BMJ/2011/892646
Cancer risk with folic acid supplements: a systematic review and meta-analysis
Tale Norbye Wien, Eva Pike, Torbjørn Wisløff, Annetine Staff, Sigbjørn Smeland, and Marianne Klemp

Decision: Reject From This Journal; **Decision Date:** 4 Jul 2011
Date Received: 17 Jun 2011
Editor: Wim Weber
Article Types: Research
Corresponding Author: Marianne Klemp
Keywords: None
Supplemental Files: 2

Dear Editor and Reviewers:

Thank you both for your comments. We have answered in green under each paragraph, as well as of course made the necessary corrections in the manuscript, tables and figures. We want to make you aware that the original page numbers (mentioned by the reviewers in their comments) not longer apply to the corrected manuscript.

Reviewer 1 Comments...

Name: Pagona Lagiou
Position: Associate Professor of Hygiene and Epidemiology, University of Athens Medical School; Adjunct Professor of Epidemiology, Harvard School of Public Health

What has apparently triggered this systematic review and metaanalysis is a paper published in JAMA in 2009 (ref 11), the authors of which combined the extended post-trial follow-up time results of two Norwegian randomized controlled clinical trials (RCTs) (ref 12 and 13) and reported an increased incidence of cancer among patients taking folic acid to reduce homocysteine levels for secondary prevention of cardiovascular events.

The authors of the present systematic review and metaanalysis did an excellent analytical work, but they have been facing a daunting task: the paper has to focus on folic acid supplementation (and in a certain dose range). Studies on folic acid fortification of foods could not be included and folate in dietary sources, abundant in vegetables, fruits and grains (all these food groups reported to be inversely associated with cancer risk), could not be accounted for. The authors distinguish between the synthetic folic acid used as supplement, and folate found in dietary sources, even though folic acid in the human body is converted to 5-methyltetrahydrofolate, which is the form found in dietary sources. Thus, interpretations are unavoidably clouded.

Another issue is that studies were included if folic acid supplements were taken at daily doses of 0.4 mg or higher (lower dose interventions were not

considered). However, increased cancer risk was found in studies with a dose from 0.4 to 1 mg per day and not in studies in which folic acid doses exceeded 1 mg per day, thus raising concerns about whether dose-response patterns were adequately captured across the range of exposures.

Answer:
In our sensitivity analyses of the RCTs, the studies with dose interval 0.4-1 mg/day were associated with a higher risk of cancer development than studies with doses above 1 mg/day, with relative risks of 1.21 (1.06-1.38) and 1.03 (0.96-1.11) respectively. Our systematic search had no dose limitation and thus allowed us to search for all dose ranges, although our eligibility criteria specified doses ≥ 0.4 mg/day. We identified no RCTs, but eleven observational studies with doses below 0.4 mg/day reporting cancer incidence, thus allowing us post hoc to evaluate a possible dose-response pattern of observational studies including doses below 0.4 mg/day. Six of these studies were already included in our review, as they contained also groups with daily intakes ≥ 0.4 mg[1-6], and five were excluded from our main analyses due to daily dose of folic acid below 0.4 mg [7-11]. No increased risk was found in the meta-analyses of doses below 0.4 mg/day, with a pooled relative risk of 1.07; 95% CI 0.92-1.26 for the cohort studies [2-9] and 1.17 (0.95% CI 0.58-2.33) for the case- control studies[1;8]. Hence, our analyses did not support any dose-response pattern between folic acid dose and cancer incidence.

As you see we take care of this dose-response question in the revised Discussion (page 17-18). This is because we want to keep to our protocol that had folic acid supplement ≥0.4 mg/day as an eligibility criterion.

A statistically significant increase in incidence was found essentially with respect to prostate cancer, for which no significant increase was found with respect to mortality. However, excess incidence was evident only after 60 months of follow-up, that is to say most likely during the post-trial period, that is, a period which is not "bias-immune" (e.g. PSA testing can bypass the issue of latency).

Answer: Our meta-analysis of prostate cancer incidence consists of six RCTs. The studies had a follow-up time from 52-84 months. All the studies had the same treatment time as follow up-time, except Ebbing 2009 (cfr Table 1. (Figueiredo is a study that studied prostate cancer occurrence in the Aspirin Folate Polyp Prevention Study, cfr Cole in table 1)).

Other minor issues:

Abstract and Results: Twelve RCTs are indicated, but in Table 1, 11 are listed. Please edit.
Answer: Please see footnote to Table 1. Ebbing 2009 is a combined analysis of two RCTs. This means 12 RCTs.

Abstract, Results, line 7: Consider replacing "most prevalent" with "most

common" to avoid confusion between incidence and prevalence.
Answer: We want to keep to prevalence since this is what we mean. We included 6 studies on prostate cancer, with 349 cancers from a population of 10256, ie a prevalence of 0.034 (in the folic acid group); correspondingly nine studies of colon and rectum cancer with 210 cancers from a population of 16291, ie a prevalence of 0.013.

What the study adds: I understand what the authors wish to convey, but the statements "moderately increase cancer incidence" and "seems safe in relation to cancer risk" appear contradictory. Consider editing.

Answer: Please see revised text were we have changed moderately to borderline. Furthermore we have included the conclusion from the observational studies showing no difference between the studied groups.

Introduction, line 8, "such as neural tube defects": it appears as if you also have other outcomes in mind. If so, please list.
Answer: OK, This has been corrected.

Introduction, paragraph 3, line 1: Although the masking effect is well-known, you still need a reference.
Answer: Yes, we have include a reference.

Methods: References 15 and later on 17 are in Norwegian. This is an editorial issue, however.

Eligibility criteria, "tablets or mixtures": Please define mixtures as contrasted to tablets.
Answer: We have rephrased in the revised manuscript.

Methodological quality, line three from the end "the very low number of events as well as broad confidence intervals": These are not distinct issues; the one leads to the other.
Answer: We have rephrased this part in our revised manuscript.

Cancer mortality, paragraph 1, last line: Do you mean "any group"?
Answer: Yes.

Discussion line 3: Mortality was not significantly increased. Please edit.
Answer: We have edited.

Discussion line 6: Consider replacing "negative effect" with "adverse effect".
Answer: This has been done in the revised manuscript.

Comparison with other studies, paragraph 2: The argument here is not strictly appropriate. Different compounds have distinct biomedical implications.
Answer: We have removed this paragraph.

Page 14 lines 3-4 vs lines 21-22: The statements are somewhat contradictory. In the first set of lines, low folate intake is reported to be protective against early carcinogenesis. In the second set of lines, lower folic acid intakes are associated with higher risk of cancer development.
Answer: Agree, we have corrected this in the revised manuscript.

Page 14, lines 23-24: Invocation of confounding is a weak argument, since most of these studies were RCTs.
Answer: Agree, we have rephrased in our revised manuscript.

Page 15, last paragraph: The study in Scottish women (ref 21) is cited as indicating a non-significant increase in the risk for breast cancer at levels of folic acid intake of 5g per day, as contrasted to the currently recommended dose of 0.4 g per day. There are two issues here: Is it 0.4g or 0.4 mg? and Since you seem to pay attention to the apparent increase in breast cancer incidence, your statement on page 18 that there is no elevated risk (as contrasted to significantly elevated risk) for breast cancer, appears somewhat at odds.
Answer: First it should be 0,4 mg. Secondly, sorry, but here we have not been concise.
On page 15 we mean incidence of total cancer mortality, which was significantly increased in Charles et al for the highest dose of 5 mg. However, this significance disappeared in the meta-analysis for total cancer mortality (6 RCTs).

Figure 1: You may wish to use a more appropriate expression than "Wrong intervention" ("ineligible intervention"?).
Answer: Done, and thank you for the suggestion.

Figure 2 c): Please clarify that you refer to colorectal adenomas.
Answer:Done.

Reviewer 2 Comments...
Name: Arduino A Mangoni
Position: Professor of Medicine of Old Age, University of Aberdeen

GENERAL COMMENTS

This work provides a useful update on the important issue of long-term safety of oral folic acid supplementation in relation to cancer risk in different cohorts

(not just pts with high cardiovascular risk). It would be of interest to the journal readership. That said, I find the data interpretation and reporting (results and discussion) quite biased towards the very limited, and of doubtful clinical significance, evidence of increased cancer incidence and mortality. Negative findings are downplayed throughout the manuscript. Even more disappointingly perhaps is the underreporting of results from observational studies. This is of concern because such studies provide valuable information, as they are not confined to strict RCTs criteria, and complement randomised studies.

Answer: We agree. We have rephrased our interpretation of the data throughout the manuscript, changing moderate to borderline and including the results from the observational studies in a much more detailed manner, as well as showing negative results more clearly.

It should be further stressed that an important limitation of the meta-analysis is the lack of information on dietary patterns in the studied populations. Significant differences in folate content among different food types (and food processing) might have influenced the reported findings.

Answer: Yes we agree to this possibility, but nevertheless this lacking information in the clinical studies might be randomly distributed across the study populations.

A better justification of why studies on food fortification were not considered is needed in both introduction and discussion. The results of RCTs/observational studies in Canada and USA are likely to be influenced by the introduction of mandatory food fortification, particularly when this occurred during the conduct of such studies. Cancer incidence and mortality should be compared between those studies in countries where mandatory folate fortification was implemented from those studies where folate fortification was not introduced.

Answer: Two of the RCTs[12;13] were done in USA after introduction of fortification. Sensitivity analysis of those two compared to the studies performed in the countries without fortification, showed no significant difference between the groups (Figure 2c); i.e populations with fortification did not show increased cancer risk with folate supplements compared to studies performed in countries before fortification/ countries without fortification.

SPECIFIC COMMENTS

Abstract_results_a: 95% CI cancer incidence data for 1-2 relevant cancer types other than prostate (e.g. colon and breast) are required. Negative data, in addition to positive associations, should be reported.

Answer: This has been performed in the revised manuscript.

Abstract_results_b: for the same reason 95% CI total cancer mortality data

should be reported.
Answer: This has been performed in the revised manuscript.

Abstract_results_c: once again, 95% CI data for cancer risk in observational studies must be reported.
Answer: This has been reported in the results chapter.

Abstract_conclusions_a: 'FA might moderately increase total cancer incidence' is clearly an overstatement. With an RR of 1.07 and CI of 1.00-1.14 the risk is very mild at best. Also, the conclusions should reflect the findings (negative) from observational studies.
Answer: we have rephrased and added findings from observational studies (please see our answer under General Comments above).

Abstract_conclusions_b: the conclusions on prostate cancer should also reflect the negative findings of observational studies.
Answer: There was no observational studies reporting on prostate cancer in our included studies. However one of the excluded studies (Shannon 2009) (excluded due to daily folic acid dose <0.4 mg) has now been analyzed and commented on in the discussion part. This study showed no significant difference between the groups. (Please see our first answer to Reviewer 1 for more details).

Abstract_conclusions_c: the rest of the para (starting with 'A major limitation....") can be removed as it's not relevant.
Answer: Done.

What this study adds: once again, the first bullet point ('moderately increase') is an overstatement.
Answer: We agree and this has been updated in the revised version.

Introduction_a: data on prevalence of folate deficiency would be useful.
Answer: We have rephrased this paragraph.

Results_a: a full list of observational studies considered should also be provided in Table format.
Answer: Done, please see table 2a and 2b.

Results_b: were there any differences in cancer incidence and mortality in studies with folic acid alone vs. combination with other B-vitamins?
Answer: No difference, please see new text in results part.

Results_c: ASA is thought to be protective. Were the results of studies on folate + acetylsalicylic acid any different?

Answer: Two of the studies administered folic acid together with aspirin[14;15]. A sensitivity analysis of those studies showed a borderline significant increased risk in the folic acid groups compared to the control groups without aspirin (RR 1.43, 95% CI 1.00-2.03).

Results_d: full results from observational studies should be presented in the text and Table/Figure format. This should include data on cancer incidence as well as cancer mortality.
Answer: Done. However, none of the observational studies reported on mortality.

Discussion_a: the first para is very biased towards the very limited positive findings of an association between folic acid and cancer. You cannot state that folate may increase mortality, when no such a link is reported in the results (!). As previously discussed data interpretation should be much more balanced and include the lack of association with the vast majority of cancer types + negative findings from observational studies.
Answer: We have corrected this in the revised manuscript.

Discussion_b: similar (major) revision is needed for the para "Our finding of a possible adverse effect from folic acid....
Answer: This paragraph has been deleted.

Discussion_c: the first 2 sentences (page 14, "folate may play a dual role...") don't make any sense and should be revised.
Answer: This paragraph has been deleted.

Discussion_d: need also to explain why no associations were found for cancers other than prostate (and the potential biological mechanisms for the lack of such associations)
Answer: We have tried to explain this paragraph better in the updated version of the discussion.

Conclusions and policy implications: as previously discussed the first sentence does not reflect the findings of the study. Emphasis on negative findings is also needed.
Answer: This has been corrected in the revised manuscript.

Reference List

(1) Freudenheim JL, Marshall JR, Vena JE, Laughlin R, Brasure JR, Swanson MK et al. Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. *J Natl Cancer Inst* 1996; 88(6):340-348.

(2) Maruti SS, Ulrich CM, White E. Folate and one-carbon metabolism nutrients from supplements and diet in relation to breast cancer risk. *Am J Clin Nutr* 2009; 89(2):624-633.

(3) Oaks BM, Dodd KW, Meinhold CL, Jiao L, Church TR, Stolzenberg-Solomon RZ. Folate intake, post-folic acid grain fortification, and pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *The American journal of clinical nutrition* 2010; 91(2):449-455.

(4) Stolzenberg-Solomon RZ, Chang S-C, Leitzmann MF, Johnson KA, Johnson C, Buys SS et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr* 2006; 83(4):895-904.

(5) Slatore CG, Littman AJ, Au DH, Satia JA, White E. Long-term use of supplemental multivitamins, vitamin C, vitamin E, and folate does not reduce the risk of lung cancer. *Am J Respir Crit Care Med* 2008; 177(5):524-530.

(6) Skinner HG, Michaud DS, Giovannucci EL, Rimm EB, Stampfer MJ, Willett WC et al. A prospective study of folate intake and the risk of pancreatic cancer in men and women. *Am J Epidemiol* 2004; 160(3):248-258.

(7) Tjønneland A, Christensen J, Olsen A, Stripp C, Nissen SB, Overvad K et al. Folate intake, alcohol and risk of breast cancer among postmenopausal women in Denmark. *Eur J Clin Nutr* 2006; 60(2):280-286.

(8) Shannon J, Phoutrides E, Palma A, Farris P, Peters L, Forester A et al. Folate intake and prostate cancer risk: a case-control study. *Nutr Cancer* 2009; 61(5):617-628.

(9) Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Micronutrient Intake and Risk of Urothelial Carcinoma in a Prospective Danish Cohort. *Eur Urol* 2009; 56(5):764-770.

(10) Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Source-specific effects of micronutrients in lung cancer prevention. *Lung Cancer* 2010; 67(3):275-281.

(11) Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Micronutrient intake and risk of colon and rectal cancer in a Danish cohort. *Cancer Epidemiology* 2010; 34(1):40-46.

(12) Jamison RL, Hartigan P, Kaufman JS, Goldfarb DS, Warren SR, Guarino PD et al. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* 2007; 298(10):1163-1170.

(13) Zhang SM, Cook NR, Albert CM, Gaziano JM, Buring JE, Manson JE. Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women: a randomized trial. *JAMA : the journal of the American Medical Association* 2008; 300(17):2012-2021.

(14) Logan RFA, Grainge MJ, Shepherd VC, Armitage NC, Muir KR. Aspirin and Folic Acid for the Prevention of Recurrent Colorectal Adenomas. *Gastroenterology* 2008; 134(1):29-38.

(15) Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA : the journal of the American Medical Association* 2007; 297(21):2351-2359.