± SD	6.1 ± 1.5	6.2 ± 1.6	5.9 ± 1.4	6.5 ± 1.6	<.001
ALBI grade (1/2/3), %	38/52/10	30/59/11	49/43/8	27/60/13	<.001
MELD score, mean ± SD	10.0 ± 4.4	10.3 ± 4.5	9.5 ± 4.2	10.4 ± 4.8	<.001

Note: hepatitis B surface antigen, HCV: positive for antihepatitis C virus antibody.

Abbreviations: AFP, alpha-fetoprotein; ALBI, albumin-bilirubin grade; BCLC, Barcelona Clínic Liver Cancer; INR of PT, international normalized ratio of prothrombin time; IQR, interquartile range; MELD, model for end-stage liver disease; SD, standard deviation.

[1]HBV, positive for HBsAg, negative for anti-HCV Ab and no alcoholism; HCV, positive for anti-HCV Ab, negative for HBsAg and no alcoholism; alcohol: negative for HBsAg, negative for anti-HCV Ab, with alcoholism; mixed: at least two aetiologies including hepatitis B, hepatitis C and alcoholism; cryptogenic: negative for HBsAg, negative for anti-HCV Ab, with no alcoholism

2.2 | Data collection

Baseline patient characteristics including demographics, liver disease aetiology, the severity of cirrhosis, performance status, complete blood count, serum biochemistries and serum alpha-fetoprotein were collected from the time of HCC diagnosis. The model for end-stage liver disease (MELD) and albumin-bilirubin (ALBI) scores and grades were calculated to assess the severity of liver dysfunction.[27,28] Hepatitis B virus (HBV) infection was defined by the presence of hepatitis B surface antigen, and hepatitis C virus (HCV) infection was based on the presence of a positive anti-HCV antibody or RNA. We collected tumour burden including the number of nodules, maximum tumour diameter, presence of vascular invasion and evidence of lymph node or distant metastases. Tumour stage was determined according to the Barcelona Clinic Liver Cancer (BCLC) staging system.[10,29] In both cohorts, patients were followed up every 3-6 months until death, liver transplantation or lost to follow-up. In the Taiwan cohort, mortality was additionally confirmed via a nationwide National Cancer Registry.

Platelet count was recorded at the time of HCC diagnosis for all patients. Thrombocytopenia was defined as a platelet count < 150×10^9/L[12] and thrombocytosis as a platelet count ≥ 300×10^9/L.[13,18,19] Normal platelet count was defined as platelet count between $150\text{-}299 \times 10^9$/L accordingly.

TABLE 2 Demographic, clinical and staging information among hepatocellular carcinoma patients stratified by blood platelet count in the USA cohort

Variables	All patients	Stratified by blood platelet count (10^9/L)			*P*
(US cohort)	(n = 1145)	<150 (n = 717)	150-299 (n = 349)	≥300 (n = 79)	value
Age (years), median (IQR)	59 (55-65)	59 (54-64)	60 (55-66)	61 (56-67)	.005
Male, %	78%	77%	78%	89%	.049
Cirrhosis	1036 (90%)	705 (98%)	280 (80%)	51 (65%)	<.001
[1]Aetiologies of chronic liver diseases (HBV/HCV/alcohol/mixed/cryptogenic), %	4/21/15/46/14	4/20/17/49/10	4/23/10/44/19	6/20/18/32/24	<.001
Race/ethnicity (NHW/H/AA/Asian/Other), %	34/28/32/5/1	37/33/25/4/1	30/19/42/7/2	25/17/52/6/0	
Performance status (0/1/2-4), %	55/27/18	57/27/16	54/29/17	36/30/34	.001
Mean/Median survival (months)	32/15	34/16	31/14	14/4	<.001
Tumour characteristics					
Maximal size 0-2/2-5/5-10/≥10 cm, %	17/40/22/21	21/45/22/12	11/36/22/31	1/14/22/63	<.001
Maximal size, median (IQR), cm	4.1 (2.4-8.7)	3.4 (2.2-6.1)	5.3 (2.8-11.8)	12.6 (7-17.4)	<.001
Multiple tumour, %	47%	44%	48%	67%	<.001
Any vascular invasion, %	29%	25%	33%	43%	.001
Distant/lymph node metastasis, %	18%	12%	24%	47%	<.001
Laboratory values					
Platelet (10^9/L), mean ± SD	145 ± 93	90 ± 33	202 ± 40	394 ± 82	<.001
Albumin (g/dL), mean ± SD	3.3 ± 0.7	3.2 ± 0.7	3.5 ± 0.7	3.4 ± 0.6	<.001
Bilirubin (mg/dL), mean ± SD	2.0 ± 3.1	2.1 ± 2.8	1.8 ± 3.6	1.5 ± 2.2	<.001
INR of PT, mean ± SD	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.4	<.001
AFP (ng/mL), median (IQR)	42 (8-981)	33 (8-572)	48 (6-1628)	451 (15-10372)	.005
Tumour staging					
BCLC staging (0/A/B/C/D), %	6/37/17/24/16	6/41/16/18/19	5/34/18/31/12	0/15/19/48/18	<.001
Liver functional reserve					
Child-Turcotte-Pugh class (A/B/C), %	49/38/13	40/44/16	63/30/7	63/32/5	<.001
Child-Turcotte-Pugh score, mean ± SD	7.1 ± 2.0	7.4 ± 2.0	6.5 ± 1.8	6.5 ± 1.4	<.001
ALBI grade (1/2/3), %	20/55/25	13/57/30	34/48/18	17/68/15	<.001
MELD score, mean ± SD	11.2 ± 5.0	11.9 ± 5.0	10.2 ± 4.9	9.9 ± 4.9	<.001

Abbreviations: AA, African American, AFP, alpha-fetoprotein, ALBI, albumin-bilirubin grade, BCLC, Barcelona Clínic Liver Cancer, INR of PT, international normalized ratio of prothrombin time, IQR, interquartile range, MELD, model for end-stage liver disease, NHW, non-Hispanics White, SD, standard deviation.

[1]HBV: positive for HBsAg, negative for anti-HCV Ab and no alcoholism; HCV: positive for anti-HCV Ab, negative for HBsAg and no alcoholism; alcohol: negative for HBsAg, negative for anti-HCV Ab, with alcoholism; mixed: at least two aetiologies including hepatitis B, hepatitis C and alcoholism; cryptogenic: negative for HBsAg, negative for anti-HCV Ab, with no alcoholism.

2.3 | Statistics

We used the Kruskal-Wallis tests to compare continuous variables between three patient groups, while the χ^2 and Fisher exact tests were utilized to compare categorical data. Median and overall survival was evaluated by the Kaplan-Meier method with log-rank tests. We employed the Cox proportional hazard modelling to examine the adjusted hazard ratios (aHR) and 95% confidence intervals (CI) for each prognostic variable. Prognostic factors including age, gender, liver disease aetiology, Child-Pugh score, maximal tumour size, tumour nodularity, vascular invasion, lymph node or distant metastasis, performance status, alpha-fetoprotein (AFP) and platelet count at diagnosis were included in univariable and multivariable analyses. Patients were censored at the time of liver transplantation or last visit if alive at the end of the study period or lost to follow-up.

As an exploratory analysis, we examined the possible non-linear relationship between platelet count and the hazards of mortality with restricted cubic splines.[30,31] To test for non-linearity, we used a likelihood ratio test to compare a model consisting of linear terms with a model consisting of both linear terms and the cubic spline terms. A two-tailed $P < .05$ was considered statistically significant

for all analyses. Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc, NC) and R 3.6.1 (R foundation, Vienna).

3 | RESULTS

3.1 | Patient and tumour characteristics

The cohort from Taiwan consisted of 3561 newly diagnosed HCC patients and the USA cohort included 1145 newly diagnosed patients. Baseline demographics are shown in Tables 1 and 2. The median age was 65 years in the Taiwan cohort and 59 years in the USA cohort, with the majority in both cohorts being male. As expected, the most common liver disease aetiology in the Taiwan cohort was HBV infection, while HCV infection accounted for most patients in the USA cohort. A total of 33% of patients in the Taiwan cohort and 41% of patients in the USA cohort had early-stage HCC (BCLC stage 0 or A).

3.2 | Thrombocytosis and tumour burden

A total of 1513 (42.5%) in the Taiwan cohort and 349 (30.4%) patients in the USA cohort had normal platelet count, while 1727 (48.5%) in the Taiwan cohort and 717 (62.7%) patients in the USA cohort had thrombocytopenia. Thrombocytosis was present among 321 (9.0%) of Taiwan patients and 79 (6.9%) of USA patients.

In both cohorts, patients with thrombocytosis were more likely to be male and had worse performance status, larger tumour size (Figure 1) and higher serum AFP levels (all $P < .05$). For patients with thrombocytosis, normal platelet count and thrombocytopenia, the median tumour size was 11.0 cm vs 6.0 cm vs 3.2 cm in the Taiwan cohort and 12.6 cm vs 5.3 cm vs 3.4 cm in the USA cohort respectively (both $P < .001$). Patients with thrombocytosis also had higher vascular invasion (54% vs 29% vs 15% in the Taiwan cohort and 42% vs 33% vs 26% in the USA cohort) and increased lymph node involvement or extra-hepatic distant metastases (28% vs 12% vs 6% in the Taiwan cohort and 48% vs 26% vs 12% in the USA cohort) respectively (all $P < .001$). Overall, patients with thrombocytosis presented more frequently with late-stage HCC (81% in Taiwan and 66% in USA cohorts), compared to 43% and 37% late-stage HCC among thrombocytopenic patients (both $P < .001$). In the USA cohort, HCC patients with thrombocytosis were less likely diagnosed by surveillance (11%) compared to 48% among thrombocytopenia patients ($P < .001$).

3.3 | Thrombocytosis, thrombocytopenia and overall survival

The median overall survival was 32 months and 15 months, respectively, for Taiwan and USA cohorts. In both cohorts, overall survival was similar between thrombocytopenic patients and patients with normal platelet count, whereas patients with thrombocytosis had significantly worse survival (Figure 2). Patients with thrombocytosis had a median overall survival of 6 and 4 months in the Taiwan cohort and USA cohort, respectively, compared with 32 and 14 months for patients with normal platelet counts, and 38 and 16 months for patients with thrombocytopenia. In univariable Cox survival analysis, thrombocytosis was associated with increased mortality compared to those with normal platelet counts in the combined cohort (HR 2.31, 95% CI 2.04-2.62). Thrombocytosis remained a significant predictor for worse survival in multivariable analysis after adjusting for known prognostic factors including age, gender, liver disease aetiology, Child-Pugh score, maximal tumour size, tumour nodularity, vascular invasion, lymph node or distant metastasis, performance status and AFP level in Taiwan cohort (aHR 1.39, 95% CI 1.20-1.61) and in the USA cohort (aHR 1.70, 95% CI 1.25-2.31, Table 3). In the combined cohort, thrombocytosis was independently associated with adverse outcome (aHR 1.40, 95% CI 1.23-1.60). Thrombocytopenia was also independently associated with worse survival in the combined cohort (aHR 1.13, 95% CI 1.04-1.23).

3.4 | Subgroup analyses: Thrombocytosis and overall survival

We combined the Taiwan and USA cohorts to examine the association between platelet count and survival among subgroups (Table 4). We found a consistent association between thrombocytosis and poor prognosis across liver disease aetiologies, including HBV infection (aHR 1.26, 95% CI 1.04-1.52), HCV infection (aHR 1.55, 95% CI 1.20-2.01), alcohol-related liver disease (aHR 1.40, 95% CI 1.09-1.79) and those with other aetiologies (aHR 1.72, 95% CI 1.28-2.31). We also found an association between thrombocytopenia and worse survival among patients with relatively preserved liver function (ALBI grade 1, aHR 1.21, 95% CI 1.02-1.42, Figure 3, Table 4). On the contrary, thrombocytosis was associated with worse survival among patients with significant liver dysfunction (ALBI grades 2-3, aHR 1.38, 95% CI 1.19-1.60).

3.5 | Non-linear analysis between platelet count and overall survival

In a post-hoc exploratory analysis, we examined the non-linear association between platelet count and overall survival among HCC patients in the combined cohort (Figure 4). Non-parametric regression suggested the platelet-survival relationship was non-linear in multivariable models (P for non-linearity < 0.001). The association appears to be non-linear among patients with relatively preserved liver function (ALBI grade 1, P for non-linearity = 0.022), and among patients with more significant liver dysfunction (ALBI grade 2 and 3, P for non-linearity = 0.041).

4 | DISCUSSION

Although thrombocytopenia has traditionally been associated with worse outcomes in patients with cirrhosis and HCC, platelets

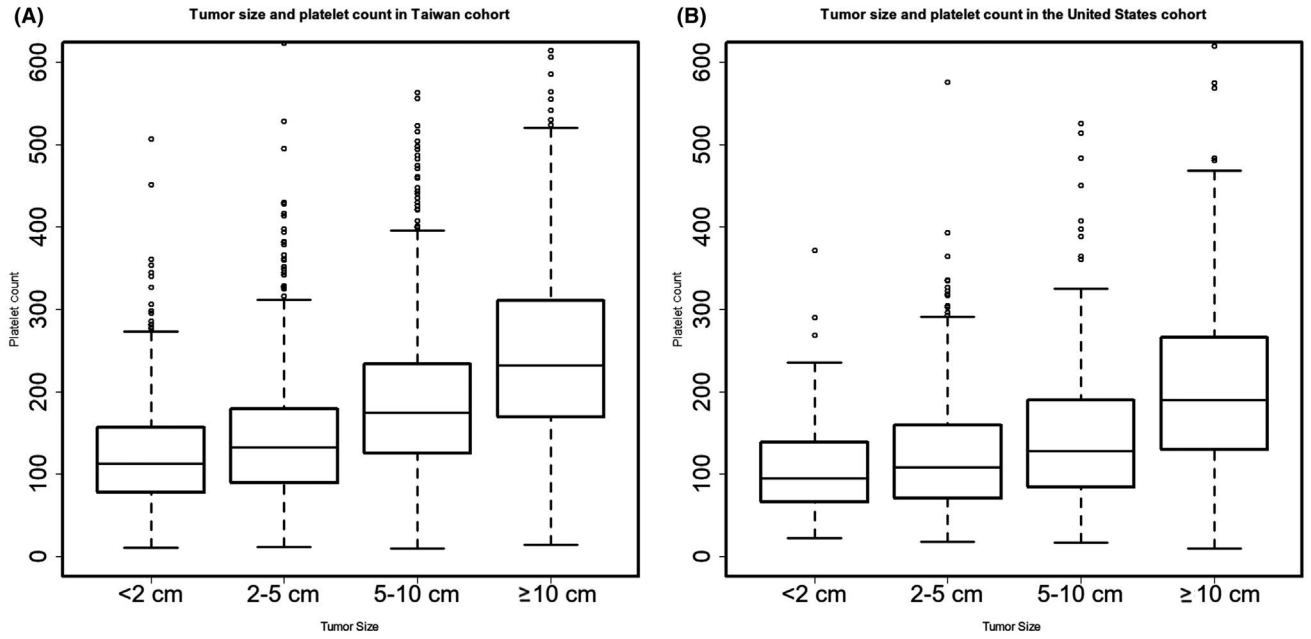

FIGURE 1 Distribution of platelet count according to maximal tumor size in (A) Taiwan cohort, and (B) USA cohort of patients with hepatocellular carcinoma. Higher platelet counts were associated with larger tumour diameter in both cohorts

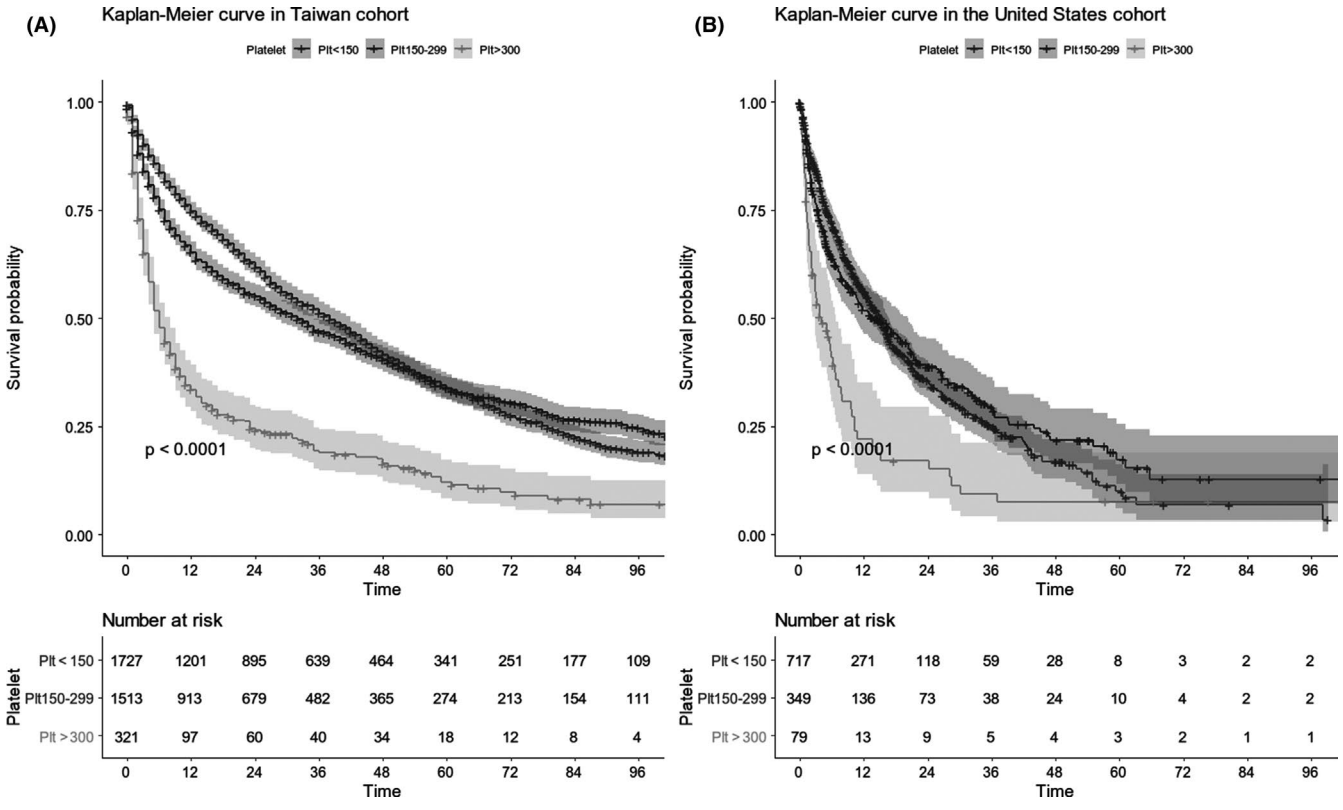

FIGURE 2 Overall survival according to blood platelet count in (A) Taiwan cohort, and (B) USA cohort of patients with hepatocellular carcinoma. Thrombocytosis (platelet count ≥ 300 × 10^9/L) was associated with decreased survival in both cohorts

are also actively involved in all aspects of carcinogenesis including tumour growth, extravasation and metastasis.[32] In this study, we recruited two large, well-characterized, HCC cohorts to examine the prognostic role of platelets in HCC patients. We found that thrombocytosis was associated with more advanced tumour burden and independently associated with worse survival after adjusting for age, gender, liver disease aetiology, Child-Pugh score, maximal tumour size, tumour nodularity, vascular invasion, lymph node or

TABLE 3 Univariable and multivariable survival analysis for patients with hepatocellular carcinoma in Taiwan, USA, and combined cohort

Risk factors (Total n = 4706)	Univariable[1] HR (95% CI)	*P* value	Multivariable[1] HR (95% CI)	*P* value	Univariable[1] HR (95% CI)	*P* value	Multivariable[1] HR (95% CI)	*P* value	Univariable[1] HR (95% CI)	*P* value	Multivariable[1] HR (95% CI)	*P* value
	Taiwan Cohort (n = 3,561)				USA Cohort (n = 1,145)				Combined Cohort (n = 4,706)			
Blood platelet count (10^9/L)												
Platelet < 150	0.95 (0.87-1.04)	.245	1.17 (1.06-1.28)	.002	0.99 (0.83-1.17)	.868	0.88 (0.73-1.05)	.149	0.95 (0.88-1.03)	.234	1.13 (1.04-1.23)	.004
Platelet 150-299	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Platelet ≥ 300	2.38 (2.07-2.73)	<.001	1.39 (1.20-1.61)	<.001	2.19 (1.64-2.91)	<.001	1.70 (1.25-2.31)	<.001	2.31 (2.04-2.62)	<.001	1.40 (1.23-1.60)	<.001
Maximal tumour size												
Tumour size ≤ 2 cm	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Tumour size 2-4.9 cm	1.33 (1.16-1.51)	<.001	1.23 (1.08-1.41)	.002	1.21 (0.93-1.56)	0.152	1.27 (0.98-1.65)	.075	1.32 (1.17-1.48)	<.001	1.24 (1.10-1.40)	<.001
Tumour size 5-9.9 cm	2.70 (2.36-3.10)	<.001	1.96 (1.69-2.27)	<.001	2.96 (2.26-3.86)	<.001	2.38 (1.79-3.17)	<.001	2.77 (2.45-3.13)	<.001	2.03 (1.78-2.32)	<.001
Tumour size ≥ 10 cm	5.45 (4.72-6.30)	<.001	2.86 (2.42-3.39)	<.001	7.23 (5.55-9.42)	<.001	3.66 (2.64-5.07)	<.001	5.94 (5.24-6.74)	<.001	3.06 (2.64-3.55)	<.001
Multiple tumours	1.53 (1.40-1.66)	<.001	1.32 (1.21-1.44)	<.001	3.00 (2.56-3.53)	<.001	1.55 (1.30-1.84)	<.001	1.74 (1.62-1.87)	<.001	1.38 (1.28-1.49)	<.001
Vascular invasion	4.71 (4.30-5.17)	<.001	2.50 (2.24-2.80)	<.001	4.74 (4.01-5.59)	<.001	1.91 (1.54-2.36)	<.001	4.69 (4.33-5.08)	<.001	2.29 (2.08-2.52)	<.001
Lymph node or distant met	4.45 (3.93-5.05)	<.001	1.58 (1.38-1.82)	<.001	4.65 (3.88-5.56)	<.001	1.59 (1.28-1.98)	<.001	4.55 (4.11-5.04)	<.001	1.61 (1.44-1.80)	<.001
ECOG performance status												
Performance status 0	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Performance status 1	2.12 (1.91-2.36)	<.001	1.29 (1.15-1.45)	<.001	2.34 (1.96-2.79)	<.001	1.42 (1.18-1.71)	<.001	2.19 (2.00-2.40)	<.001	1.33 (1.21-1.47)	<.001
Performance status 2-4	4.27 (3.85-4.73)	<.001	1.72 (1.52-1.95)	<.001	6.54 (5.38-7.94)	<.001	3.00 (2.39-3.76)	<.001	4.46 (4.07-4.88)	<.001	1.85 (1.67-2.06)	<.001
Cirrhosis	NA		NA		1.60 (1.18-2.19)	<.001	1.55 (1.09-2.21)	<.001	NA		NA	
Gender—Female	0.93 (0.83-1.03)	.159	0.94 (0.84-1.04)	.241	0.83 (0.68-1.01)	.067	1.02 (0.83-1.25)	.873	0.91 (0.83-1.00)	.004	0.95 (0.87-1.05)	.318
Age (per 10 years increase)	1.09 (1.05-1.13)	<.001	1.15 (1.11-1.19)	<.001	1.05 (0.97-1.14)	.220	1.14 (1.05-1.25)	.003	1.05 (1.02-1.08)	<.001	1.12 (1.09-1.16)	<.001
AFP (per 10^6 ng/mL increase)	2.93 (2.55-3.37)	<.001	2.07 (1.69-2.52)	<.001	6.40 (4.44-9.24)	<.001	3.32 (2.01-5.48)	<.001	3.05 (2.69-3.45)	<.001	2.11 (1.76-2.53)	<.001
CTP score (per 1 point) increase)	1.44 (1.41-1.47)	<.001	1.36 (1.32-1.40)	<.001	1.37 (1.32-1.42)	<.001	1.33 (1.27-1.39)	<.001	1.42 (1.40-1.45)	<.001	1.36 (1.33-1.39)	<.001

Abbreviations: CI, confidence interval, HR, hazard ratio.

[1]Additionally adjusted for age, gender and liver disease aetiology.

TABLE 4 Multivariable survival analysis among hepatocellular carcinoma subgroups in the combined Taiwan and USA cohort

Patient Subgroup	Definition	Number	[1]Multivariable HR (95% CI) for platelet < 150 × 10^9/L	[1]Multivariable HR (95% CI) for platelet 150-299 × 10^9/L	[1]Multivariable HR (95% CI) for platelet ≥ 300 × 10^9/L
HBV related	Positive for HBsAg	1996	1.12 (0.98-1.28)	1 [Reference]	1.26 (1.04-1.52)
HBV only	Positive for HBsAg, negative for anti-HCV antibody, and no alcoholism	1462	1.21 (1.04-1.42)	1 [Reference]	1.32 (1.06-1.65)
HCV related	Positive for Anti-HCV antibody	1804	0.99 (0.86-1.13)	1 [Reference]	1.55 (1.20-2.01)
HCV only	Positive for anti-HCV antibody, negative for HBsAg, and no alcoholism	1026	1.05 (0.88-1.27)	1 [Reference]	1.76 (1.25-2.49)
Alcohol related	Positive for alcoholism	1361	1.01 (0.86-1.19)	1 [Reference]	1.40 (1.09-1.79)
Alcohol only	Positive for alcoholism, negative for HBsAg and anti-HCV antibody	347	1.66 (1.16-2.38)	1 [Reference]	1.62 (1.02-2.56)
Cryptogenic	Negative for HBsAg and anti-HCV antibody, no alcoholism	715	1.40 (1.13-1.73)	1 [Reference]	1.72 (1.28-2.31)
No liver dysfunction	Albumin-bilirubin grade 1	1573	1.21 (1.02-1.42)	1 [Reference]	1.18 (0.89-1.57)
Liver dysfunction	Albumin-bilirubin grade 2 and 3	3133	1.03 (0.94-1.14)	1 [Reference]	1.38 (1.19-1.60)

Abbreviations: CI, confidence interval, HBV, hepatitis B virus, HBsAg, hepatitis B virus surface antigen, HCV, hepatitis C virus, HR, hazard ratio.
[1]Additionally adjusted for age, gender, liver disease aetiology, Child-Pugh score, maximal tumour size, tumour nodularity, vascular invasion, lymph node or distant metastasis, performance status and alpha-fetoprotein level.

distant metastasis, performance status and AFP level. The results were consistent across cohorts and liver disease aetiology. Further experimental and clinical studies are warranted to explore the chemoprophylactic, prognostic, and therapeutic role of platelets in HCC patients.

Our findings that thrombocytosis is associated with advanced tumour burden and potentially more aggressive tumour behaviour are in line with previous reports regarding the association between platelets and tumour stage.[13,18,19] It is unclear if this difference is related to differences in tumour biology or simply related to differences in surveillance receipt. HCC surveillance is known to be a key driver of early tumour detection and is widely underused among at-risk patients.[33-35] We only had surveillance data available for the USA cohort and patients with thrombocytosis were significantly

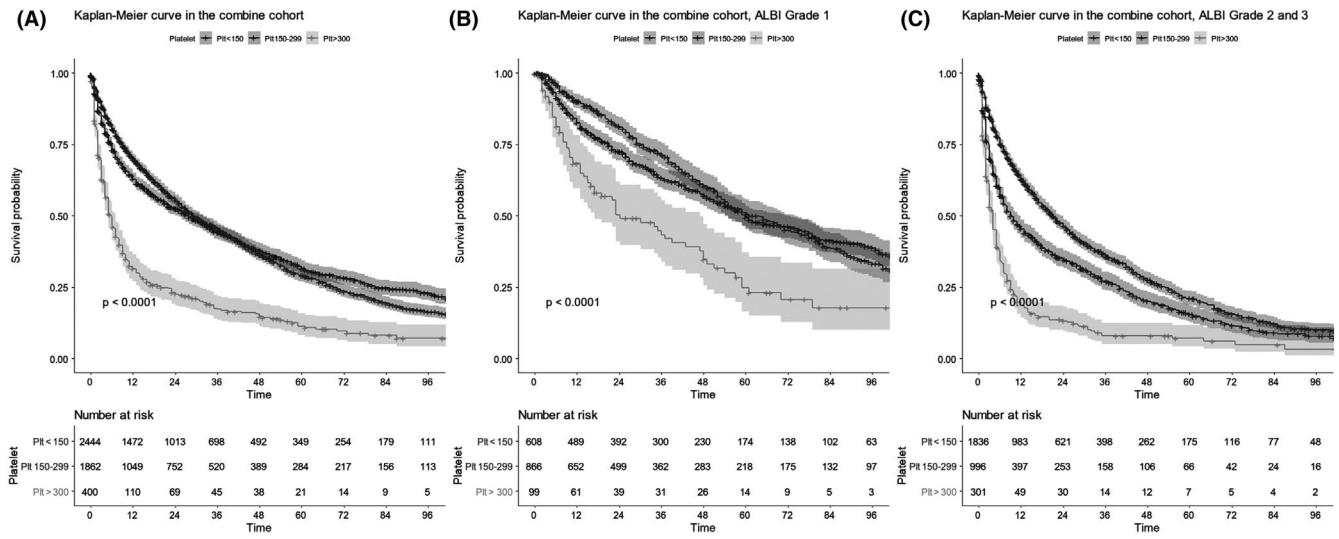

FIGURE 3 Overall survival according to blood platelet count in the combined hepatocellular carcinoma cohort. Thrombocytosis (platelet count ≥ 300 × 10^9/L) was associated with decreased survival among (A) all patients in the combined cohort, and (B) patients with preserved liver function (albumin-bilirubin grade 1). Among patients with significant liver dysfunction (C, albumin-bilirubin grade 2 and 3), platelet level was inversely related to overall survival

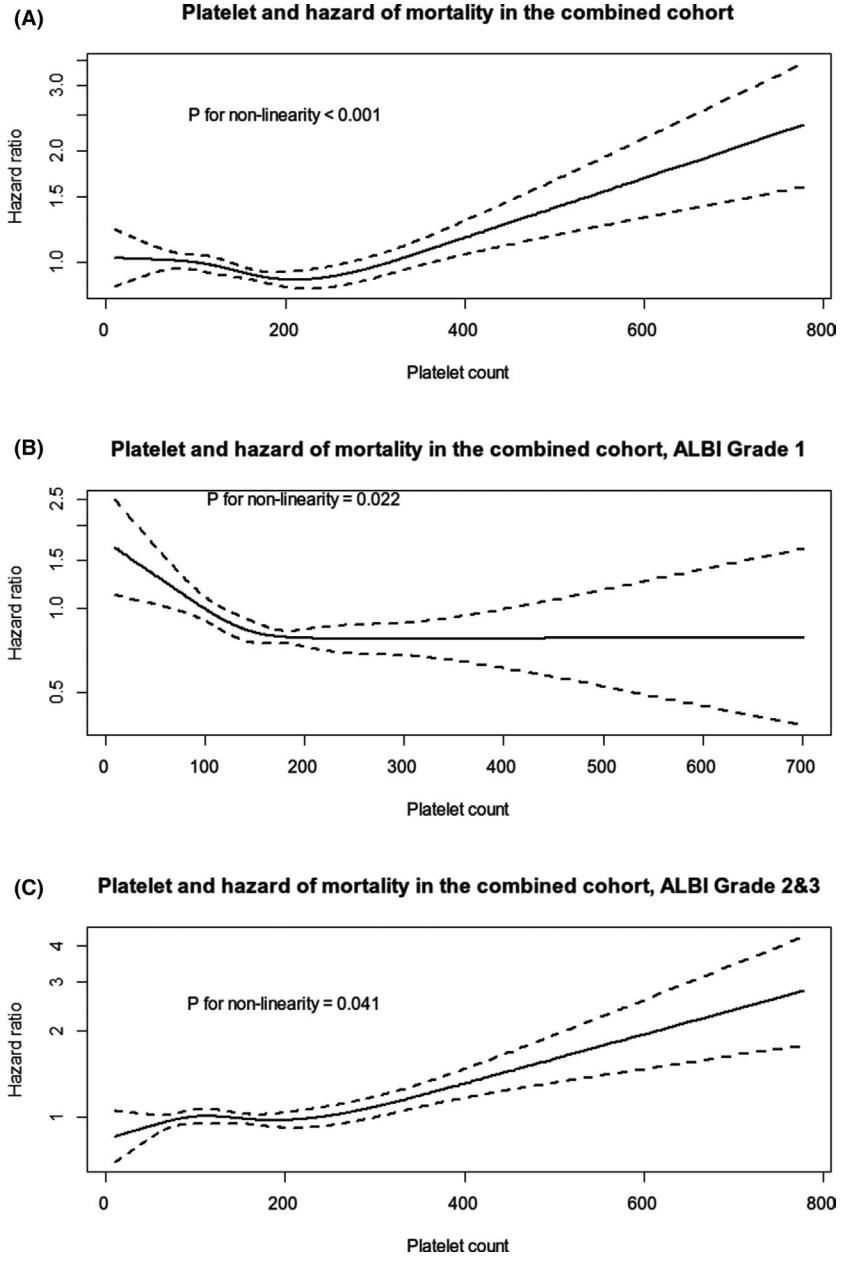

FIGURE 4 Non-parametric restricted cubic splines of platelet count and hazard for mortality in the multivariable models in the combined hepatocellular carcinoma cohort among (A) all patients, (B) patients with relatively preserved liver function (albumin-bilirubin grade 1) and (C) patients with more significant liver dysfunction (albumin-bilirubin grade 2 and 3). We adjusted for age, gender, liver disease aetiology, Child-Pugh score, maximal tumour size, tumour nodularity, vascular invasion, lymph node or distant metastasis, performance status and alpha-fetoprotein level in the multivariable models

less likely to be detected by surveillance. The lower surveillance receipt in these patients may relate to the under-recognition of cirrhosis, a common barrier to HCC surveillance in clinical practice.[36] Thrombocytosis has also been associated with worse survival among many other types of cancer[8,9,32]; however, to our knowledge, this is among the first reports confirming the prognostic implications of thrombocytosis among HCC patients. As platelet count is readily available in all HCC patients, the presence of thrombocytosis can serve as an important prognostic marker identifying patients who may benefit from more aggressive treatment, including antiplatelet therapies.[18,22]

Many existing reports on the relationship between platelet and liver diseases have focused on subjects with HBV infection. For example, antiplatelet therapy has been shown to prevent HCC both in a mouse model and in patients with chronic hepatitis B.[20,37-39] Our results confirmed the association between thrombocytosis and poor prognosis among HBV-infected patients; however, this association was independent of liver disease aetiology and consistent effect sizes were seen in patients with HCV, alcohol-related cirrhosis and other aetiologies. As platelets are closely involved in all steps of tumorigenesis,[32,40] additional research is warranted to reveal the roles of platelets in patients with HCC as a result of other aetiologies.

We also found that thrombocytopenia was a predictor of worse clinical outcomes. Thrombocytopenia, as a marker for portal hypertension, is closely associated with liver dysfunction and overall survival. We thus performed post-hoc subgroup analysis based on the degree of liver dysfunction. Among patients with relatively preserved liver function (ALBI grade 1), thrombocytopenia, but not thrombocytosis, was associated with decreased survival (Figure 4B, Table 4). On the other hand, among patients with more

significant liver dysfunction (ALBI grade 2 and 3), thrombocytosis, but not thrombocytopenia, was associated with worsening survival (Figure 4C, Table 4). One possible explanation is that among patients with preserved liver function, thrombocytopenia represented more severe portal hypertension, hence the decreased outcome. On the other hand, patients with more significant liver dysfunction would likely have background thrombocytopenia, and a "normal" platelet count could actually be an "elevated" platelet count, indicating an aggressive tumour behaviour. Taken together, these results suggest platelet count may not only serve as an indicator of portal hypertension but also potentially as a dual marker of portal hypertension *and* tumour biology, thereby having varying prognostic implications among different patients.

Our study's findings call for further evaluation of the therapeutic roles of antiplatelet therapy in HCC patients. In a mouse model of ovarian cancer, thrombocytosis was the result of increased hepatic thrombopoietin synthesis in response to tumour-derived interleukin-6.[8] The use of antiplatelet antibody reduced tumour growth in mouse models.[8] It is interesting that sorafenib, the first targeted therapy in HCC patients, also harbours antiplatelet activity by inhibiting platelet-derived growth factor receptor β.[41] The potential of antiplatelet therapy was demonstrated by studies showing that antiplatelet therapy was associated with improved HCC survival after surgical resection[22] and chemoembolization.[42] Counteracting thrombocytosis either by directly targeting platelets, eg aspirin or NSAIDs, or indirectly via cytokines may be a new avenue of therapeutic options among HCC patients.

The study has certain inherent limitations. Although the study included HCC cohorts from Taiwan and the USA encompassing wide ranges of liver disease aetiologies, our results may not be generalizable to other geographic areas. Second, although patients were enrolled consecutively in both cohorts, referral bias cannot be avoided completely. Third, platelet count was measured at a single time point rather than serial measurements. We did not have serum information of pertinent biomarkers such as thrombopoietin and interleukin-6 levels in HCC patients, and could not elucidate the complex interaction between platelet and these cytokines. Fourth, we did not collect the administration of antiplatelet therapy before or after HCC diagnosis and could not evaluate the prognostic role of antiplatelet therapy among HCC patients. Finally, we do not have information about platelet size to examine its prognostic implications. However, we feel that these limitations are outweighed by the study's strengths including its novelty, large multinational, well-characterized cohort and detailed granular data on tumour burden and overall survival.

In conclusion, thrombocytosis was independently associated with more advanced tumour stage and worse overall survival among HCC patients. Our findings add to the growing literature about the complex interaction between platelets and HCC. Further translational studies are warranted in exploring the chemoprophylactic, prognostic and therapeutic roles of platelets and antiplatelet therapy among HCC patients.

CONFLICT OF INTEREST

Dr Liu was a stockholder in Novartis and Neurocrine Biosciences. Dr Singal has served on advisory boards or as a consultant for Gilead, Abbvie, Bayer, Eisai, Bristol Meyers Squibb, Exelixis, Wako Diagnostics, Exact Sciences. Roche, Glycotest and TARGET Pharmasolutions. He has received research funding from Gilead and Abbvie. The other authors have none to declare.

AUTHOR CONTRIBUTIONS

Drs. Liu and Huo had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version of the manuscript. Study concept and design: Liu, Singal and Huo; Acquisition of data: Liu, Hsu, Su, Huang, Hou, Rich and Singal; Analysis and interpretation of data: all authors; Drafting of the manuscript: Liu; Critical revision of the manuscript for important intellectual content: all authors; Statistical analysis: Liu and Hsu; Obtained funding: Huang, Singal and Huo; Study supervision: Singal and Huo.

ROLE OF SPONSOR

The study sponsors have no role in the study design, collection, analysis and interpretation of data.

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APPENDIX A

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State-specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-7
Setting	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up and data collection	5-6
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	5-6
		(*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	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders and effect modifiers. Give diagnostic criteria, if applicable	5-6
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	4-6
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	6-7
		(*c*) Explain how missing data were addressed	6-7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	
		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	6-7
		(*e*) Describe any sensitivity analyses	6-7
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	5
		(b) Give reasons for non-participation at each stage	5
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarize follow-up time (eg average and total amount)	7

	Item No	Recommendation	Page
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
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	8-9
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarize key results with reference to study objectives	10
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	12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies and other relevant evidence	10-13
Generalizability	21	Discuss the generalizability (external validity) of the study results	10-13
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	2

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.

*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.