INCIDENCES, TEMPORAL TRENDS AND RISKS OF HOSPITALIZATION FOR GASTROINTESTINAL BLEEDING IN NEW OR CHRONIC LOW-DOSE ASPIRIN USERS AFTER TREATMENT FOR *HELICOBACTER PYLORI*: A TERRITORY-WIDE COHORT STUDY

Short Title: GIB in aspirin users after HP eradication

Chuan-Guo Guo[1], Ka Shing Cheung[1], Feifei Zhang[2], Esther W Chan[3], Lijia Chen[1], Ian CK Wong[3,4], Wai K Leung[1]

[1] Department of Medicine, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong

[2] Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, United Kingdom

[3] Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong

[4] Research Department of Practice and Policy, UCL School of Pharmacy, London, UK

Correspondence: Wai K. Leung, MD, Department of Medicine, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong

Email: waikleung@hku.hk; Fax: +852 28162863

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ABSTRACT

Objective: The risk of gastrointestinal bleeding (GIB) in aspirin users after *H. pylori* (HP) eradication remains poorly defined. We characterized the incidences and temporal trends of hospitalizations for all GIB in aspirin users after HP eradication therapy.

Design: Based on a territory-wide health database, we identified all patients who had received the first course of clarithromycin-based triple therapy between 2003 and 2012. Patients were divided into three cohorts according to aspirin use: new users (commenced after HP eradication), chronic users (commenced before and resumed after HP eradication) and non-users. The primary outcome was to determine the risk of hospitalization for GIB.

Results: We included 6,985 new aspirin users, 5,545 chronic users, and 48,908 non-users. The age- and sex-adjusted incidence of hospitalization for all GIB in new, chronic and non-users was 10.4, 7.2 and 4.6 per 1000 person-years, respectively. Upper and lower GIB accounted for 34.7% and 45.3% of all bleeding, respectively. Compared to chronic users, new users had a higher risk of GIB (hazards ratio [HR] with propensity score matching: 1.89; 95% CI 1.29-2.70). Landmark analysis showed that the increased risk in new aspirin users was only observed in the first 6-month for all GIB (HR 2.10, 95% CI 1.41-3.13) and upper GIB (HR 2.52, 95% CI 1.38-4.60), but not for lower GIB.

Conclusion: New aspirin users had a higher risk of GIB than chronic aspirin users, particularly during the initial 6-month. Lower GIB is more frequent than UGIB in aspirin users who had HP eradicated.

SIGNIFICANCE OF THE STUDY

What is already known about this subject?

- *H. pylori* eradication has been shown to reduce the risk of upper gastrointestinal bleeding (GIB) in patients newly started on aspirin.
- Aspirin is also increasingly recognized to be an important cause of lower GIB.
- However, the long term risks and temporal patterns of GIB, including upper and lower, among new or chronic aspirin users who had *H. pylori* eradicated remain uncertain.

What are the new findings?

- We found that the risk of GIB, both upper and lower, were significantly higher among new or chronic aspirin users when compared to non-users in a territory-wide cohort of *H. pylori* infected patients who had received eradication therapy.
- New aspirin users had a significantly higher risk of GIB, especially upper GIB, than chronic aspirin users, particularly during the initial 6-month of aspirin therapy.
- Although the incidence of LGIB was even more frequent than upper GIB after treatment for *H. pylori*, similar decline in the risk of lower GIB between new and chronic aspirin users was not observed.

How might it impact on clinical practice in the foreseeable future?

- New aspirin users still have an increase in risk of GIB even after eradication of *H. pylori*, particularly during the initial 6-month of aspirin therapy.
- Lower GI tract appears to be an important source of bleeding among aspirin users who had *H. pylori* eradicated.

INTRODUCTION

Acute gastrointestinal bleeding (GIB) is one of the most common causes of hospitalization and emergency visit, resulting in a substantial economic burden on the health care system. In the United States, GIB accounted for more than 500,000 hospitalizations and consumed $4.85 billion in 2012.[1] *Helicobacter pylori* (*H. pylori*) infection, non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin uses are generally considered to be the most important risk factors in the pathogenesis of peptic ulcers as well as the causes of non-variceal upper gastrointestinal bleeding (UGIB).[2, 3] With the widespread use of *H. pylori* eradication therapy, the prevalence of *H. pylori* infection had been declining globally.[4] However, with the aging population, aspirin is increasingly used in the prevention of cardiovascular or cerebrovascular events[5], giving rise to the increasing proportion of patients with UGIB due to aspirin. While both *H. pylori* infection and aspirin are risk factors for peptic ulcer and its complications, *H. pylori* eradication has been shown to reduce the risk of GIB in low-dose aspirin users.[6-8] *H. pylori* eradication is therefore recommended in long-term aspirin users, especially high-risk patients.[8-10]

Apart from UGIB, aspirin is increasingly recognized to be associated with lower gastrointestinal bleeding (LGIB).[11, 12] Although *H. pylori* eradication and the use of gastroprotective agents, including proton pump inhibitors (PPI) and histamine type-2 receptor antagonists (H2RA), could reduce the risk of UGIB, the risk of LGIB remains. There is a significant knowledge gap about the natural history of all-cause GIB,

including upper and lower GIB, among aspirin users who had *H. pylori* eradicated. The issue is further complicated by the potential adaptive effects of the gastric mucosa to aspirin, in which aspirin associated UGIB tend to occur at the early course of treatment [13,14]. As yet, whether there is similar adaptive effect to aspirin in the lower gastrointestinal tract remains unknown.

Based on a large cohort of *H. pylori*-eradicated patients from Hong Kong, we characterized the incidences, temporal trends and risks of hospitalizations for all-cause GIB, including UGIB and LGIB, in new aspirin users as compared to chronic users and non-users.

METHODS

Data source

All data were retrieved from the Clinical Data Analysis and Reporting System (CDARS) of the Hong Kong Hospital Authority. The Hospital Authority is the only public healthcare provider of Hong Kong with more than 7 million residents. The CDARS is a centralized electronic system which records all patients' clinical information including demographics, diagnoses, prescriptions, treatment, hospitalization and death.[15-17] All records were anonymized to protect patients' confidentiality, and a unique numeric identifier was assigned to each patient. The International Classification of Diseases, Ninth Revision (ICD-9) was used for disease coding and the accuracy of

coding for GIB had been previously verified.[16] This study was approved by the Institutional Review Board of the University of Hong Kong and the Hospital Authority Hong Kong West Cluster (Reference number: UW 16-545).

Study subjects and study design

We have previously identified a large cohort of *H. pylori*-infected patients who had received clarithromycin-based triple eradication therapy in Hong Kong between 1 January 2003 and 31 December 2012.[17, 18] In this study, we analyzed the risk of hospitalization for GIB in this cohort who used aspirin after *H. pylori* eradication. Patients who were newly started on aspirin after *H. pylori* eradication, but who had not used any aspirin within two years before the eradication, were classified as new users. Patients who used aspirin both before and after *H. pylori* eradication therapy were classified as chronic users, whereas those who had never used aspirin both before and after *H. pylori* eradication were labelled as non-users (**Supplementary Figure 1**). Patients who used aspirin before *H. pylori* eradication but did not resume on aspirin after the eradication therapy were excluded.

Since post-eradication *H. pylori* statuses were not available in the electronic database, we excluded patients who required re-treatment for *H. pylori* as described previously.[17] Other exclusion criteria included patients with follow up less than 7 days, patients who had gastrointestinal cancer, inflammatory bowel disease, coagulant deficiency,

gastroenteritis or colitis due to radiation, and excision of gastrointestinal tract segment. In addition,

Outcome and covariates

The primary outcome was to determine the incidences of hospitalization for GIB in new aspirin users, chronic users and non-users after *H. pylori* eradication therapy, and to compare the risk of GIB in new users with chronic users. The risk factors of GIB among all aspirin users were also evaluated. The start point of the follow-up period for all aspirin users was the date of starting or resuming aspirin after *H. pylori* eradication therapy. The end-point was the occurrence of GIB, 30 days after aspirin discontinuation, death, or the end of the study at 30 June 2016. The maximum observation period was set as 10 years. Discontinuation of aspirin was defined as an interruption for more than 30 days between two aspirin prescriptions. Since there was no definite start date for non-users, the start date was arbitrarily set as 60 days after *H. pylori* eradication. The 60 days was chosen to allow for the healing of possible peptic ulcer, which may falsely increase the bleeding rate.[19] A sensitivity analysis was also performed to use 7 days after eradication as start date for non-users.

The primary end point was hospitalization for non-variceal GIB, which was retrieved using the ICD-9 codes of UGIB, LGIB and unspecified GIB (578.xx, **Supplementary Table 1**). Hematemesis (578.0) and melena or black tarry stool (578.1) were regarded

as UGIB, whereas hematochezia from 578.1 was taken as LGIB in this study. For other diagnoses with the code of 578.xx, the specific bleeding site would be used if the description of the diagnosis had mentioned the bleeding location. Moreover, if there were new specific diagnoses within 30 days, the diagnosis of unspecified GIB would be renewed with the original index date unchanged. As a secondary outcome, the risk of in-hospital mortality was also evaluated, which was defined as death during the hospitalization for GIB.

Baseline characteristics of the patients, their comorbid medical conditions and concurrent medications were included as covariates in binary variables. Pre-existing medical conditions before enrollment were extracted using ICD-9 codes including history of GIB or peptic ulcer, hypertension, ischemic heart disease, stroke (ischemic stroke, transient ischemic attack or systemic embolism), diabetes, renal disease, intracranial hemorrhage, and liver cirrhosis. Concurrent medications (**Supplementary Table 2**) used during the follow-up period which could potentially alter the bleeding risk were also included: gastroprotective agents including PPI and H2RA, other antiplatelet drugs, NSAIDs, anticoagulants, corticosteroids, selective serotonin reuptake inhibitors (SSRI) and bisphosphonate. Drug usage was defined as more than 7 days use during the follow-up period. To reduce potential indication bias for gastroprotective agents, PPI and H2RA prescription records within the last 4 weeks of the event date or censor date were excluded.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR), while categorical variables were presented as frequencies and percentages. Mann-Whitney U test was used for continuous variables and Chi-square test or Fisher's exact test was used for categorical variables. Incidence rates and relative risks (RR) among new, chronic aspirin users and non-users were calculated. The in-hospital mortality rate of GIB was also determined.

The risks of hospitalization for GIB among new, chronic users and non-users were illustrated by fitting Kaplan-Meier curves and the differences were tested using the log-rank test. Cox proportional hazards regression model was used and the bleeding risk was expressed in hazards ratios (HR) with 95% confidence intervals (CI). When fitting a Cox regression model, the proportional hazards assumption was checked using Schoenfeld test and graphical diagnostics by plotting the scaled Schoenfeld residuals against the survival times.[20, 21] Once violation of this assumption was observed, interactions of time-dependent covariates with time would be introduced into the regression model.[21] In multivariable Cox regression model, concurrent medications were included as time-varying covariates, of which the follow-up period was split into 3-monthly intervals and drug usage was defined in each interval as more than 7 days use. In all regression models, aspirin use was included as a time-varying variable.

To balance the potential differences in the baseline characteristics between new and chronic users, the propensity score (PS) matching method was performed using the

nearest-neighbor algorithm with a ratio of 1:1 and calipers of width equaling to 0.2. In addition, matching weighting (MW), inverse probability of treatment weighting (IPTW) method were also performed.[22, 23] Absolute standardized differences (ASD) were used to compare the mean or prevalence of covariates between groups to identify for imbalance.[24] An ASD ≥0.1 denotes imbalance of baseline characteristics. Therefore, Cox regression models were also fitted with the PS matched, and weighted (MW and IPTW) samples. A competing risk analysis was also performed with PS matched samples, in which death was considered to be a competing event for GIB. To better interpret the temporal trend of bleeding risk in new *versus* chronic users, landmark analyses were performed.[25, 26] The HRs were calculated separately in each observational interval, adjusting for all other covariates. A two-sided *P* value less than 0.05 were regarded as statistically significant. The R version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria, 2017) was used in all statistical analyses.

RESULTS

Patient characteristics

Of the 74,612 subjects who had received clarithromycin-based triple therapy for *H. pylori* during the study period, we identified 6,985 new and 5,545 chronic aspirin users, as well as 48,908 non-users (**Supplementary Figure 2**). The characteristics of all eligible patients are shown in **Table 1**. The median follow-up duration of new, chronic

and non-users was 1.48 (IQR 0.42, 3.74), 4.09 (IQR 1.27-6.99) and 7.68 (IQR 5.29-10) years, respectively ($P < 0.001$). The daily dosage of aspirin, expressed in person-days, was <100 mg in 84.1%.

Incidences of hospitalization for GIB

During the follow-up period, 261 (3.74%) new aspirin users, 303 (5.46%) chronic users and 1,295 (2.65%) non-users had hospitalizations for GIB. The corresponding age- and sex-adjusted incidence rate of GIB was 10.4 (95% CI 7.9-66.4), 7.2 (95% CI 6.3-157.7) and 4.6 (95% CI 4.4-4.9) per 1,000 person-years, respectively. After stratified by bleeding sites, UGIB and LGIB accounted for 34.7% and 45.3% of all GIB, respectively. For all aspirin users, the proportion of UGIB was 37.2% and LGIB was 40.8%. The adjusted incidence rate of UGIB for new, chronic and non-users were 3.0 (95% CI 2.4-61.2), 2.6 (95% CI 2.1-155.1), 1.7 (95% CI 1.5-1.9) per 1,000 person-years, respectively. The corresponding figures of LGIB for the three groups was 5.7 (95% CI 3.5-63.1), 3.0 (95% CI 2.4-155.3) and 1.9 (95% CI 1.7-2.1) per 1,000 person-years. The detailed sources of GIB in all patients are shown in the **Supplementary Table 3.**

Both new and chronic aspirin users had higher crude incidence rates of hospitalization for all GIB, UGIB and LGIB as compared with non-users, and the difference was significant in each time intervals during follow-up (**Figure 1**). This was consistent in Kaplan-Meier curves for all GIB (log-rank test $P < 0.001$; **Figure 2A**). In the

multivariable Cox model, increased risk of GIB was observed in both new (HR 1.92, 95% CI 1.62-2.27) and chronic users (HR 1.44, 95% CI 1.19-1.74), when compared to non-users. Similar results were obtained when the start date of follow up of non-users was changed to 7 days after *H. pylori* eradication (new users vs. non-users: HR 1.95, 95% CI 1.65-2.31; chronic users vs. non-users: HR 1.44, 95% CI 1.20-1.74).

When compared to chronic users, new users had a higher incidence rate of hospitalization for all GIB (RR 1.25, 95% CI 1.06-1.47). Due to the difference in baseline characteristics between the new and chronic users, we have used various models, including PS matching, MW, IPTW and multivariable model to adjust for these differences (**Supplementary Figure 3**), to show that new users still had a higher risk of GIB when compared to chronic users (**Figure 2B,** HR with PS matching: 1.89; 95% CI 1.29-2.70 and **Table 2**). The result was also consistent in the competing risk regression (HR 1.79, 95% CI 1.09-2.97).

Data Validation

To validate the final *H. pylori* statuses of patients who had UGIB after *H. pylori* eradication, we retrieved the final *H. pylori* statuses of 51 patients from our center. Among them, only two (3.9%) were found to be positive including one patient who was negative by urea breath test post-treatment but became positive upon re-examination during GIB.

In-hospital mortality of GIB

The in-hospital mortality rate of GIB for new, chronic and non-users was 9.6% (25/261), 9.6% (29/303) and 5.3% (69/1,295), respectively. In multivariable model, new users (HR 2.23, 95% CI 1.18-4.22) were associated with a higher risk of in-hospital mortality than non-users. There was no significant difference between chronic users and non-users (HR 1.87, 95% CI 0.97-3.60), as well as between new and chronic users (HR 1.47, 95% CI 0.72-3.01).

Time trend of hospitalization for GIB in aspirin users

The crude incidence rates of hospitalization for GIB in both new and chronic users showed a declining trend with time (**Figure 1**). When comparing new with chronic users, the elevated risk of all GIB decreased over time in all models except for PS matching, where a borderline CI was noted (HR 0.89, 95% CI 0.80-1.01; **Table 2** and **Supplementary Figure 4**). Specifically, the difference in the incidences of GIB between new and chronic users was only significant in the first two years for all GIB (RR 1.35, 95% CI 1.07−1.7) and UGIB (RR 1.49, 95% CI 1.02−2.17; **Figure 1**). In the landmark analysis of all GIB, the risk of GIB associated with new aspirin use was significantly higher in the first 6 months (HR 2.10, 95% CI 1.41-3.13; **Figure 3A**), but not in the following period (HR 1.18, 95% CI 0.93-1.50). The result was consistent with

the landmark analysis of UGIB (0-6 months: HR 2.52, 95% CI 1.38-4.60; >6 months: HR 0.96, 95% CI 0.64-1.45; **Figure 3B**). Similar decline in LGIB risk between new and chronic aspirin users was not detected (Schoenfeld test in multivariable Cox model, $P = 0.934$).

Factors associated with hospitalization for GIB among aspirin users

In multivariable model with all aspirin users, we confirmed that new aspirin users had higher risk of GIB than chronic users (HR 1.74, 95% CI 1.32-2.29, **Figure 4**). Other risk factors of GIB included past history of GIB or ulcer (HR 2.78, 95% CI 2.15-3.60), renal disease (HR 2.25, 95% CI 1.65-3.08), stroke (HR 1.50, 95% CI 1.07-2.12), use of other antiplatelet drugs (HR 1.49, 95% CI 1.11-2.00), NSAIDs (HR 1.64, 95% CI 1.14-2.35), corticosteroids (HR 1.91, 95% CI 1.29-2.35) and older age (HR 1.05, 95% CI 1.03-1.06). Subgroup analysis further showed that new aspirin users had higher risk of GIB both in patients with (HR 1.93, 95% CI 1.27-2.94) or without history of GIB or ulcer (HR 1.58, 95% CI 1.09-2.28).

On the other hand, the use of gastroprotective agents was associated with a lower risk of GIB (HR 0.34, 95% CI 0.25-0.46) in aspirin users, including the use of PPI (HR 0.46, 95% CI 0.36-0.58) and H2RA (HR 0.43, 95% CI 0.32-0.56). Benefits of gastroprotective agents on lowering risk of GIB were also found in other subgroups including elderly (≥ 60 years), those who had concurrent use of aspirin with NSAIDs or other antiplatelet therapies (**Supplementary Table 4)**.

DISCUSSION

While *H. pylori* infection and aspirin are both important risk factors for UGIB,[27] elimination of *H. pylori* infection would leave aspirin and/or NSAIDs to be the major risk factor(s) for UGIB.[4, 12] Hence, study on *H. pylori* eradicated subjects could possibly delineate the natural history of aspirin related GIB. This is the first study to characterize the incidences, temporal trends and risk factors of hospitalization for all GIB, including both upper and lower GIB, in a large cohort of *H. pylori*-infected patients who had received eradication therapy and were then newly started on aspirin or continued to use aspirin. We found that the incidences of GIB, including upper and lower GIB, in both new and chronic aspirin users were significantly higher than non-users. More importantly, we showed that new aspirin users had a 1.9-fold (PS matched analysis) higher risk of GIB when compared to chronic users. The risk of GIB, particularly UGIB, was significantly increased in new aspirin users during the initial 6-month of aspirin therapy in landmark analyses.

The risk of GIB and UGIB in new aspirin users, when compared to chronic users, decreased with time in most models, suggesting that the bleeding risk in aspirin users is time-dependent. Slattery et al showed that UGIB were three times more likely to occur in the initial 152 days of aspirin treatment.[14] Apart from aspirin, current literature also suggests that GIB are more likely to occur in the early course of treatment with NSAIDs, antiplatelet drugs, or dual antiplatelet therapy.[14, 28-30] Although previous studies have demonstrated the potential gastric adaptation to aspirin[6 31, 32], these studies

failed to address the issue of concurrent *H. pylori* infection, which is an important confounding factor for UGIB. Our findings further showed that the gastric adaptive effects may not be related to *H. pylori* and remain even after eradication therapy. Arguably, the observed difference between new and chronic users could be accounted by the depletion of susceptible patients in chronic users who would have developed bleeding and then stopped aspirin treatment. In addition, the increase in early bleeding risk of new users could be explained by the effect of aspirin upon pre-existing gastric pathology, which may lead to early bleeding.

In this study, we showed that new users had a higher in-hospital mortality of GIB than non-users, but there was no difference in mortality between chronic and new users or between chronic users and non-users. Thus far, data on aspirin and GIB mortality remains conflicting. Studies have shown that aspirin use was associated with a reduction in the risk of adverse outcomes in patients with UGIB.[33, 34] On the other hand, there was reports of no increase in mortality of aspirin-related GIB.[35, 36] These discrepancies may be related to the difference in patient characteristics including *H. pylori* infection, timings of aspirin use (before or after GIB) and comorbidities (particularly underlying ischemic diseases).

It is important to note that after treatment for *H. pylori*, LGIB accounted for about 45.3% of all GIB, which was even higher than UGIB. This may be a consequence of both *H. pylori* eradication and use of gastroprotective agents. In this study, more than 74% of patients were taking gastroprotective agents. LGIB was also significantly more frequent

in both new and chronic aspirin users when compared to non-users. However, similar decline in the risk of LGIB with time between new and chronic aspirin users was not observed. Hence, similar adaptation of the small or large intestinal mucosa to aspirin may not exist. Aspirin users are therefore still at continuing risk of LGIB even years after aspirin therapy.

History of GIB or peptic ulcer are generally considered to be a risk factor for GIB. In this study, risk factors analysis also showed that history of GIB or peptic ulcer is an important risk factor of GIB in patients who used aspirin after *H. pylori* eradication, irrespective of whether they are new or chronic aspirin users. There was a 2.8-fold increase in GIB risk among those with past history of peptic ulcer or GIB, which was consistent with previous studies.[37, 38] Our study also found that, among patients without history of GIB or peptic ulcer, new aspirin users still have a higher risk of GIB than chronic users. According to current recommendations, long-term gastroprotective agent is recommended to high-risk patients including older age, previous GIB, peptic ulcer or ulcer complications, concomitant use of NSAIDs, anticoagulants, other antiplatelet drugs or other drugs increasing GIB risk.[10, 39] Our subgroup analyses also confirmed that gastroprotective agents reduced the risk of aspirin related GIB among elderly (≥ 60 years) and those with concurrent use of NSAIDs or other anti-platelet therapies. To our knowledge, there is no recommendation that specifically emphasized the higher risk of bleeding during the early course of aspirin treatment in patients after treatment for *H. pylori*. Hence, prophylactic gastroprotective agents are particularly warranted during

this initial period of aspirin treatment. As yet, gastroprotective agents could not reduce the risk of LGIB which may account for the non-declining risk of LGIB with time.

The strengths of this study are the inclusion of a large cohort of *H. pylori* subjects who had received eradication therapy based on the comprehensive healthcare database in Hong Kong which captures all bleeding episodes, concurrent medical illnesses and medications usages. In addition to UGIB, we have also demonstrated the high incidences of LGIB in aspirin users who had received treatment for *H. pylori*. To adjust for potential differences in the baseline characteristics between new and chronic aspirin users in this study, we had used multiple models including PS matching and weighting (IPTW and MW) to adjust for various potential biases. Time-dependent Cox regression model were also used to evaluate the time-dependent effect of aspirin, and other covariates in multivariable model on GIB.

Immortal time bias is an important methodological consideration which was common in observational studies.[40, 41] However, when comparing new and chronic aspirin users, there should be minimal immortal time bias as both new and chronic users have same start point as the date of first aspirin prescription after *H. pylori* eradication. To further minimize immortal bias, we have also adopted time-dependent regression models in which all medications were treated as time-varying covariates.

Our study has limitations. First, post-treatment *H. pylori* statuses were not available in the electronic database and the success of treatment was only inferred by the needs of retreatment. Some patients who failed *H. pylori* eradication might not receive further

therapy due to various reasons. Nonetheless, the overall retreatment rate of this study (11%) was comparable to the failure rate of clarithromycin-based triple therapy in a prospective study conducted in Hong Kong during the same period.[42] To verify the success of *H. pylori* eradication, we had performed a validation study of 51 bleeding patients from our centre who had been retested for *H. pylori.* Second, this study did not evaluate the independent effect of *H. pylori* on the risk of GIB or the interaction between *H. pylori* and aspirin, as only *H. pylori* eradicated subjects were included. Ideally, this study should include a control group of *H. pylori* infected patients with no prior treatment, but this may pose ethical issues not to treat infected subjects, particularly before starting aspirin therapy. The lack of a group of patients without *H. pylori* infection is another limitation of this study. However, it has been shown that the recurrent bleeding risk among low-dose aspirin users after *H. pylori* eradication did not differ from average risk individuals.[8] Third, the follow up duration of the three groups were different due to higher censoring rate from bleeding and shorter duration of aspirin usage in new users. As yet, the bleeding rate was the lowest among non-users with the longest follow up. Fourth, the electronic database could only determine the prescription and dispension but not the actual compliance to aspirin. Lastly, though various models were used to adjust for potential bias including competing risk analysis and time-dependent regression, it is possible that some residual confounders may not be adequately adjusted. Despite these potential caveats, our findings support that *H. pylori* eradication is not risk proof in preventing subsequent GIB in aspirin users, particularly among new users and for the prevention of LGIB.

CONCLUSION

In this study involving a large cohort of patients who had received *H. pylori* eradication therapy, we showed that both new and chronic aspirin users continued to have a significantly higher risk of hospitalizations for GIB than non-users. The risk of GIB, UGIB in particular, was significantly higher for new aspirin users when compared to chronic aspirin users during the initial 6-month of aspirin treatment. LGIB became more frequent than UGIB among aspirin users who had received *H. pylori* treatment. Although treatment with gastroprotective agents appeared to reduce the risk of GIB after *H. pylori* treatment, the risks of LGIB between new and chronic aspirin users continued and showed no trend of decline.

Contributions: CGG, KSC and WKL were responsible for the conception and design of this study. LC and CGG were involved in data collection. CGG and FZ were involved in data analysis and interpretation. CGG and WKL drafted the manuscript. KSC, FZ, EWC, LC and IW assisted in data interpretation and provided critical review of the manuscript. All authors approved the final version of the manuscript.

Competing interests: The authors disclose no conflicts of interest.

REFERENCES

1. Peery AF, Crockett SD, Barritt AS, et al. Burden of Gastrointestinal, Liver, and Pancreatic Diseases in the United States. Gastroenterology 2015;149:1731-1741 e3.
2. Hunt RH, Bazzoli F. Should NSAID/low-dose aspirin takers be tested routinely for H. pylori infection and treated if positive? Implications for primary risk of ulcer and ulcer relapse after initial healing. Aliment Pharmacol Ther 2004;19:9-16.
3. Laine L, Yang H, Chang SC, et al. Trends for incidence of hospitalization and death due to GI complications in the United States from 2001 to 2009. Am J Gastroenterol 2012;107:1190-5; quiz 1196.
4. Nagasue T, Nakamura S, Kochi S, et al. Time Trends of the Impact of Helicobacter pylori Infection and Nonsteroidal Anti-Inflammatory Drugs on Peptic Ulcer Bleeding in Japanese Patients. Digestion 2015;91:37-41.
5. Guirguis-Blake JM, Evans CV, Senger CA, et al. Aspirin for the Primary Prevention of Cardiovascular Events: A Systematic Evidence Review for the U.S. Preventive Services Task Force. Ann Intern Med 2016;164:804-13.
6. Konturek JW, Dembinski A, Konturek SJ, et al. Infection of Helicobacter pylori in gastric adaptation to continued administration of aspirin in humans. Gastroenterology 1998;114:245-255.
7. Konturek JW, Fischer H, Konturek PC, et al. Heat shock protein 70 (HSP70) in gastric adaptation to aspirin in Helicobacter pylori infection. J Physiol Pharmacol 2001;52:153-64.
8. Chan FK, Ching JY, Suen BY, et al. Effects of Helicobacter pylori infection on long-term risk of peptic ulcer bleeding in low-dose aspirin users. Gastroenterology 2013;144:528-35.
9. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut 2017;66:6-30.
10. Ki ELL, Chan FKL. Interaction of Helicobacter pylori infection and low-dose aspirin in the upper gastrointestinal tract: Implications for clinical practice. Best Practice & Research Clinical Gastroenterology 2012;26:163-172.

11. Lanas A, Carrera-Lasfuentes P, Arguedas Y, et al. Risk of upper and lower gastrointestinal bleeding in patients taking nonsteroidal anti-inflammatory drugs, antiplatelet agents, or anticoagulants. Clin Gastroenterol Hepatol 2015;13:906-12 e2.
12. Chen WC, Lin KH, Huang YT, et al. The risk of lower gastrointestinal bleeding in low-dose aspirin users. Aliment Pharmacol Ther 2017;45:1542-1550.
13. Rothwell PM, Price JF, Fowkes FG, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet 2012;379:1602-12.
14. Slattery J, Warlow CP, Shorrock CJ, et al. Risks of gastrointestinal bleeding during secondary prevention of vascular events with aspirin--analysis of gastrointestinal bleeding during the UK-TIA trial. Gut 1995;37:509-11.
15. Leung WK, Wong IOL, Cheung KS, et al. Effects of Helicobacter pylori Treatment on Incidence of Gastric Cancer in Older Individuals. Gastroenterology 2018;155:67-75.
16. Chan EW, Lau WC, Leung WK, et al. Prevention of Dabigatran-Related Gastrointestinal Bleeding With Gastroprotective Agents: A Population-Based Study. Gastroenterology 2015;149:586-95 e3.
17. Cheung KS, Chan EW, Wong AYS, et al. Long-term proton pump inhibitors and risk of gastric cancer development after treatment for Helicobacter pylori: a population-based study. Gut 2018;67:28-35.
18. Cheung KS, Chan EW, Wong AYS, et al. Aspirin and Risk of Gastric Cancer After Helicobacter pylori Eradication: A Territory-Wide Study. J Natl Cancer Inst 2018;110:743-749.
19. Gisbert JP, Pajares JM. Systematic review and meta-analysis: is 1-week proton pump inhibitor-based triple therapy sufficient to heal peptic ulcer? Alimentary Pharmacology & Therapeutics 2005;21:795-804.
20. Grambsch PM, Therneau TM. Proportional Hazards Tests and Diagnostics Based on Weighted Residuals. Biometrika 1994;81:515-526.
21. Collett D. Modelling survival data in medical research. Boca Raton: CRC Press, Taylor & Francis Group, 2015.
22. Austin PC. An Introduction to Propensity Score Methods for Reducing the

Effects of Confounding in Observational Studies. Multivariate Behav Res 2011;46:399-424.

23. Li L, Greene T. A weighting analogue to pair matching in propensity score analysis. Int J Biostat 2013;9:215-34.
24. Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Statistics in Medicine 2009;28:3083-3107.
25. Putter H, van Houwelingen HC. Understanding Landmarking and Its Relation with Time-Dependent Cox Regression. Stat Biosci 2017;9:489-503.
26. De Bruyne B, Pijls NH, Kalesan B, et al. Fractional flow reserve-guided PCI versus medical therapy in stable coronary disease. N Engl J Med 2012;367:991-1001.
27. Huang JQ, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet 2002;359:14-22.
28. Bonaca MP, Storey RF, Theroux P, et al. Efficacy and Safety of Ticagrelor Over Time in Patients With Prior MI in PEGASUS-TIMI 54. J Am Coll Cardiol 2017;70:1368-1375.
29. Hilkens NA, Algra A, Kappelle LJ, et al. Early time course of major bleeding on antiplatelet therapy after TIA or ischemic stroke. Neurology 2018;90:e683-e689.
30. Langman MJ, Weil J, Wainwright P, et al. Risks of bleeding peptic ulcer associated with individual non-steroidal anti-inflammatory drugs. Lancet 1994;343:1075-8.
31. Konturek JW, Dembinski A, Stoll R, et al. Mucosal adaptation to aspirin induced gastric damage in humans. Studies on blood flow, gastric mucosal growth, and neutrophil activation. Gut 1994;35:1197-204.
32. Pajdo R, Brzozowski T, Konturek PC, et al. Importance of epi-lipoxin A(4) and nitric oxide (NO) in the mechanism of adaptation of gastric mucosa to continuous aspirin administration. Gastroenterology 2008;134:A703-A703.
33. Wehbeh A, Tamim HM, Abu Daya H, et al. Aspirin Has a Protective Effect Against Adverse Outcomes in Patients with Nonvariceal Upper Gastrointestinal Bleeding. Digestive Diseases and Sciences 2015;60:2077-2087.

34. Lanas A, Aabakken L, Fonseca J, et al. Clinical predictors of poor outcomes among patients with nonvariceal upper gastrointestinal bleeding in Europe. Alimentary Pharmacology & Therapeutics 2011;33:1225-1233.
35. Elwood PC, Morgan G, Galante J, et al. Systematic Review and Meta-Analysis of Randomised Trials to Ascertain Fatal Gastrointestinal Bleeding Events Attributable to Preventive Low-Dose Aspirin: No Evidence of Increased Risk. Plos One 2016;11.
36. Mose H, Larsen M, Riis A, et al. Thirty-day mortality after peptic ulcer bleeding in hospitalized patients receiving low-dose aspirin at time of admission. The American Journal of Geriatric Pharmacotherapy 2006;4:244-250.
37. Valkhoff VE, Sturkenboom MC, Kuipers EJ. Risk factors for gastrointestinal bleeding associated with low-dose aspirin. Best Pract Res Clin Gastroenterol 2012;26:125-40.
38. Lanas A, Bajador E, Serrano P, et al. Nitrovasodilators, low-dose aspirin, other nonsteroidal antiinflammatory drugs, and the risk of upper gastrointestinal bleeding. N Engl J Med 2000;343:834-9.
39. Bhatt DL, Scheiman J, Abraham NS, et al. ACCF/ACG/AHA 2008 Expert Consensus Document on Reducing the Gastrointestinal Risks of Antiplatelet Therapy and NSAID Use. American Journal of Gastroenterology 2008;103:2890-2907.
40. van Walraven C, Davis D, Forster AJ, et al. Time-dependent bias was common in survival analyses published in leading clinical journals. Journal of Clinical Epidemiology 2004;57:672-682.
41. Targownik LE, Suissa S. Understanding and Avoiding Immortal-Time Bias in Gastrointestinal Observational Research. Am J Gastroenterol 2015;110:1647-50.
42. Gu Q, Xia HH, Wang JD, et al. Update on clarithromycin resistance in Helicobacter pylori in Hong Kong and its effect on clarithromycin-based triple therapy. Digestion 2006;73:101-6.

Figure Legends

Figure 1 Incidence rates of hospitalization for all GIB, UGIB and LGIB during the follow-up period in new aspirin users, chronic users and non-users, and the corresponding relative risks between different groups.

Figure 2 Kaplan-Meier curves for the proportion of patients who were free from GIB. A: GIB in all new aspirin users, chronic users and non-users; B: GIB in matched new aspirin users vs. chronic aspirin users.

Figure 3 Kaplan-Meier curves of GIB (A) and UGIB (B) in the landmark analysis with the splitting time of 6-month (— chronic aspirin users; --- new aspirin users)

Figure 4 Risk factors of hospitalization for GIB among aspirin users

Table 1 Baseline characteristics of new aspirin users, chronic aspirin users and non-users

Characteristics	Non-users (n = 48,908)	Before matching*		After matching*	
		New users (n = 6,985)	Chronic users (n = 5,545)	New users (n = 2,801)	Chronic users (n = 2,801)
Age at start point (year) †	51.0 (43.0, 60.0)	67.0 (59.0-77.0)	68.0 (60.0-76.0)	69.0 (59.0, 78.0)	68.0 (60.0, 76.0)
Gender (Male, %)	21,575 (44.1)	3,736 (53.5)	3,273 (59.0)	1,368 (48.8)	1,530 (54.6)
Baseline conditions (%)					
GIB or ulcer history	7168 (14.7)	1,466 (21.0)	1,293 (23.3)	655 (23.4)	589 (21.0%)
Ischemic heart disease	295 (0.6)	304 (4.4)	2,036 (36.7)	304 (10.9)	298 (10.6)
Stroke	195 (0.4)	186 (2.7)	1,380 (24.9)	186 (6.6)	191 (6.8)
Hypertension	1,985 (4.1)	1,562 (22.4)	2,060 (37.2)	818 (29.2)	749 (26.7)
Diabetes	1488 (3.0)	973 (13.9)	1,313 (23.7)	526 (18.8)	506 (18.1)
Renal disease	362 (0.7)	356 (5.1)	276 (5.0)	173 (6.2)	132 (4.7)
Intracranial hemorrhage	156 (0.3)	87 (1.3)	64 (1.2)	30 (1.1)	32 (1.1)
Cirrhosis	297 (0.6)	65 (0.9)	19 (0.3)	15 (0.5)	14 (0.5)
Medications (%)					
Gastroprotective agents	36,208 (74.0)	6,285 (90.0)	5,173 (93.3)	2,585 (92.3)	2,579 (92.1)
Other antiplatelet drugs	258 (0.5)	1,358 (19.4)	1,016 (18.3)	463 (16.5)	438 (15.6)
NSAIDs	19,636 (40.1)	1,247 (17.9)	1,142 (20.6)	596 (21.3)	574 (20.5)
Anticoagulants	405 (0.8)	301 (4.3)	270 (4.9)	136 (4.9)	146 (5.2)
Corticosteroids	2,626 (5.4)	556 (8.0)	419 (7.6)	238 (8.5)	213 (7.6)
SSRI	3,020 (6.2)	477 (6.8)	356 (0.6)	217 (7.7)	180 (6.4)
Bisphosphonate	466 (1.0)	96 (1.3)	82 (1.5)	52 (1.9)	44 (1.6)

† Variables expressed as median and interquartile range. * Absolute standardized differences between new and chronic users before or after matching (ASD) are showed in the Supplementary Figure 3.

GIB, gastrointestinal bleeding; NSAIDs, nonsteroidal anti-inflammatory drugs; SSRI, selective serotonin reuptake inhibitors; Gastroprotective agents include proton pump inhibitors and histamine type-2 receptor antagonists.

Table 2 Results of time-dependent regression models comparing new with chronic aspirin users in patients after *H. pylori* eradication

Models	Variables	GIB		UGIB		LGIB	
		HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Univariate Cox regression model with original samples	New users	1.16 (0.98-1.38)	0.080	1.12 (0.86-1.47)	0.396	1.19 (0.92-1.55)	0.187
Including a time-by-covariate interaction	New users	1.41 (1.10-1.80)	0.007	-	-	-	-
	New users*time§	0.92 (0.85-0.998)	0.04	-	-	-	-
PSM†	New users	1.89 (1.29-2.70)	<0.001	1.30 (0.85-1.99)	0.234	1.94 (1.29-2.91)	0.001
	New users*time	0.89 (0.80-1.01)	0.063	-	-	-	-
MW†	New users	1.80 (1.32-2.46)	<0.001	2.00 (1.21-3.20)	0.007	1.49 (1.07-2.07)	0.018
	New users*time	0.87 (0.80-0.95)	0.002	0.82 (0.70-0.96)	0.015	-	-
IPTW†	New users	1.81 (1.34-2.44)	<0.001	1.73 (1.09-2.73)	0.019	1.61 (1.15-2.25)	0.006
	New users*time	0.87 (0.80-0.95)	0.003	0.84 (0.72-0.97)	0.018	-	-
Multivariable Cox regression model‡	New users	1.74 (1.32-2.29)	<0.001	1.63 (1.08-2.46)	0.020	1.53 (1.11-2.10)	0.009
	New users*time	0.91 (0.84-0.99)	0.025	0.91 (0.79-1.04)	0.154	-	-

† Propensity scores or weights were calculated based on age, sex, baseline conditions and concomitant medications; ‡ Adjusted for age, sex, baseline conditions and concomitant medications which were included as time-varying covariates; § Time-by-covariate interactions in regression model, in which time indicates start points of each 3-month interval of the follow-up period, in terms of 0, 0.25, 0.5, 0.75 year, and so forth.

PSM, propensity score matching; PSMW, propensity score matching weighting; IPTW, inverse probability of treatment weighting; GIB, gastrointestinal bleeding; UGIB, upper gastrointestinal bleeding; LGIB, lower gastrointestinal bleeding; HR, hazard ratio; CI, confidence interval.