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 (Chan *et al*, 2011). Multiple large randomised clinical trials have shown that inhibition of inflammation with medications such as

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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 $\sim$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 $\sim$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. Blinded study physicians reviewed these records and recorded information on stage and important tumour characteristics. For nonrespondents, the National Death Index was used to confirm death and ascertain whether CRC contributed to death or was a secondary diagnosis. It is estimated that 96% of CRC cases were identified using these methods (Giovannucci *et al*, 2006).

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 $-160\,^{\circ}$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 (Stampfer *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 $\geqslant$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 were 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[a]				
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[b]				
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 (46)	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 (4.0–33.9)	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).

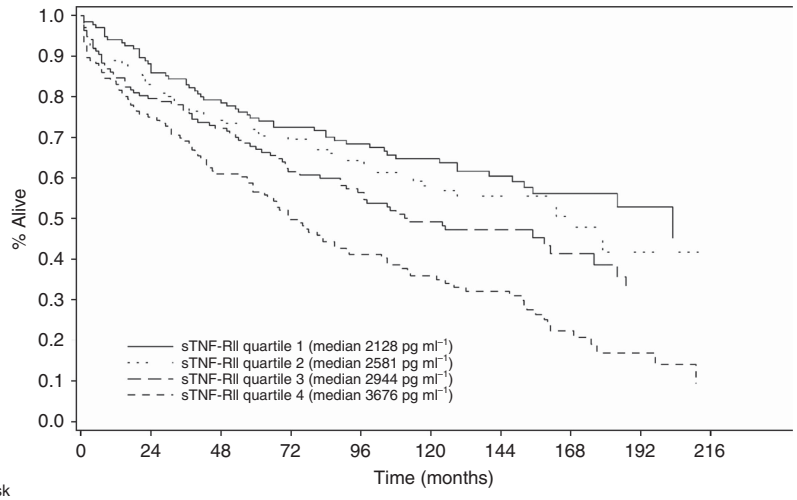
RESULTS

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.

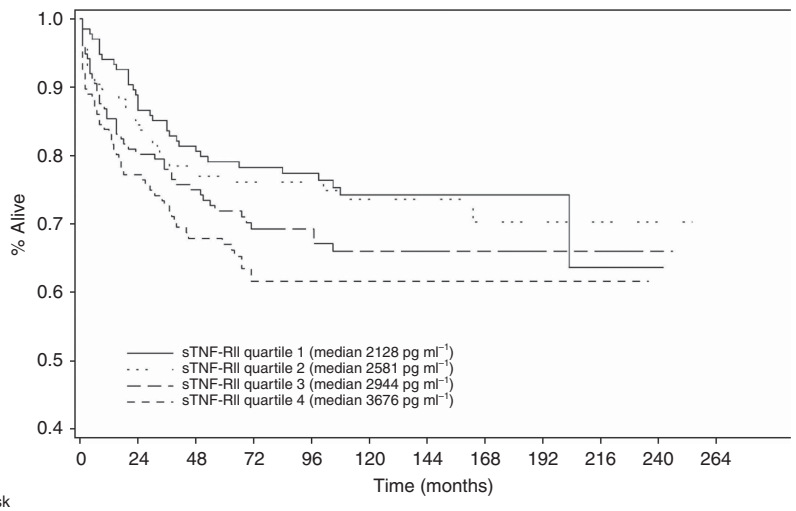
Kaplan–Meier curves for overall survival (Figure 1) and CRC-specific survival (Figure 2) by quartile of sTNF-RII are shown. Log-rank testing demonstrated a statistically significant difference in overall survival ($P<0.0001$) and CRC-specific survival ($P=0.05$)



Number at risk

sTNF-RII quartile 1	135	119	107	95	78	65	49	29	12
sTNF-RII quartile 2	136	113	101	85	66	49	33	19	9
sTNF-RII quartile 3	137	109	99	75	64	50	30	19	9
sTNF-RII quartile 4	136	102	83	65	50	38	29	14	6

Figure 1. Overall survival by quartile of sTNF-RII.



Number at risk

sTNF-RII quartile 1	135	119	107	95	78	65	49	29	12
sTNF-RII quartile 2	136	113	101	85	66	49	33	19	9
sTNF-RII quartile 3	137	109	99	75	64	50	30	19	9
sTNF-RII quartile 4	136	102	83	65	50	38	29	14	6

Figure 2. Colorectal cancer-specific survival by quartile of sTNF-RII.

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 specifically (HR = 1.84, 95% CI 0.58–5.81, P-trend = 0.15). When examined 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. Interestingly, 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).

DISCUSSION

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 cardiovascular 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)

	Quartile 1			Quartile 2			Quartile 3			Quartile 4			
Range of sTNF-RII (pg ml^{-1})													
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													
	No.	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	***P*-trend[a]**
Age adjusted[b]	135	58	Referent	136	62	1.09 (0.76–1.56)	137	76	1.28 (0.90–1.81)	136	103	1.78 (1.27–2.48)	0.0005
Multivariate[c]	135	58	Referent	136	62	0.93 (0.63–1.38)	137	76	1.17 (0.80–1.70)	136	103	1.48 (1.02–2.16)	0.006
Colorectal cancer mortality													
	No.	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	***P*-trend**
Age adjusted[b]	135	34	Referent	136	35	1.04 (0.64–1.70)	137	44	1.25 (0.77–2.01)	136	50	1.49 (0.95–2.35)	0.12
Multivariate[c]	135	34	Referent	136	35	1.05 (0.62–1.78)	137	44	1.25 (0.75–2.10)	136	50	1.23 (0.72–2.08)	0.34
Non-colorectal cancer-specific mortality													
	No.	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	**No.**	**Events**	**HR (95% CI)**	***P*-trend[a]**
Age adjusted[b]	135	24	Referent	136	27	1.12 (0.63–2.00)	137	32	1.29 (0.75–2.21)	136	53	2.18 (1.31–3.63)	0.0002
Multivariate[c]	135	24	Referent	136	27	0.84 (0.45–1.55)	137	32	1.00 (0.55–1.83)	136	53	1.91 (1.09–3.37)	0.002

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.

[a] P-trend calculated by using sTNF-RII as a continuous variable in the Cox model.

[b] HRs, 95% CIs, and P-values are adjusted for age at blood draw (years).

[c] Multivariate HRs, 95% CIs, and P-values are calculated using a meta-analysis (fixed effects model) by cohort, stratified by stage (I–III, 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.

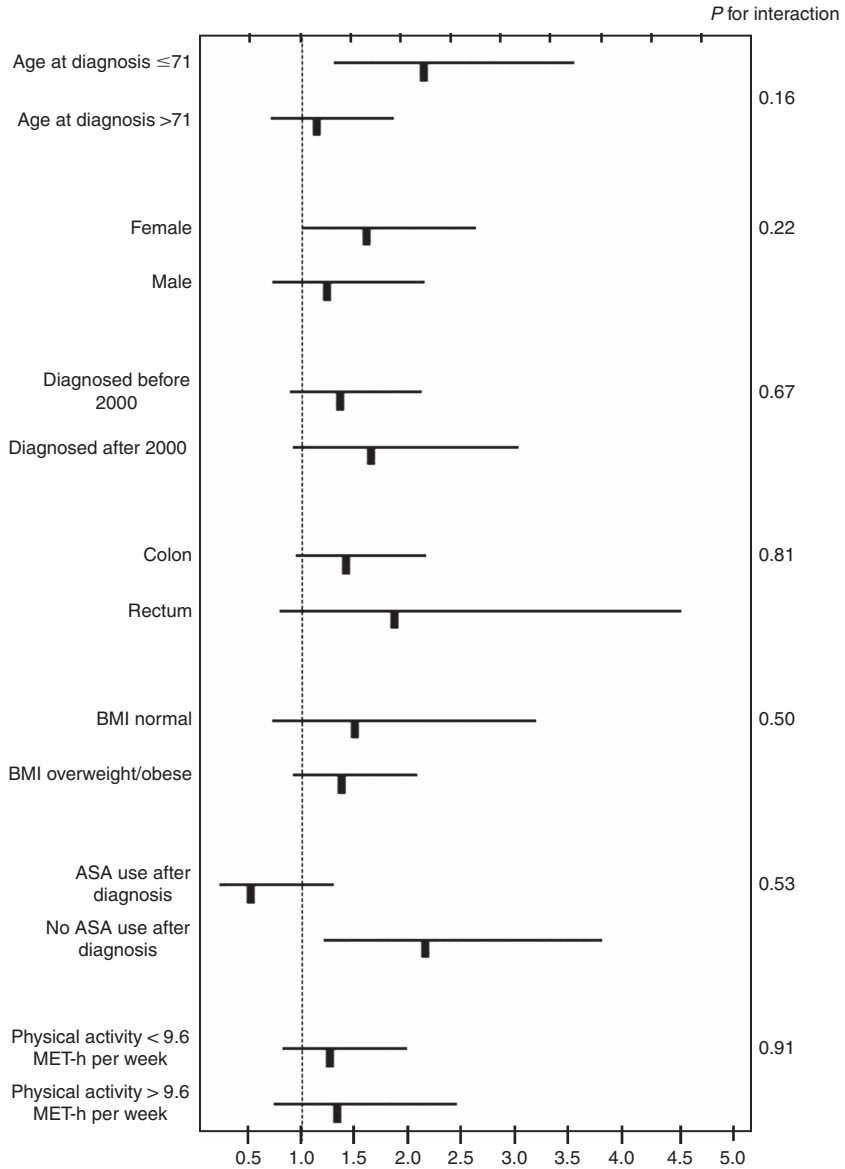


Figure 3. Relationship between sTNF-RII and overall mortality in prespecified patient subgroups. Multivariate hazard ratios (HRs) and 95% confidence intervals (CIs) for overall mortality across subgroups of various factors, comparing CRC patients in the highest quartile of plasma sTNF-RII levels with 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).

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-α-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-α 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

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 did not 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.

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