Soluble tumour necrosis factor receptor type II and survival in colorectal cancer

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Background: Chronic inflammation may play a role in colorectal cancer (CRC) pathogenesis. The relationship between soluble tumour necrosis factor receptor type II (sTNF-RII) and survival among CRC patients is not well defined.

Methods: We prospectively evaluated the association between pre-diagnosis plasma levels of sTNF-RII and mortality in 544 CRC patients from the Nurses’ Health Study and Health Professionals Follow-Up Study diagnosed from 1990 to 2010. Primary and secondary end points were overall and CRC-specific mortality, respectively. Cox proportional hazards models were used to calculate multivariate hazard ratios for mortality.

Results: Higher sTNF-RII levels were significantly associated with increased overall mortality (multivariate HR = 1.48, 95% CI 1.02–2.16, P-trend = 0.006), but not with CRC-specific mortality (HR = 1.23, 95% CI 0.72–2.08, P-trend = 0.34). In subgroup analyses, among regular aspirin users, those with higher sTNF-RII levels had an adjusted HR of 0.52 (95% CI 0.20–1.33) for overall mortality compared with those with lower sTNF-RII levels, whereas among nonregular aspirin users the adjusted HR was 2.26 (95% CI 1.23–4.01, P for interaction = 0.53).

Conclusions: Among CRC patients, higher sTNF-RII levels are associated with a significant increase in overall mortality, but not CRC-specific mortality. The role of inflammation and anti-inflammatory medications in survival of CRC patients warrants further exploration.

Chronic inflammation may play a role in the development and progression of colorectal cancer (CRC) (Bernstein et al., 2001; Kant and Hull, 2011; Song et al., 2013). Soluble tumour necrosis factor receptor type II (sTNF-RII; (HUGO Gene Nomenclature Committee (HGNC) ID TNFRSF1B) is the plasma form of the 75 kD cell surface receptor for the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) (Spoettl et al., 2007; Faustman and Davis, 2010). In CRC cells, TNF-α activates various inflammatory pathways and induces expression of cyclooxygenase-2 (COX-2), prostaglandin synthesis, increased tumour growth, and angiogenesis (Popivanova et al., 2008; Hamilton et al., 2011; Setia et al., 2013; Zhu et al., 2013). The TNF-α signalling is also associated with decreased apoptosis and chemotherapy resistance in metastatic CRC (Matsuyama et al., 2006).

Prospective observational studies have shown an association between sTNF-RII and increased risk of incident CRC in women (Chen et al., 2011). Multiple large randomised clinical trials have shown that inhibition of inflammation with medications such as
aspirin and COX-2 (PTGS2) inhibitors can effectively prevent formation of colorectal adenomas in patients with a history of polyps or CRC (Steinbach et al., 2000; Sandler et al., 2003; Arber et al., 2006; Bertagnolli et al., 2006). Moreover, regular aspirin use has also been associated with improved survival in CRC patients (Chan et al., 2009). In contrast, the role of sTNF-RII in patients with established CRC is less clear. We therefore conducted a prospective study to investigate the association between levels of sTNF-RII and mortality in CRC patients from two large, well-established cohorts with over 20 years of follow-up data.

**MATERIALS AND METHODS**

**Study population.** Participants were drawn from the Nurses' Health Study (NHS) and Health Professionals Follow-Up Study (HPFS). The NHS cohort was established in 1976 when 121,700 US female registered nurses aged 30–55 years completed a mailed questionnaire regarding their health history (Belanger et al., 1978; Colditz et al., 1997). Every 2 years, follow-up questionnaires were mailed to participants to update information on medical history, medication use, lifestyle factors, and chronic diseases. Between May 1989 and September 1990, blood samples were collected from 32,826 participants. NHS cohort follow-up is ~96%.

The HPFS cohort was established in 1986 when 51,529 male medical providers aged 45 to 70 years completed a mailed questionnaire regarding their health history (Rimm et al., 1991; Giovannucci et al., 1994). Every 2 years, follow-up questionnaires were mailed to participants to update information on medical history and medication use. Between 1993 and 1995, blood samples were also collected from 18,225 participants. HPFS cohort follow-up is ~95%.

**Inclusion criteria.** Participants were included in this analysis if they provided a blood sample and had a pathologically confirmed diagnosis of CRC between the date of blood sampling and June 2010 for NHS and January 2010 for HPFS. To identify CRC cases, participants were asked on each follow-up questionnaire whether they had a diagnosis of CRC during the previous 2 years. When a diagnosis of CRC was reported by a participant or next of kin (if the patient was deceased at the time of contact), permission was obtained to retrieve hospital records and pathology reports. Blindly studied physicians reviewed these records and recorded the patient was deceased at the time of contact), permission was obtained to retrieve hospital records and pathology reports. Blindly studied physicians reviewed these records and recorded the diagnosis until death or June 2012 (NHS) or January 2013 (HPFS), whichever came first. Confirmation of death included reporting by family or postal authorities. Names of persistent nonresponders were searched in the National Death Index. Cause of death was assigned by blinded study physicians. More than 98% of deaths have been identified by these methods (Stamper et al., 1984; Giovannucci et al., 2006). Overall mortality was defined as duration of time from CRC diagnosis to death from any cause. The CRC-specific mortality was defined as duration of time from diagnosis to death related to CRC. Non-CRC-specific mortality was defined as duration of time from diagnosis to death related to causes other than CRC. Patients not experiencing outcomes of interest were censored either at the time of death or at the end of the observation period defined above, if still alive.

**Exclusion criteria.** Patients were excluded from this analysis if they reported a diagnosis of cancer (other than non-melanoma skin cancer) or inflammatory bowel disease (IBD) before CRC diagnosis. To minimise any bias in the plasma levels of sTNF-RII associated with occult cancer, patients diagnosed with CRC within 2 years of blood collection were excluded. Based on these criteria, 319 women from the NHS cohort and 225 men from the HPFS cohort were eligible for analysis, for a total of 544 participants. All patients provided informed consent for use of their clinical data and biospecimens in the study that was approved by the institutional review board at Brigham and Women's Hospital and Harvard School of Public Health. sTNF-RII measurement. Blood samples were collected in tubes with heparin and sent to our laboratory by overnight courier in chilled containers. Blood samples were centrifuged, aliquoted, and stored in liquid nitrogen freezers at −160 °C. Levels of sTNF-RII were measured in a single run in a core laboratory using enzyme-linked immunosorbent assay (R&D Assays, Minneapolis, MN, USA) by blinded laboratory personnel. Blinded quality control samples were interspersed alongside test samples, and the intra-assay coefficient of variation for sTNF-RII was 6.7% (Chan et al., 2011).

The sTNF-RII is an accepted surrogate measurement for TNF-α because of its increased measurement stability in frozen blood samples, less diurnal variation, and its role in TNF-α signalling (Diez-Ruiz et al., 1995; Liu et al., 2007). Whereas plasma TNF-α levels tend to fluctuate, sTNF-RII levels in humans remain stable over long periods of time (Epstein et al., 2013). The stability of sTNF-RII measurements from blood samples stored for 24 h before processing compared with measurements obtained from samples frozen immediately after collection has been previously confirmed, with an assay intra-class correlation of 0.91 (Chan et al., 2011; Song et al., 2013).

**Outcome assessment.** Participants were followed from the time of diagnosis until death or June 2012 (NHS) or January 2013 (HPFS), whichever came first. Confirmation of death included reporting by family or postal authorities. Names of persistent nonresponders were searched in the National Death Index. Cause of death was assigned by blinded study physicians. More than 98% of deaths have been identified by these methods (Stamper et al., 1984; Giovannucci et al., 2006). Overall mortality was defined as duration of time from CRC diagnosis to death from any cause. The CRC-specific mortality was defined as duration of time from diagnosis to death related to CRC. Non-CRC-specific mortality was defined as duration of time from diagnosis to death related to causes other than CRC. Patients not experiencing outcomes of interest were censored either at the time of death or at the end of the observation period defined above, if still alive.

**Covariates.** Data on clinically relevant covariates were extracted from questionnaires and medical records, including age at blood draw, sex, race, tumour stage at diagnosis, primary tumour site, histologic grade of differentiation, year of diagnosis (surrogate for treatment received), time between blood draw and CRC diagnosis, body mass index (BMI) at blood draw, physical activity at blood draw, and use of aspirin or non-steroidal anti-inflammatory drug (NSAID) at blood draw and post diagnosis. Post-diagnosis covariates were taken from the first questionnaire between years 1 and 4 after diagnosis to avoid confounding by the immediate postoperative period or recent chemotherapy. Regular aspirin or NSAID users were defined as those using ≥2 tablets of regular-strength aspirin or NSAID per week. Nonregular users were defined as patients who either did not use those medications or who used <2 tablets per week.

**Statistical analysis.** We controlled for batch-to-batch variability using the method described by Rosner et al. (2008). Quartiles of sTNF-RII were determined separately within each cohort. Hazard ratios (HRs) were calculated separately for HPFS and NHS and subsequently pooled for the primary analysis using a meta-analysis with a fixed effects model. Heterogeneity across cohorts was tested using the Q-statistic (DerSimonian and Laird, 1986). Overall mortality was assessed according to quartile of sTNF-RII using Kaplan–Meier curves and the log-rank test. Cox proportional hazards models were used to calculate HRs of death from all causes or death because of CRC. Models were stratified by stage and grade at diagnosis and adjusted for the potential confounding factors listed above, with the lowest quartile of sTNF-RII as the referent. The two-tailed P-value for the linear trend test across categories was calculated using sTNF-RII as a continuous variable in the final multivariate model (Allison, 1995).

Analyses of the relationship between sTNF-RII and mortality are also conducted within subgroups of prespecified covariates using the same model as detailed above. For continuous variables (i.e., age and physical activity), cut-points were determined by the median value. Patients with missing data for the subgroup of
| Table 1. Baseline characteristics of study cohort according to quartile of sTNF-RII (n = 544) |
|---------------------------------------------------------------|
| **Baseline characteristics** | **Quartile 1 (n = 135)** | **Quartile 2 (n = 136)** | **Quartile 3 (n = 137)** | **Quartile 4 (n = 136)** |
| Mean age at blood draw, years (s.e.) | 58.5 (0.70) | 60.9 (0.64) | 62.5 (0.63) | 64.3 (0.68) |
| **Sex, no. (%)** | | | | |
| Male | 56 (41) | 56 (41) | 57 (42) | 56 (41) |
| Female | 79 (59) | 80 (59) | 80 (58) | 80 (59) |
| **Stage, no. (%)** | | | | |
| I | 44 (33) | 36 (26) | 36 (26) | 31 (23) |
| II | 32 (24) | 42 (31) | 26 (19) | 28 (21) |
| III | 26 (19) | 21 (15) | 31 (23) | 28 (21) |
| IV | 17 (13) | 16 (12) | 18 (13) | 24 (18) |
| Unknown/missing | 16 (12) | 21 (15) | 26 (19) | 25 (18) |
| **Race, no. (%)** | | | | |
| White | 128 (95) | 134 (99) | 131 (96) | 135 (99) |
| African American | 3 (2) | 1 (1) | 0 (0) | 0 (0) |
| Other | 1 (1) | 0 (0) | 0 (0) | 0 (0) |
| Missing | 3 (2) | 1 (1) | 6 (4) | 1 (1) |
| **Tumour location, no. (%)** | | | | |
| Proximal colon | 41 (30) | 67 (49) | 59 (43) | 65 (48) |
| Distal colon | 49 (36) | 35 (26) | 37 (27) | 31 (23) |
| Rectum | 40 (30) | 21 (15) | 27 (20) | 24 (18) |
| Unknown | 5 (4) | 13 (10) | 14 (10) | 16 (12) |
| **Family history of colorectal cancer, no. (%)** | | | | |
| Yes | 44 (33) | 44 (32) | 42 (31) | 41 (30) |
| No | 91 (67) | 92 (68) | 95 (69) | 95 (70) |
| **Mean time between blood draw and diagnosis, years (s.e.)** | 8.4 (0.3) | 9.8 (0.4) | 9.2 (0.4) | 8.6 (0.4) |
| **Grade of differentiation, no. (%)** | | | | |
| Well differentiated | 22 (16) | 12 (9) | 11 (8) | 9 (7) |
| Moderately differentiated | 74 (55) | 79 (58) | 80 (58) | 73 (54) |
| Poorly differentiated or undifferentiated | 22 (16) | 18 (13) | 17 (12) | 18 (13) |
| Unknown | 17 (13) | 27 (20) | 29 (21) | 36 (26) |
| **Year of diagnosis, no. (%)** | | | | |
| 1990–2000 | 83 (61) | 61 (45) | 64 (47) | 78 (57) |
| 2001–2010 | 52 (39) | 75 (55) | 73 (53) | 58 (43) |
| **Tobacco use at blood draw, no. (%)** | | | | |
| Never smoker | 48 (36) | 58 (43) | 63 (46) | 63 (46) |
| Past smoker | 77 (57) | 66 (49) | 61 (45) | 55 (40) |
| Current smoker | 10 (7) | 11 (8) | 12 (9) | 18 (13) |
| Missing/unknown | 0 (0) | 1 (1) | 1 (1) | 0 (0) |
| **Regular aspirin use** | | | | |
| At blood draw, no. (%) | | | | |
| Yes | 30 (22) | 31 (23) | 32 (23) | 31 (23) |
| No | 79 (59) | 70 (51) | 67 (49) | 58 (43) |
| Missing/unknown | 26 (19) | 35 (26) | 38 (28) | 47 (35) |
| 1–4 Years after diagnosis, no. (%) | | | | |
| Yes | 39 (29) | 39 (29) | 53 (39) | 52 (38) |
| No | 94 (70) | 94 (69) | 80 (58) | 81 (60) |
| Missing/unknown | 2 (1) | 3 (2) | 4 (3) | 3 (2) |
| **Regular anti-inflammatory medication use** | | | | |
| At blood draw, no. (%) | | | | |
| Yes | 16 (12) | 17 (13) | 17 (12) | 19 (14) |
| No | 106 (79) | 108 (79) | 107 (78) | 103 (76) |
| Missing/unknown | 13 (10) | 11 (8) | 13 (9) | 14 (10) |
| 1–4 Years after diagnosis, no. (%) | | | | |
| Yes | 13 (10) | 18 (13) | 20 (15) | 13 (10) |
| No | 103 (76) | 100 (74) | 95 (69) | 97 (71) |
| Missing/unknown | 19 (14) | 18 (13) | 22 (16) | 26 (19) |
| **BMI, kg m\(^{-2}\)** | | | | |
| At blood draw, no. (%) | | | | |
| Normal weight (<25) | 41 (30) | 30 (22) | 26 (19) | 35 (26) |
| Overweight (25–29.9) | 69 (51) | 73 (54) | 75 (55) | 69 (51) |
| Obese (≥30) | 25 (19) | 33 (24) | 36 (26) | 32 (24) |
| At diagnosis, no. (%) | | | | |
| Normal weight (<25) | 31 (23) | 30 (22) | 32 (23) | 35 (26) |
| Overweight (25–29.9) | 66 (49) | 62 (44) | 55 (40) | 59 (43) |
| Obese (≥30) | 38 (28) | 44 (32) | 50 (37) | 42 (31) |
| **Median level of physical activity, MET-h per week (IQR)** | | | | |
| 1–4 Years after diagnosis | 12.7 (6.0–27.1) | 8.5 (5.4–21.8) | 11.1 (6.9–17.7) | 8.5 (4.0–16.5) |
| At blood draw | 15.4 (6.0–29.0) | 13.5 (4.3–33.9) | 13.9 (5.9–31.1) | 11.8 (3.5–27.0) |

*Abbreviations: BMI = body mass index; IQR = interquartile range; MET = metabolic equivalent task; sTNF-RII = soluble tumour necrosis factor receptor type II. Quartile sTNF-RII ranges (pg ml\(^{-1}\)): quartile 1: 1304–2361; quartile 2: 2346–2767; quartile 3: 2755–3297; and quartile 4: 3186–9572.

\(^a\) A standard tablet contains 325 mg of aspirin, and regular aspirin use is defined as taking at least two tablets of aspirin per week.

\(^b\) Regular anti-inflammatory drug users were defined as those who used at least two tablets of a non-steroidal anti-inflammatory drug (NSAID) per week or any use of a cyclooxygenase-2 (COX-2) inhibitor.
interest were excluded from the analysis. Tests of interaction between sTNF-RII and potential effect modifiers were assessed by Wald’s test of cross-product terms created by multiplying the covariate of interest with sTNF-RII as a continuous variable. All analyses used SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Among the eligible 544 patients, there were 299 deaths, with 163 deaths (55%) because of CRC. Non-CRC causes of death included cardiovascular disease (n = 30, 22%), other malignancies (n = 23, 17%), neurologic disorders (n = 24, 18%), pulmonary disorders (n = 10, 7%), miscellaneous causes (n = 26, 19%), and unknown reasons, typically because of the inability to confidently assign a single cause (n = 23, 17%).

The median duration of follow-up of patients still alive at the end of the study was 11.5 years (range 3.9–20.5 years). The median follow-up of patients who died of any cause was 3.6 years (range 0–21.2 years). The median follow-up of those patients who died of CRC was 1.5 years (range 0–16.9 years). The sTNF-RII levels were measured at a median of 8.7 years before CRC diagnosis (range 2–16.9 years).

Baseline characteristics according to quartile of plasma sTNF-RII are shown in Table 1. Most characteristics did not differ significantly across quartiles. Patients with higher levels of sTNF-RII appeared to be older, have stage IV disease at diagnosis, have proximal rather than distal colon or rectal tumours, be diagnosed earlier than 2001, and have a higher BMI at blood draw. There was no significant difference in the mean time between blood draw and diagnosis of CRC between sTNF-RII quartiles.

Kaplan–Meier curves for overall survival (Figure 1) and CRC-specific survival (Figure 2) by quartile of sTNF-RII are shown. Log-rank testing demonstrated a statistically significant difference in overall survival (P < 0.0001) and CRC-specific survival (P = 0.05)
between quartiles of sTNF-RII. The association between pre-
diagnosis sTNF-RII levels and overall survival in CRC patients
remained significant after adjustment for potential confounders
(Table 2). Patients with higher sTNF-RII plasma levels had a HR
for overall mortality of 1.48 (95% CI 1.02–2.16, P-trend = 0.006)
compared with the lowest levels. Excluding stage IV patients at
diagnosis, who typically have higher levels of inflammation and
worse prognosis, did not appreciably alter the results (HR = 1.69,
95% CI 1.05–2.72). In contrast, after multivariate adjustment,
increasing levels of sTNF-RII were not significantly associated with
increased CRC-related mortality (HR = 1.23, 95% CI 0.72–2.08,
P = 0.34). We also found a statistically significant difference in non-
CRC-specific survival (P < 0.0001) between sTNF-RII quartiles
(Kaplan–Meier curve data not shown). The significantly increased
risk of non-CRC-related mortality among patients with higher sTNF-
RII levels persisted after multivariate adjustment (HR = 1.91, 95% CI
1.09–3.37, P-trend = 0.002; Table 2). Of note, there was no significant
association between sTNF-RII and cardiovascular mortality specifi-
cally (HR = 1.84, 95% CI 0.58–5.81, P-trend = 0.15). When exam-
ined separately by cohort, there was no significant difference in
association between sTNF-RII and overall (P-heterogeneity = 0.86),
CRC-related (P-heterogeneity = 0.19), or non-CRC-related mortality
(P-heterogeneity = 0.63; Supplementary Table 1).

To address the possibility of competing risks of non-CRC-
related death, we performed a competing risk analysis (Lunn and
McNeil, 1995) and obtained similar results. Compared with
patients with lowest sTNF-RII, patients with highest levels were
not at statistically higher risk of CRC-related death (HR = 1.29,
95% CI 0.78–2.12), but were at increased risk of non-CRC death
(HR = 2.13, 95% CI 1.22–3.70). We performed subgroup analyses
of the association of sTNF-RII with overall mortality (Figure 3).
For most subgroups, the increased risk of all-cause mortality
associated with higher levels of sTNF-RII was preserved. Interes-
tingly, however, among patients who reported regular aspirin use
after diagnosis, higher sTNF-RII levels were not associated with an
increased risk of death (multivariate HR = 0.52, 95% CI 0.20–1.33).
In contrast, nonregular aspirin users in the highest quartile of
sTNF-RII showed a multivariate HR of 2.26 (95% CI 1.23–4.04)
compared with the lowest quartile (P for interaction = 0.53).

We found that higher pre-diagnosis sTNF-RII levels were
associated with an ~48% increase in overall mortality compared
with lower sTNF-RII levels after adjustment for potential confounding factors. Among regular users of aspirin after
diagnosis, however, increasing levels of sTNF-RII were not
associated with worse mortality in exploratory subgroup analyses,
though the P for interaction was not significant. Interestingly, we
did not see a statistically significant association between higher
sTNF-RII levels and CRC-specific mortality after multivariate
adjustment.

Although decreased power and the strong association between
sTNF-RII and non-CRC-related death are potential explanations
for the nonsignificant relationship between sTNF-RII and CRC-
specific mortality, it is also plausible that higher circulating sTNF-
RII adversely affects mortality via non-CRC-related pathways.
Non-colorectal causes of death in our cohort included cardiovas-
cular disease, other malignancies, neurologic disorders, and
pulmonary disease, all of which are characterised by chronic
inflammation. Moreover, several studies have demonstrated that
elevated plasma sTNF-RII is associated with increased mortality in
these diseases (Dobrzycka et al, 2009; Heemann et al, 2012;
Schnabel et al, 2013). For example, previous analyses have clearly
shown associations between sTNF-RII and cardiovascular disease,
such as risk of incident coronary heart disease in healthy women
(Pai et al, 2004), myocardial infarction in diabetic women in the
NHS cohort (Shai et al, 2005), and cardiovascular mortality in the
Framingham Heart Study (Schnabel et al, 2013). Consistent with
this, we demonstrated a two-fold increase in risk of non-CRC-
related mortality among CRC patients with elevated sTNF-RII
levels. We also found that higher sTNF-RII levels were associated
with increased cardiovascular mortality among CRC patients, but
this was not statistically significant because of the small number of
patients in our study who died of cardiovascular disease (n = 30).
These results suggest that comorbid inflammatory conditions may
carry important prognostic implications for CRC patients (De
Marco et al, 2000), a finding that is increasingly relevant in the

| Table 2. Age-adjusted and multivariate hazard ratios for mortality by quartile of sTNF-RII (n = 544) |
|-----------------------------------------------|-------------|-------------|-------------|-------------|
| Range of sTNF-RII (pg ml⁻¹) | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 |
| NHS cohort (N = 319) | (1459–2344) | (2346–2752) | (2755–3185) | (3186–9572) |
| HPFS cohort (N = 225) | (1304–2361) | (2361–2767) | (2771–3297) | (3328–5512) |
| Overall mortality | | | | |
| Age adjusted | 1.35 | 0.78 | 1.09 (0.76–1.56) | 1.28 (0.90–1.81) |
| Multivariate | 1.35 | 0.78 | 0.93 (0.63–1.38) | 1.17 (0.80–1.70) |
| Colorectal cancer mortality | | | | |
| Age adjusted | 1.35 | 0.78 | 1.04 (0.64–1.70) | 1.25 (0.77–2.01) |
| Multivariate | 1.35 | 0.78 | 1.05 (0.62–1.78) | 1.25 (0.75–2.10) |
| Non-colorectal cancer-specific mortality | | | | |
| Age adjusted | 1.35 | 0.78 | 1.12 (0.63–2.00) | 1.29 (0.75–2.21) |
| Multivariate | 1.35 | 0.78 | 0.84 (0.45–1.55) | 1.00 (0.55–1.83) |

Abbreviations: 95% CI = 95% confidence interval; HPFS = Health Professionals Follow-Up Study; HR = hazard ratio; NHS = Nurses’ Health Study; sTNF-RII = soluble tumour necrosis factor receptor type II.

¢ Trend calculated by using sTNF-RII as a continuous variable in the Cox model.
¢ HRs, 95% CIs, and P-values are adjusted for age at blood draw (years).
¢ Multivariate HRs, 95% CIs, and P-values are calculated using a meta-analysis (fixed effects model) by cohort, stratified by stage (II, IV, or unknown) and grade (well or moderately differentiated, poorly differentiated, or unknown), and adjusted for age at blood draw (in years as a continuous variable), location of primary tumour (proximal, distal, rectum, or unknown), year of diagnosis (as a continuous variable), time between blood draw and diagnosis (in years as a continuous variable), body mass index at blood draw (in kg m⁻² as a continuous variable), physical activity at blood draw (in metabolic equivalent-h per week as a continuous variable), and regular aspirin or anti-inflammatory use at blood draw.

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context of the longer life expectancy of CRC patients in recent years.

Interestingly, our study found that higher sTNF-RII levels were not associated with worse outcome among patients who reported regular aspirin use, although the $P$-value for interaction between sTNF-RII and aspirin was not significant, possibly because of reduced power in exploratory subgroup analyses. Ample clinical data (Algra and Rothwell, 2012) suggest that initiation of aspirin after CRC diagnosis may be beneficial in reducing mortality, particularly among older patients (Lai and Liao, 2013) and COX-2-overexpressing (Chan et al., 2009) or PIK3CA-mutated tumours (Liao et al., 2012; Langley and Rothwell, 2013). Moreover, treatment with COX inhibitors leads to inhibition of TNF-$\alpha$-induced COX-2 expression and decreased proliferation of CRC cells (Ricchi et al., 1997; Paik et al., 2000), thus lending biologic plausibility to our findings. Currently, a large, randomised Intergroup study, CALGB/Alliance 80702, is testing the efficacy of celecoxib, a selective COX-2 inhibitor, for preventing disease recurrence in stage III colon cancer patients, and future studies should investigate the impact of these medications on TNF-$\alpha$ signalling and prognosis.

The strengths of our study include its prospective design, and use of two established cohorts with high rates of long-term follow-up for measurement of confounders and mortality end points. Moreover, NHS and HPFS participants are motivated medical professionals who provide accurate information about potential confounders and outcomes.

A potential study limitation is that only one measurement of sTNF-RII was drawn before diagnosis; however, previous studies have demonstrated that sTNF-RII levels stay fairly constant over time (Epstein et al., 2013). Higher sTNF-RII levels may also be a consequence of cancer, rather than a factor in the causative

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**Figure 3. Relationship between sTNF-RII and overall mortality in prespecified patient subgroups.** Multivariate hazard ratios (HRs) and 95% confidence intervals (CIs) for overall mortality across subgroups of various factors, comparing CRC patients in the highest quartile of plasma sTNF-RII levels with patients in the lowest sTNF-RII quartile. Subgroups include age at diagnosis (less than or equal to cohort median diagnosis age of 71 years, greater than cohort median diagnosis age), sex (male, female), year of diagnosis (before and including the year 2000, after the year 2000), tumour location at diagnosis (colon, rectum), BMI (kg m$^{-2}$) at diagnosis (normal weight, overweight, or obese), regular aspirin (ASA) use 1–4 years after diagnosis (yes, no), and physical activity 1–4 years after diagnosis (less than or equal to cohort median physical activity of 9.6 metabolic equivalent task (MET)-h per week, greater than median physical activity).
pathway, or reflect other poor prognostic factors. However, we adjusted our analysis for potential risk factors for CRC mortality, and attempted to control for reverse causation by excluding patients diagnosed with CRC within 2 years of blood draw. Moreover, excluding patients with stage IV disease at diagnosis (who often have the highest sTNF-RII levels and mortality rates) from the analysis as well as significantly alter our results. Although NHS and HPFS cohort questionnaire data are extensive, information about treatment is limited, and hence we adjusted the analysis for year of diagnosis as a surrogate for treatment. Another limitation is the racial homogeneity of the NHS and HPFS cohorts (>90% white), possibly reducing the generalisability of our findings, although it is unlikely that the underlying biological pathways of inflammation differ by race.

In conclusion, pre-diagnosis plasma levels of sTNF-RII among CRC patients are significantly associated with increased overall mortality. Future studies should continue to explore the role of inflammatory signalling and methods of reducing inflammation in patients with colorectal cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Algra AM, Rothwell PM (2012) Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies vs randomised trials. *Lancet Oncol* 13(5): 518–527.
Allison PD (1995) Survival Analysis Using SAS: A Practical Guide. pp 111–185. SAS Institute: Cary, NC, USA.
Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Golightly P, Tanig Y, Rosenzweig IB, Macdonald K, Bhadra P, Fowlston R, Witjes J, Zauber AG, Solomon SD, Levin B. PreSAP Trial Investigators (2006) Celecoxib for the prevention of colorectal adenomatous polyps. *N Engl J Med* 355(9): 885–895.
Belanger CF, Hennekens CH, Rosner B, Speizer FE (1978) The nurses’ health study. *Am J Nurs* 78(6): 1039–1040.
Bernstein CN, Blanchard JF, Kliever W, Wajda A (2001) Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* 91(4): 854–862.
Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, Tang J, Rosenzweig RB, Witjes J, Corle D, Hess TM, Woloj GM, Boissiere F, Anderson WF, Viner JL, Bagheri D, Burn J, Chung DC, Decwar T, Foley TR, Hoffman N, Macrae F, Pruitt RE, Saltzman JR, Salzberg B, Sylwestrowicz T, Gordon GB, Hawk ET. APC Study Investigators (2006) Celecoxib for the prevention of sporadic colorectal adenomas. *N Engl J Med* 359(7): 873–884.
Chan AT, Ogino S, Fuchs CS (2009) Aspirin use and survival after diagnosis of colorectal cancer. *JAMA* 302(6): 649–658.
Chan AT, Ogino S, Giovannucci EL, Fuchs CS (2011) Inflammatory markers are associated with risk of colorectal cancer and chemopreventive response to anti-inflammatory drugs. *Gastroenterology* 140(3): 799–808, quiz e11.
Colditz GA, Manson JE, Hankinson SE (1997) The Nurses’ Health Study: 20-year contribution to the understanding of health among women. *Jungsprache* 6(1): 49–62.
De Marco MF, Janssen-Heijnen ML, van der Heijden LH, Coehberg JH (2000) Comorbidity and colorectal cancer according to subsite and stage: a population-based study. *Eur J Cancer* 36(1): 95–99.
DerSimonian R, Laird (1986) Meta-analysis in clinical trials. *Control Clin Trials* 7(3): 177–188.
Diez-Ruiz A, Tizl GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D (1995) Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. *Eur J Haematol* 54(1): 1–8.
Dobrzycka B, Terlikowski SJ, Kowalczyk O, Kinalska M (2009) Circulating levels of TNF-alpha and its soluble receptors in the plasma of patients with epithelial ovarian cancer. *Eur J Obstet Gynecol Reprod Biol* 148(2): 131–134.
Epstein MM, Breen EC, Magapanyt L, Detels R, Lepone L, Penigonda S, Bream JH, Jacobson LP, Martinez-Maza O, Birrmann BM (2013) Temporal stability of serum concentrations of cytokines and soluble receptors measured across two years in low-risk HIV-seronegative men. *Cancer Epidemiol Biomarkers Prev* 22(11): 2009–2015.
Faustman D, Davis M (2010) TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat Rev Drug Discov* 9(6): 482–493.
Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, Willett WC (2006) Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst* 98(7): 451–459.
Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC (1994) Intake of fat, meat, and fiber in relation to risk of colon cancer in men. *Cancer Res* 54(9): 2390–2397.
Hamilton KE, Simmons JG, Ding S, Van Landeghem I, Lund PK (2011) Cytokine induction of tumor necrosis factor receptor 2 is mediated by STAT3 in colon cancer cells. *Mol Cancer Res* 9(12): 1718–1731.
Heemstra C, Kreuz M, Stoller I, Schoof N, von Bonin F, Ziepert M, Loffler M, Heemann C, Kreuz M, Stoller I, Schoof N, von Bonin F, Ziepert M, Loffler M, Gerletti P, Tang J, Rosenstein RB, PA, RI, SC, TN, TX, VA, WA, and WY. We assume full responsibility for analyses and interpretation of these data. This work was supported by the National Cancer Institute at the National Institutes of Health (K07 CA148894 to KN; R01 CA137178 to ATC; K24 DK098311 to ATC; R01 CA151993 and R35 CA179735 to SO; P50 CA127003 to CSF), the American Society of Clinical Oncology (ASCO) Career Development Award (to KN), the Entertainment Industry Foundation's National Cancer Research Alliance (NCCRA), and the Dana Farber Cancer Institute Gift Funds from the Janock Fellowship and Herndon Fellowship (to SMS). Other funding sources include UM1 CA186107 (NHS cohort infrastructure grant), P01 CA87969 (NHS program project grant), R01 CA49449 (NHS blood cohort grant), UM1 CA167552 (HPFS infrastructure grant), and P01 CA 55075 (HPFS program project grant). The sponsors did not participate in the design and conduct of the study; collection, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the article for publication. The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health, ASCO, The Conquer Cancer Foundation, the Damon Runyon Cancer Research Foundation, or the NCCRA.

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REFERENCES

Algra AM, Rothwell PM (2012) Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies vs randomised trials. *Lancet Oncol* 13(5): 518–527.
Allison PD (1995) Survival Analysis Using SAS: A Practical Guide. pp 111–185. SAS Institute: Cary, NC, USA.
Lunn M, McNeil D (1995) Applying Cox regression to competing risks. *Biometrics* 51(2): 524–532.

Matsuyama R, Togo S, Shimizu D, Momiyama N, Ishikawa T, Ichikawa Y, Endo I, Kunisaki C, Suzuki H, Hayasizaki Y, Shimada H (2006) Predicting 5-fluorouracil chemosensitivity of liver metastases from colorectal cancer using primary tumor specimens: three-gene expression model predicts clinical response. *Int J Cancer* 119(2): 406–413.

Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB (2004) Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 351(25): 2599–2610.

Paik JH, Ju JH, Lee JY, Boudreau MD, Hwang DH (2000) Two opposing effects of non-steroidal anti-inflammatory drugs on the expression of the inducible cyclooxygenase. Mediation through different signaling pathways. *J Biol Chem* 275(36): 28173–28179.

Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N (2008) Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest* 118(2): 560–570.

Ricchi P, Pignata S, Di Popolo A, Memoli A, Apicella A, Zarrilli R, Acquaviva AM (1997) Effect of aspirin on cell proliferation and differentiation of colon adenocarcinoma Caco-2 cells. *Int J Cancer* 73(6): 880–884.

Rimm EB, Giovannucci EL, Willett WC, Ascherio A, Rosner B, Stampfer MJ (1991) Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet* 338(8765): 464–468.

Rosner B, Cook N, Portman R, Daniels S, Falkner B (2008) Determination of blood pressure percentiles in normal-weight children: some methodological issues. *Am J Epidemiol* 167(6): 653–666.

Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, Petrelli N, Pippas JM, Karp DD, Loprinzi CL, Steinbach G, Schilsky R (2003) A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med* 348(10): 883–890.

Schnabel RB, Yin X, Larson MG, Yamamoto JF, Fontes JD, Kathiresan S, Rong J, Levy D, Keaney Jr JF, Wang TJ, Murabito JM, Vasan RS, Benjamin EJ (2013) Multiple inflammatory biomarkers in relation to cardiovascular events and mortality in the community. *Arterioscler Thromb Vasc Biol* 33(7): 1728–1733.

Setia S, Nehru B, Sanwal SN (2013) Activation of NF-kappaB: bridging the gap between inflammation and cancer in colitis-mediated colon carcinogenesis. *Biomed Pharmacother* 68(1): 119–128.

Shai I, Schulze MB, Manson JE, Rexrode KM, Stampfer MJ, Mantzoros C, Hu FB (2005) A prospective study of soluble tumor necrosis factor-alpha receptor II (sTNF-RII) and risk of coronary heart disease among women with type 2 diabetes. *Diabetes Care* 28(6): 1376–1382.

Song M, Wu K, Ogino S, Fuchs CS, Giovannucci EL, Chan AT (2013) A prospective study of plasma inflammatory markers and risk of colorectal cancer in men. *Br J Cancer* 108(9): 1891–1898.

Spoettl T, Hausmann M, Klebl F, Dirmeier A, Klump B, Hoffmann J, Herfarth H, Timmer A, Rogler G (2007) Serum soluble TNF receptor I and II levels correlate with disease activity in IBD patients. *Inflamm Bowel Dis* 13(6): 727–732.

Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B, Hennekens CH (1984) Test of the National Death Index. *Am J Epidemiol* 119(5): 837–839.

Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B, Godio I, Patterson S, Rodriguez-Bigas MA, Jester SL, King KL, Schumacher M, Abbruzzese J, DuBois RN, Hittel WN, Zimmerman S, Sherman JW, Kelloff G (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 342(26): 1946–1952.

Zhu M, Zhu Y, Lance P (2013) TNFalpha-activated stromal COX-2 signalling promotes proliferative and invasive potential of colon cancer epithelial cells. *Cell Prolif* 46(4): 374–381.

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