Auto-antibodies to p53 and the Subsequent Development of Colorectal Cancer in a U.S. Prospective Cohort Consortium

Julia Butt[1,2], William J. Blot[3], Kala Visvanathan[4], Loïc Le Marchand[5], Lynne R. Wilkens[5], Yu Chen[6], Howard D. Sesso[7,8], Lauren Teras[9], Marc D. Ryser[10], Terry Hyslop[2,11], Sylvia Wassertheil-Smoller[12], Lesley F. Tinker[13], John D. Potter[14,15], Mingyang Song[8,16,17], Sonja I. Berndt[18], Tim Waterboer[1], Michael Pawlita[1], and Meira Epplein[2]

ABSTRACT

Background: Auto-antibodies to tumor suppressor p53 are found in a subset of patients with colorectal cancer. A recent prospective study in the United States has reported a significant 1.8-fold increased odds for colorectal cancer development with prediagnostic seropositivity to p53. In this study, we sought to examine this association in a U.S. colorectal cancer cohort consortium to evaluate the potential utility of p53 auto-antibodies as an early biomarker for colorectal cancer.

Methods: Auto-antibodies to p53 were measured in prediagnostic blood samples of 3,702 incident colorectal cancer cases and 3,702 controls, matched by age, race, and sex, from 9 U.S. prospective cohorts. The association of seropositivity to p53 with colorectal cancer risk, overall and by time between blood draw and diagnosis, was determined by conditional logistic regression.

Results: Overall, 5% of controls and 7% of cases were seropositive to p53, resulting in a statistically significant 33% increased colorectal cancer risk [odds ratio (OR), 1.33; 95% confidence interval (CI), 1.09–1.61]. By follow-up time, the association was only significant with colorectal cancer diagnoses within 4 years after blood draw (OR, 2.27; 95% CI, 1.62–3.19), but not thereafter (OR, 0.97; 95% CI, 0.76–1.24).

Conclusions: In this large consortium of prospective cohorts, we found that prediagnostic seropositivity to tumor suppressor p53 was significantly associated with an over 2-fold increased odds of developing colorectal cancer within 4 years after blood draw.

Impact: Our finding suggests that p53 seropositivity may not be a useful predictor of long-term colorectal cancer risk; however, it might be considered as a marker to aid in the early diagnosis of colorectal cancer.

Introduction

Colorectal cancer is the third most common cancer and the second most common cause of cancer death worldwide with more than 1.8 million newly diagnosed cases and 880,000 deaths in 2018 (1). Diagnosis of the cancer at an early stage has been shown to be associated with better survival (2), and screening by measurement of blood in stool as well as colonoscopy or sigmoidoscopy have been reported to be effective in reducing colorectal cancer mortality (3, 4). However, although colonoscopy is the gold standard for the detection of colorectal cancer and precursor lesions, it is also a costly and invasive procedure (5). The less invasive blood-in-stool test is often impaired by the quality of the self-sampled specimen (6). Moreover, the current best performing assay for testing blood in stool (fecal immunochemical testing) still has limited sensitivity in detecting high-risk precancerous lesions, that is, advanced adenomas (7, 8). Thus, there is an ongoing search for new biomarkers that may identify individuals at risk of developing colorectal cancer, ideally in easily obtainable samples, such as blood.

It is well known that a subset of patients with colorectal cancer present with auto-antibodies directed against the tumor suppressor p53 (9). Missense mutations in the *TP53* gene lead to a loss of function of the protein and accumulation of the protein in the cancer cells to which 20%–40% of patients develop antibodies (9, 10). Various case–control studies have addressed the diagnostic performance of p53 auto-antibodies in detecting prevalent colorectal cancer and a recent

[1]Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany. [2]Cancer Control and Population Sciences Program, Duke Cancer Institute, and Department of Population Health Sciences, Duke University, Durham, North Carolina. [3]Division of Epidemiology, Vanderbilt University Medical Center, Nashville, Tennessee. [4]Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, Maryland. [5]Epidemiology Program, University of Hawai'i Cancer Center, Honolulu, Hawaii. [6]Department of Population Health, New York University School of Medicine, New York, New York. [7]Brigham and Women's Hospital, Boston, Massachusetts. [8]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [9]Behavioral and Epidemiology Research Group, American Cancer Society, Atlanta, Georgia. [10]Department of Population Health Sciences, and Department of Mathematics, Duke University, Durham, North Carolina. [11]Department of Biostatistics and Bioinformatics, Duke University, Durham, North Carolina. [12]Department of Epidemiology & Population Health, Albert Einstein College of Medicine, Bronx, New York. [13]Cancer Prevention Program, Division of Public Health Sciences at Fred Hutchinson Cancer Research Center, Seattle, Washington. [14]Centre for Public Health Research, Massey University, Wellington, New Zealand. [15]Fred Hutchinson Cancer Research Center, Seattle, Washington. [16]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [17]Clinical and Translational Epidemiology Unit and Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. [18]Infections and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Julia Butt, German Cancer Research Center, Im Neuenheimer Feld 280, Heidelberg 69120, Germany. Phone: 49-6221-424605; E-mail: j.butt@dkfz-heidelberg.de

Cancer Epidemiol Biomarkers Prev 2020;29:2729–34

doi: 10.1158/1055-9965.EPI-20-0780

meta-analysis reported a pooled sensitivity of 19% and specificity of 93% (11). There is, however, only very little information available that addresses how long before diagnosis the association between p53 auto-antibodies and colorectal cancer can be observed and, thus, whether p53 serology might aid in identifying patients with colorectal cancer at an early or even premalignant state. Pedersen and colleagues, 2013, tested prediagnostic serial samples from 97 women who developed colorectal cancer and compared these with samples from 97 controls and found auto-antibodies to p53 peptides in 26% of the cases compared with 5% of controls up to 4 years prior to cancer diagnosis (12). A larger, prospective study by Teras and colleagues, 2018, including 392 colorectal cancer cases and 784 controls reported that p53 seropositivity was associated with an overall statistically significant 1.8-fold increased risk for a subsequent colorectal cancer diagnosis and a 2.3-fold increased risk when the analyzed blood sample was drawn within 3 years before colorectal cancer diagnosis (13).

In this study, we sought to assess and potentially validate previous findings on the association of p53 auto-antibodies with the odds of developing colorectal cancer, by time from blood draw to diagnosis, in a consortium of 9 prospective cohort studies in the United States.

Materials and Methods

Study population

This study was conducted within a colorectal cancer cohort consortium designed as described previously (14). Briefly, the overall consortium included 4,063 prospectively ascertained colorectal cancer cases (C180-189, C100, and C209, International Classification of Diseases for Oncology, 3rd edition) and 1:1 matched controls nested within 10 U.S. cohort studies: Campaign Against Cancer and Stroke (CLUE), Cancer Prevention Study II (CPSII), Health Professionals Follow-up Study (HPFS), Multiethnic Cohort Study (MEC), Nurses' Health Study (NHS), NYU Women's Health Study (NYUWHS), Physician's Health Study (PHS), Prostate, Lung, Colorectal, and Ovarian Screening Study (PLCO), Southern Community Cohort Study (SCCS), and Women's Health Initiative (WHI). Controls were selected using incidence density sampling based on age at diagnosis and date of blood draw. Within each cohort, one control was chosen at random for each colorectal cancer case from the appropriate risk sets consisting of all cohort members who were alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case and had provided blood specimens. Matching criteria included sex, race, and date of birth. Because the association of p53 seropositivity and colorectal cancer has already been assessed in the CPSII study (13), we excluded samples from CPSII for this analysis, resulting in a total number of serum samples and sociodemographic data for 3,702 colorectal cancer cases and 3,702 controls. The median time between blood draw and diagnosis for cases was 7 years (range 0–40 years).

p53 Serology

Antibody responses to p53 were measured as part of a larger panel of antigens, including bacterial and viral proteins, using multiplex serology as described previously (13–16). Briefly, full-length p53 (NCBI accession number: NM_000546) was recombinantly expressed as GST-tag fusion protein in *Escherichia coli* BL21 Rosetta (Novagen-Merck) and affinity purified on fluorescently labeled glutathione-coupled beads (Luminex Corp). Distinctly labeled bead types, each carrying a different antigen, were mixed and incubated with serum (diluted 1:1,000). Serum antibodies bound to the antigens were detected using a biotin-labeled secondary antibody (IgA/IgM/IgG) and subsequent incubation with the reporter fluorescent streptavidin-R-phycoerythrin. A Luminex 200 analyzer (Luminex Corp.) then distinguished between the bead type and, consequently, the bound antigen as well as quantified the amount of bound serum antibody. Individuals with a median fluorescence intensity of 200 or more against p53 were defined as p53 seropositive (13). All personnel performing the serology assay were blinded for case–control status of the samples and all samples were analyzed in one batch to avoid potential batch-to-batch effects.

Statistical analysis

Differences in baseline sociodemographic factors between cases and controls, as well as between p53 seronegative and -positive controls, were assessed using χ^2test for categorical variables and Wilcoxon rank-sum test for continuous variables.

Conditional logistic regression was used to determine OR and 95% confidence intervals (CI) for the association of seropositivity to p53 with the odds of developing colorectal cancer. The analysis was performed in the overall sample set, as well as by time between blood draw and diagnosis in 2-year increments. On the basis of these findings, we then divided time between blood draw and diagnosis in two categories (as <4 years or ≥4 years) to summarize the results. Apart from the matching variables age at blood draw, sex, and race/ethnicity, we assessed the effect of *a priori* determined potential confounders, including body mass index (BMI), smoking, education, a history of colonoscopy or sigmoidoscopy, and a family history of colorectal cancer. None of these variables changed the main OR by more than 10% and we therefore present the results from a conditional logistic model, with matched case–control sets as the strata, without further adjustment in the model.

In a subgroup analysis, we assessed the association of p53 seropositivity with odds of developing colorectal cancer by cancer site, stage, and age at diagnosis in cases diagnosed within 4 years after blood draw and their matched controls using conditional logistic regression models. We also assessed whether the association varied by study, sex, and race/ethnicity by applying a multiplicative interaction term for the respective variable with p53 seropositivity. Finally, we assessed the inclusion of data on a previous colonoscopy/sigmoidoscopy (available for $n = 807$ controls and $n = 809$ cases) on the association of p53 seropositivity with colorectal cancer using an unconditional logistic regression model with adjustment for: age, sex, race/ethnicity; stratification by study; and the addition of a multiplicative interaction term.

All statistical analyses were performed using SAS 9.4 (SAS Institute).

Results

Baseline characteristics

At baseline, colorectal cancer cases compared with controls were more frequently obese (BMI ≥30 kg/m^2; 27% and 22%, respectively), more likely to have a family history of colorectal cancer (16% and 13%, respectively), and less likely to have undergone a colonoscopy or sigmoidoscopy prior to study recruitment (43% and 46%, respectively; **Table 1**).

P53 seroprevalence among controls varied by study, ranging from the highest in the SCCS (10%) to the lowest in WHI (2%). It was higher in males (7%) than females (4%) and in non-whites (8%−10%) than whites (4%; **Table 2**).

Association of p53 seropositivity with colorectal cancer risk

Seven percent of all colorectal cancer cases ($n = 242$) were p53 seropositive compared with 5% of controls ($n = 185$), resulting in a 33% increased odds of developing colorectal cancer in the overall

Table 1. Sociodemographic factors in colorectal cancer cases and controls.

Variable	Total ($n = 7,404$)	Controls ($n = 3,702$)	Cases ($n = 3,702$)
Study, *n* (%)			
CLUE	982 (13)	491 (13)	491 (13)
HPFS	302 (4)	151 (4)	151 (4)
MEC	1,510 (20)	755 (20)	755 (20)
NHS	576 (8)	288 (8)	288 (8)
NYUWHS	572 (8)	286 (8)	286 (8)
PHS	360 (5)	180 (5)	180 (5)
PLCO	1,240 (17)	620 (17)	620 (17)
SCCS	252 (3)	126 (3)	126 (3)
WHI	1,610 (22)	805 (22)	805 (22)
Age at blood draw, years			
Median (range)	63 (18–89)	63 (18–88)	64 (18–89)
Sex, *n* (%)			
Female	4,744 (64)	2,372 (64)	2,372 (64)
Male	2,660 (36)	1,330 (36)	1,330 (36)
Race/ethnicity, *n* (%)			
White	5,422 (73)	2,711 (73)	2,711 (73)
African American	792 (11)	396 (11)	396 (11)
Asian American	612 (8)	306 (8)	306 (8)
Latino	422 (6)	211 (6)	211 (6)
Other/unknown/multiracial	156 (2)	78 (2)	78 (2)
Education, *n* (%)			
Less than HS	950 (13)	458 (13)	492 (14)
Completed HS or GED	1,515 (21)	750 (20)	765 (21)
Post HS training other than college	304 (4)	157 (4)	147 (4)
Some college	1,492 (20)	748 (20)	744 (20)
College graduate	1,330 (18)	682 (19)	648 (18)
Graduate school	1,707 (23)	865 (24)	842 (23)
BMI (kg/m^2), *n* (%)			
<30	4,770 (75)	2,470 (78)	2,300 (73)
≥30	1,564 (25)	701 (22)	863 (27)
Smoking, *n* (%)			
Never	3,303 (45)	1,685 (46)	1,618 (44)
Former	2,983 (41)	1,439 (39)	1,544 (42)
Current	1,030 (14)	536 (15)	484 (14)
Ever had colonoscopy/sigmoidoscopy, *n* (%)			
No	3,003 (56)	1,453 (54)	1,550 (57)
Yes	2,404 (44)	1,252 (46)	1,152 (43)
Family history of colorectal cancer, *n* (%)			
No	4,558 (85)	2,329 (87)	2,229 (84)
yes	794 (15)	356 (13)	438 (16)

Abbreviations: GED, general educational development test; HS, high school.

consortium (OR, 1.33; 95% CI, 1.09–1.61). Analysis of the association by time between blood draw and diagnosis showed that p53 seroprevalence remained fairly stable in controls over time, varying between 3% and 6% (average 5%). In colorectal cancer cases, however, p53 seroprevalence was highest (15%) in those with a colorectal cancer diagnosis within 2 years and lowest (3%) in those with a colorectal cancer diagnosis 14 or more years after blood draw (**Fig. 1A**). The association of p53 seropositivity with odds of developing colorectal cancer was statistically significant only in the subgroups diagnosed within less than 2 years (OR, 2.73; 95% CI, 1.67–4.45) and between 2 and 4 years (OR, 1.89; 95% CI, 1.17-3.03) after blood draw (**Fig. 1B**). Combining these two time-interval categories, the OR for developing colorectal cancer within 4 years after blood draw associated with p53 seropositivity was 2.27 (95% CI, 1.62–3.19). For any longer period between blood draw and diagnosis, there was no statistically significant association between p53 seropositivity and odds of developing colorectal cancer (**Fig. 1B**; OR for colorectal cancer diagnosis more than 4 years after blood draw: 0.97; 95% CI, 0.76–1.24).

Analysis by case characteristics, including site, stage, and age at diagnosis, as well as by demographic factors of study, sex, and race, did not identify a specific group of colorectal cancer cases that was particularly strongly associated with baseline p53 seropositivity (**Table 3**; Supplementary Table S1). Moreover, stratification by screening history of colonoscopy or sigmoidoscopy had no statistically significant effect on the association of p53 seropositivity and odds of developing colorectal cancer within 4 years after blood draw (no screening history: OR, 2.44; 95% CI, 1.46–4.10; history of screening:

Table 2. Variables associated with p53 seropositivity among controls.

Variable	Total *n*	p53 negative	p53 positive
Study, *n* (%)			
CLUE	491	477 (97)	14 (3)
HPFS	151	138 (91)	13 (9)
MEC	755	688 (91)	67 (9)
NHS	288	273 (95)	15 (5)
NYUWHS	286	274 (96)	12 (4)
PHS	180	171 (95)	9 (5)
PLCO	620	597 (96)	23 (4)
SCCS	126	114 (90)	12 (10)
WHI	805	785 (98)	20 (2)
Age at blood draw, years			
Median (range)	3,702	63 (18–88)	65 (29–87)
Sex, *n* (%)			
Female	2,372	2,281 (96)	91 (4)
Male	1,330	1,236 (93)	94 (7)
Race/ethnicity, *n* (%)			
White	2,711	2,615 (96)	96 (4)
African American	396	362 (91)	34 (9)
Asian American	306	274 (90)	32 (10)
Latino	211	195 (92)	16 (8)
Other/unknown/multiracial	78	71 (91)	7 (9)
Education, *n* (%)			
Less than HS	458	438 (96)	20 (4)
Completed HS or GED	750	712 (95)	38 (5)
Post HS training other than college	157	151 (96)	6 (4)
Some college	748	711 (95)	37 (5)
College graduate	682	640 (94)	42 (6)
Graduate school	865	824 (95)	41 (5)
BMI (kg/m^2), *n* (%)			
<30	2,470	2,335 (95)	135 (5)
≥30	701	667 (95)	34 (5)
Smoking, *n* (%)			
Never	1,685	1,607 (95)	78 (5)
Former	1,439	1,363 (95)	76 (5)
Current	536	507 (95)	29 (5)
History of colonoscopy/sigmoidoscopy, *n* (%)			
No	1,453	1,385 (95)	68 (5)
Yes	1,252	1,172 (94)	80 (6)
Family history of colorectal cancer, *n* (%)			
No	2,329	2,211 (95)	118 (5)
Yes	356	333 (94)	23 (6)

Abbreviations: GED, general educational development test; HS, high school.

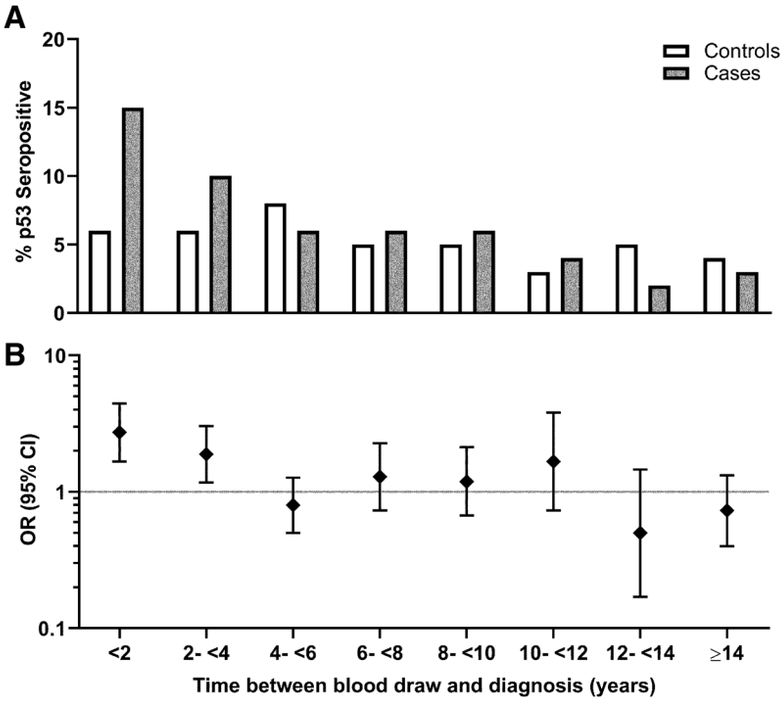

Figure 1.

p53 seroprevalence and association with colorectal cancer risk by time interval between blood draw and diagnosis. **A,** The p53 seroprevalence in colorectal cancer cases and controls. **B,** The association of p53 seropositivity with colorectal cancer risk, both in 2-year increments of time interval between blood draw and diagnosis. Controls are matched to cases by study, age, sex, and race/ethnicity. OR (diamond) and 95% CI (whiskers) in **B** were estimated by conditional logistic regression without further adjustment, the horizontal line at an OR of 1 serves as a reference for null association.

OR, 2.08; 95% CI, 1.26–3.43, $P_{\text{interaction}} = 0.496$; Supplementary Table S1).

Discussion

In this large colorectal cancer cohort consortium, we found that auto-antibodies to the tumor suppressor p53 were statistically significantly associated with an over 2-fold increased odds of developing colorectal cancer within 4 years after the analyzed blood sample was drawn. There was no association of p53 auto-antibodies with colorectal cancer when colorectal cancer was diagnosed 4 or more years after blood draw.

Our study is concordant with previously published studies to the extent that the observed association of p53 auto-antibodies with colorectal cancer is limited to a relatively short time period prior to colorectal cancer diagnosis. Pedersen and colleagues, 2013, reported seroconversion to p53 in longitudinal samples as early as 4 years and on average 2 years prior to colorectal cancer diagnosis (12). Teras and colleagues analyzed their prospective study in time intervals of <3, 3 to <6, and ≥6 years between blood draw and diagnosis. P53 seropositivity was found to be statistically significantly associated with an increased 2.3-fold colorectal cancer risk only within the shortest time interval of <3 years (13). With our larger numbers, we were able to analyze our consortium data in

Table 3. p53 seropositivity association with risk of developing colorectal cancer within less than 4 years after blood draw, by case characteristics.

	N controls/ cases	p53 seropositive controls, *n* (%)	p53 seropositive cases, *n* (%)	OR[a]	95% CI
Diagnosed within <4 years after blood draw	944/944	55 (6)	116 (12)	**2.27**	**1.62–3.19**
By site					
Right colon	443/443	21 (5)	46 (10)	**2.47**	**1.41–4.34**
Left colon	268/268	19 (7)	34 (13)	**1.83**	**1.03–3.26**
Rectum	157/157	11 (7)	28 (18)	**2.89**	**1.35–6.17**
By stage					
I/II	395/395	22 (6)	44 (11)	**2.05**	**1.22–3.45**
III/IV	274/274	23 (8)	41 (15)	**1.90**	**1.11–3.27**
By age at diagnosis					
≤65	339/339	19 (6)	46 (14)	**2.69**	**1.51–4.77**
66–75	391/391	23 (6)	35 (9)	1.57	0.91–2.72
>75	214/214	13 (6)	35 (16)	**3.00**	**1.52–5.94**

[a]Conditional logistic regression, adjusted for the matching factors only. A *P* value <0.05 was considered statistically significant (marked in bold font).

shorter time intervals (2-year increments) between blood draw and diagnosis. We replicated the findings of the two previous studies in that the association of p53 seropositivity with colorectal cancer risk is particularly strong the shorter the interval between blood draw and diagnosis, with a 2.7-fold and 1.9-fold increased risk with a diagnosis within 2 years and between 2 and 4 years after blood draw, respectively. Expression of mutated p53 is hypothesized to be a late event in colorectal carcinogenesis, most likely at the transition from adenoma to cancer (17–19), which would explain the absence of association of colorectal cancer risk with auto-antibodies to p53 in blood samples drawn with a longer time interval before diagnosis. This is further supported by a study that assessed p53 seroprevalence in nonadvanced and advanced adenoma cases and did not find associations (20). Thus, p53 serology is unlikely to be applied as a biomarker for precancerous lesions.

The potential diagnostic performance of p53 auto-antibodies in detecting colorectal cancer is further limited because of the low prevalence in colorectal cancer cases. A recent meta-analysis reported a pooled seroprevalence of 19% among cases and 7% among controls in 11 case–control studies (11). The p53 seroprevalence in our study is in line with these findings with 15% within 2 years prior to diagnosis and only 10% for 2 to 4 years prior to diagnosis, while on average 5% of controls were seropositive. Teras and colleagues, using the same methodology to measure auto-antibodies to p53, reported a similar seroprevalence of 13% in cases that were diagnosed within 3 years after blood draw (13). Pedersen and colleagues, however, analyzed their study using an array of p53 15-mer peptides and reported a seroprevalence of 26% among cases and 5% among controls with the best performing peptides (12). Thus, modification of the p53 antigen might lead to higher seroprevalences among cases. Previous literature has also discussed the inclusion of p53 into a panel of auto-antigens. Werner and colleagues, for example, combined p53 with carcinoembryonic antigen, osteopontin, seprase, and ferritin and detected 50% of colonoscopy-confirmed colorectal cancer cases compared with 10% of confirmed healthy controls. (21). A systematic review by Chen and colleagues, showed marker panels including p53 that detected up to 88% of colorectal cancer cases; however, as the authors concluded, these panels warrant further validation in screening settings to assess noninferiority to gold-standard screening (22). In addition, auto-antibodies to p53 have been found with other cancers besides those of the colorectum, for example in individuals with breast, lung, and gastric cancer, as well as ovarian cancer and others (9); incorporating other auto-antigens could increase specificity to detect certain cancers.

A limitation of our study is the lack of knowledge of the underlying reasons for auto-antibodies to p53 in our control population. We observed differences in seroprevalence varying between 2% and 10% by sex, race/ethnicity, and concordantly by study, which could suggest prevalent cancers in our control population, biasing the OR toward the null. The highest seroprevalence was observed in the SCCS (10%), HPFS (9%), and MEC (9%). MEC and HPFS did not exclude controls who had a cancer diagnosis (except colorectal cancer) after diagnosis of the index case. As pointed out above, other cancers have been described to be associated with auto-antibodies to p53 (9), which could explain the presence of p53 seropositivity in a proportion of controls. However, for example, in the MEC, only 6 of the 67 p53 seropositive controls were linked to a cancer diagnosis after diagnosis of the index case. This does not explain the substantially higher number of p53 seropositives compared with other studies. Another explanation could be differences in the level of immune response by sex and/or race. It has been shown that sex and race influence the immune response elicited by an individual (23), which could also relate to differences in the adaptive immune response against auto-antigens. This hypothesis needs to be further elucidated and should be considered when attempting to implement antibody markers in cancer screening. As mentioned above controls selected by the majority of cohorts in this consortium potentially developed cancer after diagnosis of the index case. In four cohorts, however, (WHI, PHS, NYUWHS, and SCCS) controls were selected as supercontrols, in that they did not develop cancer within the follow-up of the study. This, in turn, may introduce a bias that leads to an overestimation of our results. A sensitivity analysis excluding these cohorts showed that the results did not differ: overall 8% of cases were p53 seropositive compared with 6% of controls (OR, 1.38; 95% CI, 1.10–1.74); within 4 years after blood draw 15% of cases were seropositive versus 7% controls (OR, 2.20; 95% CI, 1.51–3.20). This suggests that selection of supercontrols in some of the cohorts did not overestimate our findings. Major strengths of our study, however, are the large sample size; data on stage, tumor site, and age at diagnosis for the majority of cases; and evidence that the association was present across all categories of case characteristics. We moreover showed that a prior colonoscopy or sigmoidoscopy did not modify the association of p53 seropositivity with odds of developing colorectal cancer. However, we lacked information on the time since the last colorectal cancer screening for the majority of study participants and were thus unable to perform a more detailed analysis of screening.

In conclusion, this study provides evidence that auto-antibodies directed against p53 are associated with increased odds of developing colorectal cancer but only for a subset of cancer cases and within a short time period before diagnosis. Thus, auto-antibodies to p53 may not be a useful predictor of long-term colorectal cancer risk, but could be considered within a marker panel for the early detection of colorectal cancer.

Disclosure of Potential Conflicts of Interest

W.J. Blot reports grants from NIH during the conduct of the study. K. Visvanathan reports grants from NIH during the conduct of the study. L. Le Marchand reports grants from NCI (to institution) during the conduct of the study. T. Hyslop reports grants from NCI during the conduct of the study, as well as personal fees from AbbVie (not oncology related) outside the submitted work. M. Epplein reports grants from NCI during the conduct of the study. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

J. Butt: Conceptualization, data curation, formal analysis, visualization, methodology, writing–original draft, writing–review and editing. **W.J. Blot:** Resources, data curation, writing–review and editing. **K. Visvanathan:** Resources, data curation, writing–review and editing. **L. Le Marchand:** Resources, data curation, writing–review and editing. **L.R. Wilkens:** Resources, data curation, writing–review and editing. **Y. Chen:** Resources, data curation, writing–review and editing. **H.D. Sesso:** Resources, data curation, writing–review and editing. **L. Teras:** Resources, data curation, writing–review and editing. **M.D. Ryser:** Formal analysis, writing–review and editing. **T. Hyslop:** Data curation, formal analysis, writing–review and editing. **S. Wassertheil-Smoller:** Resources, data curation, writing–review and editing. **L.F. Tinker:** Resources, data curation, writing–review and editing. **J.D. Potter:** Resources, data curation, writing–review and editing. **M. Song:** Resources, data curation, writing–review and editing. **S.I. Berndt:** Resources, data curation, writing–review and editing. **T. Waterboer:** Resources, data curation, methodology, writing–review and editing. **M. Pawlita:** Resources, data curation, methodology, writing–review and editing. **M. Epplein:** Conceptualization, resources, data curation, formal analysis, supervision, writing–original draft.

Acknowledgments

The NCI, NIH, U.S. Department of Health and Human Services funds this consortium (R01 CA190428 to M. Epplein); the Southern Community Cohort Study (U01 CA202979 to W.J. Blot); the NYU Women's Health Study (U01 CA182934 to Y. Chen); the MEC (U01 CA164973 to L. Le Marchand); the NHS/HPFS (U01 CA167552, UM1 CA186107, P01 CA087969, and UM1CA167552); the PHS (R01 CA097193, R01 CA040360, and R01 HL034595); and R00 CA215314 to M. Song. The WHI program is funded by the National Heart, Lung, and Blood Institute, NIH, U.S. Department of Health and Human Services through contracts, HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C. CLUE thanks the participants and staff for their contributions, as well as the Maryland Cancer Registry, Center for Cancer Surveillance and Control, Department of Health and Mental Hygiene (http://phpa.dhmh.maryland.gov/cancer).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 20, 2020; revised August 1, 2020; accepted September 21, 2020; published first September 24, 2020.

References

1. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. 2018 Global Cancer Observatory: Cancer Today. Lyon (France): International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today.
2. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. CA Cancer J Clin 2017;67:177–93.
3. Brenner H, Stock C, Hoffmeister M. Effect of screening sigmoidoscopy and screening colonoscopy on colorectal cancer incidence and mortality: systematic review and meta-analysis of randomised controlled trials and observational studies. BMJ 2014;348:g2467.
4. Hewitson P, Glasziou P, Watson E, Towler B, Irwig L. Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update. Am J Gastroenterol 2008;103:1541–9.
5. Pawa N, Arulampalam T, Norton JD. Screening for colorectal cancer: established and emerging modalities. Nat Rev Gastroenterol Hepatol 2011;8:711–22.
6. Gies A, Gruner LF, Schrotz-King P, Brenner H. Effect of imperfect compliance with instructions for fecal sample collection on diagnostic performance of 9 fecal immunochemical tests. Clin Gastroenterol Hepatol 2019;17:1829–39.
7. Brenner H, Tao S. Superior diagnostic performance of faecal immunochemical tests for haemoglobin in a head-to-head comparison with guaiac based faecal occult blood test among 2235 participants of screening colonoscopy. Eur J Cancer 2013;49:3049–54.
8. Wong MC, Ching JY, Chan VC, Lam TY, Shum JP, Luk AK, et al. Diagnostic accuracy of a qualitative fecal immunochemical test varies with location of neoplasia but not number of specimens. Clin Gastroenterol Hepatol 2015;13:1472–9.
9. Soussi T. p53 Antibodies in the sera of patients with various types of cancer: a review. Cancer Res 2000;60:1777–88.
10. Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. Nat Rev Cancer 2009;9:701–13.
11. Meng R, Wang Y, He L, He Y, Du Z. Potential diagnostic value of serum p53 antibody for detecting colorectal cancer: a meta-analysis. Oncol Lett 2018;15:5489–96.
12. Pedersen JW, Gentry-Maharaj A, Fourkala EO, Dawnay A, Burnell M, Zaikin A, et al. Early detection of cancer in the general population: a blinded case-control study of p53 autoantibodies in colorectal cancer. Br J Cancer 2013;108:107–14.
13. Teras LR, Gapstur SM, Maliniak ML, Jacobs EJ, Gansler T, Michel A, et al. Prediagnostic antibodies to serum p53 and subsequent colorectal cancer. Cancer Epidemiol Biomarkers Prev 2018;27:219–23.
14. Butt J, Varga MG, Blot WJ, Teras L, Visvanathan K, Le Marchand L, et al. Serologic response to helicobacter pylori proteins associated with risk of colorectal cancer among diverse populations in the United States. Gastroenterology 2019;156:175–86.e2.
15. Butt J, Blot WJ, Teras LR, Visvanathan K, Le Marchand L, Haiman CA, et al. Antibody responses to streptococcus gallolyticus subspecies gallolyticus proteins in a large prospective colorectal cancer cohort consortium. Cancer Epidemiol Biomarkers Prev 2018;27:1186–94.
16. Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. Clin Chem 2005;51:1845–53.
17. Baker SJ, Preisinger AC, Jessup JM, Paraskeva C, Markowitz S, Willson JK, et al. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res 1990;50:7717–22.
18. Laurent-Puig P, Blons H, Cugnenc PH. Sequence of molecular genetic events in colorectal tumorigenesis. Eur J Cancer Prev 1999;8:S39–47.
19. Srivastava S, Verma M, Henson DE. Biomarkers for early detection of colon cancer. Clin Cancer Res 2001;7:1118–26.
20. Chen H, Werner S, Butt J, Zornig I, Knebel P, Michel A, et al. Prospective evaluation of 64 serum autoantibodies as biomarkers for early detection of colorectal cancer in a true screening setting. Oncotarget 2016;7:16420–32.
21. Werner S, Krause F, Rolny V, Strobl M, Morgenstern D, Datz C, et al. Evaluation of a 5-marker blood test for colorectal cancer early detection in a colorectal cancer screening setting. Clin Cancer Res 2016;22:1725–33.
22. Chen H, Werner S, Tao S, Zornig I, Brenner H. Blood autoantibodies against tumor-associated antigens as biomarkers in early detection of colorectal cancer. Cancer Lett 2014;346:178–87.
23. Brodin P, Davis MM. Human immune system variation. Nat Rev Immunol 2017;17:21–9.