ORIGINAL RESEARCH—BASIC

The CancerenD24: A Novel Blood Test That Can Prevent Colorectal Cancer

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BACKGROUND AND AIMS: Colorectal cancer (CRC) typically develops over 10–15 years, providing a critical window for early detection and prevention. Precursor lesions, including neoplastic (adenomatous) and non-neoplastic (hyperplastic) polyps, can be detected via colonoscopy, which remains the only current modality capable of identifying precancerous lesions. However, colonoscopy is limited by its invasiveness, cost, and accessibility. This study evaluates CancerenD24, a simple blood-based assay measuring cluster of differentiation 24 (CD24) expression, for its ability to distinguish healthy individuals from those with adenomas or CRC. **METHODS:** A total of 652 participants were enrolled at Tel Aviv Medical Center: 505 healthy controls, 22 with hyperplastic polyps, 25 with adenomatous polyps, and 100 with CRC. Healthy individuals underwent full screening, including colonoscopy, at the Integrated Cancer Prevention Center. Adenoma and CRC patients were recruited from gastroenterology, surgery, and oncology departments. CD24 expression was assessed by staining 10^6 leukocytes with anti-cluster of differentiation 11b/anti-CD24 antibodies, followed by flow cytometry. Data were integrated into the Pan-cancer Screening Probability Index, incorporating epidemiologic and laboratory parameters. The study was conducted under good laboratory practice and approved by the local institutional review board and Ministry of Health. **RESULTS:** Mean CancerenD24 scores were significantly lower in healthy individuals (0.11 ± 0.01) compared to those with adenomas (0.26 ± 0.04) or CRC (0.45 ± 0.03), correlating with polyp size and histology. No gender-based differences were observed. **CONCLUSION:** CD24 levels increase early in CRC carcinogenesis, detectable already at the level of adenomas >5 mm. CancerenD24, as a noninvasive screening tool, shows promise as an aid to clinicians in identifying at-risk individuals and may reduce reliance on colonoscopy. Further validation is ongoing.

Keywords: CD24; Premalignant Lesions; Cancer; Early Detection; PANDEX

Introduction

Cancer is the second leading cause of death globally. The latest estimates by the World Health Organization predict a 77% global increase in new cancer cases in 2050, up from the 20 million estimated in 2022. The number of cancer deaths worldwide is expected to double by 2050 to an estimated 18.5 million compared to 9.7 million in 2022.[1]

Colorectal cancer (CRC) is a major health concern and a leading cause of cancer mortality in the Western world, with an estimated lifetime risk of 5%–6% and a sharp risk increase after the age of 50 years.[1,2]

CRC is a classical example of a cancer that follows a multistage process of carcinogenesis. It begins with focal molecular changes in the colon (aberrant crypt foci), progresses through benign and precancerous polyps, and ultimately develops into localized and then metastatic cancer. This multistep process spans several decades, with metastatic seeding typically occurring only in the final few years.[3–7] This phase is asymptomatic, and individuals generally feel in perfect health. Symptoms usually appear only when a large tumor has formed or metastases have developed. At that stage, therapy is rarely effective, and a cure is often no longer a realistic goal.[8–12] Prevention and early detection of cancer can reduce mortality by up to 90%.[9]

The ideal cancer test should be acceptable and accessible to the entire population, simple, safe, reliable, inexpensive, sensitive, specific, and capable of detecting premalignant lesions. Currently, only one approved blood test exists for the early diagnosis of cancer, eg, prostate-specific antigen for prostate cancer, and even its utility remains controversial.[13]

About 75% of new cases of CRC occur in individuals at average risk. Despite diagnostic and therapeutic advances,

Abbreviations used in this paper: **AP, adenomatous polyp; CD11b, cluster of differentiation 11b; CD24, cluster of differentiation 24; CRC, colorectal cancer; EDTA, ethylenediaminetetraacetic acid; HP, hyperplastic polyp; FIT test, fecal immunochemical test; PANDEX, Pan-cancer Screening Probability Index; PBS, phosphate-buffered saline; RPM, revolutions per minute.**

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long-term survival has not improved significantly over the past 3 decades, and nearly 50% of CRC patients ultimately die from the disease.[2,14] CRC has become an important issue for physicians and the general population over the past decade, as preventive medicine has emerged as a central focus in health care.

CRC ideally fulfills the criteria a disease must have for organizing a mass-screening program.

A) It is common and associated with high rates of mortality and morbidity.
B) CRC is preceded by a premalignant precursor lesion (ie, adenomatous polyp [AP]).
C) The transition from precursor to malignant lesion develops over many years, providing an opportunity for screening and prevention.
D) Identification and removal of premalignant lesions (polypectomy) prevents cancer and cancer-related death. It is well established that patients who are kept adenoma-free through endoscopic polypectomy are generally protected from CRC.[15,16]

Currently, the acceptable approaches for primary screening include fecal occult blood test (fecal immunochemical test [FIT]), sigmoidoscopy, colonoscopy, and virtual colonoscopy.

The overall reported reduction of CRC mortality with annual FIT is approximately 15%–33%. It is a simple, inexpensive, noninvasive, safe, and widely studied test. However, the efficacy of the test is achieved only by 3 annual samples,[17] the compliance rate of 15%–40% decreases over the years. FIT has a sensitivity of 66%–92% and specificity of 90%–92% for CRC, but a low sensitivity of 17%–24% for large polyps.[17,18] Altogether, this test offers a low reduction and neither reliably rules out nor confirms colorectal adenomas.

Sigmoidoscopy reduces the overall CRC mortality up to 40% over a 5-year period.[17] Compared to colonoscopy, it is a simple, less expensive, minimally invasive, safe, and widely studied test. It does not require extensive bowel preparation, and it can be performed without sedation. On the other hand, more than half of colonic neoplasia are missed by this procedure. Indeed, Lieberman and Weiss have shown that 52% of patients with advanced neoplasia in the proximal colon had no distal lesion.[19] Altogether, this examination offers evaluation of about 50% or less of the large bowel and is comparable to performing a mammography on only 1 breast.

Colonoscopy is the most accurate and definitive test to identify polyps (accuracy of 95%–97%).[17] It also combines a definitive therapy by polyp removal. The National Polyp Study has found a 76%–90% decrease in CRC incidence after colonoscopic polypectomy, compared with appropriately selected controls.[16] However, colonoscopy has several limitations: it is more invasive, not widely available, relatively expensive, and associated with a higher rate of complications.[20]

Cluster of differentiation 24 (CD24), a small glycosylphosphatidylinositol-anchored highly glycosylated protein. It has an oncogenic role in the progression of numerous human malignancies. The overexpression of CD24 in ovarian, breast, prostate, bladder, renal, and nonsmall cell carcinomas, as well as CRC indicates that CD24 could be a significant marker in tumor prognosis and diagnosis.[21] CD24 overexpression plays a role in the early stages of the multistep process of CRC carcinogenesis.[22–24] CD24 is defined as an oncogene and an important determinant in gastrointestinal carcinogenesis.[25] Finally, we have shown that measuring CD24 levels in peripheral blood leukocytes can serve as a promising screening tool for the early detection of colorectal neoplasia with high sensitivity of 70.5% (95% confidence interval [CI], 54.8%–83.2%) and specificity of 83.8% (95% CI, 74.6%–92.7%) for distinguishing CRC from healthy individuals.

Further evidence supporting the possible role of CD24 in peripheral blood leukocytes as a biomarker for malignancy is still under investigation with promising results.[26]

Herein, we demonstrate that CancerenD24, a new screening blood test, can detect CRC and even the premalignant lesions such as colonic adenomas. CancerenD24 is an advanced development of the CD24 score (based on the expression levels of CD24 and cluster of differentiation 11b (CD11b) and other epidemiological and laboratory parameters, utilizing a newly developed Pan-cancer Screening Probability Index (PANDEX), a composite biomarker-based algorithm designed to estimate the likelihood of an individual having any type of cancer, regardless of tissue origin. The PANDEX aims to serve as a noninvasive screening tool for early cancer detection by identifying specific biomarkers. This test is currently under evaluation for its ability to detect other types of cancers and its use as a universal pan-cancer screening assay.

Materials, Methods, and Study Population

Subjects

The study was conducted at the Tel Aviv Sourasky Medical Center, a tertiary referral center. Written informed consent was obtained from all eligible participants prior to entry into the study. The study was approved by the Institutional Review Board of Tel Aviv Sourasky Medical Center and the Israeli Ministry of Health. The studies were compliant with Israeli Ministry of Health and Good Clinical Practice guidelines. Eligible subjects completed a detailed questionnaire on medical history, including personal and familial cancer diagnoses, origin, demographic data, and other epidemiologic information. Blood specimens were drawn using a standard operating procedure so that collection and handling would occur uniformly. Patient information was deidentified, and only anonymized data were available to the investigators. The trial was registered on ClinicalTrials.gov (NCT06656728).

Blood samples were collected from consenting individuals in Tel Aviv Medical Center across various age groups: (1) healthy subjects recruited from the Integrated

Cancer Prevention Center; (2) patients with polyps recruited from the department of gastroenterology; and (3) CRC patients from the departments of gastroenterology, surgery, and oncology.

Eligible subjects completed a detailed questionnaire on medical history and other epidemiologic information, including personal and family history of cancer, background diseases, body mass index, physical activity, smoking status, and demographic characteristics.

Blood was collected into 3 standard 9 mL ethylenediaminetetraacetic acid (EDTA) collection tubes (Vacuette, Greiner Bio-One, Cat. No. 455036), and one test tube (Vacuette, Greiner Bio-One, Cat. No. 455036). Each of the tubes was assigned a consecutive serial internal laboratory number in a blind manner, without any knowledge of the patient's status or diagnosis.

Isolation of Peripheral Blood Leukocytes

Three 9 mL EDTA blood test tubes were centrifuged for 10 minutes at 3000 revolutions per minute (RPM) at 4 °C. The plasma layer was collected carefully. A Pasteur pipette was used to collect the "buffy coat" by aspirating the whitish layer in a circular movement, ensuring all white cells were gathered. After collection, all white cells were transferred to 15 mL tubes. Each 15 mL tube was filled up to 10 mL with cooled erythrocyte lysis buffer, containing 155 mM NH4Cl, 0.1 mM EDTA, and 10 mM NaHCO3.

All tubes were vortexed vigorously and immediately incubated on ice for 30–40 minutes. Following incubation, the samples were centrifuged for 5 minutes at 2000 RPM at 4 °C. The pellet underwent 2 washes—first with erythrocyte lysis buffer and then with phosphate-buffered saline (PBS) buffer to obtain a clean, white pellet. Leukocytes were counted using a COULTER cell counter, and all cells were collected for fixation. Each fixation tube was treated with 2% formaldehyde in PBS and incubated at room temperature for 15 minutes. Following incubation, the samples were centrifuged for 5 minutes at 2000 RPM at 4 °C. Finally, all samples were resuspended with PBS and stored in a 4 °C refrigerator until staining the next day.

Flow Cytometry

Three test samples in Eppendorf were prepared for each subject: unstained, single-stained, and double-stained; 1×10^6 cells were added to each tube; 0.05 μg of fluorescein isothiocyanate-conjugated anti-CD24 antibody mAB and 0.5 μg of anti-CD11b-PerCp-Cy5.5. After adding the calculated volume of antibodies, all samples were incubated, protected from light, for 30 minutes. Following incubation, all samples were washed twice with 1 mL FACs buffer (0.01% sodium azide, 10% fetal bovine serum in PBS). It was centrifuged for 5 minutes at 2000 RPM at 4 °C and kept in the dark at 4 °C until analysis.

For each sample, 30,000 cells were analyzed using the BD FACS Canto II device. Prior to reading, 0.6 mL of 1× PBS was added to each sample and thoroughly mixed by pipetting. The samples were then immediately read.

Forward scatter data files were analyzed using FCS Express 5 Flow software.

Analysis and Final CancerenD24 Test Result Calculation

The CD24 score was calculated by subtracting the percentage of CD24-positive cell population from the percentage of the double-positive population for CD24 and CD11b. This score is then incorporated into the PANDEX together with other parameters. The resulting value from this algorithm is designated as the CancerenD24 test result.

Statistics

Descriptive statistics (N, mean, standard deviation, and standard error of the mean) were calculated for all variables and are presented in tables categorized by CRC, healthy, polyps, and other variables. A t-test was conducted to evaluate the CancerenD24 test results alongside other variables (diagnosis, polyp size, polyp number, and gender) as diagnostic tools for the early detection of CRC and polyps. All tests were two-tailed, and a P value of < 0.05 was considered significant. All authors had access to the study data and reviewed and approved the final manuscript.

Results

A total of 652 individuals enrolled between January 2024 and October 2024 were analyzed and categorized into 4 groups: healthy individuals (505), those with hyperplastic polyps (HPs) (22), individuals with APs (25), and CRC patients (100) (Figure 1, Table 1). A schematic representation of the CancerenD24 test results for the various categories is shown in Figure 2.

There was a statistically significant difference in the CancerenD24 scores between healthy individuals and CRC patients ($P < .0001$), as well as between healthy individuals and those with AP ($P = .0006$) (Table 2).

There were also significant differences in CancerenD24 scores between CRC patients and individuals with AP ($P = .0005$) and with HP ($P = .0003$). There was no significant difference in CancerenD24 scores between AP and HP ($P = .35$).

The CancerenD24 did not show a significant difference between HP and healthy subjects, with values of 0.2 ± 0.05 and 0.11 ± 0.01, respectively ($P = .1$), suggesting their nonmalignant nature. These lesions do not appear to pose a direct cancer risk. Conversely, subjects with AP exhibited a CancerenD24 score of 0.26 ± 0.04, significantly higher than healthy subjects ($P = .0006$). It is impressive that CancerenD24 can distinguish between malignant and nonmalignant polyps (Figure 2, Table 2).

With a cutoff of 0.15, CancerenD24 demonstrates promising performance, showing high specificity (80.4%)

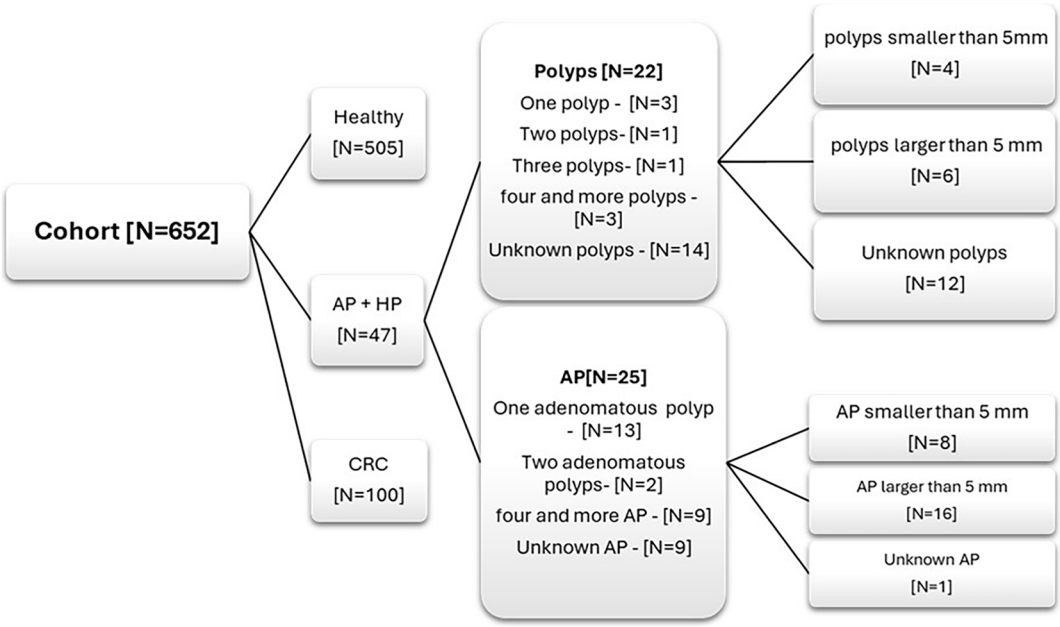

Figure 1. Schematic illustration of the study cohort. N represents the number of participants.

and sensitivity (72%) for CRC, as well as 60% for AP and 40.9% for HP with high negative predictive values: 93.6%, 97.6%, and 96.9%, respectively.

CancerenD24 and Polyp Size

Polyps can vary widely in size. In this study, HP and AP were categorized into 2 major groups according to their size: less than 5 mm or greater than 5 mm. CancerenD24 analysis highlighted size-dependent differences. Table 3 presents a classification of participants based on both polyp type and CancerenD24 results, further subdivided by polyp size.

A t-test analysis revealed a statistically significant difference in CancerenD24 scores between medium and large AP patients and healthy individuals ($P = .004$). The risk of malignancy increases with the size of APs, making size an important factor in clinical decisions regarding monitoring and treatment. When the cohort was divided into 2 major groups, healthy individuals, HP, and small AP (CancerenD24 score: 0.12 $\pm$ 0.007), and CRC patients along with medium and large AP (CancerenD24 score: 0.43 $\pm$ 0.03), CancerenD24 successfully distinguished between the groups ($P < .001$). A cutoff value of 0.25 was determined with exact Clopper-Pearson CIs. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated as 65.3%, 88%, 55%, and 91.5%, respectively.

Table 1. Participants Diagnosis and CancerenD24 Test Result

Diagnosis	Number of participants	CancerenD24 (mean $\pm$ SEM)
Healthy subjects	505	0.11 $\pm$ 0.01
CRC patients	100	0.45 $\pm$ 0.03
AP & HP	47	0.24 $\pm$ 0.03
AP	25	0.26 $\pm$ 0.04
HP	22	0.20 $\pm$ 0.05

SEM, standard error of the mean.

CancerenD24 and Gender

Of the 652 participants, 426 were male and 226 were female. Among the male participants, 338 were healthy, 63 had CRC, and 25 had APs or other polyps.

Statistically significant differences in CancerenD24 scores were observed between healthy males and those with CRC ($P = 1.78E-05$) or AP ($P = .01$) (Table 4). Males with CRC exhibited significantly higher values compared to those with AP ($P = .04$) or HP ($P = .002$). However, no significant difference was found between males with AP and those with HP.

For the female participants, 167 were healthy, 37 had CRC, and 22 had AP or HP (Table 4). A significant difference was observed between healthy females and those with CRC ($P = 6.98E-06$). Additionally, females with CRC had significantly higher CancerenD24 scores than those with HP ($P = 1.08E-09$).

When comparing the 2 genders across the different groups, no significant differences in CancerenD24 scores were found between males and females (Table 5).

Discussion

The CancerenD24 marker is based on CD24, a small, heavily glycosylated cell surface protein involved in

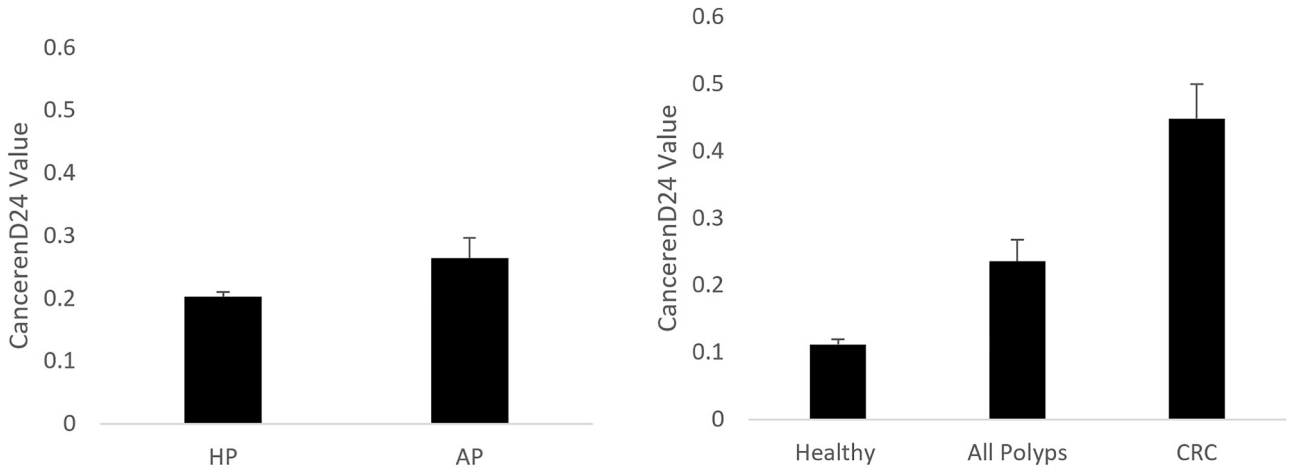

Figure 2. Comparison of CancerenD24 test results between (A) hyperplastic and adenomatous polyps and (B) healthy individuals, all polyps (HP + AP), and colorectal cancer (CRC) patients. Each column represents the mean value ± standard error of the mean. The left panel shows the division of the polyps' column into AP and HP.

immune modulation and known to play a role in tumor immune evasion. The clinical utility of CD24 has been explored in our laboratory at the Tel Aviv Sourasky Medical Center for the last 3 decades, where it was identified as a potential diagnostic and therapeutic biomarker in a range of cancers due to its consistent overexpression in malignant tissues.

The CancerenD24 test leverages peripheral blood mononuclear cell profiling and an algorithm to create a pan-cancer screening tool.

The noticeable increase in CancerenD24 test results among individuals diagnosed with AP and CRC is a pivotal revelation in the presented study. It is a proof-of-concept evidence that patients with colorectal neoplasia have significantly higher CancerenD24 test results than healthy individuals (0.45 ± 0.03 vs 0.11 ± 0.01, $P < .0001$). CancerenD24 test results in healthy subjects with a clean colonoscopy (0.11 ± 0.01) are significantly lower than in patients with AP (0.26 ± 0.04, $P = .0006$), providing further evidence that increased expression of CD24 is an early event in the multistep process of CRC carcinogenesis. Notably, CancerenD24 can serve as a powerful tool, not only for the early detection of CRC but also as a preventive tool.

Delving further into the intricacies of CancerenD24, the test does not distinguish individuals with HP (0.2 ± 0.05, $P = .1$). The idea that CancerenD24 test result is contingent on polyp histology introduces a compelling dimension to our study. This is advantageous, since distinguishing between a population with and without colorectal neoplasia potential is important, and there is no need to detect harmless polyps with no malignant potential.

As we explore these implications, it becomes evident that integrating CancerenD24 into screening protocols has the potential to revolutionize current practices and address

Table 2. CancerenD24 Comparison According to Polyp's Type

Diagnosis	PANDEX – mean ± SEM	*P*-value
CRC vs healthy subjects	0.45 ± 0.03 vs 0.11 ± 0.01	.0001
CRC patients vs adenomatous polyps	0.45 ± 0.03 0.26 ± 0.04	.0005
CRC vs (AP + HP)	0.45 ± 0.03 0.24 ± 0.03	7.16E-06
Healthy vs AP + HP	0.11 ± 0.01 0.24 ± 0.03	.0005
CRC vs hyperplastic polyps	0.45 ± 0.03 0.2 ± 0.05	.0003
Healthy subjects vs hyperplastic polyps	0.11 ± 0.01 0.2 ± 0.05	.1
Healthy subjects vs adenomatous colon polyps	0.11 ± 0.01 0.26 ± 0.04	.0006
Adenomatous colon polyp/s vs hyperplastic polyps	0.26 ± 0.04 0.20 ± 0.05	.35

SEM, standard error of the mean.

Table 3. CancerenD24 Comparison According to Polyps' Size

Polyps size	PANDEX – mean ± SEM	*P*-value
Medium & large AP vs Healthy subjects	0.29 ± 0.05 0.11 ± 0.01	.004
Medium & large polyps vs Healthy subjects	0.39 ± 0.14 0.11 ± 0.01	.097
Small AP vs Healthy subjects	0.24 ± 0.06 0.11 ± 0.01	.062
Small polyps vs Healthy subjects	0.11 ± 0.1 0.11 ± 0.01	.986
Healthy & HP & small AP vs CRC + medium & large AP	0.12 ± 0.007 0.43 ± 0.03	.0001

SEM, standard error of the mean.

existing challenges in colorectal neoplasia detection. This nuanced understanding has promising implications for selectively targeting screening colonoscopy toward individuals with positive CancerenD24 results. By doing so, we can enhance the precision and effectiveness of CRC screening protocols, enabling early detection and prevention.

The convenience and noninvasiveness of the CancerenD24 test have the potential to significantly improve the accessibility and acceptability of CRC screening. Existing screening methods, notably colonoscopy, are limited by invasiveness, discomfort, and the need for meticulous preparation, leading to suboptimal adherence rates among eligible individuals. The CancerenD24 blood test presents a compelling alternative, offering the prospect of overcoming these barriers and encouraging greater participation in CRC screening programs.

The noninvasive nature of the CancerenD24 screening test holds promise for improving overall screening effectiveness. By providing a simpler and less burdensome means of identifying individuals at risk of colorectal neoplasia, such a test could contribute to the earlier detection of precancerous lesions or tumors, facilitating timely interventions and potentially enhancing patient outcomes [69]. This is particularly crucial, as detection and removal of AP can directly prevent CRC.

CancerenD24 provides comparable results across genders, reinforcing that it is not a gender-specific screening test and can be reliably used for both men and women.

The correlation between CD24 expression levels and the specific stage of cancer and location in the colon has been previously evaluated by our group. We have shown that increased expression of CD24 is an early event in the multistep process of carcinogenesis regardless of age, gender, and location in the colon. Nonetheless, it is important to emphasize that this is not an epidemiological study. It is a proof-of-concept and validation of a breakthrough screening test that not only can detect cancer but also can identify premalignant lesion, the AP.

Additionally, the feasibility of integrating a CD24-based test into existing screening programs is both cost-effective

Table 4. Statistical Analysis of CancerenD24 Results in Males and Females

	Male		Female	
Diagnosis of male participants	PANDEX – mean ± SEM	*P*-value	PANDEX – mean ± SEM	*P*-value
CRC vs healthy subjects	0.43 ± 0.04 0.07 ± 0.006	1.78E-05	0.47 ± 0.05 0.19 ± 0.02	6.98E-06
CRC patients vs adenomatous colon polyps	0.43 ± 0.04 0.27 ± 0.065	.04	0.47 ± 0.05 0.26 ± 0.047	.004
CRC vs (AP + HP)	0.43 ± 0.04 0.22 ± 0.04	.0007	0.47 ± 0.05 0.26 ± 0.047	.003
Healthy vs (AP + HP)	0.07 ± 0.006 0.22 ± 0.04	.003	0.19 ± 0.02 0.26 ± 0.047	.21
CRC vs hyperplastic polyps	0.43 ± 0.04 0.17 ± 0.06	.002	0.47 ± 0.05 0.25 ± 0.097	1.08E-09
Healthy subjects vs hyperplastic polyps	0.07 ± 0.006 0.17 ± 0.06	.13	0.19 ± 0.02 0.25 ± 0.097	.59
Healthy subjectsvsadenomatous colon polyps	0.07 ± 0.006 0.27 ± 0.065	.01	0.19 ± 0.02 0.26 ± 0.047	.18
Adenomatous colon polyp/s vs hyperplastic polyps	0.27 ± 0.065 0.17 ± 0.06	.29	0.26 ± 0.047 0.25 ± 0.097	.89

SEM, standard error of the mean.

Table 5. CancerenD24 Results in Both Genders

Diagnosis	CD24 score – mean ± SEM	*P*-value
Male healthy subjects vsfemale healthy subjects	0.07 ± 0.006 0.19 ± 0.02	1.03E-10
Male CRC patients vs female CRC patients	0.43 ± 0.04 0.47 ± 0.05	.56
Male AP + HP vs female AP + HP	0.22 ± 0.04 0.26 ± 0.05	.57
Males with HP vs females with HP	0.17 ± 0.06 0.25 ± 0.097	.53
Males with adenomatous colon polyps vs females with adenomatous colon polyps	0.27 ± 0.065 0.26 ± 0.047	.92

SEM, standard error of the mean.

and cost-saving. Nevertheless, there are inherent limitations associated with our study. It is essential to emphasize that incorporating CancerenD24 as a diagnostic test into clinical practice requires thorough validation and further investigation. Large-scale studies [56, 58] are essential to confirm the reliability and reproducibility of the test and to establish standardized thresholds for risk stratification based on polyp characteristics. While our sample offered a foundational understanding of the CancerenD24 test in healthy and CRC patients, it is important to recognize that the diversity within colorectal neoplasia cases may not be fully captured. The demographic characteristics, genetic predispositions, and environmental factors may vary across populations; however, the key factors incorporated into the PANDEX algorithm should normalize it. Obviously, there are still many parameters that need to be addressed and evaluated in future studies, such as the location in the colon, cancer stage, and other parameters. Indeed, to attain a more thorough comprehension, it is imperative that larger-scale studies involving diverse cohorts systematically investigate the CancerenD24 test, considering factors such as drug influences, genetic predispositions, and lifestyle variables. Another limitation of this study is the relatively small sample size in several of the noncolon cancer and non-normal mucosa groups; therefore, interpretation should be made with caution, and validation of the observed trends is needed. We are currently expanding these cohorts significantly, providing additional support for the initial findings and increasing our confidence in the robustness of the observed patterns.

In the near future, our intention is to conduct subsequent studies to bolster the reliability of our findings. Currently, our primary focus is to broaden the scope of the test. We aim to assess the CancerenD24's performance in detecting CRC as well as other cancer types, ensuring it demonstrates high sensitivity and specificity across the different malignancies.

Another critical limitation stems from the subjects voluntarily attending the Integrated Cancer Prevention Center, which is located in the center of the country. While this reflects the sample composition of study subjects at our institution, the generalizability of these results to the entire country, or other countries, may be limited.

Building upon the significant findings of our study, several key recommendations emerge to guide future research endeavors and translate our insights into actionable strategies for colorectal neoplasia screening and early detection. These recommendations span various research domains, from validation studies and mechanistic investigations to practical considerations for integrating CancerenD24 screening test into existing CRC screening programs.

First and foremost, it is imperative to conduct further research to validate and refine CancerenD24 as a screening test for colorectal neoplasia. The validation process should extend beyond the confines of our initial study, encompassing more extensive populations that reflect the broad spectrum of colorectal neoplasia cases. Furthermore, investigations into the molecular mechanisms underlying CD24 expression in colorectal neoplasia represent a promising avenue for deeper insights. Elucidating the intricate signaling pathways and biological processes that regulate CD24 expression could uncover novel therapeutic targets and contribute to the development of more targeted interventions. In parallel, efforts to explore the feasibility of incorporating the CancerenD24 screening test into existing CRC screening programs are warranted. Assessing the practical aspects of implementing CancerenD24 as a screening test, such as cost-effectiveness, ease of sample collection, and scalability, is essential for its eventual integration into routine clinical practice.

In this study, we have shown that the CancerenD24 test can detect even small AP, but not HP. Larger APs have a higher likelihood of harboring cancer. As such, their size is a critical factor in determining the management and follow-up strategies.

Small polyps, often under 5 millimeters, usually pose a lower risk of becoming cancerous, especially if they are hyperplastic. Polyps over 5 mm are typically more concerning

and carry a higher risk of malignant transformation, and this is exactly the population that we wish to identify.

Conclusion

Identifying CancerenD24 as a potential screening test for colorectal neoplasia opens new avenues for noninvasive, blood-based screening methods. The journey from discovery to clinical application requires a concerted effort to address key research questions and overcome practical challenges. The recommendations outlined above form a roadmap for future investigations, emphasizing the need for validation studies, mechanistic insights, and feasibility assessments. By pursuing these avenues, we can unlock the full potential of CD24 as a valuable tool in the early detection and risk stratification of colorectal neoplasia, ultimately contributing to more effective and personalized CRC screening strategies.

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The data, analytic methods, and study materials will not be made available to other researchers. The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

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