Supplemental Materials

Supplement to: Cao *et al.*, Regular Aspirin Use Associates with Lower Risk of Colorectal Cancers With Low Numbers of Tumor-infiltrating Lymphocyte

Supplemental Table 1. Age-standardized characteristics comparing cases with and without immunity data

Characteristic	NHS		HPFS	
	Cases with immunity data	Cases without immunity data	Cases with immunity data	Cases without immunity data
Cases, No.	830	928	628	632
Age at diagnosis, y*	68.3 (61.4-73.5)	66.6 (60.0-73.6)	72.3 (65.4-77.6)	70.7 (64.0-77.6)
Family history of cancer, %	20	20	18	17
History of diabetes, %	10	10	10	13
BMI, kg/m²	25.1 (22.7-28.6)	24.9 (22.5-28.2)	25.7 (24.0-27.6)	25.9 (24.2-28.2)
Postmenopause, %	91	92	-	-
Menopausal hormone therapy, %	23	18	-	-
History of colonoscopy/sigmoidoscopy, %	37	32	45	45
Current use of multivitamin, %	52	49	49	46
Regular use of NSAIDs, %	24	20	15	12
Physical activity, MET-hrs/wk	11.1 (5.4-20.1)	11.3 (4.6-20.9)	23.0 (10.1-41.2)	19.9 (8.8-37.1)
Pack-year among ever smokers	24 (10-43)	25 (11-45)	23 (13-40)	24 (13-43)
Total calorie, kcal/d	1673 (1394-1956)	1615 (1358-1936)	1878 (1584-2312)	1859 (1563-2287)
Alcohol intake, g/d	2.0 (0.4-7.8)	2.5 (0.3-8.7)	8.1 (1.8-21.0)	7.4 (1.3-17.4)
Red and processed meat, servings/wk	5.8 (3.9-7.9)	5.9 (4.1-8.3)	5.8 (3.6-9.1)	5.9 (3.2-8.9)
Calcium, mg/d	880 (681-1114)	850 (648-1110)	834 (679-1100)	831 (645-1102)
Folate, µg/d/	386 (297-531)	376 (274-516)	467 (342-656)	478 (347-679)
Alternate Healthy Eating Index (AHEI)† 2010†	46.4 (40.6-52.6)	46.7 (40.5-53.4)	47.6 (41.1-55.0)	47.8 (41.1-55.0)
Stage, %				
I	24	24	28	26
II	35	26	28	17
III	27	25	29	21
IV	14	25	14	37
Tumor differentiation, %				
Well or moderately differentiated	88	93	93	96
Pooly differentiated	12	7	7	4
Tumor location, %				
Rectum	21	23	23	28
Distal colon	28	31	31	39
Proximal colon	51	47	46	33

*All values other than age have been directly standardized to age distribution (in 5-year age group) of all the participants. Median (25th-75th percentile) was presented for continuous variables.

†Without alcohol intake

Supplemental Table 2. Regular aspirin use and risk of colorectal cancer by stage and tumor-infiltrating lymphocytes

			Aspirin use	
			Nonregular users	**Regular users**
Stage	**Stage I/II**	Cases, No. (n=769)	459	310
		Multivariable RR (95% CI)*	1 (reference)	0.76 (0.66, 0.89)
	Stage III/IV	Cases, No. (n=554)	333	221
		Multivariable RR (95% CI)*	1 (reference)	0.76 (0.64, 0.91)
	Stage I/II	**Tumor-infiltrating lymphocytes**		
		Low		
		Cases, No. (n=533)	333	200
		Multivariable RR (95% CI)*	1 (reference)	0.68 (0.57, 0.82)
		Intermediate/high		
		Cases, No. (n=236)	126	110
		Multivariable RR (95% CI)*	1 (reference)	0.97 (0.75, 1.26)
	Stage III/IV	**Tumor-infiltrating lymphocytes**		
		Low		
		Cases, No. (n=449)	277	172
		Multivariable RR (95% CI)*	1 (reference)	0.71 (0.59, 0.87)
		Intermediate/high		
		Cases, No. (n=105)	56	49
		Multivariable RR (95% CI)*	1 (reference)	1.03 (0.70, 1.52)

*Adjusted for the same set of covariates as in Table 2.

Supplemental Table 3. Regular aspirin use, tumor-infiltrating lymphocytes and colorectal cancer-specific mortality

	Nonregular users	Regular users
Tumor-infiltrating lymphocytes (TILs)		
Low		
Cases, No.	665	422
Events, No.	219	126
Univariate RR (95% CI)	1 (reference)	0.93 (0.74-1.15)
Multivariable RR (95% CI)*	1 (reference)	0.88 (0.70-1.10)
Intermediate/high		
Cases, No	220	147
Events, No	36	37
Univariate RR (95% CI)	0.51 (0.36-0.73)	0.66 (0.47-0.94)
Multivariable RR (95% CI)*	0.53 (0.36-0.76)	0.66 (0.46-0.96)
$P_{interaction}$†		
Univariate model		0.20
Multivariable model		0.17

*The multivariable Cox regression model initially included sex, age at diagnosis, year of diagnosis, family history of colorectal cancer, prediagnosis body mass index, tumor differentiation, tumor location, microsatellite instability status, CpG island methylator phenotype-specific promoter status, long interspersed nucleotide element-1 hypomethylation level, PTGS2 expression, and *KRAS*, *BRAF*, *PIK3CA* mutations. A backward elimination with a threshold *P* of 0.05 was used to select variables for the final models.

†$P_{interaction}$ (two-sided) was calculated using the Wald test for the cross-product of regular aspirin use (regular vs. nonregular) and TILs (intermediate/high vs low) in the Cox regression model.

Supplemental Table 4. Regular use of NSAIDs and risk of colorectal cancer overall and by components of lymphocytic reaction

	NHS		HPFS		Combined		
	Nonregular users*	Regular users*	Nonregular users*	Regular users*	Nonregular users*	Regular users*	$P_{heterogenity}$
Total colorectal cancer							
Person-years	1237311	1184473	453472	522072	1690783	1706545	
Cases, No. (n=1458)	426	404	286	342	712	746	
Age-adjusted RR (95% CI)	1 (reference)	0.82 (0.71, 0.94)	1 (reference)	0.89 (0.75, 1.04)	1 (reference)	0.85 (0.76, 0.94)	
Multivariable RR (95% CI)†	1 (reference)	0.82 (0.71, 0.94)	1 (reference)	0.99 (0.79, 1.25)	1 (reference)	0.85 (0.76, 0.94)	
Tumor-infiltrating lymphocytes (TILs)							
Low							
Cases, No. (n=1087)	311	280	234	262	545	542	
Age-adjusted RR (95% CI)	1 (reference)	0.78 (0.67, 0.92)	1 (reference)	0.82 (0.69, 0.99)	1 (reference)	0.80 (0.71, 0.90)	0.03
Multivariable RR (95% CI)†	1 (reference)	0.78 (0.66, 0.92)	1 (reference)	0.83 (0.69, 1.00)	1 (reference)	0.80 (0.71, 0.91)	0.04
Intermediate							
Cases, No. (n=221)	70	72	36	43	106	115	
Age-adjusted RR (95% CI)	1 (reference)	0.86 (0.62, 1.20)	1 (reference)	0.94 (0.60, 1.50)	1 (reference)	0.89 (0.68, 1.16)	
Multivariable RR (95% CI)†	1 (reference)	0.87 (0.62, 1.21)	1 (reference)	0.95 (0.60, 1.50)	1 (reference)	0.89 (0.68, 1.17)	
High							
Cases, No. (n=146)	44	51	15	36	59	87	
Age-adjusted RR (95% CI)	1 (reference)	0.99 (0.66, 1.49)	1 (reference)	1.73 (0.94, 3.19)	1 (reference)	1.18 (0.85, 1.66)	
Multivariable RR (95% CI)†	1 (reference)	1.00 (0.66, 1.50)	1 (reference)	1.70 (0.92, 3.14)	1 (reference)	1.18 (0.85, 1.65)	
Intratumoral periglandular reaction							
Low							
Cases, No. (n=191)	61	52	34	44	95	96	0.08
Age-adjusted RR (95% CI)	1 (reference)	0.73 (0.50, 1.06)	1 (reference)	0.74 (0.47, 1.16)	1 (reference)	0.73 (0.55, 0.98)	0.09
Multivariable RR (95% CI)†	1 (reference)	0.72 (0.50, 1.05)	1 (reference)	0.74 (0.47, 1.17)	1 (reference)	0.73 (0.55, 0.97)	
Intermediate							
Cases, No. (n=1077)	307	295	227	248	534	543	
Age-adjusted RR (95% CI)	1 (reference)	0.84 (0.71, 0.98)	1 (reference)	0.85 (0.71, 1.03)	1 (reference)	0.84 (0.75, 0.95)	
Multivariable RR (95% CI)†	1 (reference)	0.84 (0.71, 0.98)	1 (reference)	0.86 (0.72, 1.04)	1 (reference)	0.85 (0.75, 0.96)	
High							
Cases, No. (n=186)	54	57	25	50	79	107	
Age-adjusted RR (95% CI)	1 (reference)	0.89 (0.61, 1.29)	1 (reference)	1.40 (0.86, 2.30)	1 (reference)	1.05 (0.78, 1.41)	
Multivariable RR (95% CI)†	1 (reference)	0.89 (0.61, 1.30)	1 (reference)	1.36 (0.83, 2.23)	1 (reference)	1.05 (0.78, 1.41)	
Peritumoral lymphocytic							

reaction							
Low							
Cases, No. (n=205)	60	55	39	51	99	106	
Age-adjusted RR (95% CI)	1 (reference)	0.79 (0.55, 1.14)	1 (reference)	0.76 (0.50, 1.17)	1 (reference)	0.78 (0.59, 1.03)	0.06
Multivariable RR (95% CI)†	1 (reference)	0.79 (0.54, 1.14)	1 (reference)	0.77 (0.50, 1.18)	1 (reference)	0.78 (0.59, 1.03)	0.07
Intermediate							
Cases, No. (n=1012)	297	279	216	220	513	499	
Age-adjusted RR (95% CI)	1 (reference)	0.82 (0.69, 0.97)	1 (reference)	0.82 (0.67, 0.99)	1 (reference)	0.82 (0.72, 0.93)	
Multivariable RR (95% CI)†	1 (reference)	0.82 (0.69, 0.97)	1 (reference)	0.83 (0.68, 1.01)	1 (reference)	0.82 (0.72, 0.93)	
High							
Cases, No. (n=231)	63	70	30	68	93	138	
Age-adjusted RR (95% CI)	1 (reference)	0.93 (0.66, 1.30)	1 (reference)	1.50 (0.96, 2.33)	1 (reference)	1.12 (0.86, 1.46)	
Multivariable RR (95% CI)†	1 (reference)	0.93 (0.66, 1.31)	1 (reference)	1.46 (0.94, 2.27)	1 (reference)	1.11 (0.85, 1.45)	
Crohn's-like lymphoid reaction							
Low							
Cases, No. (n=899)	271	254	177	197	448	451	
Age-adjusted RR (95% CI)	1 (reference)	0.81 (0.68, 0.96)	1 (reference)	0.80 (0.65, 0.98)	1 (reference)	0.80 (0.70, 0.92)	0.24
Multivariable RR (95% CI)†	1 (reference)	0.81 (0.68, 0.96)	1 (reference)	0.80 (0.65, 0.99)	1 (reference)	0.80 (0.70, 0.92)	0.30
Intermediate							
Cases, No. (n=205)	58	53	39	55	97	108	
Age-adjusted RR (95% CI)	1 (reference)	0.79 (0.54, 1.15)	1 (reference)	1.14 (0.74, 1.74)	1 (reference)	0.93 (0.70, 1.23)	
Multivariable RR (95% CI)†	1 (reference)	0.79 (0.54, 1.15)	1 (reference)	1.10 (0.72, 1.68)	1 (reference)	0.91 (0.69, 1.21)	
High							
Cases, No. (n=86)	26	27	12	21	38	48	
Age-adjusted RR (95% CI)	1 (reference)	0.87 (0.51, 1.50)	1 (reference)	1.21 (0.59, 2.49)	1 (reference)	0.98 (0.64, 1.51)	
Multivariable RR (95% CI)†	1 (reference)	0.87 (0.51, 1.50)	1 (reference)	1.15 (0.56, 2.39)	1 (reference)	0.96 (0.63, 1.48)	

*The reference group included individuals who regularly used neither aspirin nor other NSAIDs, and the comparison group included individuals who used aspirin and/or other NSAIDs regularly.

†Adjusted for family history of colorectal cancer (*yes/no*), history of diabetes (*yes/no*), body mass index (*quartile*), history of colonoscopy/sigmoidoscopy (*yes/no*), smoking in pack-years (*never, 0.1-4.9, 5-19.9, 20-39.9, ≥40*), physical activity (*quartile*), alcohol intake (*0, 0.1-4.9, 5-14.9, 15-29.9, ≥30 g/d*), current multivitamin use (*yes/no*), total energy intake (*quartile*), folate (*quartile*), calcium (*quartile*), red and processed meat intake (*quartile*), and Alternate Healthy Eating Index-2010 without alcohol (*quartile*). For women, we additionally adjusted for menopause status/menopausal hormone therapy (MHT) (*premenopausal, postmenopausal and never use of MHT, postmenopausal and past use of MHT, postmenopausal and current use of MHT*). The Cox models were also conditioned on age in months, calendar year of the questionnaire cycle and sex/cohort (in the combined cohort analysis).