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Original Research Article

Intake and biomarkers of folate and folic acid as determinants of chemotherapy-induced toxicities in patients with colorectal cancer: a cohort study

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A B S T R A C T

Background: Capecitabine is an oral chemotherapeutic drug showing antitumor activity through inhibition of thymidylate synthase, an enzyme involved in folate metabolism. There are concerns about the high intake of certain vitamins, and specifically folate, during chemotherapy with capecitabine. Whether folate or folic acid, the synthetic variant of the vitamin, impact treatment toxicity remains unclear.

Objective: We studied associations between intake and biomarkers of folate as well as folic acid and toxicities in patients with colorectal cancer (CRC) receiving capecitabine.

Methods: Within the prospective COLON (Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that influence recurrence, survival, and quality of life) cohort, 290 patients with stage II to III CRC receiving capecitabine were identified. Dietary and supplemental intake of folate and folic acid were assessed at diagnosis and during chemotherapy using questionnaires (available for 280 patients). Plasma folate and folic acid levels were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) and were available for 212 patients. Toxicities were defined as toxicity-related modifications of treatment, including dose reductions, regimen switches, and early discontinuation. Associations of intake and biomarkers of folate and folic acid with toxicities were determined using Cox proportional hazards regression adjusted for age and sex.

Results: In total, 153 (53%) patients experienced toxicities leading to modification of capecitabine treatment. Folate intake and plasma folate levels were not associated with risk of toxicities. However, use of folic acid-containing supplements during treatment (hazard ratio (HR) 1.81 and 95% confidence interval (CI) 1.15-2.85) and presence of folic acid in plasma at diagnosis (HR 2.09, 95% CI: 1.24, 3.52) and during treatment (HR 2.31, 95% CI: 1.29, 4.13) were associated with an increased risk of toxicities.

Conclusions: This study suggests a potential association between folic acid and capecitabine-induced toxicities, providing a rationale to study diet-drug interactions and raise further awareness of the use of dietary supplements during oncological treatment.

Clinical trial details: This trial was registered at clinicaltrials.gov as NCT03191110.

Keywords: folate, folic acid, dietary supplements, diet, biomarker, colorectal cancer, chemotherapy, toxicity, capecitabine

Abbreviations: 5-FU, 5-fluorouracil; 5mTHF, 5-methyl-tetrahydrofolate; 95% CI, 95% confidence interval; ASA, American Society of Anesthesiologists; BMI, body mass index; CAP, capecitabine; CAPOX, capecitabine and oxaliplatin; COLON, COlorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that influence colorectal tumor recurrence, survival and quality of life; CRC, colorectal cancer; FOLFOX, 5-fluorouracil, leucovorin, oxaliplatin; DCRA, Dutch colorectal audit; DFE, dietary folate equivalents; DHFR, dihydrofolate reductase; HR, hazard ratio; IQR, interquartile range; LC-MS/MS, liquid chromatography tandem mass spectrometry; MeFox, pyrazino-s-triazine derivative of 4-alpha-hydroxy-5-methyl-tetrahydrofolate; RDI, relative dose intensity; TS, thymidylate synthase.

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Introduction

The B-vitamin folate plays a central role in the mode of action of several anticancer drugs, including fluoropyrimidines such as capecitabine and 5-fluorouracil (5-FU). Capecitabine is an oral chemotherapeutic agent for colorectal cancer (CRC) [1, 2]. After conversion to 5-FU, capecitabine inhibits the enzyme thymidylate synthase (TS) [3, 4]. Inhibition of TS results in disturbed nucleotide pools and consequently impairs DNA synthesis and repair, causing antitumor effects [5]. Reduced folate species can contribute to the stability of a ternary complex formed with TS and active fluoropyrimidine drug metabolites, which could theoretically enhance the efficacy but also toxicity and, thus, side effects of treatment [3, 5, 6].

The toxicity profile of capecitabine is well-characterized, with hand-foot syndrome and diarrhea being common toxicities [1, 7, 8]. However, determinants of capecitabine-induced toxicities remain poorly understood [1]. Given the mode of action of capecitabine and the central role of folate metabolism therein, it is hypothesized that folate intake and status are potential determinants of toxicity [1, 9]. This hypothesis is substantiated by clinical applications of the folate vitamer leucovorin (5-formyltetrahydrofolate or folinic acid) to increase the efficacy of 5-FU due to enhanced binding of the drug with TS [10, 11]. Coadministration of leucovorin is not recommended for capecitabine, as this has been associated with excessive toxicity [12, 13].

Some evidence suggests that a higher folate status is associated with an increased risk of toxicities of capecitabine [14–16]. Most studies conducted so far were restricted to relatively small (N=38-150) or heterogenous populations or did not consider potential confounding factors, such as age, sex, or capecitabine dose. Moreover, it remains unclear whether associations might differ for natural folate versus its synthetic form, folic acid. Folate naturally occurs in foods such as green leafy vegetables, whereas folic acid, which is more stable and has a higher bioavailability, is used in dietary supplements and fortified foods [17, 18]. We have previously shown that higher circulating levels of folic acid, but not folate, at the time of diagnosis were associated with an increased risk of CRC recurrence [19].

Further disentangling dietary and supplemental exposures to folate is important since dietary supplement use is common among individuals with CRC [20–22], and a substantial part of the worldwide population is exposed to folic acid through fortification programs [23]. Here, we studied intake and biomarkers of folate and folic acid in relation to chemotherapy-induced toxicities in patients with stage II to III CRC undergoing treatment with capecitabine.

Methods

Patients were recruited through the prospective "Colorectal Longitudinal, Observational study on Nutritional and lifestyle factors" (COLON) study, which started in 2010 [24]. Design and patient recruitment have been described before [24]. Briefly, adult patients with any CRC cancer stage were recruited between August 2010 and August 2018 from 11 hospitals in the Netherlands. Patients were not included if they were nonDutch speaking, had a history of CRC or (partial) bowel resection, inflammatory bowel disease, hereditary CRC syndromes, or a mental condition affecting abilities to complete questionnaires.

From the COLON study, we identified those patients with stage II-III CRC who underwent adjuvant chemotherapy and for whom toxicity data were available at the time of analyses (N=309). Patients who received regimens without capecitabine (N=8) were excluded. Also, patients with missing data on dietary or supplemental intake and circulating folate and folic acid (N=6) were excluded. Finally, patients for whom the toxicity status was undefined (N=5) were excluded, resulting in a study population of 290 patients (Figure 1). As the patients were not recruited as part of a clinical trial but via a hospital-based cohort, prescribed doses of capecitabine might have varied, but generally aligned with national treatment protocols (capecitabine: 800–1000 mg/m^2, administered twice daily for 14 d followed by a 7-d rest period)[25, 26]. All patients provided written informed consent. The COLON study was approved by the Committee on Research involving Human Subjects, Arnhem-Nijmegen, the Netherlands and was registered at clinicaltrials.gov (NCT03191110).

Dietary and supplemental intake

Habitual dietary intake, reflecting intake 1 mo before diagnosis, was assessed through a validated 204-item food frequency questionnaire completed shortly after diagnosis and before start of treatment [27]. The same questionnaire was also completed 6 mo after diagnosis, reflecting intake (in the past month) during chemotherapy. Dietary folate intake (of natural folate and folic acid, in μg dietary folate equivalents (DFE) per d) was calculated based on the frequency of intake, number of portions and standard portion sizes of the relevant items using data from the 2011 Dutch food composition table [28]. Supplemental intake of folic acid was assessed through a questionnaire on dietary supplements developed at Wageningen University [24]. This questionnaire was also completed shortly after diagnosis (reflecting use in the past 12 mo) and 6 mo after diagnosis (reflecting use in the past 6 mo, i.e., during chemotherapy). Use of folic acid supplements, as well as folic acid-containing multivitamins, were considered. For multivitamins, reported brand names were checked to verify whether the supplement contained folic acid.

Biomarkers

Nonfasted blood samples were collected in EDTA tubes at diagnosis and 6 mo after diagnosis. One patient with samples collected after start of chemotherapy and one patient with the second sample collected before start of chemotherapy were excluded from biomarker analyses. Patients who switched to FOLFOX (N=6) were also excluded from these analyses. Plasma was stored at -80 °C until analyses at the Laboratory of BEVITAL, Bergen, Norway (www.bevital.no) were performed as part of the FOCUS consortium [29]. Folate status was determined as the sum of 5-methyl-tetrahydrofolate (5mTHF) and its degradation product MeFox [30, 31] measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described previously [19, 32]. Levels of folic acid were also determined using LC-MS/MS [32]. In the same samples, levels of creatinine were assessed by LC-MS/MS, providing an approximation of renal function.

Capecitabine-induced toxicities

Data on chemotherapy-induced toxicities were collected from medical records. To focus on clinically relevant side effects, we defined toxicities as modifications of capecitabine treatment as described previously [33]. Toxicity-induced modifications of treatment include dose reductions or early discontinuation of the capecitabine treatment, as well as switches from capecitabine-containing regimens to other regimens due to toxicities. Treatment delays were not uniformly reported in the medical records and were, therefore, not considered. Planned treatment modifications or modifications in case of progressive disease

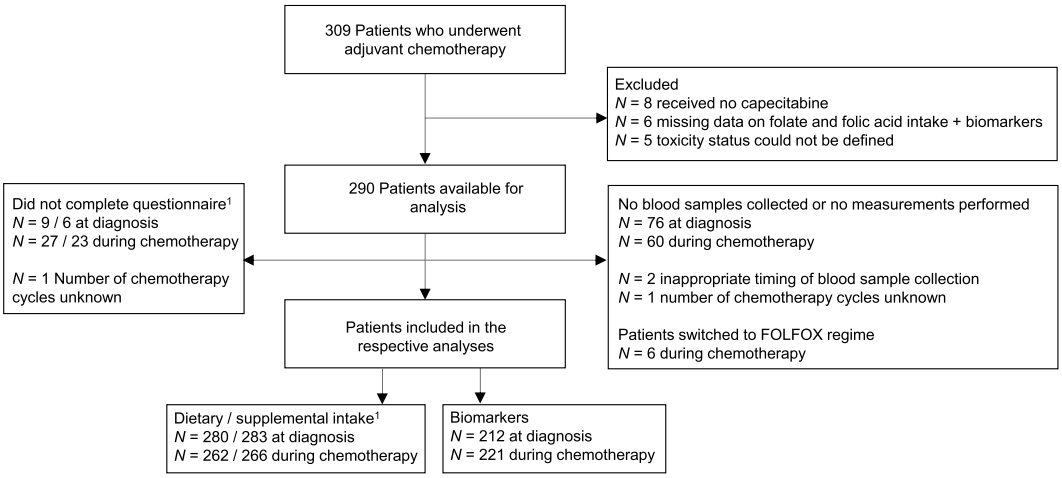

Figure 1. Flow diagram for patient selection within the COLON study. [1] These numbers represent information on completion of the food frequency questionnaire (number reported before the slash) and the dietary supplement questionnaire (number reported after the slash).

were not classified as toxicities and were not considered as outcomes for this study. We calculated the relative dose intensity (RDI) of the total given dose as a percentage of the total planned dose. For most patients, 8 cycles (6 mo) of CAPOX (capecitabine + oxaliplatin) were planned according to protocols operational at that time [34]. However, in the exceptional case of fewer cycles planned, for example due to updated protocols in more recent years (4 cycles or 3 mo of CAPOX [34, 35]), the RDI was calculated based on the actual number of planned cycles.

Descriptive data and covariates

Clinical data were obtained through linkage with the Dutch colorectal audit (DCRA) [36]. Apart from cancer stage (II or III) and tumor location (colon, rectum), data on the American Society of Anesthesiologists (ASA) physical status (I–III) assessed before surgery were collected to assess medical comorbidities. Further information on general and lifestyle factors was obtained through the questionnaires completed at time of diagnosis or 6 mo after diagnosis. Body mass index (BMI) was calculated based on self-reported weight and height.

Data analyses

Patients' characteristics are presented as descriptive data using medians and interquartile ranges (IQRs) or numbers and percentages. Associations between the different exposures and risk of toxicity-induced modifications of treatment were determined through Cox proportional hazards regression analyses with the number of completed, unmodified cycles as time variable. Dietary folate intake was considered continuously (per 10 μg DFE/d). Circulating levels of folate (in nmol/L) were considered as log2-transformed levels, and the hazard ratios (HRs) should, therefore, be interpreted per doubling in folate levels. Use of folic acid-containing supplements was compared with nonuse as the reference category. Since folic acid was only detected in a relatively small proportion of our population (10% at diagnosis), we used information on whether folic acid was detected (yes or no) as an exposure variable for folic acid status. Patients with missing data on any of these exposure variables were excluded from the analyses.

Analyses were adjusted for age and sex. Other potential confounders, including BMI (kg/m^2), alcohol use (g/d), smoking status (current, former, never), ASA physical status (I, II or III), cancer stage (II or III), and plasma creatinine (μmol/L) as marker for renal function, were identified based on literature [37–41]. These potential confounders were added one by one to the models (Supplemental Table 1). Since none of these variables changed the HRs by >10%, the final adjusted models included age and sex as covariates.

Given the importance of drug exposure in toxicity outcomes, we performed sensitivity analyses additionally adjusted for the starting dose of capecitabine. All analyses were performed in SPSS version 28.0.1.1 (IBM SPSS Statistics, New York, USA). Statistical significance was defined as 95% confidence intervals (CIs) not containing 1.

Results

Median (IQR) age of the patients was 64 (60–69) y, 38% were female, and most patients (90%) had stage III disease, and the tumor located in the colon (95%). Capecitabine was predominantly given in combination with oxaliplatin (87%), with a minority receiving capecitabine monotherapy (13%) (Table 1).

Median time between first blood collection and start of chemotherapy was 47 (38–57) d. The second blood sample was collected (median and IQR) 125 (110–143) d after start of chemotherapy, with a planned chemotherapy duration of 168 d (8 cycles of 3 wk) for most patients. Median intake of dietary folate was 239 (199–302) μg DFE/day, plasma folate levels were 11.6 (8.0–16.5) nmol/L, 19% reported use of dietary supplements containing folic acid, and 10% of the population had folic acid detected in their plasma at time of diagnosis (Table 2). At time of diagnosis, patients who used dietary supplements with folic acid (*N*=54, 19%) had higher plasma folate levels (14.9 [8.4–26.7] versus 10.8 [7.9–15.6] nmol/L) and had more often folic acid detected (19% versus 7%) compared with nonsupplement users (Supplemental Table 2).

Patients with plasma folic acid detected (*N*=21) tended to have higher plasma folate levels (at diagnosis 19.8 [13.8–29.4] nmol/L) compared with patients without folic acid (10.6 [7.7–15.4] nmol/L). Patients switching to FOLFOX (5-FU, leucovorin, oxaliplatin) (*N*=6) because of capecitabine-related toxicities showed an increase in plasma folate levels over time (+60.4 [39.2–74.3] nmol/L versus -0.4 [-4.0–3.3] nmol/L in patients without switch). These patients were excluded in further analyses where biomarkers were considered since these levels most likely increased due to administration of leucovorin.

TABLE 1
Demographic and clinical characteristics presented for the total study population and by occurrence of capecitabine-related toxicities

	Total population *N*=290	Toxicity[1] *N*=290	
		Yes *N*=153 (53%)	No *N*=137 (47%)
Age at diagnosis (y)	64 (60–69)	64 (61–69)	64 (58–69)
Sex, female (*N*,%)	111 (38)	62 (41)	49 (36)
BMI at diagnosis (kg/m^2), missing *N*=6	26.3 (23.9–29.0)	26.2 (23.8–28.7)	26.6 (23.9–29.4)
Smoking status at diagnosis (*N*,%), missing *N*=9			
Current	20 (7)	11 (7)	9 (7)
Former	163 (58)	83 (56)	80 (60)
Never	98 (35)	54 (37)	44 (33)
Tumor location (*N*,%)[2]			
Colon	274 (95)	144 (94)	130 (95)
Rectum	16 (6)	9 (6)	7 (5)
Tumor stage (*N*,%), missing *N*=2			
II	28 (10)	14 (9)	14 (10)
III	260 (90)	137 (91)	123 (90)
ASA physical status (*N*,%), missing *N*=13			
I	107 (39)	56 (39)	51 (39)
II	150 (54)	81 (56)	69 (52)
III	20 (7)	8 (6)	12 (9)
Plasma creatinine (μmol/L), missing *N*=77	77 (69–88)	76 (69–90)	78 (69–88)
Chemotherapeutic regimen (*N*,%)			
CAP monotherapy	37 (13)	26 (17)	11 (8)
CAP + oxaliplatin (CAPOX)	253 (87)	127 (83)	126 (92)
Relative dose intensity CAP (*N*,%), missing *N*=15	98 (80–100)	81 (63–90)	100 (100–100)

Data are presented as median and interquartile range or numbers and percentages, unless indicated otherwise.
Abbreviations: ASA: American Society of Anesthesiologists, BMI: body mass index, CAP: capecitabine, CAPOX: capecitabine and oxaliplatin.
[1] Toxicity was defined as any toxicities resulting in regimen switch, dose reduction, or early discontinuation of the CAP regimen.
[2] Colon = cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon. Rectum = rectosigmoid junction and rectum.

In total, 53% (*N*=153) of the population had a toxicity-induced modification of the capecitabine schedule. Dose reductions were the main first toxicity-induced treatment modifications, whereas after cycle 7, early discontinuations became most common (Figure 2).

Dietary intake of folate (per 10 μg DFE/d) at diagnosis (HR 1.00; 95%CI: 0.98,1.01) and during treatment (HR 0.99; 95%CI: 0.97,1.01) was not associated with risk of capecitabine-related toxicities. Also, plasma levels of folate (per doubling in nmol/L) at diagnosis and during treatment were not associated with toxicities (HR 1.04, 95%CI: 0.82,1.30 and HR 0.94, 95%CI: 0.72,1.23, respectively). Use versus nonuse of dietary supplements with folic acid during treatment (HR 1.81, 95%CI: 1.15,2.85), but not at diagnosis (HR 1.14, 95%CI: 0.75,1.72), was associated with an increased risk of toxicities. Also, the detection (versus no detection) of folic acid in plasma was associated with an increased risk of toxicities (HR 2.09, 95%CI: 1.24,3.52 and HR 2.31, 95%CI: 1.29,4.13 at diagnosis and during treatment, respectively)

TABLE 2
Dietary and supplemental intake and plasma levels of folate and folic acid at diagnosis and during chemotherapy

	Total population *N*=290	Toxicity[1] *N*=290	
		Yes *N*=153 (53%)	No *N*=137 (47%)
At diagnosis			
Dietary folate intake (μg DFE/day), missing *N*=9	239 (199-302)	232 (198-305)	244 (200-296)
Use of supplements with folic acid (*N*,%), missing *N*=6	54 (19)	31 (21)	23 (17)
Plasma folate levels (nmol/L)[2,3] missing *N*=76	11.6 (8.0-16.5)	11.6 (7.5-17.9)	11.3 (8.3-16.2)
Plasma folic acid detected (*N*,%)[3], missing *N*=76	21 (10)	17 (15)	4 (4)
During chemotherapy			
Dietary folate intake (μg DFE/day), missing *N*=27	230 (191-281)	229 (186-281)	233 (192-280)
Use of supplements with folic acid (*N*,%), missing *N*=23	32 (12)	24 (17)	8 (6)
Plasma folate levels (nmol/L)[2,3,4], missing *N*=60	12.1 (8.7-16.1)	12.5 (7.9-17.0)	11.9 (9.0-15.6)
Plasma folic acid detected[3,4](*N*,%), missing *N*=60	17 (8)	14 (12)	3 (3)

Data are presented as median and interquartile range or numbers and percentages.
Abbreviations: DFE: dietary folate equivalents, FOLFOX: folinic acid/leucovorin, 5-fluorouracil, and oxaliplatin, MeFox: pyrazino-s-triazine derivative of 4-alpha-hydroxy-5-methyl-tetrahydrofolate.
[1] Toxicity was defined as any toxicities resulting in regimen switch, dose reduction, or early discontinuation of the CAP regimen.
[2] Sum of 5-methyl-tetrahydrofolate and MeFox.
[3] One patient with blood samples collected after start of chemotherapy and one patient with the second blood sample collected before start of chemotherapy were excluded for biomarker analyses.
[4] Patients who switched to FOLFOX (*N*=6) were excluded here, since for these patients folate metabolism may be impacted by administration of leucovorin (folinic acid).

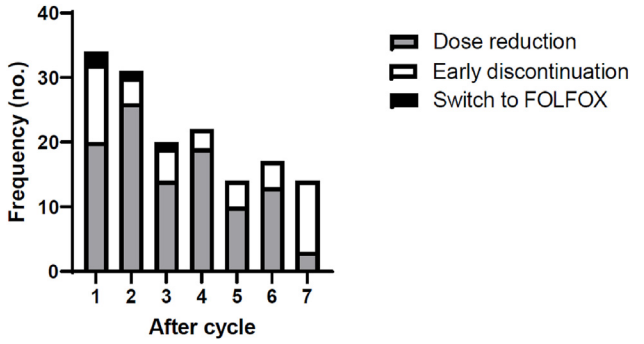

Figure 2. Treatment modifications of the capecitabine schedule. Toxicity-induced modifications of the capecitabine treatment occurred in 153 of 290 patients. The treatment modifications that occurred first in each individual patient are presented here according to the cycle after which they occurred. For one patient, it was unknown after which cycle the treatment modification took place, therefore data for 152 patients are presented here. Abbreviations: no.: number.

(Table 3). Sensitivity analyses adjusted for initial dose of capecitabine showed similar findings (Supplemental Table 3). Model adjustment for plasma creatinine, reflecting renal function, did not impact the findings (Supplemental Table 1). Also, analyses that considered the two folate species (5mTHF and MeFox) separately showed similar findings (Supplemental Table 4).

Discussion

In this prospective cohort of patients undergoing chemotherapy with capecitabine for CRC, use of dietary supplements with folic acid and presence of folic acid in the circulation were associated with an increased risk of toxicities related to capecitabine. Dietary folate intake or plasma levels of folate were not associated with risk of these toxicities.

Folate vitamers are involved in various chemotherapeutic mechanisms, including those of the fluoropyrimidines [4]. The presence of reduced folate species contributes to stability of a ternary complex formed with the active fluoropyrimidine drug metabolite and TS [6, 42]. Hypothetically, higher availability of reduced folate species might thus lead to enhanced TS inhibition and more toxicities. In support of this hypothesis, earlier small studies suggested that patients with a higher folate status before start of treatment have a higher risk of capecitabine-related toxicities [9, 14–16]. Sharma *et al.* demonstrated that a higher pretreatment serum folate was associated with a higher risk of grade 2 to 3 toxicities in 38 patients with advanced CRC [15]. Similarly, pretreatment serum folate levels were associated with grade ≥2 toxicity in 126 patients with CRC [14]. Yap *et al.* [16] showed that higher serum folate levels were associated with grade ≥2 capecitabine-induced hand-foot syndrome in 149 patients with various cancers. Similar findings have been reported for 5-FU-containing chemotherapy [43], although not all studies were able to confirm associations between folate status and 5-FU-related toxicities [44, 45]. Only one study reported on intake of dietary folate in relation to risk of toxicities in 132 gastric cancer patients receiving 5-FU [46]. In this retrospective cohort, dietary folate intake of >260 μg/d (versus ≤260 μg/d) was associated with an increased risk of hematologic toxicities [46]. Within these studies, no distinction between folate and folic acid was made, nor was intake or exposure to folate or folic acid during treatment examined. In our study, we observed an association between plasma folic acid, but not folate, and capecitabine-induced toxicities. In contrast to the previous studies, we were able to differentiate between folate and folic acid. The observation that folate was not associated with toxicities might be explained by the relatively low plasma folate levels (median at diagnosis =11.6 nmol/L) in our population, which might be inherent to the fact that there is no mandatory fortification program in the Netherlands. Similarly, the other 2 European studies, not providing clear indications for an association between folate and 5-FU-related toxicities, showed relatively low folate levels (~8 and 14 nmol/L) [44, 45]. Serum folate levels were higher (~16–27 nmol/L) in

TABLE 3
Associations between intake and biomarkers of folate and folic acid and risk of capecitabine-related toxicities

	No. of events / Population size	Hazard ratio	95% confidence interval
At diagnosis			
Dietary folate intake (per 10 μg DFE/d)	145/280	1.00	0.98–1.01
Use of supplements with folic acid			
No	117/230	Ref	
Yes	30/53	1.14	0.75–1.72
Plasma folate levels (per doubling in nmol/L)[1,2]	115/212	1.04	0.82–1.30
Plasma folic acid detected[2]			
No	98/191	Ref	
Yes	17/21	2.09	1.24–3.52
During chemotherapy			
Dietary folate intake (per 10 μg DFE/d)	134/262	0.99	0.97–1.01
Use of supplements with folic acid			
No	114/235	Ref	
Yes	23/31	1.81	1.15–2.85
Plasma folate levels (per doubling in nmol/L)[1,2,3]	112/221	0.94	0.72–1.23
Plasma folic acid detected[2,3]			
No	99/205	Ref	
Yes	13/16	2.31	1.29–4.13

The Cox proportional hazards regression analyses were adjusted for age (in y) and sex.
[1] Plasma folate levels were log2-transformed, and the hazard ratios should therefore be interpreted as per doubling in folate level.
[2] One patient with blood samples collected after start of chemotherapy and one patient with the second blood sample collected before start of chemotherapy were excluded for biomarker analyses.
[3] Patients who switched to FOLFOX (N=6) were excluded for these analyses. Abbreviations: DFE: dietary folate equivalents.

the studies from Canada, Australia, China, and Singapore that reported associations with chemotherapy-induced toxicity [14–16, 43], which might be due to mandatory fortification policies, dietary supplement use or dietary traditions in those populations [18, 47, 48].

Detection of folic acid is often due to saturation of the enzyme dihydrofolate reductase (DHFR)[17, 49] and high intake of folic acid itself, with supplement use being considered the primary source of excessive intake [17]. As such, circulating folic acid may be considered a proxy for an overall higher folate status. Indeed, we observed higher folate levels in patients with versus those without plasma folic acid. We have now shown for the first time that folic acid, as a supplemental intake or circulating marker, is associated with risk of capecitabine-related toxicities.

Theoretically, enhanced TS inhibition by higher availability of reduced folate species leads to higher efficacy of treatment [3]. This concept underlies the mode of action of leucovorin in treatment schedules with 5-FU [10, 50]. The higher treatment efficacy may, however, also lead to toxicities and resulting treatment modifications that could impair prognosis [51, 52]. In a multicohort consortium, including the COLON study, we have previously shown that higher levels of folic acid, but not folate, were associated with an increased risk of CRC recurrence [19]. Besides direct impact of folic acid on growth of residual cancer cells [17, 53], interference of folic acid with treatment, possibly resulting in more toxicities and treatment modifications, could be an explanation for these findings.

The current study has some limitations. First, inherent to the observational nature, we cannot establish causal relationships, and reverse causation cannot be ruled out. Patients with worse clinical conditions, which might predispose them to more toxicities, may take dietary supplements more often. It should be noted, however, that patients with and patients without toxicity did not markedly differ in terms of age, sex, tumor stage, or ASA classification. Especially during chemotherapy, patients may also start using dietary supplements or (temporarily) change dietary habits to relieve treatment-related symptoms [20, 54, 55]. However, the observation that folic acid detected at time of diagnosis was associated with an increased risk of toxicities makes this possibility less likely. Also, although we have considered and evaluated a set of relevant confounders, such as age, sex, lifestyle, and clinical factors, residual confounding cannot be ruled out completely. Second, for patients with (early) toxicities, the planned collection of blood samples during chemotherapy might have occurred after the event (i.e., toxicity-induced treatment modification) took place. Again, also plasma folic acid measured at time of diagnosis was associated with toxicity, suggesting that this option did not substantially impact our findings. Third, blood samples were collected in nonfasting conditions. Very recent dietary or supplemental intake of folate or folic acid could have impacted circulating levels [56], although potential misclassification is most likely non-differential for patients with versus those without toxicities and, is unlikely to overestimate the associations observed. Fourth, although this study reports on a large and well-characterized patient population, the number of patients in some subgroups is relatively small. Most patients received capecitabine combined with oxaliplatin, which could have impacted toxicity profiles, although we were able to distinguish treatment modifications for both regimens. We were not able to consider types (e.g., hand-foot syndrome or diarrhea) and grades of toxicities. This aligns, however, with our aim to focus on all clinically relevant toxicities and provided us with the opportunity to circumvent interindividual differences in toxicity profiles and inappropriate grading [57].

Strengths of this relatively large study include comprehensive assessment of both intake and status of folate as well as folic acid in a well-defined population. To the best of our knowledge, dietary and supplemental intake of folate, as well as folic acid, have not been studied separately in relation to capecitabine-induced toxicities before. Providing new evidence in this area is important, as it can guide health care professionals and cancer patients in directing their choices regarding dietary supplement use. We measured folate as the sum of the main circulating form 5mTHF and the degradation product MeFox, to allow for recovery of 5mTHF [30]. Previous studies mostly relied on assays that measured total folate levels and that were unable to distinguish folate from folic acid [14–16, 43–45].

In conclusion, we report an association between supplemental folic acid intake as well as detection of plasma folic acid and toxicities in patients treated with capecitabine for CRC. Results of this study may contribute to awareness on this topic for researchers and health care professionals and provide an evidence base for future studies on diet-drug interactions.

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Author contributions

The authors responsibilities were as follows—DK and EK designed the research; DK, FvD, FL, JMcK, CU, AU, PU, and EK obtained funding for any aspect of the research; DK, FvD, ASL, RW, EW, HvH, JdW, AU and PU contributed to data collection; DK performed formal analysis and wrote the draft of the paper. All authors contributed to the study and have read and approved the final manuscript.

Conflict of interest

None of the authors declared a conflict of interest.

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Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request, pending (inter)national regulations. Because the data consist of identifying cohort information, some access restrictions apply and not all data can be made publicly available. Requests for data and other information can be sent to Dr. Dieuwertje Kok, Division of Human Nutrition and Health, Wageningen University & Research, the Netherlands (e-mail: dieuwertje.kok@wur.nl).

Appendix A. Supplementary data

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