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Research Article

Cancer Prevention Research

# Distinct Chemopreventive Effects of Aspirin in Diffuse and Intestinal-Type Gastric Cancer

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## Abstract

**Introduction:** Although aspirin/NSAIDs may have potential preventive effects on several cancers, it remains unclear on gastric cancer. The purpose of this study is to compare the risk of developing gastric cancer and the histologic changes of intestinal metaplasia and neutrophil infiltration, between aspirin/NSAID users and nonusers.

**Methods:** Using an electronic endoscopy database in two hospitals from 1996 to 2017, we analyzed the data from patients with chronic gastritis who received aspirin or NSAIDs prior to upper gastrointestinal endoscopy. One-to-one propensity score matching was performed to compare the proportion of gastric cancer, intestinal metaplasia, and neutrophil infiltration between these drug users and nonusers.

**Results:** We analyzed 2,082 aspirin users and 2,082 nonusers as well as 898 NSAID users and 898 nonusers. Six diffuse-type and 19 intestinal-type gastric cancer, 1,243 intestinal metaplasia, and 1,503 neutrophil infiltration patients were identified. The proportion of diffuse-type gastric cancer (0.05%) was 80% lower in aspirin users compared with the nonusers (0.24%), and there was no case of diffuse-type cancer in patients who took aspirin for more than 2 years. In contrast, intestinal-type gastric cancer incidence was significantly higher in aspirin users (0.72%) compared with nonusers (0.14%). No significant differences in the incidence of gastric cancer were found between NSAID use and nonusers. NSAID use was significantly associated with decreased proportion of neutrophil infiltration compared with nonusers.

**Conclusion:** Aspirin may have distinct effects between intestinal-type and diffuse-type gastric cancer development. *Cancer Prev Res; 11(5); 279–86. ©2018 AACR.*

## Introduction

Although the incidence of gastric cancer in western countries has been decreasing, it remains one of the most common cancers and is one of the leading causes of cancer mortality worldwide (1). *Helicobacter pylori* (*H. pylori*) is the most important carcinogen for gastric cancer. In the stomach, *H. pylori* induces chronic inflammation with a massive infiltration of neutrophils and other immune cells. This is followed by gastric atrophy and intestinal metaplasia and culminates in the development of gastric cancer through the Correa pathway, a multistep histopathologic cascade. The presence of neutrophil infiltration and intestinal metaplasia in gastric biopsy samples is an independent risk factor for gastric cancer (2, 3). Thus, the inhibition of chronic inflammation and subsequent histopathologic changes may lead to the prevention of gastric cancer.

Aspirin, including buffered and enteric-coated forms, are widely used for the treatment of various types of diseases due to their anti-inflammatory and antiplatelet functions. Aspirin and NSAIDs inhibit inflammatory signals through suppression of cyclooxygenase (Cox) activity and prostaglandin (PG) synthesis (4). Experimentally, Cox inhibition by these drugs may lead to the induction of apoptosis (5) and the inhibition of angiogenesis (6), resulting in an anticarcinogenic effect in several cancer types (7). Indeed, it has been reported that aspirin exhibits a protective effect on colorectal cancer development (8, 9), and several studies have suggested that it may also have a protective effect on gastric cancer development (10–14). However, other observational studies and randomized controlled trials reported a nonsignificant association between aspirin use and the development of gastric cancer or intestinal

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metaplasia; thus, the effect of aspirin or NSAIDs use on gastric carcinogenesis remains undetermined (15, 16).

In previous studies, differences in age, sex, and the presence of comorbidities, and potential confounding factors in aspirin/NSAID users and nonusers were often not appropriately adjusted, which may have led to biased results that influenced the conclusions. These confounding factors include the use of drugs that may alter the background histopathologic status of the patient. To reduce these biases and confounding factors, we performed a propensity score matching analysis to specifically evaluate the protective effect of these drugs on gastric cancer development and compared the risk of gastric cancer, intestinal metaplasia, and neutrophil infiltration between aspirin users and nonusers and between NSAID users and nonusers.

## Materials and Methods

### Data source

We developed the Gastric Cancer Endoscopy Database of Tokyo University (Tokyo, Japan) and the Institute for Adult Diseases, Asahi Life Foundation (Tokyo, Japan), which is a retrospectively recorded database of 53,143 patients from 1996 to 2017 who underwent upper gastrointestinal endoscopy. This database included the following information: patient characteristics, indications for endoscopy, endoscopy findings, histologic findings, gastric cancer data, *H. pylori* infection status, and drug use. The indications for upper gastrointestinal endoscopy included gastric cancer surveillance and observed gastrointestinal symptoms. For patients who underwent repeated endoscopies, we used the data from the first examination record.

### Study design, setting, and participants

We performed two retrospective cohort analyses using our database during 1996 to 2017. We extracted data from patients who had undergone upper endoscopy for gastric cancer surveillance without a previous 5-year diagnosis of gastric cancer.

We selected a case group who received daily aspirin (100 mg enteric-coated or 81 mg buffered aspirin) for at least 30 days prior to initial upper gastrointestinal endoscopy, and a control group who did not receive aspirin (Fig. 1A). Indications for aspirin use included a history of ischemic heart disease, cerebrovascular disease, and thromboembolism. In another analysis, we selected a case group who received daily NSAIDs, including ibuprofen, indomethacin, indomethacin farnesyl, sulindac, diclofenac sodium, naproxen, etodolac, mefenamic acid, meloxicam, or loxoprofen, for at least 30 days prior to initial upper gastrointestinal endoscopy, and a control group who did not receive NSAIDs (Fig. 1B). We calculated the duration of aspirin and NSAID use as the period between first prescription and last prescription

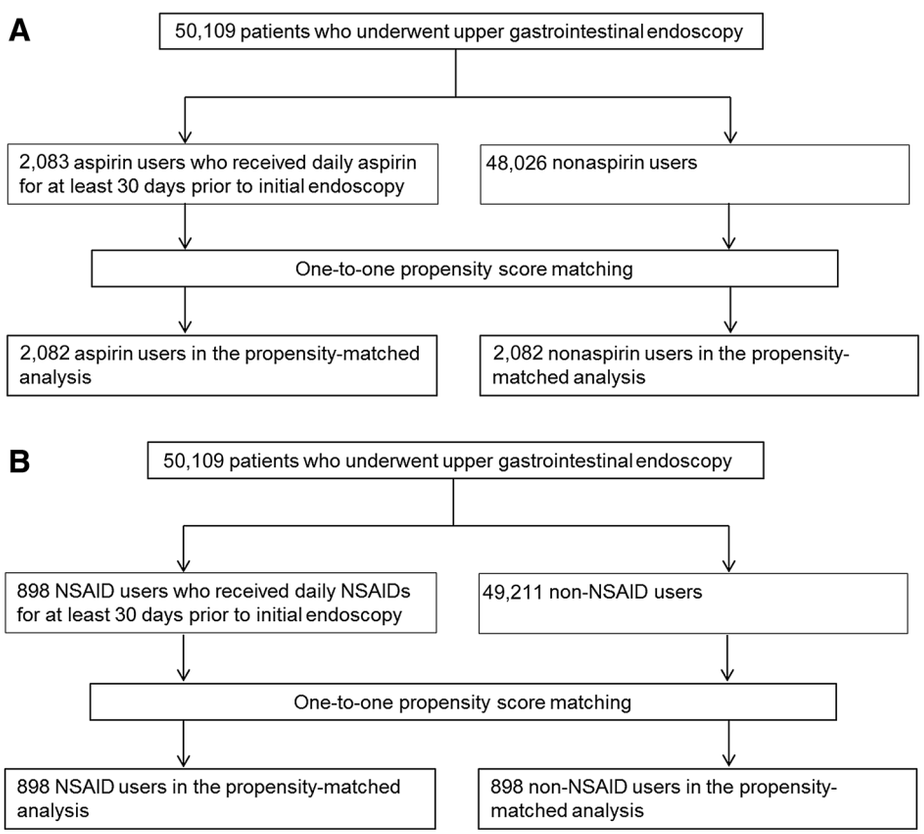


**Figure 1.**
Study flow chart. **A,** Aspirin use and nonuse group. **B,** NSAID use and nonuse group.

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data of drugs. Follow-up period is calculated by measuring the time between first visit and last visit to our hospitals: Median follow-up period was 4.2 years.

The human investigations were performed after approval by an Institutional Review Board at the University of Tokyo and the Institute for Adult Diseases, Asahi Life Foundation and in accordance with an assurance filed with and approved by the Japanese Ministry of Health, Labor and Welfare. Written informed consent from patients was obtained from each subject in accordance with Declaration of Helsinki.

### Upper gastrointestinal endoscopy and histologic examination

All upper gastrointestinal endoscopies were performed using an electronic video endoscope (Olympus Optical) by experienced endoscopists. Biopsy was performed to evaluate intestinal metaplasia and neutrophil infiltration for all patients except who could not discontinue antithrombotic drugs such as warfarin, thienopyridine, and direct oral anticoagulants. Biopsy specimens were obtained from two points, the antrum and middle corpus of greater curvature. Gastric cancer was diagnosed by pathologic evaluation of biopsy and/or resected specimens. Diffuse-type gastric cancer was defined by the presence of signet-ring cancer cells. Intestinal metaplasia was diagnosed by the presence of goblet cells, absorptive cells, and cells resembling colonocytes (17). Neutrophil infiltration was diagnosed by the presence of polymorphonuclear leukocytes in the lamina propria of the gastric mucosa based on a previous report (18). All histologic findings were diagnosed by experienced pathologists. In the case of discordant diagnoses between pathologists, they discussed findings and a consensus was reached.

### Outcomes and variables

The primary outcome was the risk of gastric cancer. All gastric cancer cases were diagnosed by upper gastrointestinal endoscopy and confirmed by pathologic examination. The secondary outcome was the risk of diffuse-type gastric cancer, intestinal-type gastric cancer, intestinal metaplasia, and neutrophil infiltration.

We evaluated data on age, sex, comorbidities, *H. pylori* infection status, location of cancer, and cancer stage. Age was categorized into quintiles. Comorbidities were evaluated using the Charlson comorbidity index (19). The *H. pylori* infection status was categorized as "positive," "negative," or "unknown." *H. pylori* status was defined as the latest result from serological testing, a urea breath test, or a stool antigen test.

### Statistical analysis

We performed a one-to-one propensity score matching analysis between aspirin users and nonusers and between NSAID users and nonusers using estimated propensity scores from each patient (20). To estimate the propensity score, we fit a logistic regression model for aspirin and NSAID use as a function of patient demographic factors, including age, sex, 15 comorbidities, and *H. pylori* infection status. We calculated the c-statistic to evaluate the goodness-of-fit. Each aspirin and NSAID user was matched with a nonaspirin or NSAIDs user, respectively. Furthermore, patients were matched to those who had the most similar estimated propensity score on the logit scale within a specified range ($\leq$0.2 of the pooled SD of estimated logits) to reduce differences between the two groups.

After propensity matching, we compared the proportions of gastric cancer, diffuse-type gastric cancer, intestinal-type gastric cancer, intestinal metaplasia, and neutrophil infiltration in the aspirin and NSAID use and nonuse groups. Categorical data comparisons between the two groups were performed using the $\chi^2$ test or Fisher exact test as appropriate.

Logistic regression analysis was performed to estimate the OR and 95% confidence interval for the risk of gastric cancer, diffuse-type gastric cancer, intestinal-type gastric cancer, intestinal metaplasia, and neutrophil infiltration in the 2- to 6-year and $\geq$6-year groups relative to the $\leq$2-year group in patients with aspirin.

Statistical analyses were performed using SAS software version 9.4 (SAS Institute). $P < 0.05$ was considered to indicate statistical significance.

## Results

### Aspirin use versus nonuse

A total of 2,082 patients who received aspirin were included. The median duration of aspirin use was 4.9 years. After matching, we selected 2,082 controls who did not use aspirin from 48,026 patients. The c-statistic for goodness-of-fit was 0.932 in the propensity score model. Table 1 shows the patient backgrounds in the unmatched and propensity score–matched groups. Patient distributions were closely balanced between matched groups.

Gastric cancer was observed in 17 patients in the aspirin user group and 8 patients in the nonuser group, and the proportion of gastric cancer between the groups was not significantly different (0.82% vs. 0.38%, $P = 0.071$); the characteristics of gastric cancer cases are shown in Supplementary Table S1. When we analyzed the proportion of intestinal-type and diffuse-type gastric cancers separately, we found that there was an 80% lower relative risk of diffuse-type gastric cancer in the aspirin user group (0.05 %) compared with the nonuser group (0.24 %), although the difference did not reach statistically significance. In contrast, the proportion of intestinal-type gastric cancer was significantly higher in the aspirin user group (0.72%) compared with that in the nonuser group (0.14%; $P = 0.004$). No significant association was observed between aspirin use and cancer location and stage (Supplementary Table S2). No significant difference was observed in the

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**Table 1.** Baseline characteristics of unmatched and propensity score–matched patients in aspirin user and nonuser groups

| | Unmatched | | | Propensity score matched | | |
|---|---|---|---|---|---|---|
| | **Aspirin use *n* = 2,083 (%)** | **Nonuse *n* = 48,026 (%)** | ***P*** | **Aspirin use *n* = 2,082 (%)** | **Nonuse *n* = 2,082 (%)** | ***P*** |
| Age, years | | | | | | |
| <50 | 168 (8.07) | 10,205 (21.25) | <**0.001** | 168 (8.07) | 144 (6.92) | 0.184 |
| 50–60 | 179 (8.59) | 9,607 (20.00) | | 179 (8.60) | 190 (9.13) | |
| 60–70 | 557 (26.74) | 13,853 (28.84) | | 556 (26.71) | 586 (28.15) | |
| 70–80 | 852 (40.90) | 11,018 (22.94) | | 852 (40.92) | 877 (42.12) | |
| ≥80 | 327 (15.70) | 3,343 (6.96) | | 327 (15.71) | 285 (13.69) | |
| Sex, male | 1,380 (66.25) | 27,075 (56.38) | <**0.001** | 1,380 (66.28) | 1,374 (65.99) | 0.844 |
| Comorbidities | | | | | | |
| Ischemic heart disease | 1,478 (70.96) | 4,374 (9.11) | <**0.001** | 1,477 (70.94) | 1,493 (71.71) | 0.584 |
| Chronic heart failure | 1,514 (72.68) | 5,803 (12.08) | <**0.001** | 1,513 (72.67) | 1,518 (72.91) | 0.862 |
| Peripheral vascular disease | 501 (24.05) | 1,921 (4.00) | <**0.001** | 500 (24.02) | 461 (22.14) | 0.151 |
| Cerebrovascular disease | 681 (32.69) | 2,020 (4.21) | <**0.001** | 681 (32.71) | 668 (32.08) | 0.667 |
| Dementia | 126 (6.05) | 754 (1.57) | <**0.001** | 126 (6.05) | 120 (5.76) | 0.693 |
| COPD | 134 (6.43) | 1,017 (2.12) | <**0.001** | 134 (6.44) | 132 (6.34) | 0.899 |
| Collagen diseases | 248 (11.91) | 2,636 (5.49) | <**0.001** | 248 (11.91) | 261 (12.54) | 0.539 |
| Peptic ulcers | 1,109 (53.24) | 13,628 (28.38) | <**0.001** | 1,108 (53.22) | 1,147 (55.09) | 0.225 |
| Diabetes | 1,616 (77.58) | 12,998 (27.06) | <**0.001** | 1,615 (77.57) | 1,657 (79.59) | 0.113 |
| Chronic kidney disease | 314 (15.07) | 1,123 (2.34) | <**0.001** | 313 (15.03) | 293 (14.07) | 0.379 |
| Hemiplegia or paraplegia | 137 (6.58) | 1,090 (2.27) | <**0.001** | 137 (6.58) | 115 (5.52) | 0.153 |
| Leukemia | 44 (2.11) | 363 (0.76) | <**0.001** | 44 (2.11) | 49 (2.35) | 0.600 |
| Malignant lymphoma | 91 (4.37) | 1,331 (2.77) | <**0.001** | 91 (4.37) | 90 (4.32) | 0.939 |
| Liver cirrhosis | 72 (3.46) | 2,279 (4.75) | **0.007** | 72 (3.46) | 73 (3.51) | 0.933 |
| AIDS | 4 (0.19) | 78 (0.16) | 0.743 | 4 (0.19) | 1 (0.05) | 0.180 |
| *H. pylori* infection status | | | | | | |
| Negative | 55 (2.64) | 1,671 (3.48) | <**0.001** | 55 (2.64) | 69 (3.31) | 0.408 |
| Positive | 405 (19.44) | 6,030 (12.56) | | 405 (19.45) | 392 (18.83) | |
| Unknown | 1,623 (77.92) | 40,325 (83.96) | | 1,622 (77.91) | 1,621 (77.86) | |

NOTE: Bold indicates statistical significance ($P < 0.05$).
Abbreviations: AIDS, acquired immunodeficiency syndrome; COPD, chronic obstructive pulmonary disease.

proportion of intestinal metaplasia (40.44% vs. 41.61%) and neutrophil infiltration (49.71% vs. 49.86%) between the two groups (Table 2). No significant association was observed between the duration of aspirin use and gastric cancer, intestinal metaplasia, and neutrophil infiltration in patients with aspirin; however, it is noteworthy that no diffuse-type cancer was observed in patients who received aspirin for more than 2 years (Supplementary Table S3).

### NSAID use versus nonuse

We included 898 patients who received NSAIDs and selected 867 controls who did not receive NSAIDs from 49,211 patients using one-to-one propensity score matching (the c-statistic of the model was 0.932). Patient distributions were closely balanced between the two groups (Table 3). The median duration of NSAID use was 0.3 years.

No significant differences were observed in the proportions of gastric cancer (0% vs. 0.33%) and intestinal metaplasia (42.81% vs. 39.79%) between NSAID users and nonusers, whereas NSAID use was significantly associated with decreased proportion of neutrophil infiltration in the antrum (30.86% vs. 36.38%, $P = 0.015$) compared with nonusers (Table 4). No significant association was observed between aspirin use and cancer location and stage (Supplementary Table S4).

**Table 2.** Gastric cancer, intestinal metaplasia, and neutrophil infiltration in propensity score–matched patients with aspirin

| | **Aspirin use *n* = 2,082 (%)** | **Nonuse *n* = 2,082 (%)** | ***P*** |
|---|---|---|---|
| Gastric cancer | 17 (0.82) | 8 (0.38) | 0.071 |
| Diffuse-type | 1 (0.05) | 5 (0.24) | 0.218 |
| Intestinal-type | 16 (0.77) | 3 (0.14) | **0.004** |
| Intestinal metaplasia[a,b] | 628 (40.44) | 615 (41.61) | 0.512 |
| Antrum | 524 (33.63) | 529 (35.70) | 0.232 |
| Corpus | 248 (15.69) | 219 (14.80) | 0.494 |
| Neutrophil infiltration[a,b] | 770 (49.71) | 733 (49.86) | 0.932 |
| Antrum | 540 (34.82) | 532 (36.02) | 0.489 |
| Corpus | 675 (42.94) | 636 (43.12) | 0.920 |

NOTE: Bold indicates statistical significance ($P < 0.05$).
[a]Allows duplicated.
[b]Includes missing data.

## Discussion

We performed a propensity score matching analysis and found different effects by aspirin on intestinal-type and diffuse-type gastric cancers. It has been suggested that aspirin and other NSAIDs may prevent gastrointestinal cancers, including gastric cancer (14, 16, 21). Cox-2 expression, which could be suppressed by aspirin and NSAIDs, is often upregulated in inflamed gastrointestinal mucosa and may promote epithelial cell proliferation and carcinogenesis (22–24). Indeed, Cox-2 overexpression induces gastric cancer development, and anti-inflammatory drugs have been found to inhibit gastric cancer progression in several mouse models (3, 25–29). Thus, a potential chemopreventive approach to inhibit gastric cancer could involve the

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**Table 3.** Baseline characteristics of unmatched and propensity score–matched patients in NSAID user and nonuser groups

| | Unmatched | | | Propensity score matched | | |
|---|---|---|---|---|---|---|
| | **NSAIDs use** $n = 898$ (%) | **Nonuse** $n = 49{,}211$ (%) | ***P*** | **NSAID use** $n = 898$ (%) | **Nonuse** $n = 898$ (%) | ***P*** |
| Age, years | | | | | | |
| <50 | 161 (17.93) | 10,212 (20.75) | <**0.001** | 161 (17.93) | 146 (16.26) | 0.534 |
| 50—60 | 179 (19.33) | 9,607 (19.52) | | 179 (19.93) | 160 (17.82) | |
| 60—70 | 304 (33.85) | 14,106 (28.66) | | 304 (33.85) | 331 (36.86) | |
| 70—80 | 228 (25.39) | 11,642 (23.66) | | 228 (25.39) | 232 (25.84) | |
| ≥80 | 26 (2.90) | 3,644 (7.40) | | 26 (2.90) | 29 (3.23) | |
| Sex, male | 449 (50.00) | 28,006 (56.91) | <**0.001** | 449 (50.00) | 445 (49.55) | 0.850 |
| Comorbidities | | | | | | |
| Ischemic heart disease | 227 (25.28) | 5,625 (11.43) | <**0.001** | 227 (25.28) | 249 (27.73) | 0.240 |
| Chronic heart failure | 319 (35.52) | 6,998 (14.22) | <**0.001** | 319 (35.52) | 325 (36.19) | 0.768 |
| Peripheral vascular disease | 111 (12.36) | 2,311 (4.70) | <**0.001** | 111 (12.36) | 103 (11.47) | 0.560 |
| Cerebrovascular disease | 130 (14.48) | 2,571 (5.22) | <**0.001** | 130 (14.48) | 128 (14.25) | 0.893 |
| Dementia | 36 (4.01) | 844 (1.72) | <**0.001** | 36 (4.01) | 30 (3.34) | 0.452 |
| COPD | 58 (6.46) | 1,093 (2.22) | <**0.001** | 58 (6.46) | 60 (6.68) | 0.850 |
| Collagen diseases | 198 (22.05) | 2,686 (5.46) | <**0.001** | 198 (22.05) | 190 (21.16) | 0.647 |
| Peptic ulcers | 625 (69.60) | 14,112 (28.68) | <**0.001** | 625 (69.60) | 627 (69.82) | 0.918 |
| Diabetes | 568 (62.35) | 14,046 (28.54) | <**0.001** | 568 (63.25) | 573 (63.81) | 0.806 |
| Chronic kidney disease | 30 (3.34) | 1,407 (2.86) | 0.391 | 30 (3.34) | 24 (2.67) | 0.407 |
| Hemiplegia or paraplegia | 63 (7.02) | 1,164 (2.37) | <**0.001** | 63 (7.02) | 63 (7.02) | 1.000 |
| Leukemia | 13 (1.45) | 394 (0.80) | **0.032** | 13 (1.45) | 12 (1.34) | 0.840 |
| Malignant lymphoma | 82 (9.13) | 1,340 (2.72) | <**0.001** | 82 (9.13) | 81 (9.02) | 0.935 |
| Liver cirrhosis | 98 (10.91) | 2,253 (4.58) | <**0.001** | 98 (10.91) | 93 (10.36) | 0.702 |
| AIDS | 3 (0.33) | 79 (0.16) | 0.202 | 3 (0.33) | 2 (0.22) | 1.000 |
| *H. pylori* infection status | | | | | | |
| Negative | 169 (18.82) | 1,557 (3.16) | <**0.001** | 169 (18.82) | 172 (19.15) | 0.981 |
| Positive | 667 (74.28) | 5,768 (11.72) | | 667 (74.28) | 665 (74.05) | |
| Unknown | 62 (6.90) | 41,886 (85.12) | | 62 (6.90) | 61 (6.79) | |

NOTE: Bold indicates statistical significance ($P < 0.05$).
Abbreviations: AIDS, acquired immunodeficiency syndrome; COPD, chronic obstructive pulmonary disease.

suppression of the Cox/PG pathway by aspirin or NSAIDs. However, our current results showed that aspirin use increased the risk of intestinal-type gastric cancer unexpectedly. In contrast, long-term (>2 years) aspirin use may suppress diffuse-type gastric cancer development, suggesting that chemopreventive effects by aspirin might be specific for this type of histologic cancer.

The pathogenesis of diffuse-type and intestinal-type gastric cancers is quite different. Intestinal-type gastric cancers usually develop through the Correa pathway and are found in the atrophic and metaplastic stomach after long-term *H. pylori* infection. In contrast, diffuse-type cancers sometimes arise even in younger patients without *H. pylori* infection. On a molecular level, approximately half of intestinal-type gastric cancer cases are characterized by catastrophic chromosomal instability, with frequent mutations in the *TP53* gene (1). Microsatellite instability–related cancers and EBV-related immunologic type cancers are also part of a subset of intestinal-type cancers with characteristic gene mutation signatures. In contrast, the majority of diffuse-type gastric cancers do not have evident genetic or chromosomal alterations and are therefore classified as "genome stable" type. Thus, it is not surprising that aspirin/NSAIDs may have distinct effects on these two subtypes. In addition, although the presence of intestinal metaplasia is a significant risk factor for intestinal-type gastric cancer, it is uncertain whether intestinal metaplasia is a direct precursor of gastric cancer. Thus, it would be possible that the developmental mechanisms of metaplasia and gastric cancer are distinct; thus, the effects by aspirin can be different between these two, as seen in our current analysis.

**Table 4.** Gastric cancer, intestinal metaplasia, and neutrophil infiltration in propensity score–matched patients with NSAIDs

| | **NSAID use** $n = 898$ (%) | **Nonuse** $n = 898$ (%) | ***P*** |
|---|---|---|---|
| Gastric cancer | 0 (0) | 3 (0.33) | 0.250 |
| Diffuse-type | 0 (0) | 1 (0.11) | 1.000 |
| Intestinal-type | 0 (0) | 2 (0.22) | 0.500 |
| Intestinal metaplasia[a,b] | 367 (42.58) | 344 (39. 91) | 0.261 |
| Antrum | 311 (36.00) | 293 (33.79) | 0.337 |
| Corpus | 132 (15.12) | 121 (14.04) | 0.523 |
| Neutrophil infiltration[a,b] | 388 (45.27) | 428 (49.94) | 0.053 |
| Antrum | 266 (30.86) | 314 (36.38) | **0.015** |
| Corpus | 351 (40.72) | 367 (42.82) | 0.376 |

NOTE: Bold indicates statistical significance ($P < 0.05$).
[a]Allows duplicated.
[b]Includes missing data.

Findings in humans and mice strongly suggest that loss of E-cadherin expression or *CDH1* gene mutations are key triggers of the production of signet-ring cancer cells, but accumulating evidence suggests that chronic inflammation, with or without additional genetic events, may be required for progression to invasive diffuse-type cancer (2–4, 30, 31). Although we would propose the possibility that long-term use of aspirin may prevent diffuse-type cancer by suppressing chronic inflammatory responses in the stomach, further study is required in a larger and longer cohort (32).

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Previous randomized controlled trials failed to prevent the development of gastric cancer in patients with chronic gastritis by long term use of Cox-2 inhibitors (33, 34). *H. pylori* sometimes disappears in stomachs with advanced atrophy, but gastric cancers, in particular intestinal-type cancers, can still develop without active *H. pylori* infection (35). In these cases, genetic mutations and epigenetic changes may play more important roles for cancer progression than inflammatory signals, such as the Cox/PG pathway (32). Our current results showed an inverse relationship between aspirin use and intestinal-type cancer risk. Given that aspirin has apparent effects on mucosal damage in the stomach, such mucosal injury might influence epithelial proliferation and transformation; however, further validation and elucidation of the underlying mechanisms are warranted.

Recently, a retrospective epidemiologic study reported that long-term proton pump inhibitor (PPI) was significantly associated with increased risk of gastric cancer compared with non-PPI use, in patients after *H. pylori* eradication (36). In fact, the proportion of PPI users in aspirin users was higher (32.7 %) than that in aspirin nonusers (4.3%). Thus, we performed additional propensity score matching analysis to evaluate the effect by PPI on gastric cancer. Unlike the previous report (36), PPI use was not associated with gastric cancer, intestinal metaplasia, and neutrophil infiltration (Supplementary Tables S5 and S6). Nevertheless, we do not exclude the possibility that the greater proportion of PPI users in aspirin use group might affect the increase of intestinal-type gastric cancer cases in this group. It is likely that the effect by PPI in gastric cancer development may be restricted in *H. pylori*–eradicated patients; thus, further analysis with detailed information of *H. pylori* status is required to evaluate an exact association between PPI use and gastric carcinogenesis.

Our study had several strengths. We are the first to perform propensity score matching for studying the association between aspirin/NSAIDs and gastric cancer, by following >50,000 patients who received upper endoscopy over 10 years in two separate hospitals. We also included detailed histopathologic findings in our analysis, such as histologic type of cancers and the presence of intestinal metaplasia and neutrophil infiltration. However, our study also had several limitations. The duration of aspirin/NSAID use was relatively short compared with previous studies. Previous observational studies reported that patients who took aspirin for more than 5 years had lower relative odds and HRs of gastric cancer compared with those that did not take aspirin (14, 16). However, the median duration of aspirin and NSAID use in our cohort was 4.9 and 0.3 years, and this may have affected our statistics to evaluate a protective effect of aspirin/NSAIDs on gastric cancer development. In addition, there is a possibility that aspirin/NSAIDs may be prescribed from other hospitals, or that these drugs are used as off-prescription drugs that we cannot include in our database. However, all types of enteric-coated aspirin are restricted to prescriptional use and not commercially available in Japan. Thus, there is limited chance of off-prescription use of aspirin. Furthermore, the number of gastric cancer events was relatively small, which may lead to statistically insignificant results. Finally, we included data from patients with unknown *H. pylori* status.

In conclusion, aspirin/NSAID use may affect the development of gastric cancers, but it should be validated in future comprehensive cohort or randomized control studies.

## Disclosure of Potential Conflicts of Interest

M. Fujishiro reports receiving commercial research grants from EA Pharma Co., HOYA-Pentax Co., and Takeda Pharmaceutical Co., has received speakers bureau honoraria from AstraZeneka Pharmaceuticals, Daiichi-Sankyo Co., Takeda Pharmaceutical Co., and Zeria Pharmaceutical Co. No potential conflicts were disclosed by the other authors.

## Disclaimer

The funders had no role in the design of the study, data collection and analysis, decision to publish, or preparation of the manuscript.

## Authors' Contributions

**Conception and design:** R. Niikura, Y. Hayakawa
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Cancer Prevention Research

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