ASPIRIN AND THE RISK OF NON-DIGESTIVE TRACT CANCERS: AN UPDATED META-ANALYSIS TO 2019

Short title: Aspirin and non-digestive tract cancer risk

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Abbreviations: CI: Confidence Interval; COX: Cyclooxygenase; RCT: Randomized Controlled Trial; RR: Relative Risk; WHS: Women's Health Study.

Novelty and Impact: This comprehensive meta-analysis provides limited supports to a protective role of aspirin on the risk of lung, breast, endometrium, ovary, and prostate cancer.

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ABSTRACT

Aspirin has been associated to a reduced risk of colorectal and other selected digestive tract cancers, but the evidence other neoplasms is still controversial. In order to provide an up-to-date quantification of the role of aspirin on lung, breast, endometrium, ovary, prostate, bladder, and kidney cancer, we conducted a systematic review and meta-analysis of all observational studies published up to March 2019. We estimated pooled relative risk (RR) of cancer or cancer death for regular aspirin use versus non-use using random-effects models, and, whenever possible, we investigated dose- and duration-risk relations. A total of 148 studies were considered. Regular aspirin use was associated to a reduced risk of lung (RR=0.88, 95% confidence interval, CI=0.79-0.98), breast (RR=0.90, 95% CI=0.85-0.95), endometrial (RR=0.91, 95% CI=0.84-0.98), ovarian (RR=0.91, 95% CI=0.85-0.97), and prostate (RR=0.93, 95% CI=0.89-0.96) cancer. However, for most neoplasms nonsignificant risk reductions were reported in cohort and nested case-control studies, and there was between-study heterogeneity. No association was reported for bladder and kidney cancer. No duration-risk relations for most neoplasms were observed, except for an inverse duration-risk relation for prostate cancer. The present meta-analysis confirms the absence of appreciable effect of regular aspirin use on cancers of the bladder and kidney, and quantifies small and heterogeneous inverse associations for other cancers considered.

INTRODUCTION

Aspirin has long been investigated for its possible chemo-preventive role on cancer [1], and has been associated in particular to a reduced risk of colorectal and other digestive tract cancers [2-8]. A recent meta-analysis, based on over 110 epidemiological studies, reported pooled relative risks (RRs) of 0.73 for colorectal cancer, 0.67 for squamous-cell oesophageal cancer, 0.61 for stomach cancer, 0.64 for adenocarcinoma of the oesophagus and gastric cardia, 0.62 for hepato-biliary tract cancer, and 0.78 for pancreatic cancer [8].

A favorable effect of aspirin has also been reported for a few other common neoplasms, including lung, breast, and prostate cancer [2, 7], but the evidence is still controversial. Moreover, only a few studies investigated the association with dose and duration of aspirin use.

In order to provide up-to-date and comprehensive evidence of the role of aspirin on selected non-digestive tract cancers (i.e., lung, breast, endometrium, ovary, prostate, bladder, and kidney), we updated a systematic review and meta-analysis of observational studies published in 2012 [2], evaluating also dose- and duration-risk relations.

MATERIALS AND METHODS

The details of present systematic review and meta-analysis have already been provided in a companion paper on digestive tract cancers [8]. Briefly, we carried out a literature search to identify all original study publications from observational studies on aspirin and cancer risk, published between January 1 2011 and March 18 2019, and indexed in Pubmed/MEDLINE, Embase, and the Cochrane Library (see **Supplementary Table 1** for the search strings used in

each database). Additional articles were identified through manual check of the references of selected papers or other systematic reviews/meta-analyses.

We included studies if they: (i) were cohort studies, pooled analyses of cohort studies, nested case-control studies, case-control studies, or pooled analyses of case-control studies; (ii) provided data on humans in the general population; (iii) provided information on regular aspirin use (i.e., use of at least 1-2 tablets per week) or, alternatively, on any use; (iv) focused on 14 selected neoplasms; (v) reported RR estimates – including odds ratios and hazard ratios – for the selected neoplasms, in relation to aspirin use versus non-use, and the corresponding 95% confidence interval (CI), or provided sufficient information to compute them; and (vi) were published as original articles in English.

Two reviewers (CS and MM) independently screened the titles and/or abstracts of the identified publication records in order to exclude those that did not meet the eligibility criteria. Subsequently, they retrieved and assessed the full text of the selected articles. Any disagreement was solved by consensus between the two reviewers or with the help of a third one (CB). No quality score was assigned to the studies and no study was excluded *a priori*.

For each eligible study, we abstracted information on: first author, publication year, study design, country, cancer site and/or subsite, endpoint, type of controls, number of cases and controls (or subjects at risk/person years for cohort studies), and RR estimates for aspirin use versus non-use, with the corresponding 95% CI. When available, we also retrieved information on aspirin formulation, dose (mg/day), frequency (times per month, week or day), and duration (years) of aspirin use. When the results of the same study were reported on multiple publications, we abstracted data only from the most recent or informative one. We

also checked overlapping information between pooled-analyses and original studies, and, for some pooled-analyses, we only included information not provided in separate publications.

Statistical analysis

For each cancer site of interest, we pooled RRs for regular aspirin use compared to non-use, overall and by study design using random-effect models [9, 10]. For cohort studies providing estimates for both incidence and mortality, in the main analysis we pooled data on incidence, unless the results on mortality were more recent and included a larger number of cases. When required, we computed estimates of the RR for regular aspirin use by pooling the RRs for various categories of frequency or duration of use, using the method described by Hamling et al. [11]. Heterogeneity between studies was assessed using the Cochran χ^2 test and the inconsistencies was quantified using the I^2 statistic [12]. To evaluate publication bias, we examined the funnel plots and applied the Egger's and Begg's tests for funnel plot asymmetry [10, 12]. We also performed stratified analyses according to various study and population characteristics, such as study design, year of publication, geographic area, sex, endpoint, type of controls, and conducted sensitivity analyses excluding studies on prescription data. For neoplasms with adequate information, we investigated both linear and nonlinear relations between daily dose and duration of aspirin use and the log-RR, testing the log-linearity using the Wald test. Dose-response relationships between dose and duration of aspirin and log RR of each cancer site were evaluated using one-stage random-effects log-linear models, in case of linearity, or restricted cubic splines with three knots, when linearity was rejected [13-16].

All statistical analyses were performed using the SAS software version 9.4 (SAS Institute Inc., Cary, North Carolina) and R version 3.4.1 (Development Core Team, 2017).

RESULTS

Supplementary Figure 1 shows the flow-chart of study selection. From 1575 records on aspirin and cancer risk published between 2011 and 2019, after removing duplicate or ineligible records and considering 150 additional articles published before 2012 [2], we included 242 eligible articles. One-hundred and forty-eight focused on lung, breast, endometrium, ovary, prostate, bladder, and kidney and were considered in the present systematic review. **Supplementary Tables 2-8** summarize the main characteristics of the included studies, while Supplementary Tables **9-15** list the eligible studies excluded from the meta-analysis, since their results have already been reported in other more recent or complete publications**.**

Lung cancer

Twenty-one studies comprising 106 814 cases contributed to estimate the relation between aspirin use and lung cancer risk (**Supplementary Table 2** and **Table 1**). Of these, six studies, including 96 883 cases, were added to the previous meta-analysis [2] (**Supplementary Table16**). The summary RR was 0.88 (95% CI=0.79-0.98) for all studies combined (p-heterogeneity <0.001; **Table 1** and **Figure 1**). The corresponding figures were 0.73 (95% CI=0.58-0.92; p-heterogeneity=0.002) for case-control studies, 0.95 for cohort studies (95% CI=0.82-1.09; p-heterogeneity<0.001), and 0.93 for nested case-control studies

(95% CI=0.72-1.22; p-heterogeneity=0.15). The RR was 0.86 (95% CI=0.75-0.98) after excluding three studies conducted on prescription databases (data not shown).

The funnel plot suggested some publication bias, confirmed by the Begg's test (P=0.007), but not the Egger's test (p=0.98; **Supplementary Figure 2**). Risk estimates were consistent across strata of geographic area, type of controls, endpoint, year of publication, and sex (**Supplementary Table 17**). Only three studies provided estimates of lung cancer risk in relation to dose, indicating a modest and nonsignificant increased risk for increasing dose (**Supplementary Figure 3**). Twelve studies analyzed duration of aspirin use and reported a nonsignificant linear decrease in lung cancer risk with increasing years of use (**Supplementary Figure 4**).

Breast cancer

Thirty-three studies analyzed the association between aspirin use and breast cancer risk (**Supplementary Table 3** and **Table 1**). Of these, seven studies, including 19 880 cases, were added to the previous meta-analysis [2] (**Supplementary Table 16**). Overall the pooled RR was 0.90 (95% CI=0.85-0.95; p heterogeneity between studies <0.001; **Table 1** and **Figure 2**). Corresponding estimates were 0.77 (95% CI=0.69-0.87; p-heterogeneity=0.03) in case-control, 0.85 (95% CI=0.73-0.99; p-heterogeneity=0.004) in nested case-control, and 0.96 (95% CI=0.91-1.02; p-heterogeneity<0.001) in cohort studies. After excluding two studies on prescription databases the RR was 0.91 (95% CI=0.86-0.96; data not shown). The RRs were consistent in women with a positive hormone receptor status (RR=0.92, 95% CI=0.83-1.03) and in those with a negative one (RR=1.03, 95% CI=0.89-1.19; p-heterogeneity across

strata=0.25).

The funnel plot, the Egger's test (p=0.005), and the Begg's test (p=0.025) reported some publication bias (**Supplementary Figure 5**). Risk estimates were consistent across strata of the covariates considered (**Supplementary Table 18**). Only three studies provided estimates of breast cancer risk in relation to aspirin dose, indicating a modest and nonsignificant increased risk for increasing dose (**Supplementary Figure 6**). Sixteen studies, giving RR estimates of breast cancer risk in relation to duration of aspirin use, showed a nonsignificant linear reduction in the risk with increasing years of regular aspirin use (**Supplementary Figure 7**).

Endometrial cancer

Nineteen studies (of which four deriving from a pooled analysis [17]), for a total of 13 912 cases, contributed estimating the risk of endometrial cancer in relation to aspirin use (**Supplementary Table 4** and **Table 1**). Of these, 11 studies on 11 088 cases were added to our previous meta-analysis [2] (**Supplementary Table 16**). Overall, a reduced risk was reported (RR=0.91, 95% CI=0.84-0.98). The RR was 0.93 (95% CI=0.85-1.02) in cohort studies, 0.77 (95% CI=0.64-0.93) in case-control studies, and 0.98 (95% CI=0.91-1.05) in nested case-control studies (**Table 1** and **Figure 3**). There was no heterogeneity between studies nor across study design. The RR was 0.88 (95% CI=0.81-0.96) after removing two studies conducted on prescription databases (data not shown).

The funnel plot displayed some publication bias, confirmed by the Egger's test (P=0.038), but not by the Begg's test (P=0.112; **Supplementary Figure 8**). The RRs were

consistent across type of controls and year of publication, but no across geographic areas (RR=0.91 in the USA, 0.98 in Europe, and 0.54 in other areas; p-heterogeneity=0.005; **Supplementary Table 19**). Seven studies reported information on duration of aspirin use and showed no relation between duration of use and the risk of endometrial cancer (**Supplementary Figure 9**).

Ovarian cancer

Twenty-two studies (of which five deriving from a pooled analysis [18]) including 18 403 cases estimated the risk of ovarian cancer for aspirin use (**Supplementary Table 5** and **Table 1**). Of these, 10 studies including 11 260 cases, were added to our previous meta-analysis [2] (**Supplementary Table 16**). Overall (RR=0.91, 95% CI=0.85-0.97) and in 18 case-control studies (RR=0.88, 95% CI=0.80-0.97) an inverse relation was found, while no association was observed in two cohort (RR=0.95, 95% CI=0.88-1.02) and two nested case-control studies (RR=0.92, 95% CI=0.75-1.12; **Table 1** and **Figure 4**). There was no heterogeneity between studies nor across study design. The RR was 0.90 (95% CI=0.83-0.97) after excluding one study conducted on prescription databases (data not shown).

No indication of publication bias emerged (Egger's test P=0.05; Begg's test P=0.10; **Supplementary Figure 10**). The RRs were consistent across strata of the study characteristics considered (**Supplementary Table 20**). Eleven studies reported RR estimates for duration of aspirin use and suggested a decline of ovarian cancer risk up to five years of aspirin use, but not for longer duration of use (**Supplementary Figure 11**).

Prostate cancer

Thirty studies considered aspirin use and prostate cancer risk, including 107 521 cancer cases (**Supplementary Table 6 and Table 1**). Of these, nine studies including 74 332 cases were added to the previous meta-analysis [2] (**Supplementary Table 16**). The summary RR was 0.93 (95% CI=0.89-0.96; p-heterogeneity<0.001) in all studies combined, 0.97 (95% CI=0.93-1.01; p-heterogeneity=0.08) in cohort studies, 0.88 (95% CI=0.77-1.00; p-heterogeneity=0.03) in case-control studies, and 0.88 (95% CI=0.81-0.95; p-heterogeneity<0.001) in nested case-control studies (p-heterogeneity across study design =0.043; **Table 1** and **Figure 5**). The RRs were 0.87 (95% CI=0.80-0.94) for highly aggressive cancers and 0.94 (95% CI=0.87-1.02) less aggressive ones, but the two estimates were not heterogeneous (p=0.152; data not shown). The RR was 0.93 (95% CI=0.89-0.98) after excluding five studies conducted on prescription databases

There was no evidence of publication bias (Egger's test P=0.231; Begg's test P=0.341; **Supplementary Figure 12**). The risk estimates were consistent across strata of all the covariates considered, except for types of controls (RR=1.00 for hospital controls, and 0.79 for population controls; p-heterogeneity=0.044; **Supplementary Table 21**). Thirteen studies displayed a linear decrease in the risk of prostate cancer with increasing years of regular aspirin use, with a RR of 0.94 (95% CI=0.89-0.99) for five and a RR of 0.89 (95% CI=0.78-0.99) for 10 years of use (**Supplementary Figure 13**).

Bladder cancer

Fifteen studies including 14 108 cases contributed to the risk of bladder cancer in relation

to aspirin use (**Supplementary Table 7** and **Table 1**). Of these, seven studies on 9755 cancer cases were added to the previous meta-analysis [2] (**Supplementary Table 16**). Overall, there was no meaningful associations (RR=1.03, 95% CI=0.99-1.08, overall, RR=1.04, 95% CI=0.99-1.10 in nine cohort, and RR=0.98, 95% CI=0.80-1.16, in six case-control studies; **Table 1** and **Figure 6**). Estimates were not significantly heterogeneous between studies, except among case-control studies (p=0.04), nor across study design. The RR was 1.06 (95% CI=0.95-1.18) after excluding one study conducted on prescription databases (data not shown).

No evidence of publication bias was detected (Egger's test P=0.29; Begg's test P=0.40; **Supplementary Figure 14**). No differences were observed across strata of various study characteristics (**Supplementary Table 22**). There was no meaningful association between duration of aspirin use and the risk of bladder cancer based on six studies (**Supplementary Figure 15**).

Kidney cancer

Seventeen studies including 20 703 cases analyzed aspirin use in relation to kidney cancer risk (**Supplementary Table 8** and **Table 1**). Of these, eight studies including 16 042 cases were added to the previous meta-analysis [2] (**Supplementary Table 16**). No association was found overall (RR=1.06, 95% CI=0.96-1.16; p-heterogeneity<0.001), in nine cohort (RR=1.05, 95% CI=0.91-1.22; p-heterogeneity=0.02), or in seven case-control (RR=1.11, 95% CI=0.96-1.29; p-heterogeneity=0.07), while an inverse association was found in one nested case-control study (RR=0.93, 95% CI=0.88-0.98; **Table 1** and **Figure 7**). There was

significant heterogeneity across study design (P=0.04). The RR was 1.03 (95% CI=0.97-1.10) after excluding two studies conducted on prescription databases (data not shown).

Evidence of publication bias was found by visual inspection of the funnel plot and from the Egger's test (P=0.045), but not from the Begg's test (p=0.51; **Supplementary Figure 16**). The RRs were consistent across strata of the covariates considered (**Supplementary Table 23**). There was a direct nonsignificant linear relationship between the duration of aspirin use and kidney cancer risk, based on seven studies (**Supplementary Figure 17**).

DISCUSSION

The present comprehensive meta-analysis on aspirin and non-digestive tract cancers confirms that the absence of any meaningful association with bladder and kidney cancer risk, and reports small and heterogeneous inverse associations with cancers of the lung, breast, endometrium, ovary, and prostate. Moreover, there were no duration-risk relations for most neoplasms, except for an inverse duration-risk relation for prostate cancer.

A modest overall reduction (around 12%) in lung cancer risk was reported in over 20 studies, on more than 100 000 cases. This risk reduction was, however, restricted to case-control studies and was characterized by significant between-studies heterogeneity. The duration-risk analysis suggested a higher risk reduction for longer duration of aspirin use, although the relation was not significant. These results further confirm those of a previous meta-analysis, reporting a modest and inconsistent benefit of aspirin on lung cancer risk [19]. Among randomized clinical trials (RCTs) for primary or secondary prevention of cardiovascular disease, the Women's Health Study (WHS) showed a nonsignificant benefit

for alternate-day, low-dose aspirin use for an average 10 years on lung cancer incidence and a significant one on mortality [20]. However, in a post-trial follow-up of more than 10 years the association with lung cancer incidence was not confirmed [21]. A pooled analysis of RCTs of daily aspirin use for cardiovascular prevention found a significant reduction in lung cancer mortality only in patients with at least five years of treatment and after a latent period of five years or more, and the benefit was limited to adenocarcinomas [22].

More than 30 studies, on approximately 55 000 cases, indicated a reduction by about 10% of breast cancer risk for regular aspirin use. However, the inverse relation was stronger and significant only in case-control and nested case-control studies, and study-specific estimates were heterogeneous. A higher reduction in risk – though not significant – was observed for a higher dose and longer duration of use. A meta-analysis of 13 cohort studies reported a nonsignificant inverse association between aspirin and breast cancer risk, but found significant dose- and duration-risk relations [23]. No effect of aspirin on breast cancer incidence or mortality was found in the WHS trial on low dose aspirin, as well as in the post-trial follow-up [20, 21].

Several studies have been published over the last few years on aspirin and endometrial cancer, and overall evidence from about 20 studies, on approximately 14 000 cases, suggested a modest (~ 10%) reduction in risk for regular aspirin use. Such an inverse relation was mainly reported in case-control studies and there was no indication of an inverse duration-risk relation. Two previous meta-analyses [24, 25] also found small and inconsistent inverse relations between aspirin and endometrial cancer risk.

With reference to ovarian cancer, 22 studies on about 18 000 cases indicated that regular aspirin use was associated with an overall 10% reduction of risk. Again, the evidence was largely driven from case-control studies, while cohort and nested case-control studies were scanty and did indicate a reduced risk. Moreover, the duration-risk analysis showed a reduced risk up to five years of aspirin use, which, however, levelled off for longer duration of use. In the WHS trial, a modest and nonsignificant reduction of ovarian cancer risk was reported for long-term use of alternate-day, low-dose aspirin use [20, 21].Thirty studies, on more than 100 000 cases, indicated that aspirin was associated to a small (about 7%) reduced risk of prostate cancer risk. Study-specific estimates were, however, significantly heterogeneous and there was no evidence of any meaningful association in cohort studies. The inverse association was somewhat stronger for highly aggressive cancers and for long-term use. Our findings further confirm those of a meta-analysis, which, however, did not evaluate long-term aspirin use [26]. In the pooled analysis of RCTs, there was a nonsignificant and late reduced risk of prostate cancer death [22].

Only a few studies reported an effect of regular aspirin on bladder cancer risk [27-29], and 15 studies on about 14 000 cases overall did not support an association. Moreover, there was no evidence of any duration-risk relation. Similarly, evidence from 17 studies on more than 21 000 cases indicated that regular aspirin use was not associated to the risk of kidney cancer. There was an overall nonsignificant linear increase for increasing duration of aspirin use. The absence of an association between aspirin use and bladder and kidney cancer supports the results of previous meta-analyses [30, 31]. These findings, however, allow to reassure against a possible increased risk, as reported for phenacetin, an analgesic substance which has been

banned in the USA since 1983 because of acute and chronic injuries at the urinary tract [32-34]. The apparent excess risk of kidney cancer reported in some studies [35-38] and in relation to long-term use may indeed be due to residual misclassification of exposure or mixed exposure with phenacetin-based analgesics. The WHS trial reported a nonsignificant protective effect of aspirin on kidney cancer and no meaningful association on bladder cancer [20], while the pooled analysis of RCTs reported a small nonsignificant increase in the risk of bladder and kidney cancer combined during the trial treatment period and a nonsignificant inverse association in the post-trial follow-up [22].

Among possible limitations of the present meta-analysis, there are inherent limitations of observational studies. Stronger inverse associations between regular aspirin use and cancer risk were found in case-control than cohort and nested case-control studies. This can be due to potential recall bias in case-control studies. However, a possible more careful reporting of aspirin use in cases than controls should have biased risk estimates towards the null. Cohort studies are considered less prone to recall or selection bias than case-control studies, although they often collect data only at baseline and lack information of exposure changes over time, with consequent possible misclassification of aspirin exposure. Potential misclassification of aspirin exposure is also possible in a few cohort or nested case-control studies based on prescription databases, due to less precise assessment of aspirin use and lack of information on over-the-counter use. However, we found consistent results after excluding studies conducted on prescription database. Among other limitations of our meta-analysis, there is the fact that for most neoplasms information was extremely scanty to assess the dose-risk relations. Observational studies - particularly of aspirin use - are subject to confounding, and

it is difficult to estimate how well each study has controlled for possible confounders. Further, aspirin non users might have used other types of nonsteroidal anti-inflammatory drugs, this causing a potential underestimated effect of aspirin. Moreover, we found significant between-study heterogeneity, likely due to different study populations, variable baseline risk of cancer, different prevalence of aspirin use and aspirin dose, as well as the high variability in the definition of "regular" use. In addition, in many cases we found evidence of publication bias, with many small studies reporting the strongest inverse associations.

Although the exact biological mechanisms of the chemo-preventive effect of aspirin are not fully understood, it has been mainly attributed to its inhibition of cyclooxygenase (COX), the enzymes responsible for the synthesis of prostaglandins. The COX-2 isoform has been found to be abnormally expressed in many cancer cell lines, and has been involved in the processes of carcinogenesis, tumor growth, apoptosis, and angiogenesis [1, 39-42]. Additional of COX-independent mechanisms has been proposed, including the inhibition of the nuclear factor-kappaβ, the PIK3CA pathway, the upregulation of tumor suppression genes, as well as the involvement of platelets, and the inhibition of other platelet-derived growth factors [41-43].

In conclusion, the present meta-analysis – including a uniquely large number of epidemiological studies – confirms the absence of any effect of regular aspirin use on neoplasms of the bladder and kidney and shows modest inverse associations for cancers of the lung, breast, endometrium, ovary, and prostate. However, for most neoplasms nonsignificant risk reductions were reported in cohort and nested case-control studies, and there was significant between-study heterogeneity. Therefore, further results from primary prevention RCTs are necessary to confirm a protective role of aspirin on non-digestive tract

neoplasms [5]. In addition, the issue of risk-benefit should be considered, given the excess risk of bleeding in aspirin users [6], together with the protection of thrombotic events and likely reduction of colorectal and other digestive tract neoplasms.

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Data availability statement

Data available on request from the authors.

Conflict of interest

All authors declare that they have no conflict of interest to disclose.

REFERENCES

1. Patrignani P, Patrono C. Aspirin and Cancer. *J Am Coll Cardiol* 2016;**68**: 967-76.

2. Bosetti C, Rosato V, Gallus S, Cuzick J, La Vecchia C. Aspirin and cancer risk: a quantitative review to 2011. *Ann Oncol* 2012;**23**: 1403-15.

3. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol* 2012;**13**: 518-27.

4. Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni MC, Tognoni G, Lee R, Belch JF, Wilson M, Mehta Z, Meade TW. 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.

5. Brotons C, Benamouzig R, Filipiak KJ, Limmroth V, Borghi C. A Systematic Review of Aspirin in Primary Prevention: Is It Time for a New Approach? *American Journal of Cardiovascular Drugs* 2014.

6. Cuzick J, Thorat MA, Bosetti C, Brown PH, Burn J, Cook NR, Ford LG, Jacobs EJ, Jankowski JA, La Vecchia C, Law M, Meyskens F, et al. Estimates of benefits and harms of prophylactic use of aspirin in the general population. *Ann Oncol* 2015;**26**: 47-57.

7. Qiao Y, Yang T, Gan Y, Li W, Wang C, Gong Y, Lu Z. Associations between aspirin use and the risk of cancers: a meta-analysis of observational studies. *BMC Cancer* 2018;**18**: 288.

8. Bosetti C, Santucci C, Gallus S, Martinetti M, La Vecchia C. Aspirin and the risk of colorectal and other digestive tract cancers: an updated meta-analysis to 2019. *Ann Oncol* 2020; 31(5):558-568.

9. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;**7**: 177-88.

10. Cochrane. *Handbook for Systematic Reviews of Interventions (Version 6 September 2018). Available from: https://training.cochrane.org/handbook*
2018.

11. Hamling J, Lee P, Weitkunat R, Ambuhl M. Facilitating meta-analyses by deriving relative effect and precision estimates for alternative comparisons from a set of estimates presented by exposure level or disease category. *Stat Med* 2008;**27**: 954-70.

12. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;**21**: 1539-58.

13. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med* 2010;**29**: 1037-57.

14. Orsini N, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. *Am J Epidemiol* 2012;**175**: 66-73.

15. Lugo A, Peveri G, Bosetti C, Bagnardi V, Crippa A, Orsini N, Rota M, Gallus S. Strong excess risk of pancreatic cancer for low frequency and duration of cigarette smoking: A comprehensive review and meta-analysis. *Eur J Cancer* 2018;**104**: 117-26.

16. Crippa A, Discacciati A, Bottai M, Spiegelman D, Orsini N. One-stage dose-response meta-analysis for aggregated data. *Stat Methods Med Res* 2019;**28**: 1579-96.

17. Webb PM, Na R, Weiderpass E, Adami HO, Anderson KE, Bertrand KA, Botteri E, Brasky TM, Brinton LA, Chen C, Doherty JA, Lu L, et al. Use of aspirin, other nonsteroidal

anti-inflammatory drugs and acetaminophen and risk of endometrial cancer: the Epidemiology of Endometrial Cancer Consortium. *Ann Oncol* 2019;**30**: 310-6.

18. Trabert B, Ness RB, Lo-Ciganic WH, Murphy MA, Goode EL, Poole EM, Brinton LA, Webb PM, Nagle CM, Jordan SJ, Risch HA, Rossing MA, et al. Aspirin, nonaspirin nonsteroidal anti-inflammatory drug, and acetaminophen use and risk of invasive epithelial ovarian cancer: a pooled analysis in the Ovarian Cancer Association Consortium. *J Natl Cancer Inst* 2014;**106**: djt431.

19. Jiang HY, Huang TB, Xu L, Yu J, Wu Y, Geng J, Yao XD. Aspirin use and lung cancer risk: a possible relationship? Evidence from an updated meta-analysis. *PLoS One* 2015;**10**: e0122962.

20. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. *JAMA* 2005;**294**: 47-55.

21. Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. *Ann Intern Med* 2013;**159**: 77-85.

22. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet* 2011;**377**: 31-41.

23. Lu L, Shi L, Zeng J, Wen Z. Aspirin as a potential modality for the chemoprevention of breast cancer: A dose-response meta-analysis of cohort studies from 857,831 participants. *Oncotarget* 2017;**8**: 40389-401.

24. Zhang D, Bai B, Xi Y, Zhao Y. Can Aspirin Reduce the Risk of Endometrial Cancer?: A Systematic Review and Meta-analysis of Observational Studies. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society* 2016;**26**: 1111-20.

25. Verdoodt F, Friis S, Dehlendorff C, Albieri V, Kjaer SK. Non-steroidal anti-inflammatory drug use and risk of endometrial cancer: A systematic review and meta-analysis of observational studies. *Gynecologic oncology* 2016;**140**: 352-8.

26. Shang Z, Wang X, Yan H, Cui B, Wang Q, Wu J, Cui X, Li J, Ou T, Yang K. Intake of Non-steroidal Anti-inflammatory Drugs and the Risk of Prostate Cancer: A Meta-Analysis. *Frontiers in oncology* 2018;**8**: 437.

27. Castelao JE, Yuan JM, Gago-Dominguez M, Yu MC, Ross RK. Non-steroidal anti-inflammatory drugs and bladder cancer prevention. *Br J Cancer* 2000;**82**: 1364-9.

28. Jacobs EJ, Thun MJ, Bain EB, Rodriguez C, Henley SJ, Calle EE. A large cohort study of long-term daily use of adult-strength aspirin and cancer incidence. *J Natl Cancer Inst* 2007;**99**: 608-15.

29. Fortuny J, Kogevinas M, Zens MS, Schned A, Andrew AS, Heaney J, Kelsey KT, Karagas MR. Analgesic and anti-inflammatory drug use and risk of bladder cancer: a population based case control study. *BMC Urol* 2007;**7**: 13.

30. Zhang H, Jiang D, Li X. Use of nonsteroidal anti-inflammatory drugs and bladder cancer risk: a meta-analysis of epidemiologic studies. *PLoS One* 2013;**8**: e70008.

31. Choueiri TK, Je Y, Cho E. Analgesic use and the risk of kidney cancer: a meta-analysis of epidemiologic studies. *Int J Cancer* 2014;**134**: 384-96.

32. McCredie M, Stewart JH, Ford JM, MacLennan RA. Phenacetin-containing analgesics and cancer of the bladder or renal pelvis in women. *Br J Urol* 1983;**55**: 220-4.

33. Piper JM, Tonascia J, Matanoski GM. Heavy phenacetin use and bladder cancer in women aged 20 to 49 years. *N Engl J Med* 1985;**313**: 292-5.

34. McCredie M, Stewart JH, Day NE. Different roles for phenacetin and paracetamol in cancer of the kidney and renal pelvis. *Int J Cancer* 1993;**53**: 245-9.

35. Paganini-Hill A, Chao A, Ross RK, Henderson BE. Aspirin use and chronic diseases: a cohort study of the elderly. *BMJ* 1989;**299**: 1247-50.

36. Gago-Dominguez M, Yuan JM, Castelao JE, Ross RK, Yu MC. Regular use of analgesics is a risk factor for renal cell carcinoma. *Br J Cancer* 1999;**81**: 542-8.

37. Ratnasinghe LD, Graubard BI, Kahle L, Tangrea JA, Taylor PR, Hawk E. Aspirin use and mortality from cancer in a prospective cohort study. *Anticancer Res* 2004;**24**: 3177-84.

38. Kang M, Ku JH, Kwak C, Kim HH, Jeong CW. Effects of Aspirin, Nonsteroidal Anti-inflammatory Drugs, Statin, and COX2 Inhibitor on the Developments of Urological Malignancies: A Population-Based Study with 10-Year Follow-up Data in Korea. *Cancer research and treatment : official journal of Korean Cancer Association* 2018;**50**: 984-91.

39. Taketo MM. Cyclooxygenase-2 inhibitors in tumorigenesis (part I). *J Natl Cancer Inst* 1998;**90**: 1529-36.

40. Taketo MM. Cyclooxygenase-2 inhibitors in tumorigenesis (Part II). *J Natl Cancer Inst* 1998;**90**: 1609-20.

41. Elwood PC, Gallagher AM, Duthie GG, Mur LA, Morgan G. Aspirin, salicylates, and cancer. *Lancet* 2009;**373**: 1301-9.

42. Langley RE, Burdett S, Tierney JF, Cafferty F, Parmar MK, Venning G. Aspirin and cancer: has aspirin been overlooked as an adjuvant therapy? *Br J Cancer* 2011;**105**: 1107-13.

43. Haemmerle M, Stone RL, Menter DG, Afshar-Kharghan V, Sood AK. The Platelet Lifeline to Cancer: Challenges and Opportunities. *Cancer cell* 2018;**33**: 965-83.

FIGURE LEGENDS

Figure 1. Forest plot of study-specific and pooled relative risk (RR) of lung cancer, and corresponding 95% confidence interval (CI), for regular aspirin use versus non-use, overall and by study design.

Figure 2. Forest plot of study-specific and pooled relative risk (RR) of breast cancer, and corresponding 95% confidence interval (CI), for regular aspirin use versus non-use, overall and by study design.

Footnote: L: low-dose aspirin; R: regular dose aspirin.

Figure 3. Forest plot of study-specific and pooled relative risk (RR) of endometrial cancer, and corresponding 95% confidence interval (CI), for regular aspirin use versus non-use, overall and by study design.

Footnote: L: low-dose aspirin; H: high-dose aspirin; BCDDP: Breast Cancer Detection Demonstration Project; BWHS: Black Women's Health; CONN: Connecticut Endometrial Cancer Study; SWLHS: Swedish Women's Lifestyle and Health Study; PLCO: Prostate, Lung, Colorectal, and Ovarian cancer screening trial.

Figure 4. Forest plot of study-specific and pooled relative risk (RR) of ovarian cancer, and corresponding 95% confidence interval (CI), for regular aspirin use versus non-use, overall and by study design.

Footnote: CON: Connecticut Ovarian Cancer Study; MAL: Malignant Ovarian Cancer Study; NJO: New Jersey Ovarian Cancer Study; UCI: University of California, Irvine Ovarian Cancer Study; UKO: United Kingdom Ovarian Cancer Population Study.

Figure 5. Forest plot of study-specific and pooled relative risk (RR) of prostate cancer, and corresponding 95% confidence interval (CI), for regular aspirin use versus non-use, overall and by study design.

Footnote: L: low-dose aspirin; R: regular dose aspirin.

Figure 6. Forest plot of study-specific and pooled relative risk (RR) of bladder cancer, and corresponding 95% confidence interval (CI), for regular aspirin use versus non-use, overall and by study design.

Figure 7. Forest plot of study-specific and pooled relative risk (RR) of kidney cancer, and corresponding 95% confidence interval (CI), for regular aspirin use versus non-use, overall and by study design.

Footnote: PLCO: Prostate, Lung, Colorectal, and Ovarian cancer screening trial.

Table 1. Pooled relative risks (RR) and corresponding 95% confidence intervals (CI) for regular aspirin use versus non-use by cancer site, and study design.

Cancer site, study design	N. of studies	N. of cases	Pooled RR (95% CI)	*P*-value for between-study heterogeneity	I^2 (%)	*P*-value for heterogeneity across study design
Lung						
Cohort	11	93 768	0.95 (0.82-1.09)	<0.001	96	
Nested case-control	4	4 543	0.93 (0.72-1.22)	0.148	44	0.163
Case-control	6	8 503	0.73 (0.58-0.92)	0.002	73	
Overall	21	106 814	0.88 (0.79-0.98)	<0.001	93	
Breast						
Cohort	19	28 769	0.96 (0.91-1.02)	<0.001	72	
Nested case-control	3	12 145	0.85 (0.73-0.99)	0.037	70	0.002
Case-control	11	14 417	0.77 (0.69-0.87)	0.028	50	
Overall	33	55 331	0.90 (0.85-0.95)	<0.001	74	
Endometrium						
Cohort	7	2 522	0.93 (0.85-1.02)	0.452	0	
Nested case-control	6	8 054	0.98 (0.91-1.05)	0.590	0	0.074
Case-control	6	3 336	0.77 (0.64-0.93)	0.147	39	
Overall	19	13 912	0.91 (0.84-0.98)	0.126	27	
Ovary						
Cohort	2	3 357	0.95 (0.88-1.02)	0.697	0	
Nested case-control	2	4 171	0.92 (0.75-1.12)	0.296	9	0.543
Case-control	18	10 875	0.88 (0.80-0.97)	0.079	34	
Overall	22	18 403	0.91 (0.85-0.97)	0.154	24	
Prostate						
Cohort	14	38 854	0.97 (0.93-1.01)	0.078	36	
Nested case-control	6	62 428	0.88 (0.81-0.95)	<0.001	85	0.043
Case-control	10	6 239	0.88 (0.77-1.00)	0.031	51	
Overall	30	107 521	0.93 (0.89-0.96)	<0.001	63	

Bladder						
Cohort	9	10 226	1.04 (0.99-1.10)	0.986	0	
Case-control	6	3 882	0.99 (0.84-1.16)	0.039	57	0.572
Overall	15	14 108	1.03 (0.99-1.08)	0.465	0	
Kidney						
Cohort	9	4 660	1.05 (0.91-1.22)	0.016	58	
Nested case-control	1	10 377	0.93 (0.88-0.98)	-	-	0.036
Case-control	7	5 666	1.11 (0.96-1.29)	0.065	49	
Overall	17	20 703	1.06 (0.96-1.16)	<0.001	63