Cancer Pathogenesis and Therapy 3 (2025) 60–67

Contents lists available at ScienceDirect

Cancer Pathogenesis and Therapy

journal homepage: www.journals.elsevier.com/cancer-pathogenesis-and-therapy

Research article

Fecal methylated syndecan-2 (*SDC2*) testing for early screening of colorectal cancerous and precancerous lesions: A real-world retrospective study in China☆

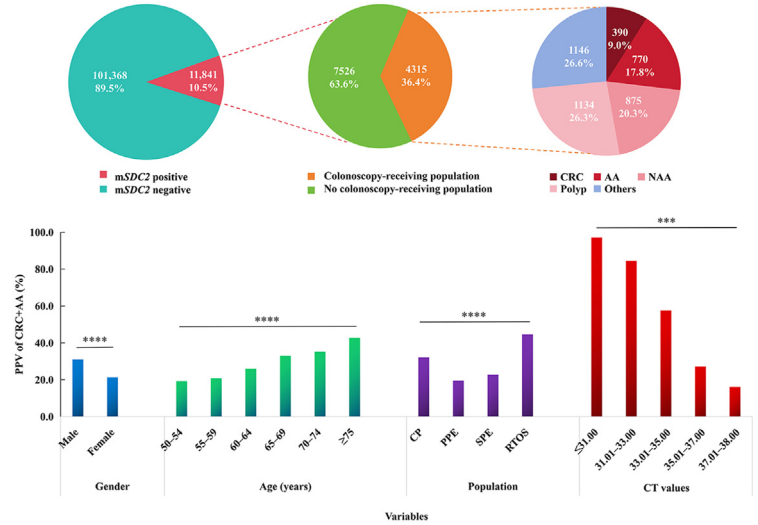
Boyu Qin [a,1], Haitao Niu [b,1], Lupeng Qiu [c,1], Hongfeng Zhou [d,**], Peng Lyu [e,f,g,*]

[a] *Department of Oncology, The Fifth Medical Center of PLA General Hospital, Beijing 100071, China*
[b] *National Health Commission of the People's Republic of China, Beijing 100044, China*
[c] *Medical School of Chinese PLA, Beijing 100853, China*
[d] *Health Management Department, Foresea Life Insurance Guangzhou General Hospital, Guangzhou, Guangdong 510000, China*
[e] *Cancer Pathogenesis and Therapy, Chinese Medical Association Publishing House, Beijing 100052, China*
[f] *Key Laboratory of Knowledge Mining and Service for Medical Journals, National Press and Publication Administration, Beijing 100052, China*
[g] *Beijing Beiya Hospital of Traditional Chinese Medicine, Beijing 100029, China*

HIGHLIGHTS

- A stool-based single-target syndecan-2 (*SDC2*) methylation test showed robust performance in detecting colorectal neoplasms based on a comprehensive analysis of the testing data of >110,000 participants.
- A detailed analysis of the testing performance associated with clinical and pathological characteristics was conducted for populations stratified as clinical patients, personal physical examinations, staff physical examinations, and rural town-based organized screening.
- The positive rate and positive predictive value for colorectal cancer and advanced adenomas combined were 10.5% (2002/19,082) and 26.7% (197/738), respectively, and tended to increase with age.
- The overall colonoscopy compliance rate was 36.4% (4315/11,841), which significantly increased to 62.7% (547/872) in the organized colorectal cancer screening program.
- The cycle threshold values can be used to indicate the likelihood of detecting colorectal neoplasms.

GRAPHICAL ABSTRACT



Schematic representation of performance of fecal *SDC2* methylation test in detecting advanced neoplasms in real-world practice. Upper panel: pie charts showing positive detection rate, colonoscopy compliance rate, and actual number of colorectal lesions found. Lower panel: bar graphs showing PPV of advanced neoplasms (CRC + AA) in different genders, age categories, classified populations, and strata of CT values. ***$P < 0.001$,****$P < 0.0001$. AA: Advanced adenoma; CP: Clinical patients group; CRC: Colorectal cancer; CT: Cycle threshold; m*SDC2*: Methylated syndecan-2; NAA: Non-advanced adenoma; PPE: Personal physical examination group; PPV: Positive predictive value; RTOS: Rural town-based organized screening group; SPE: Staff physical examination group.

☆ Given his role as Managing Editor, Dr.Peng Lyu had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Prof. Yuankai Shi.

* Corresponding author: *Cancer Pathogenesis and Therapy*, Chinese Medical Association Publishing House; Key Laboratory of Knowledge Mining and Service for Medical Journals, National Press and Publication Administration, Beijing 100052, China; Beijing Beiya Hospital of Traditional Chinese Medicine, Beijing 100029, China.

** Corresponding author: Health Management Department, Foresea Life Insurance Guangzhou General Hospital, Guangzhou, Guangdong 510000, China
E-mail addresses: 13609737049@139.com (H. Zhou), lvpeng51@yeah.net (P. Lyu).

[1] Boyu Qin, Haitao Niu and Lupeng Qiu contributed equally to this work.

https://doi.org/10.1016/j.cpt.2024.02.002
Received 25 November 2023; Received in revised form 3 February 2024; Accepted 20 February 2024

ARTICLE INFO

Managing Editor: Peng Lyu

Keywords:
Colorectal cancer
Syndecan-2
DNA methylation
Diagnosis

ABSTRACT

Background: Colorectal cancer (CRC) is a major public health concern and the second leading cause of cancer-related deaths worldwide. However, challenges remain in deploying effective screening strategies for early-stage CRC. This study aimed to evaluate the effectiveness of a fecal-based syndecan-2 (*SDC2*) methylation test for the detection of colorectal lesions and CRC.

Methods: We retrospectively collected data on participants who underwent fecal *SDC2* methylation testing from January 1, 2019, to May 30, 2023. Patients with positive results were recommended to undergo colonoscopy. Performance indicators associated with certain clinical characteristics, including positive rate (PR), positive predictive value (PPV), and colonoscopy compliance rate (CCR), were subjected to statistical analysis.

Results: We analyzed data from 113,209 participants, of whom 11,841 (10.4% PR) had positive fecal *SDC2* methylation test results. A total of 4315 participants with positive results adhered to the colonoscopy recommendations, and the CCR was 36.4%. Finally, 3169 colorectal lesions were detected, including 1134 polyps, 875 non-advanced adenomas (NAAs), 770 advanced adenomas (AAs), and 390 CRCs, with PPV values of 26.3% (1134/4315), 20.3% (875/4315), 17.8% (770/4315), and 9.0% (390/4315), respectively. Notably, the PPV for CRC increased significantly with age ($\chi^2 = 164.40$, $P < 0.0001$). In addition, as the cycle threshold (CT) values increased, the PPVs of AAs and CRCs generally decreased, whereas those of NAAs and polyps significantly increased. Moreover, the clinical patient group had the highest incidence of late-stage CRC (stage II and higher), whereas asymptomatic populations from the staff physical examination group and rural town-based screening programs had the highest number of stage 0 and I CRCs detected ($P = 0.0107$).

Conclusions: This study indicates that fecal *SDC2* methylation testing combined with colonoscopy may be an effective screening method for colorectal lesions and CRC.

Introduction

Colorectal cancer (CRC) is a common malignancy globally.[1,2] According to GLOBOCAN 2020, the incidence and mortality rates of CRC are increasing, with an estimated 555,000 new cases and 286,000 deaths in China.[1] Furthermore, the 5-year survival rate of patients with stage I and II CRC is approximately 90%, whereas survival rates decline to 71% and 14% in patients with stage III and IV disease, respectively.[3–5] Therefore, the lower rate of early CRC diagnosis greatly affects the prognosis of these patients.[6,7]

Most CRCs develop over 10–15 years, and early screening and intervention are the most effective ways to reduce the incidence and mortality thereof.[8–10] There are currently several screening methods for CRC, including colonoscopy and fecal tests. Colonoscopy is considered the gold standard for CRC screening; however, the invasive nature thereof and the low compliance rate of patients make it unsuitable for large-scale population screening. Chen et al.[11] reported that 182,927 participants aged 40–69 years from 16 provinces in China were screened for CRC from 2012 to 2015. Only 25,593 participants undertook colonoscopy as recommended, with a participation rate of 14.0%.

A systematic review and meta-analysis revealed that colonoscopy cannot detect all colonic lesions and may even miss the diagnosis of CRC.[12] Commonly used fecal-based tests include the guaiac-based fecal occult blood test (gFOBT) and fecal immunochemical test (FIT); however, the sensitivity of these tests remains relatively low.[13,14] Therefore, there is a need to develop a non-invasive and highly accurate CRC screening tool. Developing and implementing strategies to increase colonoscopy compliance is the key to improving the efficiency of CRC screening in high-risk populations.

Colonic epithelial cells are shed into the gut lumen and excreted with stool daily, and CRC cells are more likely to fall off owing to factors such as abnormal proliferation and reduced adhesion between cells or basement membranes. Therefore, fecal samples from CRC patients contain a large number of abnormally proliferating cells, which can provide a stable source of material for fecal detection.[15,16] Syndecan-2 (SDC2) is a transmembrane proteoglycan located on the cell surface that plays a vital role in CRC.[17] Recently, methylated *SDC2* (m*SDC2*) has emerged as a biomarker to screen colorectal lesions and early-stage CRC. Furthermore, the fecal *SDC2* methylation test is non-invasive, convenient, and consistent for the detection of malignancy.[18,19] In a multicenter clinical trial, Wang et al.[20] reported that hypermethylation of *SDC2* could be detected in fecal samples of CRC patients with a sensitivity of 83.8% and specificity of 98.0%, suggesting that m*SDC2* may be used as a robust biomarker for CRC detection. Consequently, a methylation detection kit for human *SDC2* was developed and approved by the National Medical Product Administration (NMPA) of China in 2018. A recent quality assessment evaluated the ability of clinical laboratories in China to accurately detect *SDC2* methylation in fecal deoxyribonucleic acid (DNA), showing that 90.6% of the participating laboratories provided satisfactory results.[21] Furthermore, Li et al.[22] reported that the area under the curve (AUC) value of fecal *SDC2* methylation in CRC screening was 0.981, which was significantly higher than those of serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19–9 (CA19-9). However, to date, no large-scale data are available for the performance analysis of single-target stool-based methylation tests in real-world practice.

We, therefore, aimed to analyze fecal-based *SDC2* methylation test results of >110,000 test-takers, spanning a period of >4 years. We evaluated the positive rate (PR), colonoscopy compliance rate (CCR), and positive predictive value (PPV), stratified by sex, age, and cycle threshold (CT) values. We further investigated the tumor node metastasis (TNM) stage, tumor location, and state of differentiation of CRCs detected in the pre-defined screening populations. Our findings highlight the fecal *SDC2* methylation test as a new and attractive modality for CRC screening and provide new insights into effective strategies for both opportunistic and organized CRC screening programs.

Methods

Participants and samples

We retrospectively collected testing data from participants who provided stool samples for *SDC2* methylation testing between January 1, 2019, and May 30, 2023, at the Medical Testing Laboratory of Creative Biosciences, Inc. (Guangzhou, China). The inclusion criteria for these participants were (1) age of 50 years or older; and (2) all participants with qualified fecal *SDC2* methylation test results. The exclusion criteria were as follows: (1) inadequate amount or insufficient quality of stool samples; (2) stool samples that failed fecal *SDC2* methylation testing; and (3) colorectal lesions treated and excised within half a month prior to fecal testing. In total, data from 113,209 eligible participants were analyzed.

Eligible participants were generally classified into four detection groups according to the different ways in which they were involved in

testing. The clinical patient group included patients with various alimentary symptoms who visited hospitals and clinics. They were high-risk CRC patients who underwent fecal *SDC2* methylation testing and received a final diagnosis at these clinical sites. The personal physical examination group included participants who personally purchased fecal *SDC2* methylation tests via physical examination centers, offline and online pharmacies, and other retail channels. Participants in this group generally had a stronger tendency toward active health management and awareness of cancer screening. The staff physical examination group refers to employees from enterprises and institutions who participate in regular physical examination programs offered by their employers. The subjects in this group elected to undergo fecal *SDC2* methylation tests during their health checkups. The rural town-based organized screening population refers to the residents of Shipai Town, Dongguan City, Guangdong Province, and Shitan Town, Guangzhou City, Guangdong Province who participated in the organized screening activities for early detection of CRC using fecal *SDC2* methylation test in a period of time spanning 2021 and 2022.

Clinical characteristics

We collected data on gender, age, laboratory indicators, colonoscopy results, and pathology reports if available of the included participants, and then analyzed the association between fecal-based *SDC2* methylation test results and colorectal lesions including polyps, NAAs, AAs, and CRC. CRC cases with known TNM stage, location, and state of differentiation, as well as those with unknown stage, location, and state of differentiation, were included in the final analysis. We also analyzed the relationship between *SDC2* methylation CT values and the distribution of intestinal lesions in *SDC2* methylation-positive participants.

Fecal sample collection, processing, and testing

Fresh fecal samples (4.5 g) were collected from each participant as required, placed in a stool preservation solution, and mailed to the Medical Testing Laboratory of Creative Biosciences at room temperature. Stool samples were subsequently subjected to nucleic acid extraction and sequence-specific capture of target genes using magnetic beads and bisulfite treatment, followed by fluorescence quantitative methylation-specific polymerase chain reaction (PCR) (qMSP). The CT value of the qMSP was used as the primary outcome measure.[20] CT values $\leq$38.00 were considered positive according to the manufacturer's instructions.

Colonoscopy examinations

Participants with positive test results were recommended to undergo colonoscopy examinations within 3 months. Endoscopic procedures with or without anesthesia were performed after standard bowel preparation by registered colonoscopists. A pathological biopsy is used to identify any suspicious lesions annotated according to size, morphology, and location during colonoscopy, which can provide a basis for diagnosis and treatment options. Colorectal lesions were categorized as polyps, NAAs, AAs, and CRCs.[11] TNM stage was determined according to the eighth edition of the American Joint Committee on Cancer TNM staging system.[23] An adenoma with a size $\geq$10 mm in diameter, or with sufficient tubulovillous or villous components, or with high-grade dysplasia in the absence of invasive CRC is commonly referred to as an AA.[24] All colonoscopic examinations were performed.

Statistical analysis

The primary outcomes of the current study included the fecal *SDC2* methylation test PR, CCR, and PPV for detecting CRC and precancerous lesions. Categorical variables were reported as counts and proportions and comparison of groups were compared using the χ^2 test. Stratified data analyses according to age and CT values were conducted using the Cochran–Armitage test. All analyses were performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). Results from the statistical data analysis were visualized using R version 4.2 for Windows (R Software, Vienna, Austria, www.R-project.org). $P < 0.05$ was considered as statistically significant.

Results

Clinical features and test results

For the final analysis, we included 113,209 participants (52.6% males) with valid fecal *SDC2* methylation test results [Table 1]. All enrolled participants were at least 50 years old, with a mean age of 58.4. Most participants were in the age ranges of 50–54 and 55–59 years, accounting for 30.5% and 26.5% of the entire population, respectively, suggesting that these two age groups were more active in methylation testing for CRC. The age category of $\geq$75 years had the least number of participants, accounting for only 7.7% of all the participants, showing the reluctance of people in this age group to take the test. More than half of the participants (54.4%) were from the personal physical examination group, in which people went to physical examination institutions and online or offline pharmacies for routine physical examinations. The clinical patient group was the next largest, accounting for 20.9% of the population. Only 24.8% of the participants were from organized screening programs, further categorized as the staff physical examination group and rural town-based screening population [Table 1].

Our results further showed that 11.6% of males were *SDC2* methylation-positive compared to 9.2% of females, and the difference was statistically significant ($\chi^2 = 161.40$, $P < 0.0001$), which is in accordance with a higher rate of colorectal lesions in males than in females in China. When participants were divided into the six strata of 50–54, 55–59, 60–64, 65–69, 70–74, and $\geq$75 years of age, fractions of *SDC2* methylation-positive patients grow substantially with increasing age categories, reaching 22.7% for participants aged 75 years or older ($\chi^2 = 2306.00$, $P < 0.0001$ [Figure 1 and Table 1]). The PRs of *SDC2* methylation were highest in the clinical patient group (14.0%), followed by the staff physical examination group (10.5%), rural town-based screening group (9.8%), and personal physical examination group (9.2%), demonstrating that the clinical patient group had the highest risk of being tested m*SDC2* methylation [Figure 2 and Table 1].

Colonoscopy examinations in syndecan-2 methylation-positive participants

In total, 4315 participants underwent complete colonoscopy after testing positive for *SDC2* methylation, including 2463 males (35.8%) and 1852 females (37.4%). CCR was the highest in the age range of 60–64 years (42.2%), followed by those aged 55–59 years (41.9%) and 50–54 years (40.1%) [Figure 1 and Table 1]. The age group 75 years or older had the lowest CCR of 18.7%. The rural town-based screening population showed the highest compliance (62.7%), followed by clinical patients (37.6%), staff physical examinations (36.9%), and personal physical examinations (31.6%) [Figure 2 and Table 1]. Significantly higher CCRs in the rural town-based screening population and staff physical examination group may be due to the organized nature of the testing.

In total, 3169 participants (3169/4315, 73.4%) who underwent colonoscopy examinations were diagnosed with colorectal lesions, of whom 1980 were males and 1189 were females ($\chi^2 = 142.00$, $P < 0.0001$). The PPV of colorectal lesions in participants aged 65 years or older was 79.3% (1252/1578), compared with 70.6% (1917/2737) in participants aged 50–64 years. The rural-town-based screening group demonstrated the highest PPV of 81.7%, whereas the personal physical examination group had the lowest PPV of 68.1%, corresponding to the highest and lowest CCR rates, respectively.

Table 1
Analyses of PRs and PPVs for different kinds of colorectal lesions.

Variables	No. of m*SDC2* detection, *n* (%)	No. of m*SDC2*+, *n* (PR, %)	Receiving-colonoscopy population of m*SDC2*+, *n* (CCR, %)	CRC, *n* (PPV, %)	AA, *n* (PPV, %)	NAA, *n* (PPV, %)	Polyp, *n* (PPV, %)	Colorectal lesion, *n* (PPV, %)
Gender								
Male	59,582 (52.6)	6885 (11.6)	2463 (35.8)	247 (10.0)	518 (21.0)	531 (21.6)	684 (27.8)	1980 (80.4)
Female	53,627 (47.4)	4956 (9.2)	1852 (37.4)	143 (7.7)	252 (13.6)	344 (18.6)	450 (24.3)	1189 (64.2)
	χ^2	161.40	3.17	6.84	39.75	5.83	6.58	142.00
	P	<0.0001	0.0751	0.0089	<0.0010	0.0160	0.0100	<0.0001
Age (years)								
50–54	34,553 (30.5)	2387 (6.9)	957 (40.1)	36 (3.8)	148 (15.5)	181 (18.9)	262 (27.4)	627 (65.5)
55–59	30,032 (26.5)	2510 (8.4)	1052 (41.9)	66 (6.3)	153 (14.5)	231 (22.0)	301 (28.6)	751 (71.4)
60–64	16,039 (14.2)	1726 (10.8)	728 (42.2)	62 (8.5)	127 (17.4)	160 (22.0)	190 (26.1)	539 (74.0)
65–69	14,902 (13.2)	1863 (12.5)	695 (37.3)	72 (10.4)	157 (22.6)	137 (19.7)	181 (26.0)	547 (78.7)
70–74	8912 (7.9)	1361 (15.3)	511 (37.5)	61 (11.9)	119 (23.3)	106 (20.7)	125 (24.5)	411 (80.4)
≥75	8771 (7.7)	1994 (22.7)	372 (18.7)	93 (25.0)	66 (17.7)	60 (16.1)	75 (20.2)	294 (79.0)
	χ^2	2306.00	344.30	164.40	32.60	8.41	11.64	61.85
	P	<0.0001	<0.0001	<0.0001	<0.0010	0.1350	0.0400	<0.0001
	χ^2 for trend	45.83	−14.48	11.56	4.18	−0.88	−2.91	7.43
	P for trend	<0.0010	<0.0010	<0.0010	<0.0010	0.3800	0.0040	<0.0010
Population								
CP	23,640 (20.9)	3302 (14.0)	1241 (37.6)	207 (16.7)	192 (15.5)	225 (18.1)	303 (24.4)	927 (74.7)
PPE	61,564 (54.4)	5665 (9.2)	1789 (31.6)	111 (6.2)	238 (13.3)	340 (19.0)	530 (29.6)	1219 (68.1)
SPE	19,082 (16.9)	2002 (10.5)	738 (36.9)	47 (6.4)	121 (16.4)	197 (26.7)	211 (28.6)	576 (78.0)
RTOS	8923 (7.9)	872 (9.8)	547 (62.7)	25 (4.6)	219 (40.0)	113 (20.7)	90 (16.5)	447 (81.7)
	χ^2	419.20	320.00	125.30	214.74	24.17	41.86	54.04
	P	<0.0001	<0.0001	<0.0001	<0.0010	<0.0010	<0.0010	<0.0001

AA: Advanced adenoma; CCR: Colonoscopy compliance rate; CP: Clinical patients group; CRC: Colorectal cancer; m*SDC2*: Methylated syndecan-2; NAA: Non-advanced adenoma; PPE: Personal physical examination group; PPV: Positive predictive value; RTOS: Rural town-based organized screening group; SPE: Staff physical examination group.

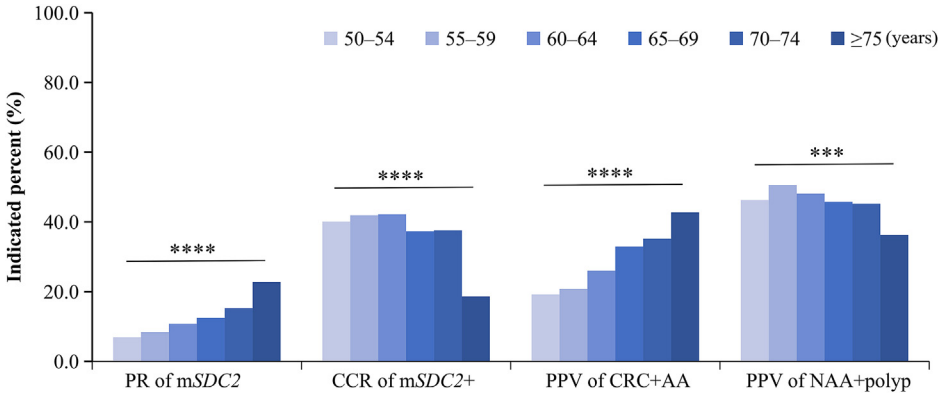


Figure 1. Changes of PRs, CCRs, and PPVs of colorectal neoplasms with categorical age groups. ***$P < 0.001$,****$P < 0.0001$. AA: Advanced adenoma; CCR: Colonoscopy compliance rate; CRC: Colorectal cancer; m*SDC2*: Methylated syndecan-2; NAA: Non-advanced adenoma; PPV: Positive predictive value; PR: Positive rate.

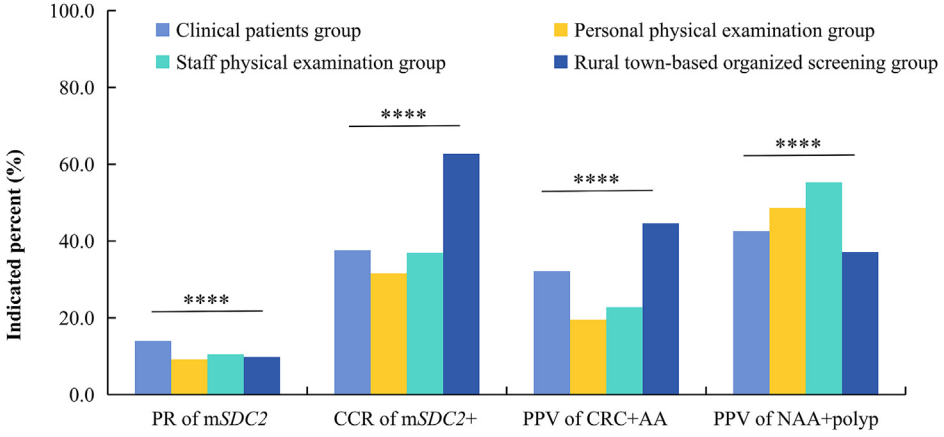


Figure 2. PRs, CCRs, and PPVs of colorectal neoplasms in four pre-defined populations. ****$P < 0.0001$. AA: Advanced adenoma; CCR: Colonoscopy compliance rate; CRC: Colorectal cancer; m*SDC2*: Methylated syndecan-2; NAA: Non-advanced adenoma; PPV: Positive predictive value; PR: Positive rate.

A total of 390 participants (9.0%) with *SDC2* methylation-positive results were diagnosed with CRC after colonoscopy, including 247 males (10.0%) and 143 females (7.7%). Overall, the PPV for CRC progressively increased with age ($\chi^2 = 164.40$, $P < 0.0001$). The PPV of CRC for participants aged 75 years or older was as high as 25.0%, while it declined to only 3.8% for those aged 50–54 years. Almost half of the CRC diagnoses were from the clinical patient group (16.7%), followed by the staff physical examination group (6.4%), the personal physical examination group (6.2%), and the rural town-based screening group (4.6%). Additionally, we analyzed the PPVs of advanced adenomas (AAs), non-advanced adenomas (NAAs), and polyps according to sex and age. Overall, the PPVs of these lesions were higher in males than females ($\chi^2 = 142.00$, $P < 0.0001$). The group of participants aged 65 years or older had a significantly higher PPV for AAs than the group aged 50–64 years (21.7% *vs.* 15.6%, $P = 0.0005$). However, there were no statistically significant differences between the different age groups in NAAs ($\chi^2 = 5.65$, $P = 0.3421$) and polyps ($\chi^2 = 6.98$, $P = 0.2219$), as shown in Table 1.

Association of syndecan-2 methylation level with colorectal lesions

The mean CT value of the 4315 participants who were *SDC2* methylation-positive and underwent colonoscopy was 36.5 (95% confidence interval [CI]: 30–38). Among all the CT values, only 2.5% were ≤31.00, 2.7% were >31.01 and ≤ 33.00, 7.0% were >33.01 and ≤ 35.00, 36.6% were >35.01 and ≤ 37.00, and 51.2% were >37.01 and ≤ 38.00 [Table 2]. As the CT values increased, the PPVs of colorectal lesions ($\chi^2 = 126.83$, $P < 0.0010$) and CRC ($\chi^2 = 1427.07$, $P < 0.001$) decreased significantly as shown in Figure 3. In participants with CT values ≤ 31.00, the PPVs of colorectal lesions and CRC were 100.0% and 81.1%, respectively, whereas they decreased to 68.4% and 2.0% in participants with CT values > 37.01 and ≤ 38.00 [Table 2 and Figure 3], suggesting that CT values might be used to predict the likelihood of being diagnosed with CRC. However, for NAAs and polyps, the PPVs increased while CT values increased from CT values ≤ 31.00 (1.9% and 0.9%) to CT values > 37.01 and ≤ 38.00 (22.0% and 30.4%) (NAAs: $\chi^2 = 57.69$, $P < 0.0010$; polyps: $\chi^2 = 90.05$, $P < 0.0010$) [Table 2 and Figure 3].

Clinical and pathological features of colorectal cancer in four types of groups

We subsequently analyzed the data on the TNM stage, tumor location, and tumor differentiation states of the 390 CRC patients [Table 3]. Among them, 118 participants had stage 0 and I tumors (30.3%), 65 had stage II and III tumors (16.7%), two had stage IV tumors (0.5%), and 205 had unknown stage tumors (52.6%). The tumor locations were 11.8% (46/390) proximal, 75.6% (295/390) distal, and 12.6% (49/390) unknown. The majority of tumors had either moderate differentiation (155/390 patients, 39.7%) or unknown states (212 patients, 54.4%). Only a small proportion of tumors were either well or poorly differentiated. As for the four pre-defined groups, the clinical patient group had the highest incidence of stage II and higher CRC (42/99, 42.4%), followed by the personal physical examination group (21/45, 46.7%), rural town-based screening group (2/24, 8.3%), and staff physical examination group (2/17, 11.8%). The latter two groups organized screening programs intended for asymptomatic participants. Regarding tumor location and differentiation status, the differences among the four pre-defined groups were not statistically significant ([Table 3] $P = 0.1978$ and 0.8817, respectively).

Discussion

The fecal *SDC2* methylation test has been shown to have robust sensitivity and superior specificity in independent clinical trials involving high-risk hospital-based cohorts.[20] However, its performance in large-scale screening of average-risk asymptomatic populations has not been reported. The current study reported PRs in different pre-defined asymptomatic populations, including the personal physical examination group (9.2%), staff physical examination group (10.5%), and rural town-based screening group (9.8%) for the single-target DNA methylation test. These values are generally higher than those reported for gFOBT, FIT, and FOBTs, which are most commonly used in CRC screening in average-risk populations.[25,26] The test PPVs for CRC and AA were 26.9% (1160/4315) for all participants and 24.8% (761/3074) for the three asymptomatic groups combined, which were comparable to the values reported for FIT.[25,26] More importantly, the proportion of stage 0 and I CRC dramatically increased to 46.8% and 60.0% in the staff physical examination and rural town-based screening groups, respectively. When CRCs of unknown stages were excluded, they further increased to 91.7% (22/24) and 88.2% (15/17), respectively, suggesting that significantly more early-stage CRCs were detected. Furthermore, an even larger proportion of AAs were detected, particularly in the rural town-based screening group, a screening-naïve population, with PPV values as high as 40%. Consequently, these patients were given timely surgical interventions and treatment, which may have led to an increased 5-year survival rate, and hence, a reduced CRC mortality rate. Hence, our analysis of data from over 110,000 participants clearly shows that the fecal *SDC2* methylation test is an effective screening tool for the early detection of CRC and advanced colorectal neoplasms in both high-risk symptomatic and average-risk asymptomatic populations.

The adherence rate to colonoscopy can have a large impact on the final outcomes using non-invasive screening modalities such as the fecal *SDC2* methylation test and FIT.[27] The adherence rate to colonoscopy as the sole screening modality has been notoriously low in China, even among high-risk populations in urban areas,[28,29] and the adherence rate to colonoscopy referral after a single FIT-positive result has only marginally improved to 30.7%, as reported by a large-scale screening of over half a million Chinese individuals.[30] In the current study, among the 11,814 participants who tested positive for *SDC2* methylation, 4315 participants subsequently received colonoscopy recommendations and completed the procedures. The overall CCR of 36.5% (4315/11,814) was

Table 2
Distribution pattern of different kinds of colorectal lesions associated with CT values.

CT values	Receiving-colonoscopy population of m*SDC2*+, *n* (%)	CRC, *n* (PPV, %)	AA, *n* (PPV, %)	NAA, *n* (PPV, %)	Polyp, *n* (PPV, %)	CRC + AA, *n* (PPV, %)	Colorectal lesion, *n* (PPV, %)
≤31.00	106 (2.5)	86 (81.1)	17 (16.0)	2 (1.9)	1 (0.9)	103 (97.2)	106 (100.0)
31.01–33.00	116 (2.7)	77 (66.4)	21 (18.1)	5 (4.3)	10 (8.6)	98 (84.5)	113 (97.4)
33.01–35.00	302 (7.0)	87 (28.8)	87 (28.8)	38 (12.6)	48 (15.9)	174 (57.6)	260 (86.1)
35.01–37.00	1581 (36.6)	95 (6.0)	335 (21.2)	344 (21.8)	404 (25.6)	430 (27.2)	1178 (74.5)
37.01–38.00	2210 (51.2)	45 (2.0)	310 (14.0)	486 (22.0)	671 (30.4)	355 (16.1)	1512 (68.4)
	χ^2	1427.07	59.03	57.69	90.05	739.03	126.83
	P	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010
	χ^2 for trend	−33.99	−3.86	7.21	9.47	−27.04	−11.14
	P for trend	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010

AA: Advanced adenoma; CRC: Colorectal cancer; CT: Cycle threshold; m*SDC2*: Methylated syndecan-2; NAA: Non-advanced adenoma; PPV: Positive predictive value.

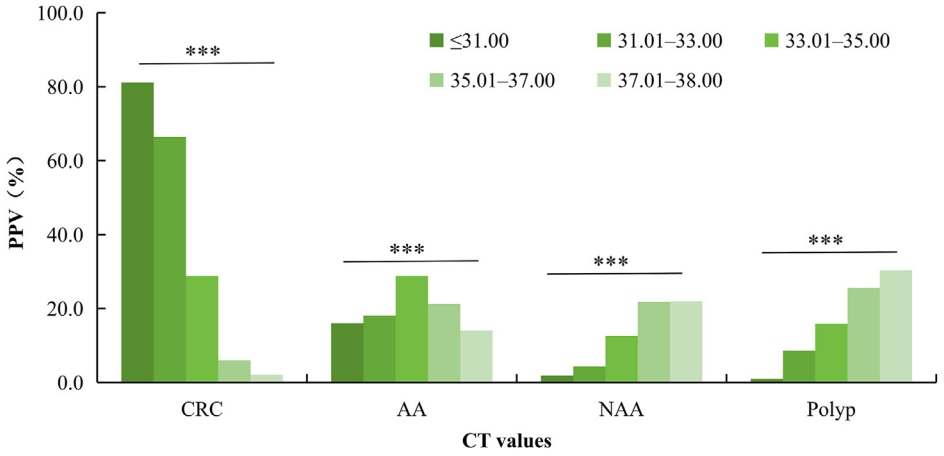


Figure 3. Trends of PPVs of colorectal neoplasms stratified according to CT values. ***$P < 0.001$. AA: Advanced adenoma; CRC: Colorectal cancer; CT: Cycle threshold; NAA: Non-advanced adenoma; PPV: Positive predictive value.

Table 3
Clinicopathological features of CRCs in four population groups.

Variables	CRC, *n* (%)	CP, *n* (%)	PPE, *n* (%)	SPE, *n* (%)	RTOS, *n* (%)	*P**
TNM stage						
0–I	118 (30.3)	57 (27.5)	24 (21.6)	22 (46.8)	15 (60.0)	0.0107
II–III	65 (16.7)	41 (19.8)	20 (18.0)	2 (4.3)	2 (8.0)	
IV	2 (0.5)	1 (0.5)	1 (0.9)	0 (0)	0 (0)	
Unknown	205 (52.6)	108 (52.2)	66 (59.5)	23 (48.9)	8 (32.0)	
Location						
Proximal	46 (11.8)	22 (10.6)	15 (13.5)	3 (6.4)	6 (24.0)	0.1978
Distal	295 (75.6)	161 (77.8)	77 (69.4)	38 (80.9)	19 (76.0)	
Unknown	49 (12.6)	24 (11.6)	19 (17.1)	6 (12.8)	0 (0)	
Differentiation						
High	12 (3.1)	7 (3.4)	4 (3.6)	1 (2.1)	0 (0)	0.8817
Moderate	155 (39.7)	82 (39.6)	47 (42.3)	17 (36.2)	9 (36.0)	
Low	11 (2.8)	5 (2.4)	5 (4.5)	1 (2.1)	0 (0)	
Unknown	212 (54.4)	113 (54.6)	55 (49.5)	28 (59.6)	16 (64.0)	

**P* values were calculated by excluding unknown cases. CP: Clinical patients group; CRC: Colorectal cancer; PPE: Personal physical examination group; RTOS: Rural town-based organized screening group; SPE: Staff physical examination group; TNM: Tumor node metastasis.

comparable to that of the clinical patient group (37.6%), personal physical examination group (31.6%), and staff physical examination group (36.9%). The CCRs for the three asymptomatic groups were the lowest in the personal physical examination group (31.6%), medium in the staff physical examination group (36.9%), and highest in the rural town-based screening population (62.7%). Sequentially improved colonoscopy compliance from opportunistic to organized screening activities was positively correlated with increased PPVs of CRCs and AAs in the three groups of 19.5% (349/1789), 22.8% (168/738), and 44.6% (244/547), respectively. In the organized screening program for the rural town-based group, participants with *SDC2*-positive results were brought into a colonoscopy referral, support, and follow-up system to improve their CCR. In addition, all colonoscopic examinations were conducted under anesthesia and were fully subsidized by the fecal *SDC2* screening program. Therefore, the 62.7% adherence rate to colonoscopy recommendations is more than double the published rates.[28–30] However, the highest CCR observed in the current study still lags behind 80% and 91% reported for randomly controlled trials of FIT screening.[25,26] More work, including persistent patient education to increase public awareness of screening effectiveness, additional monetary incentives for completing colonoscopy examinations, and keen promotion of active participation in organized screening programs by well-respected physicians and colonoscopists need to be conducted to further improve colonoscopy adherence.

To some extent, CT values can indicate the relative abundance of *SDC2* methylation in stool. Zhang et al.[31] found that the CT values can improve the sensitivity of the CRC detection than the methylation level based on the $2^{-\Delta\Delta Ct}$ value. In another study, CT value was used as an indicator of *SDC2* methylation, which could improve CRC screening efficiency.[32] In the current study, the majority of CRCs were detected with CT values at or below 37.00 (345/390, 88.5%), and the highest PPV of CRC was observed in the CT range of ≤31.00 (86/106, 81.1%). However, most AAs had CT values between 33.01 and 38.00 (732/770, 95.1%), and the highest PPV was observed in the range of $33.01 < CT \leq 35.00$ (87/770, 28.8%). Nevertheless, these data stratified according to CT values suggest that a CT of 38.00 to dichotomize the test results as positive and negative, is an appropriate threshold value that could detect a large number of CRCs and AAs in real-world clinical practice.

The current study has certain limitations. First, data presented here for analysis were collected retrospectively. The accuracy and coverage of some stratified analyses were affected by the participants' limited and incomplete information. The impact of fecal *SDC2* methylation testing on the reduction in mortality and other long-term effects could not be estimated and analyzed because of the lack of follow-up data from patients with confirmed CRC diagnosis. Future prospective studies should be conducted to accurately assess the effectiveness of fecal *SDC2* methylation testing in detecting CRC and reducing mortality. Second, a large proportion of *SDC2*-positive participants did not undergo colonoscopy. Colonoscopy adherence rates were particularly low in the clinical patient (37.6%), personal physical examination (31.6%), and staff physical examination (36.9%) groups. An effective colonoscopy referral and supportive system were not available for people in these groups compared to the organized rural town-based screening program (62.7%). The deployment of such responsive and robust service tools may significantly increase the number of detected CRC, AA, and other colorectal lesions and further improve the efficacy of fecal *SDC2* methylation testing as a result

of improved colonoscopy compliance. Third, although most of the participants were continuously enrolled over a period of 4 years, a selection bias may not be completely avoidable, which was not adjusted accordingly in the current analysis. Fourth, the current study focused on analyzing the data on those participants 50 years or older who had much higher incidence and mortality rates of CRC and were recommended for periodic screening by various expert consensus and screening guidelines.[33] As the incidence rate of young CRC has increased over the past decade and that of old CRC has decreased,[2] further evaluation of the screening efficacy of young people <50 years old is clearly warranted.

In conclusion, this study evaluated the effectiveness of fecal-based *SDC2* methylation tests for the early detection of CRC in a real-world practice setting in China. Our findings indicate that robust fecal *SDC2* methylation testing combined with high-level adherence to colonoscopy examinations may be an effective screening method for colorectal lesions and CRC. Future prospective population studies with similar or larger sample sizes are required to validate these findings.

Authors contribution

Peng Lyu and Hongfeng Zhou designed and supervised this study, and critically reviewed the manuscript. Boyu Qin, Haitao Niu, and Lupeng Qiu performed the analyses, drafted and revised the manuscript. All the authors have read and approved the final version of the manuscript.

Ethics statement

This study was performed in accordance with the principles of the *Declaration of Helsinki* and was approved by the Institutional Review Board of the main participating hospital (No. QHRSLL03-2022-03-011). As this was a retrospective study and data analysis was performed anonymously, this study was exempt from obtaining informed consent from patients.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that generative artificial intelligence (AI) and AI assisted technologies were not used in the writing process or any other process during the preparation of this manuscript.

Funding

None.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to all the participants and researchers who participated in this study.

Data availability statement

The datasets used in the current study are available from the corresponding author on reasonable request.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209–249. https://doi.org/10.3322/caac.21660.
2. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J Clin*. 2023;73:233–254. https://doi.org/10.3322/caac.21772.
3. Allemani C, Matsuda T, Di Carlo V, et al. Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet*. 2018;391:1023–1075. https://doi.org/10.1016/S0140-6736(17)33326-3.
4. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Przegląd Gastroenterol*. 2019;14:89–103. https://doi.org/10.5114/pg.2018.81072.
5. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: a review. *JAMA*. 2021;325:669–685. https://doi.org/10.1001/jama.2021.0106.
6. Gupta S. Screening for colorectal cancer. *Hematol Oncol Clin N Am*. 2022;36:393–414. https://doi.org/10.1016/j.hoc.2022.02.001.
7. Bretthauer M, Løberg M, Wieszczy P, et al. Effect of colonoscopy screening on risks of colorectal cancer and related death. *N Engl J Med*. 2022;387:1547–1556. https://doi.org/10.1056/NEJMoa2208375.
8. Sninsky JA, Shore BM, Lupu GV, Crockett SD. Risk factors for colorectal polyps and cancer. *Gastrointest Endosc Clin N Am*. 2022;32:195–213. https://doi.org/10.1016/j.giec.2021.12.008.
9. Corley DA, Levin TR, Doubeni CA. Adenoma detection rate and risk of colorectal cancer and death. *N Engl J Med*. 2014;370:2541. https://doi.org/10.1056/NEJMc1405329.
10. Kanth P, Inadomi JM. Screening and prevention of colorectal cancer. *BMJ*. 2021;374:n1855. https://doi.org/10.1136/bmj.n1855.
11. Chen H, Li N, Ren J, et al. Participation and yield of a population-based colorectal cancer screening programme in China. *Gut*. 2019;68:1450–1457. https://doi.org/10.1136/gutjnl-2018-317124.
12. Zhao S, Wang S, Pan P, et al. Magnitude, risk factors, and factors associated with adenoma miss rate of tandem colonoscopy: a systematic review and meta-analysis. *Gastroenterology*. 2019;156:1661–1674. https://doi.org/10.1053/j.gastro.2019.01.260.
13. Li JN, Yuan SY. Fecal occult blood test in colorectal cancer screening. *J Dig Dis*. 2019;20:62–64. https://doi.org/10.1111/1751-2980.12712.
14. Zorzi M, Antonelli G, Barbiellini Amidei C, et al. Adenoma detection rate and colorectal cancer risk in fecal immunochemical test screening programs : an observational cohort study. *Ann Intern Med*. 2023;176:303–310. https://doi.org/10.7326/M22-1008.
15. Zygulska AL, Pierzchalski P. Novel diagnostic biomarkers in colorectal cancer. *Int J Mol Sci*. 2022;23:852. https://doi.org/10.3390/ijms23020852.
16. Lee JK, Liles EG, Bent S, Levin TR, Corley DA. Accuracy of fecal immunochemical tests for colorectal cancer: systematic review and meta-analysis. *Ann Intern Med*. 2014;160:171. https://doi.org/10.7326/M13-1484.
17. Jang B, Song HK, Hwang J, et al. Shed syndecan-2 enhances colon cancer progression by increasing cooperative angiogenesis in the tumor microenvironment. *Matrix Biol*. 2022;107:40–58. https://doi.org/10.1016/j.matbio.2022.02.001.
18. Müller D, Győrffy B. DNA methylation-based diagnostic, prognostic, and predictive biomarkers in colorectal cancer. *Biochim Biophys Acta Rev Cancer*. 2022;1877:188722. https://doi.org/10.1016/j.bbcan.2022.188722.
19. Han YD, Oh TJ, Chung TH, et al. Early detection of colorectal cancer based on presence of methylated syndecan-2 (*SDC2*) in stool DNA. *Clin Epigenet*. 2019;11:51. https://doi.org/10.1186/s13148-019-0642-0.
20. Wang J, Liu S, Wang H, et al. Robust performance of a novel stool DNA test of methylated *SDC2* for colorectal cancer detection: a multicenter clinical study. *Clin Epigenet*. 2020;12:162. https://doi.org/10.1186/s13148-020-00954-x.
21. Fan X, Shi C, Wei M, Xiao Y, Wang X. External quality assessment for detection of methylated syndecan 2 (*SDC2*) in China. *Clin Chem Lab Med*. 2022;60:1570–1576. https://doi.org/10.1515/cclm-2022-0206.
22. Li N, Li C, Zhang X, et al. Diagnostic value of human fecal *SDC2* gene in colorectal cancer. *Am J Transl Res*. 2023;15:2843–2849.
23. Weiser MR. AJCC 8th edition: Colorectal cancer. *Ann Surg Oncol*. 2018;25:1454–1455. https://doi.org/10.1245/s10434-018-6462-1.
24. Gupta S, Lieberman D, Anderson JC, Burke CA, Dominitz JA, Kaltenbach T, et al. Recommendations for follow-up after colonoscopy and polypectomy: A consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastrointest Endosc*. 2020;91:463–485. https://doi.org/10.1016/j.gie.2020.01.014.
25. Grobbee EJ, van der Vlugt M, van Vuuren AJ, et al. A randomised comparison of two faecal immunochemical tests in population-based colorectal cancer screening. *Gut*. 2017;66:1975–1982. https://doi.org/10.1136/gutjnl-2016-311819.
26. Passamonti B, Malaspina M, Fraser CG, et al. A comparative effectiveness trial of two faecal immunochemical tests for haemoglobin (FIT). Assessment of test performance and adherence in a single round of a population-based screening programme for colorectal cancer. *Gut*. 2018;67:485–496. https://doi.org/10.1136/gutjnl-2016-312716.
27. D'Andrea E, Ahnen DJ, Sussman DA, Najafzadeh M. Quantifying the impact of adherence to screening strategies on colorectal cancer incidence and mortality. *Cancer Med*. 2020;9:824–836. https://doi.org/10.1002/cam4.2735.
28. Chen HD, Li N, Ren JS, et al. Compliance rate of screening colonoscopy and its associated factors among high-risk populations of colorectal cancer in urban China (in Chinese). *Chin J Prev Med*. 2018;52:231–237. https://doi.org/10.3760/cma.j.issn.0253-9624.2018.03.004.

29. Chen HD, Lu M, Liu CC, et al. Rates on the acceptance of colonoscopy, fecal immunochemical test and a novel risk-adapted screening approach in the screening programs of colorectal cancer as well as related associated factors (in Chinese). *Chin J Prev Med*. 2020;41:1655–1661. https://doi.org/10.3760/cma.j.cn112338-20200227-00196.
30. Wu WM, Wang Y, Jiang HR, et al. Colorectal cancer screening modalities in Chinese population: practice and lessons in Pudong new area of Shanghai, China. *Front Oncol*. 2019;9:399. https://doi.org/10.3389/fonc.2019.00399.
31. Zhang L, Dong L, Lu C, et al. Methylation of *SDC2*/TFPI2 and its diagnostic value in colorectal tumorous lesions. *Front Mol Biosci*. 2021;8:706754. https://doi.org/10.3389/fmolb.2021.706754.
32. Kim CW, Kim H, Kim HR, et al. Colorectal cancer screening using a stool DNA-based *SDC2* methylation test: a multicenter, prospective trial. *BMC Gastroenterol*. 2021;21:173. https://doi.org/10.1186/s12876-021-01759-9.
33. National Clinical Research Center for Digestive Diseases (Shanghai). National Early Gastrointestinal-Cancer Prevention & Treatment Center Alliance (GECA), Chinese Society of Digestive Endoscopy, Chinese Society of Health Management, Digestive Endoscopy Professional Committee of Chinese Endoscopist Association, Endoscopic Health Management And Medical Examination, *et al.* Chinese consensus of early colorectal cancer screening (2019, Shanghai) (in Chinese). *Chin J Intern Med*. 2019;58:736–744. https://doi.org/10.3760/cma.j.issn.0578-1426.2019.10.004.