

Review

PTGS2 (Cyclooxygenase-2) Expression and Survival among Colorectal Cancer Patients: A Systematic ReviewAndrew T. Kunzmann^{1,2}, Liam J. Murray^{1,2}, Chris R. Cardwell², Charlene M. McShane², Úna C. McMenamin², and Marie M. Cantwell^{1,2}**Abstract**

Background: Studies have examined whether tumor expression of PTGS2 (also known as COX-2), an enzyme inhibited by nonsteroidal anti-inflammatory drugs such as aspirin, is associated with prognosis in patients with colorectal cancer. However, results to date have been mixed.

Methods: Using terms for PTGS2 and colorectal cancer, the Medline, Embase, and Web of Science databases were systematically searched for studies published, in any language, until December 2011. Random effects meta-analyses were used to calculate pooled HRs [95% confidence intervals (CI)] for the association between PTGS2 expression and tumor recurrence, colorectal cancer-specific survival, and overall survival.

Results: In total, 29 studies, which had prognostic data on 5,648 patients, met the inclusion criteria. PTGS2-positive patients were at an increased risk of tumor recurrence ($n = 9$ studies; HR, 2.79; 95% CI, 1.76–4.41; $P < 0.001$) and had poorer colorectal cancer-specific survival ($n = 7$; HR, 1.36; 95% CI, 1.02–1.82; $P = 0.04$). However, there was funnel plot asymmetry, possibly due to publication bias, for the association with cancer-specific survival but less so for recurrence. PTGS2 expression was not associated with overall survival [($n = 16$; pooled unadjusted HR, 1.30; 95% CI, 0.94–1.79; $P = 0.11$) and ($n = 9$; pooled adjusted HR, 1.02; 95% CI, 0.72–1.45; $P = 0.91$)].

Conclusions: PTGS2 expression was associated with an increased risk of tumor recurrence and poorer colorectal cancer-specific survival but not overall survival among patients with colorectal cancer. However, confounding by tumor characteristics such as tumor stage seems likely.

Impact: There is insufficient evidence to recommend PTGS2 expression as a prognostic marker in patients with colorectal cancer. Furthermore, studies providing adjusted results are required. *Cancer Epidemiol Biomarkers Prev*; 22(9); 1490–7. ©2013 AACR.

Introduction

Prostaglandin endoperoxide synthases (also referred to as COX) are the rate-limiting enzymes in the conversion of arachidonic acid, a product of damaged cell membranes, into prostaglandins and they exist in at least 2 isoforms, PTGS1 (COX-1), and PTGS2 (COX-2). Unlike PTGS1, which is expressed constitutively, PTGS2 expression is induced by cytokines and growth factors and is upregulated during inflammation. Among other prostaglandins, PTGS2 activation produces prostaglandin E₂ (PGE₂),

which acts on a number of cell signaling pathways involving cell proliferation, angiogenesis, apoptosis, invasion, and immunosuppression which could increase tumor progression (1).

PTGS2 has been shown to be expressed in most solid tumor types (2–5). PTGS2 upregulation seems to be particularly common in colorectal cancer, with approximately 85% of these tumors expressing PTGS2 compared with 20% of normal colorectal mucosal samples (6–8).

A large number of studies have examined the association between PTGS2 expression in colorectal tumors and patient prognosis; however, there was a large degree of heterogeneity in terms of study design, quality, and outcomes measured. Unsurprisingly, these studies have reported inconsistent findings and the nature of the association between PTGS2 expression in colorectal cancer and cancer prognosis therefore remains uncertain. This, however, is an important question as PTGS2 activity is readily modifiable by nonsteroidal anti-inflammatory drugs (NSAID), such as aspirin and ibuprofen, or selective PTGS2 inhibitors (COXIBs) such as celecoxib. Evidence is emerging that the use of NSAIDs, especially aspirin, may reduce risk of colorectal cancer (9–11), but few studies

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have examined whether the use of these drugs after a diagnosis of colorectal cancer affects disease progression and/or is dependent on tumor expression of PTGS2 (12). Some NSAIDs, particularly aspirin, may also affect tumor progression through COX-independent mechanisms (13) such as antiplatelet activity (9). NSAIDs may present a valuable treatment opportunity in colorectal cancer but the benefits need to be weighed against the risk of serious gastrointestinal bleeding associated with use of these drugs and the increased risk of cardiovascular events associated with selective PTGS2 inhibitors (14). Clarification of the association between tumor PTGS2 expression and colorectal cancer progression will assist in the development and targeting of therapeutic options for patients with colorectal cancer. In this article, we present the first systematic review and meta-analysis of published data relating to the association between PTGS2 expression and colorectal cancer prognosis.

The aim of the study was to systematically identify all studies that analyzed the association between PTGS2 expression in colorectal tumors at diagnosis and subsequent survival and/or recurrence and to clarify the associations using meta-analyses.

Materials and Methods

Search strategy

A systematic search of the literature was conducted to identify primary studies, published in any language, investigating the association between PTGS2 expression and colorectal cancer prognosis. The Embase (from 1988), Medline (from 1966), and Web of Science (from 1950) databases were searched through to December 2011, using keywords and exploded Medical Subject Headings. We used the following search strategy:

1. (Prostaglandin endoperoxide synthase.mp. or exp Prostaglandin endoperoxide synthase/) or (COX-2 or Cox-ii or cyclooxygenase-2 or cyclooxygenase-ii or cyclo-oxygenase-2 or cyclo-oxygenase-ii).af.
2. (Cancer or Cancers or neoplasm or neoplasms or tumor or tumors or tumour or tumours or carcinoma or carcinomas or adenocarcinoma or adenocarcinomas).af.
3. (Colorectal or colon or rectum or rectal or colonic).af.
4. 1 and 2 and 3

Eligibility criteria

The principal reviewer (A.T. Kunzmann) screened all titles and abstracts independently, whereas 4 other reviewers (L.J. Murray, M.M. Cantwell, C.M. McShane, and U.C. McMenamin) independently screened a quarter of the titles and abstracts each. In cases where the title and abstract were not sufficient for a decision to be made, the full text was acquired for further inspection. Those identified by any investigator for possible inclusion, were then independently reviewed by 2 other investigators with disagreements about inclusion settled by discussion according to the eligibility and exclusion criteria.

The eligibility criteria were that: (i) the study assessed PTGS2 expression in human colorectal tumors before commencement of radiotherapy or chemotherapy; (ii) the study assessed the relationship of PTGS2 expression with a clinically relevant prognostic outcome including overall survival, disease-free survival, colorectal/colon/rectal cancer-specific survival, relapse-free survival, and time to, or risk of, recurrence/metastasis; and (iii) the study provided sufficient statistical information, on at least one of these outcomes, to be included in a meta-analysis. Tumor regression or tumor downstaging were not included as relevant outcomes because of difficulty in precisely measuring these outcomes. Studies where all patients were classified as stage IV or Duke's D, at baseline, were excluded as these patients would all have tumors elsewhere in the body which could likely influence survival. Conference abstracts and poster presentations were also excluded. When studies had overlapping populations and reported on the same outcomes, the smaller study was excluded. Finally, the reference lists of included articles were examined for other potentially relevant articles.

Data extraction

The following data were extracted, where available, from the full text of the included articles: first author's name, year of publication, location of the study, number of participants, mean age of participants, number of males and females, primary tumor site, tumor-node-metastasis/Duke stage at enrolment, cancer treatments received, methods used to measure PTGS2 expression, antibody used [if immunohistochemistry (IHC)], number of patients with PTGS2-positive and -negative tumors, median follow-up time, survival, and/or recurrence results including reported HRs.

Statistical analysis

Stata 11 software (Stata Corporation) was used for data analysis. Random effects meta-analyses were conducted to produce pooled estimates from unadjusted study results for all outcomes: recurrence (studies reporting estimates for either local recurrence only or both local and distant recurrences combined were included), distant recurrence, disease-free survival, cancer-specific survival, and overall survival. Because of limited data, a separate meta-analysis using study results adjusted for various other prognostic factors, often including age, sex, tumor stage and tumor grade, was only possible for overall survival. The published HRs and confidence intervals (CI) were used where available and where these were not available, estimates of the HR and SE were produced using the indirect and survival curve methods proposed by Parmar and colleagues, (1998; ref. 15). A χ^2 test for heterogeneity was calculated and the I^2 statistic determined to estimate the proportion of variation between study results attributable to heterogeneity rather than chance (16). Heterogeneity was considered high if I^2 statistic was above 50% (17). Because of the large heterogeneity in study design, random effects models were

used. Funnel plots were produced according to the Begg and Egger methods (18, 19), and Egger tests were conducted when more than 10 studies were included in the meta-analyses, to assess the likelihood of publication bias. The trim and fill method (20) was used to estimate the overall effect sizes after adjustment for potential publication bias.

Results

Study characteristics

The literature search yielded a total of 10,665 citations (Embase $n = 4,074$; Medline $n = 2,273$; Web of Science $n = 4,318$). After removal of duplicates, 6,093 potentially relevant citations remained. After application of the exclusion criteria, 29 studies remained (7, 21–45; Fig. 1; Supplementary Table S1). These studies published survival/recurrence data on 5,648 patients.

The median follow-up times reported ranged from 32.4 to 76 months. Sample sizes ranged from 42 to 871. Most studies were from Asian ($n = 10$) and European ($n = 18$) populations, with only 1 study from a North American population.

In 22 of the 29 studies, the association between PTGS2 expression and prognosis in combined colon and rectal cancer populations was reported without subgroup analysis based on tumor location. Of the remaining 7 studies, 3 studies analyzed the association in patients with rectal cancer, whereas 4 studies assessed the association in patients with colon cancer. Because of differences in outcomes reported, sensitivity analysis to investigate the association between PTGS2 expression and prognosis by tumor location was not possible. Only 5 of the 29 studies reported the results of subgroup analyses based on tumor stage, which meant that formal sensitivity analysis based on tumor-stage was not possible.

IHC was the most widely used method to assess PTGS2 expression (28/29 studies), with only one study using a PCR technique. However, the type of antibody (monoclonal or polyclonal) used for IHC differed between studies. Fourteen studies reported use of monoclonal antibodies, 6 reported use of polyclonal antibodies, and 8 studies did not report the type used. The definition of PTGS2 status also varied between studies: the majority used a combination of intensity and extent of PTGS2 staining; however, the cut-off point varied and some only used intensity or extent alone.

Association with cancer recurrence

Eight studies, including 1,577 patients, provided sufficient data (unadjusted for age, sex, and other tumor characteristics) to be included in a meta-analysis to assess the association between PTGS2 status and colorectal cancer recurrence. One study (40) provided results from a subgroup analysis based on tumor-stage. The results for each subgroup were combined using a random effects meta-analysis and the pooled estimate was added to the main meta-analysis. The pooled HR for recurrence in patients with PTGS2-positive tumors compared with patients with PTGS2-negative tumors was 2.79 (95% CI, 1.76–4.41; $P < 0.001$; Fig. 2A). Moderate heterogeneity was observed ($I^2 = 36\%$).

The funnel plot had minimal asymmetry (Fig. 2B). Use of the trim and fill method did not alter the result (data not shown).

Association with distant recurrence/metastasis

Five studies, including 1,010 patients, provided sufficient unadjusted data to be included in a meta-analysis to assess the association between PTGS2 status and distant recurrence/metastasis. The pooled HR for patients with PTGS2-positive tumors compared with those with

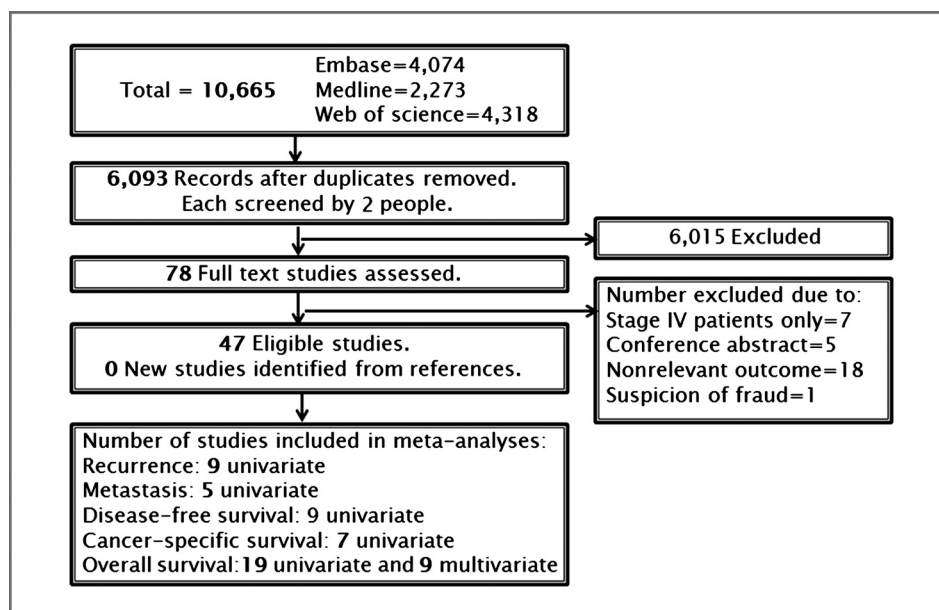


Figure 1. A flow chart of study selection.

PTGS2-negative tumors was 2.09 (95% CI, 0.78–5.60; $P = 0.14$; Fig. 2C). The I^2 value was 81% suggesting a high degree of heterogeneity.

The funnel plot showed some asymmetry suggesting possible publication bias (Fig. 2D) and using the trim and fill method an attenuated estimate was obtained; (HR, 1.45; 95% CI, 0.55–3.85; $P = 0.45$).

Association with disease-free survival

Nine studies, including 2,435 patients, provided sufficient unadjusted data to be included in a meta-analysis to assess the association between PTGS2 status and disease-free survival. The pooled HR for patients with PTGS2-positive tumors compared with those with PTGS2-negative tumors was 1.19 (95% CI, 0.87–1.62; $P = 0.29$); however, a high-degree of heterogeneity was evident ($I^2 = 65\%$; Fig. 2E).

The funnel plot was asymmetrical, suggesting publication bias is likely (Fig. 2F) and use of the trim and fill method produced an attenuated estimate HR 0.88 (95% CI, 0.63–1.22; $P = 0.445$).

Association with colorectal cancer-specific survival

Seven studies, including 1,768 patients, provided sufficient unadjusted data to be included in the meta-analysis assessing the association between PTGS2 status and colorectal cancer-specific survival. The pooled HR was 1.36 (95% CI, 1.02–1.82; $P = 0.04$) and moderate heterogeneity was observed ($I^2 = 41\%$; Fig. 2G).

The funnel plot was asymmetrical suggesting publication bias is possible (Fig. 2H) and use of the trim and fill method, which imputed 3 values, attenuated the estimate HR to 1.10 (95% CI, 0.80–1.51; $P = 0.599$).

Association with overall survival

Sixteen studies, including 3,657 patients, provided sufficient unadjusted data to be included in a meta-analysis assessing the association between PTGS2 status and overall survival. The I^2 value was 77% suggesting heterogeneity was large. The estimated pooled HR was 1.30 (95% CI, 0.94–1.79; $P = 0.11$; Fig. 2I).

The funnel plot showed asymmetry and the Egger test was statistically significant ($t = 2.96$, $P = 0.01$), indicative of publication bias (Fig. 2J). Use of the trim and fill method changed the estimate HR to 0.97 (95% CI, 0.69–1.36; $P = 0.87$).

Nine studies, involving 2,121 patients, provided overall survival estimates adjusted for age, sex, and various tumor characteristics (see Table 1 for adjustments). This estimated the pooled HR as 1.02 (95% CI, 0.72–1.45; $P = 0.91$; Fig. 2K). The I^2 value was 63% suggesting heterogeneity was large.

Funnel plot asymmetry was hard to gauge (Fig. 2L) as only large studies tended to publish adjusted analyses but use of the trim and fill method moved the estimate for overall survival from adjusted study results to 0.92 (95% CI, 0.63–1.35; $P = 0.67$).

Discussion

This systematic review with meta-analyses has shown that tumor PTGS2 expression is associated with an increased risk of colorectal cancer recurrence and poorer colorectal cancer-specific survival. However, it was not possible to conduct a meta-analysis of estimates for risk of cancer recurrence and cancer-specific survival that adjusted for other prognostic factors such as tumor stage. Adjustment for potential publication bias also meant the association with colorectal cancer-specific survival was no longer observed. In addition, PTGS2 expression was not associated with overall survival in patients with colorectal cancer, especially after adjustment for age, sex, and other tumor characteristics. Therefore, from summation of the existing literature, it remains unclear whether there is any independent association between tumor PTGS2 expression and colorectal cancer progression.

Patients with tumors that express PTGS2 were at an increased risk of colorectal cancer recurrence and colorectal cancer-specific survival when unadjusted estimates from 9 and 7 studies, respectively, were pooled. The trim and fill method showed that the results for recurrence were unlikely due to publication bias, as the results were unchanged using this additional method (46). However, the estimate for colorectal cancer-specific survival could be subject to publication bias based on asymmetry of the funnel plot and a lack of statistical significance after using the trim and fill method.

The apparent associations between PTGS2 expression and risk of recurrence and colorectal cancer-specific survival could result from confounding by other prognostic tumor characteristics such as tumor stage and tumor grade. This is particularly important as a number of studies in the current review found associations between PTGS2 expression and tumor stage/grade (8, 30, 33, 36–38, 42, 44, 47) which could reflect a causal effect of tumor stage, or an associated factor on PTGS2 expression. Conversely, however, it could indicate that increased tumor stage is caused by increased PTGS2 expression in which case adjustment for stage would not be appropriate. Nevertheless, too few studies provided adjusted analyses to adequately assess any potential confounding. This prevents a definitive conclusion as to whether there is an independent association between PTGS2 and recurrence or colorectal cancer-specific survival from being made.

Despite the increased risk of recurrence and reduced colorectal cancer-specific survival among patients with PTGS2-positive tumors, patients do not seem to be at an increased risk of either overall survival or disease-free survival. This was particularly apparent for overall survival when pooling study results that were adjusted for other prognostic characteristics such as age, sex, tumor stage, and tumor grade. Publication bias also seemed apparent and adjustment for this using the trim and fill method moved the estimates for both overall survival and disease-free survival closer to 1. A number of studies (48–51) identified in the search, which met the first two

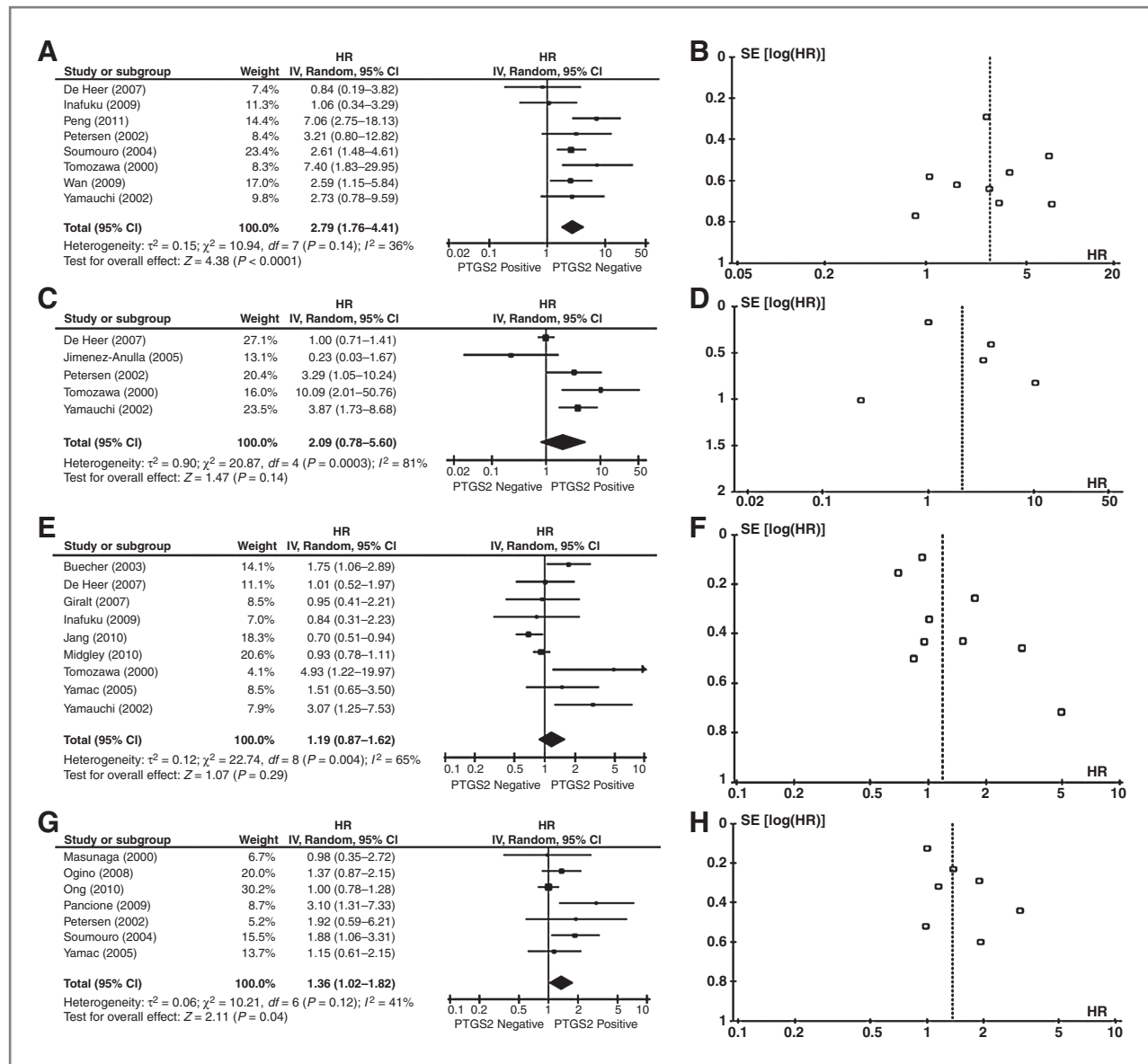


Figure 2. Forest and funnel plots of PTGS2 expression against prognostic indicators. Recurrence (A and B), metastasis (C and D), disease-free survival (E and F), and cancer-specific survival (G and H); continued on next page.

inclusion criteria, but did not provide sufficient information to be included in the meta-analysis, reported that the results were non-statistically significant which provides further support that reporting bias, a form of publication bias, had occurred. The moderate to large degree of heterogeneity among studies for each of the outcomes measured indicates that PTGS2 expression may be of prognostic value under certain conditions, but not others. These conditions may differ based on the use of different antibodies to assess PTGS2 expression or differences in the composition of each study population based on ethnicity, gender, stage of disease, or tumor locations. The cut-off point used by studies to determine PTGS2 positivity may also be important. Altering the cut-off point

used to determine positive expression could alter both the strength and direction of the association between PTGS2 positivity and survival (24). However, due to the limited number of studies included, which separated participants based on these factors, sensitivity analyses could not be conducted. Furthermore, studies comparing the effects in different subgroups would be useful to identify subgroups where PTGS2 expression may have prognostic value.

The measures of PTGS2 expression at surgery or biopsy may also not reflect PTGS2 expression in cells remaining after surgery for 2 reasons. First, as PTGS2 expression is inducible by cytokines and surgery may cause a large degree of inflammation (16), accurate classification of a

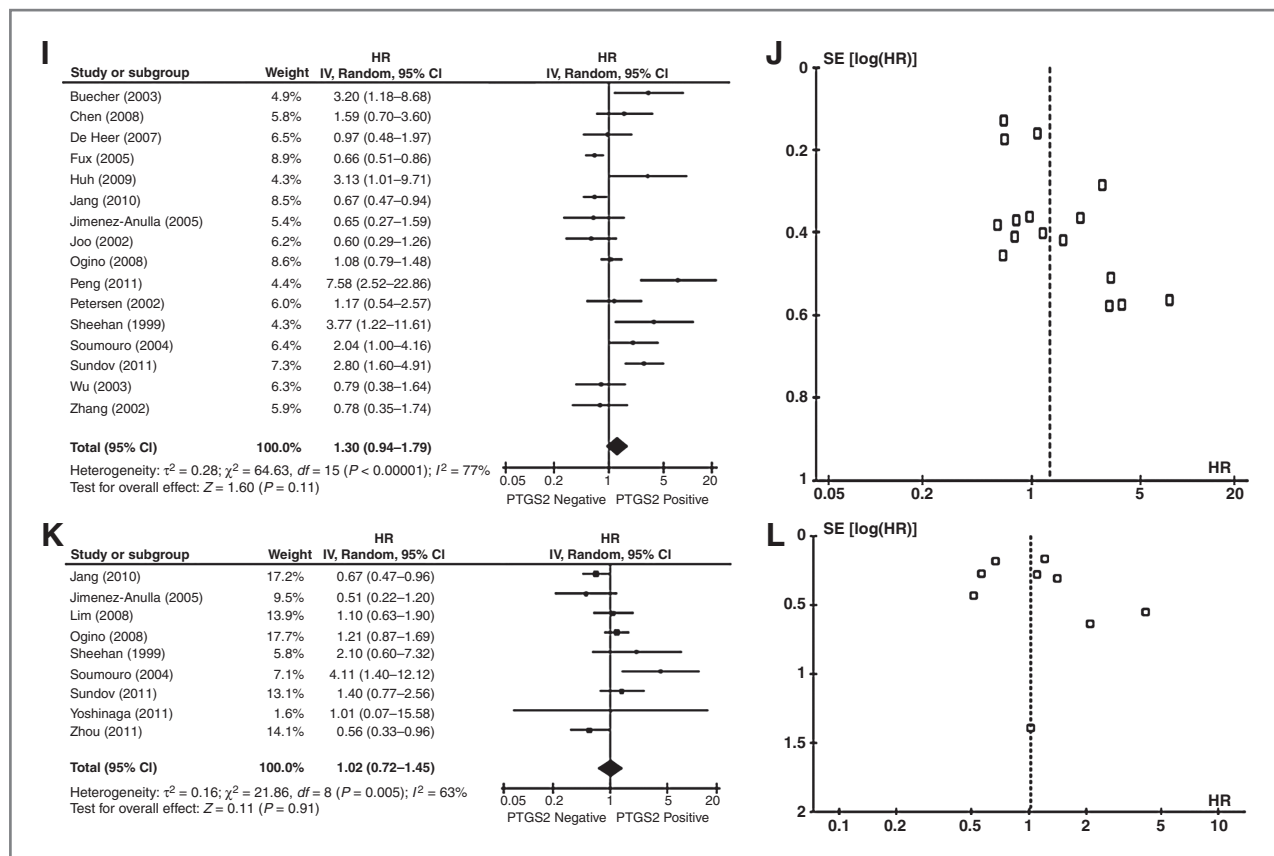


Figure 2. (Continued) Overall survival (I and J; all unadjusted) and adjusted overall survival (K and L).

patients' level of PTGS2 expression from samples taken at this time may be difficult. Second, PTGS2 expression in tumor cells remaining after surgery may also be altered by radiotherapy or chemotherapy treatments received after samples were taken (52, 53). This would mean the measure of PTGS2 expression at surgery or biopsy may not reflect a patients' PTGS2 expression throughout the whole postsurgery period.

Although adjustment for confounding by tumor characteristics may move the estimates closer to null, adjustment for the use of NSAIDs may also move the estimate, but in the opposite direction. Postdiagnostic use of NSAIDs such as aspirin may be common, for example, 8% to 43% of patients with colorectal cancer in the VICTOR trial and Nurses' Health study were classed as aspirin users, respectively (12, 31). The Nurses' Health study also found that PTGS2 expression might be needed for aspirin to affect survival in patients with colorectal cancer (12). Therefore, aspirin use may selectively improve survival among patients with PTGS2-positive tumors and thus mask any association between PTGS2 expression and survival.

Cancer survival may be influenced by a number of interacting molecular pathways. PTGS2 expression is associated with the expression of a number of potentially prognostic signaling proteins, including phos-

phoinositide 3-kinase and protein 53 (54, 55). PTGS2 is also inversely associated with the presence of microsatellite instabilities (MSI; 54, 56), which itself seems to be associated with improved survival (57, 58) and could therefore mask an association between PTGS2 and survival. Only one study within this review adjusted for MSI status (7) and found that this adjustment strengthened the association between PTGS2 and survival. Therefore, it could be speculated that the association between PTGS2 and survival observed in this review would be stronger if more studies had adjusted for MSI status. Studies testing coexpression of multiple biomarkers have also found evidence of synergistic or antagonistic effects between PTGS2 and other biomarkers on survival (7, 33). Therefore, assessment of multiple molecular pathways may allow better adjustment for confounding and may also allow a better determination of prognosis than measurement of any individual marker alone.

Systematic reviews rely on the quality of the primary studies within the existing literature. Unfortunately, the majority of the studies eligible for this review had small sample sizes which likely precluded adjustment for potential confounders and increased the risk of publication or reporting bias. More larger-scale studies, that individually have sufficient power to adjust for potential

confounders, would be more useful as they would better allow the true independent association to be determined.

It has been hypothesized that NSAIDs such as aspirin may improve survival among patients with colorectal cancer and that this effect may be mediated by the inhibition of PTGS2 expression in the tumor. The inconsistency of the current results means they neither support nor refute this hypothesis. It is also possible that NSAIDs may improve survival via alternative biologic mechanisms, including antiplatelet activity. Recent studies suggest that low-dose aspirin may improve survival among patients with colorectal cancer (12, 59), despite the selectivity of the inhibition of PTGS to platelets at these doses (60).

In summary, due to the inconsistent results between outcomes and insufficient data from studies adjusting for relevant prognostic indicators, it is unclear from the current literature whether PTGS2 expression has an independent association with prognosis. Therefore, there is insufficient evidence to recommend prognostic testing of PTGS2 expression in patients with colorectal cancer. Furthermore, better designed studies using larger sample sizes and conducting adjusted analyses are needed.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A.T. Kunzmann, L.J. Murray, M.M. Cantwell
Development of methodology: A.T. Kunzmann, L.J. Murray, M.M. Cantwell
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.T. Kunzmann, L.J. Murray
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.T. Kunzmann, L.J. Murray, C.R. Cardwell, M.M. Cantwell
Writing, review, and/or revision of the manuscript: A.T. Kunzmann, L.J. Murray, C.R. Cardwell, C.M. McShane, M.M. Cantwell
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.T. Kunzmann, L.J. Murray, C.M. McShane, Ú.C. McMenamin
Study supervision: L.J. Murray, M.M. Cantwell

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