

**Development and clinical validation of a blood test based on 29-gene
expression for early detection of colorectal cancer**

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ABSTRACT

Purpose: A blood test for early detection of colorectal cancer (CRC) is a valuable tool for testing asymptomatic individuals and reducing CRC-related mortality. The objective of this study was to develop and validate a novel blood test able to differentiate patients with CRC and adenomatous polyps (AP) from individuals with a negative colonoscopy.

Experimental Design: A case-control, multicenter clinical study was designed to collect blood samples from patients referred for colonoscopy or surgery. Predictive algorithms were developed on 75 controls, 61 large AP (LAP) ≥ 1 cm, 45 CRC, and independently validated on 74 controls, 42 LAP, 52 CRC (23 Stages I-II) as well as on 245 cases including other colorectal findings and diseases other than CRC. The test is based on a 29-gene panel expressed in peripheral blood mononuclear cells alone or in combination with established plasma tumor markers.

Results: The 29-gene algorithm detected CRC and LAP with a sensitivity of 79.5% and 55.4%, respectively, with 90.0% specificity. Combination with the protein tumor markers CEA and CYFRA21-2 resulted in a specificity increase (92.2%) with a sensitivity for CRC and LAP detection of 78.1% and 52.3%, respectively.

Conclusions: We report the validation of a novel blood test Colox[®] for the detection of CRC and LAP based on a 29-gene panel and the CEA and CYFRA21-1 plasma biomarkers. The performance and convenience of this routine blood test provides physicians a useful tool to test average risk individuals unwilling to undergo upfront colonoscopy.

TRANSLATIONAL RELEVANCE

Early detection of colorectal cancer (CRC) dramatically improves outcome of the disease. However, physicians face a challenge in convincing average risk individuals to be screened for CRC due to the unpleasant nature of existing methods. Here we report a novel blood test that effectively differentiates patients with CRC and large adenomatous polyps (LAP) from individuals with negative colonoscopy. The test is based on a 29-gene panel expressed in peripheral blood mononuclear cells, alone or in combination with the established plasma tumor markers CEA and CYFRA21-1. The test's performance is consistent with its use as a CRC detection tool for average risk individuals reluctant to undergo upfront colonoscopy. This study is being followed by a prospective study to monitor and further evaluate performance of the test in daily clinical practice. Finally, this test might become a useful option to increase compliance of average risk individuals to CRC screening.

INTRODUCTION

Colorectal cancer (CRC) is the second most common malignancy in women, the third one in men, and is the second leading cause of cancer mortality in Europe and North America (1). Each year, more than 1.4 million patients are diagnosed with CRC globally, and approximately half of these patients die from the disease. CRC represents therefore a significant public health issue, especially in industrialized countries. When diagnosed early, CRC has a good therapeutic response with high survival rates, however advanced and metastatic stages of the disease are associated with poor prognosis (2). The development from pre-malignant lesions, such as adenomatous polyps (AP), and the long pre-symptomatic course of the disease highlight the importance of screening for CRC in asymptomatic average risk individuals.

Recent studies have confirmed that regular screening by guaiac fecal occult blood tests (gFOBT) or flexible sigmoidoscopy significantly reduces CRC mortality (3-5). Moreover, systematic CRC screening is cost-effective as it lowers global healthcare costs in comparison to no screening (6-8). Average risk individuals are recommended to be tested at regular intervals above the age of 50 by either guaiac FOBT (gFBOT), fecal immunochemical test (FIT), colonoscopy or sigmoidoscopy (3). Despite these recommendations, physicians today are facing a challenge in convincing average risk asymptomatic individuals to be screened for CRC due to the invasive nature of colonoscopy and the reluctance of individuals to manipulate stool samples. There is therefore an unmet need for an effective and routine test for asymptomatic average risk individuals. A blood-based test is highly attractive due to its minimal invasiveness, convenience and high acceptance by individuals at average risk (9-11).

Myelomonocytic cells from the bone marrow are recruited at the tumor site to create an inflammatory-like microenvironment promoting tumor cell proliferation, survival, motility, angiogenesis and immune escape (12-15). Based on preclinical and clinical observations, we hypothesized that mobilized cells circulating in the blood may be used as a biomarker for the presence of a tumor (16-18). We have recently reported a novel blood-based approach to detect for AP and CRC based on this hypothesis (19). In a follow up study, we reported a 29-gene panel in peripheral blood mononuclear cells (PBMC) for the detection of patients with CRC or AP (20).

The objective of the present study was to use this 29-gene panel to develop and validate a blood test that discriminates patients with CRC and AP from individuals without lesions. The clinical sensitivity and specificity of the test were validated in a multicenter, case-control study. Finally, we investigated whether combining the newly developed 29-gene test with protein tumor markers already in routine clinical use for cancer management, would benefit the test's accuracy.

METHODS

Study design

The DGNP-COL-0310 was a multicenter, case-control clinical study in which, from June 2010 to February 2012, 1579 participants were enrolled in seven Swiss and three South Korean hospitals (Supplementary Materials and Methods). In order to have a second independent sample set to prospectively validate the algorithm, an extension to the study was conducted from November 2012 to April 2013 in four Swiss centers, which enrolled 85 additional subjects (Fig. 1). The study protocol (DGNP-COL-0310) was approved by the competent ethics committees and institutional review boards for research on human subjects (Supplementary Materials and Methods). All participants provided a written informed consent.

Study population

The study recruited subjects older than 50 years who were referred by general practitioners for the great majority (~80%) for a diagnostic colonoscopy and for a minority (~20%) for a screening colonoscopy. Most of the CRC cases (64%) were recruited among subjects who were scheduled for surgery.

Subjects with a personal history of polyps, CRC, other types of cancer, or with family history indicating high risk for CRC (≥ 2 first-degree relatives with CRC or ≥ 1 with CRC at the age < 50 years, familial polyposis or Lynch syndrome) were not eligible for the study.

Following diagnosis, the enrolled participants were retrospectively allocated to clinically pre-defined groups as summarized in Fig. 2. Main study groups included: controls, large AP (LAP) and CRC. Subjects in the control group were clear from any colorectal lesions, including hyperplastic polyps and small adenomatous polyps. The LAP group included patients with at least one adenoma ≥ 1 cm and no CRC lesion.

Categorization of adenomas into the standard classification (i.e. advanced and non-advanced adenoma) could not be performed due to the fact that the histological grading of small adenomas was not collected in the case report form. Nevertheless, since adenomas larger than 1 cm constitute the vast majority of the advanced adenomas, the result relative to this group could be generalized with a good approximation for the advanced adenomas. The CRC group was diagnosed with adenocarcinoma or, rarely, mucinous adenocarcinoma, ranging from stages I to IV, (AJCC system, 7th edition). CRC with unknown stages were collected but not included in the per-protocol analysis. The remaining study groups included patients with small AP (<1cm), hyperplastic polyps, inflammatory bowel diseases (IBD), other inflammatory diseases (e.g. psoriasis, rheumatoid arthritis, hepatitis), other gastrointestinal disorders (e.g. gall stones, diverticulitis), infectious viral diseases (e.g. HIV, Herpes Simplex) and malignancies other than CRC.

Enrolled subjects meeting all inclusion and exclusion criteria, including group-specific criteria, were designated as per-protocol subjects and used for per-protocol analysis. The study also enrolled subjects not meeting all the protocol criteria, but deemed to be valuable for the algorithm evaluation. For instance, some of them presented with co-morbidities involving more than one medical condition/disease that defined the study group (e.g. LAP and inflammatory disease, CRC and other GI disorder). These subjects were classified as “non per-protocol” and intended to be analyzed separately to test the algorithm response on a larger spectrum of medical conditions and co-morbidities.

Clinical procedures

A colonoscopy was performed for all study patients, except for those with malignancies other than CRC, due to ethical reasons. Colonoscopy to the cecum

was required in all cases except for CRC patients with a stenosis caused by a tumor mass. Resected LAP or CRC tissue were sent to a central pathology board composed of three independent pathologists blinded to all subject information, group allocation, and assessments made by other pathologists (Supplementary Materials and Methods).

For each participant, 16 ml of peripheral blood was collected into 4 x 4 ml Vacutainer[®] CPT[™] tubes (Becton Dickinson, Franklin Lakes, NJ), before or immediately after colonoscopy. Most of the samples from subjects diagnosed with CRC requiring a surgery were collected up to 12 weeks after colonoscopy. In all cases, blood was collected prior to polyp resection and prior to any measures in preparation for surgery or any cancer-specific treatment.

Laboratory procedures

Sample preparation and analysis were performed in a blinded manner by laboratory personnel. PBMC separation was performed locally within 6 hours according to Vacutainer[®] CPT[™] tube manufacturer's instructions. PBMC pellets were resuspended and stored at -80°C. Plasma from each sample was collected and stored at -80°C.

Automated purification of total RNA from PBMC pellets was performed at a central laboratory. RNA integrity was analyzed and, on average, RNA samples showed a RNA integrity number (RIN) of 8.6 ± 0.7 . RNA samples with RIN < 5 were considered of poor quality and discarded. RNA samples were aliquoted and stored at -80°C.

For each sample 200 ng of total RNA was reverse-transcribed into cDNA using the SuperScript[®] VILO cDNA Synthesis Kit according to the manufacturer's instructions. Real-time PCR analysis was performed on the Lightcycler 480

instruments using a 29-gene panel previously reported (20). Briefly, PCR reactions were carried out on a 384-well plate, pre-loaded with RealTime ready Custom RT-qPCR assays using RealTime Ready™ DNA Probes Master Mix. Gene expression values (Cp) were normalized by the Δ CT method using the mean of the three housekeeping genes RPLP0, NACA and TPT1.

Plasma concentration of CEA, CYFRA21-1, CA125 and CA19-9 tumor biomarkers were measured for each patient on the Architect immunoassay analyzer platform according to the manufacturer's instructions.

Details on the equipment used during the laboratory procedures and relative manufacturers can be found in Supplementary Materials and Methods.

Statistical design and modeling

The study aimed at enrolling 1600 participants in order to have 1400 evaluable subjects' samples. Of those, 900 were expected to be equally distributed between the 3 main study groups (control, LAP and CRC) for the development and clinical validation of the test. The remaining subjects, presenting with other pre-defined diseases, were allocated to secondary groups for testing the algorithm specificity. In order to reach a significance level $\alpha=0.05$ and a power $1-\beta=0.80$ the study required at least 200 controls, 190 CRC and 240 LAP. Out of those, 120 controls, 70 CRC and 95 LAP had to be collected at the Swiss sites. Samples from the three main groups were stratified by gender, age, site, CRC stage and LAP size, and randomly distributed into training, validation and Test Set 1, in the proportion of 40%, 20% and 40% respectively (Fig. 2). Training and validation sets were used in the discovery phase to define the predictive algorithms, including 45 samples previously used for the identification of the 29-gene panel (20). In the testing phase, the algorithms were validated on Test Set 1, Test Set 2, collected in the extension study, and Test Set 3,

which included “non per-protocol” control, LAP and CRC subjects. Samples from subjects with other colorectal lesions or diseases were used to test the positive rate of the predictive algorithms.

Classifiers and multiple classifier systems were generated by penalized logistic regression (21-23) and fuzzy logic (24, 25) modeling techniques. Firstly, an algorithm based on the 29-gene signature was defined following a multiple classifier combination strategy. We refer to it as a multi-gene multi-classifier (MGMC) algorithm. The MGMC algorithm was then combined with CEA and CYFRA21-1 proteins, using a decision tree classification approach (MGMC-P algorithm). Both algorithms release a binary result, which suggests the presence or absence of advanced colorectal neoplasia. The methodology used to develop the predictive algorithms is detailed in the Supplementary Materials and Methods. The complete test was named Colox®.

Specificity was estimated as 1–positivity among subjects belonging to the control group. For test characteristics, 95% confidence intervals were computed with the use of an exact binomial test.

The R statistics environment was used for all statistical analyses.

RESULTS

Study population

Out of 1665 enrolled subjects, 1405 were considered fully evaluable (84.4%). Of those, 782 were recruited in Switzerland and 623 in South Korea. This report focuses on the Swiss dataset only. Korean data will be presented separately.

In the Swiss collective, the per-protocol population was constituted of 594 participants (Fig. 1) including 149 control subjects, 103 LAP and 97 CRC patients from stages I to IV, as well as 245 subjects diagnosed with other types of colorectal lesions (e.g. small adenomas, hyperplastic polyps), solid cancers or other diseases such as inflammatory bowel diseases or diverticulitis, for testing the algorithm specificity. In addition, 188 cases classified as “non per-protocol” were enrolled for testing the algorithm behavior on a larger spectrum of medical conditions and co-morbidities. Demographic and clinical characteristics of the subjects are reported in Table 1. Subjects in the control, LAP, and CRC groups had a mean age of 60.6, 67.3 and 69.4, respectively. The control group enrolled 54% women and 46% men, whereas the LAP and CRC group enrolled 64% and 62% of men, respectively.

29-gene expression analysis

All blood samples were profiled with a 29-gene panel (Supplementary Table S1) previously reported, and discovered through a combined univariate and multivariate approach (20). No differences in normalized gene expression were observed across collecting centers (data not shown) or across different age categories within the control or the LAP group (Supplementary Table S2). A few genes were found to be differentially expressed between individuals of different age classes in the CRC group, as well as between men and women within all 3 groups. These differences in

individual genes had no impact on the output of the 29-gene algorithms as shown by the results described further below. The most significant genes for CRC discrimination ($p < 0.0008$) were S100A8, IL1B, CCR1 and PTGS2, with 1.7 to 2.1 fold increase in gene expression (linear scale, Supplementary Table S1). Some of these genes (S100A8/Calprotectin and PTGS2/COX2) are well-known players in CRC (13, 26).

Clinical performance of the MGMC Algorithm

The MGMC algorithm was applied to Test Set 1 showing a specificity of 89.6% and a sensitivity of 75.9% for CRC detection (Table 2). These performance characteristics were confirmed when the algorithm was tested on a second independent sample set (Test Set 2), showing the stability of the algorithm (Table 2). When the 2 test sets were combined, the algorithm showed a sensitivity of 75.0% for CRC and of 54.8% for LAP detection with specificity of 89.2%. The test performed well on early stage-CRC with a sensitivity of 60.9% for CRC stages I-II, which increased to 75.6% when CRC stage III was included. No differences were observed in LAP detection in patients with only one vs. more than one LAP (data not shown). Small AP (<1 cm) and hyperplastic polyps were also detected by the test but with lower sensitivity (29.9% and 29.5%, respectively) (Table 3).

The MGMC algorithm was also tested on subjects with other diseases (Table 3 and Supplementary Table S3). Non-gastrointestinal (GI) inflammatory and viral diseases showed a low rate of positivity, comparable to the controls, whereas IBD and other GI diseases had a higher positivity rate. The other cancers showed a very heterogeneous behavior ranging from a positive rate of 41.7% for pancreatic cancer to 80.0% for other GI cancers.

The algorithm was further tested on the Test Set 3 and the results are presented in Supplementary Table S4. They confirmed the findings of the per-protocol analyses. When these samples were added to the per-protocol samples, MGMC sensitivity for LAP and CRC was 55.4% and 79.5%, respectively with a specificity of 90.0% (Table 2).

Combination of the MGMC algorithm with protein tumor markers

Among the 4 tumor markers analyzed, CEA and CYFRA21-2 showed the most significant p-values (less than 0.001) for discrimination between the CRC and control groups, and the highest area under the curve (AUC) for CRC and control classification (data not shown). In the training set, these tumor markers displayed a specificity of 86.0% and 94.0% and sensitivity for CRC detection of 63.3% and 43.3%, respectively. These results were confirmed in the validation set with a specificity of 88.0% and 96.0% and sensitivity for CRC of 60.0% and 26.7%, respectively. CEA and CYFRA21-1 sensitivity was directly correlated to CRC stage (data not shown).

In order to explore whether tumor specific markers could complement and strengthen the MGMC algorithm, we added the variables CEA and CYFRA21-1. The resulting overall specificity (Test Sets 1+2) was 91.9%, higher than the MGMC algorithm alone (Table 4), although this difference was not statistically significant ($p > 0.05$). Sensitivity for CRC (75.0%) and LAP (52.4%) was similar to the one reported by MGMC (Table 4). When the Test Set 3 (Supplementary Table S4) was also taken into consideration, the test reached a sensitivity of 52.3% and 78.1%, respectively, with a specificity of 92.2% (Table 4). When CRC cases were stratified according to the time of recruitment and blood draw, i.e. before surgery or at colonoscopy, a

slightly higher detection rate was observed in the cases collected before surgery (79% vs 74%). However this difference was not statistically significant ($p>0.05$).

Due to the observed differences in age and sex distribution between the control and the CRC group and differences in the expression levels of some individual biomarkers, statistical testing was carried out to evaluate a possible confounding effect of age or sex on CRC discrimination by the algorithm based on the 29-gene panel. None of the considered factors showed an association with the test result ($p\text{-value}>0.05$) (Supplementary Table S5). Moreover, age and sex were tested as covariates in multivariate logistic regression analysis. None of these two variables played a statistically significant role in differentiating the control from CRC group.

DISCUSSION

Early detection of CRC dramatically improves the clinical outcome of the disease and regular screening has proven to reduce mortality (2). The “gold standard” for AP and CRC detection is colonoscopy. It has the highest sensitivity for AP or CRC among all screening tests, and has the advantage to allow direct visualization of the entire colon and removal of precancerous lesions. However, due to the unpleasant bowel preparation required by the method as well as its invasive nature, compliance to screening colonoscopy remains unsatisfactorily low. Other recommended options are stool-based tests, such as the guaiac or immunochemical FOBTs or the recently FDA-approved stool DNA test (Cologuard[®]) (27-31). Stool tests require stool manipulation at home, which is unappealing and inconvenient to some, thus contributing to the low screening compliance despite their promising performances.

An accurate blood test would be an attractive alternative for asymptomatic, average risk individuals reluctant to undergo screening by a stool test or

colonoscopy. This is supported by a Dutch study, based on a population survey and mathematical simulations which concluded that the target population preferred to be screened using a blood- rather than a stool-based test (26% versus 17%) (32). Another study reported that of 172 age-eligible individuals who were indicated for CRC screening by colonoscopy, 63% refused the procedure, while 97% of those who refused colonoscopy agreed to be screened by a less invasive test such as a blood test (83%) or a stool test (17%) (10). It is therefore reasonable to expect that a blood test can help convincing average risk individuals to be tested for CRC, and in the long term to contribute to reduction of CRC-related mortality.

A blood test based on detecting aberrantly methylated DNA in the Septin9 gene (Epi proColon[®]) has recently been approved by the FDA. In a large prospective screening study the test showed sensitivity for CRC of 68% at specificity of 79%. The test was not able to detect advanced adenomas, showing sensitivity equivalent to the false positive detection rate in the control group (22% vs 21%) (33).

Here we report a validation study for a newly developed blood test which can identify 78% of CRC and 52% of large adenomas. The latter results is of particular interest as large adenoma are one the most clinically-relevant precursor lesions of CRC. Two different predictive algorithms were developed: one based on a 29-gene panel expressed in PBMC (MGMC algorithm) and one combining the MGMC algorithm with the plasma tumor markers CEA and CYFRA21-1 (MGMC-P algorithm). While both algorithms had a comparable sensitivity for CRC, MGMC-P demonstrated a slightly higher specificity (92.2% versus 90.0%). The clinical advantage of higher specificity is that the number of false-positive individuals sent to colonoscopy would be lower thus avoiding unnecessary invasive procedures.

The test was also evaluated in patients presenting other colorectal lesions and a various spectrum of different diseases. The test detected around 30% of AP

smaller than 1 cm. It would be of interest to evaluate how this detection rate correlates with the histology, i.e. non-advanced versus advanced AP, the latter representing approximately 13% of the small AP (34).

The test detected also 28-30% of subjects with hyperplastic polyps which have traditionally not been considered as a relevant risk factor for CRC, unless present as hyperplastic polyposis syndrome (35). Whether the detection of these lesions is an added value for the test may be debatable. Under the scenario that considers the detection of hyperplastic polyps and small adenomas a false positive event, the test's specificity would be estimated at 83%, based on the study data and published prevalence data (36, 37). The full biological and clinical relevance of isolated hyperplastic polyps to CRC development, however, has not been thoroughly investigated (38).-Emerging evidence indicates that hyperplastic polyps share similar morphological and molecular features with sessile serrated adenoma, which are now recognized as precancerous lesions that can rapidly progress into invasive carcinoma (39). KRAS and BRAF mutations and aberrant CpG-island methylation are observed in a significant fraction of hyperplastic polyps (approx. 30-60%) consistent with the notion that hyperplastic polyps are a subset of serrated adenoma (40-42). On the basis of these considerations, and in the context of a more personalized medicine the detection of hyperplastic polyps with carcinogenic potential would be a desirable feature for such a test, although it may come with the burden of detecting also benign hyperplastic polyps. The clinical significance of a positive test in individuals with hyperplastic polyps will be explored in future studies.

The positive rate of the test in patients with IBD and other GI benign diseases was close to 30%. Since these patients are normally under close surveillance and/or are presenting symptoms they are considered ineligible for testing by this kind of test.

In clinical practice this test will not be applied to individuals diagnosed with other cancers. We nevertheless evaluated a limited number of patients with malignancies other than CRC and found different degrees of positivity. These exploratory observations did not yield relevant conclusions since the study was not designed to evaluate the test for the early detection of cancers other than CRC and most patients were not investigated by colonoscopy.

In summary, a new blood test has been developed and clinically validated for detection of LAP and CRC based on a 29-gene expression panel and the CEA and CYFRA21-1 plasma biomarkers. The test's performance is consistent with its use as a CRC detection tool for average risk individuals reluctant to undergo upfront colonoscopy. This convenient and routine blood test can help identify individuals who will benefit the most from a colonoscopy. A prospective study is currently on-going to monitor and further evaluate the performance of the test in daily clinical practice.

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FIGURE LEGENDS

Figure 1. Flowchart showing patient disposition.

Figure 2. Study design diagram. The Training, Validation, Test Set 1 and 2 and part of subjects with other findings and diseases (n1) constituted the per-protocol cases. Test Set 3 and remaining patients with other findings and diseases (n2) were enrolled as non per-protocol cases.

Table Legends

Table 1. Clinical and demographic characteristics of the study participants.

Table 2. Specificity and sensitivity of the MGMC algorithm for CRC and large AP detection.

Table 3. Positive rate of the MGMC and MGMC-P algorithms for other colorectal lesions and diseases.

Table 4. Specificity and sensitivity of the MGMC-P algorithm for CRC and large AP detection.

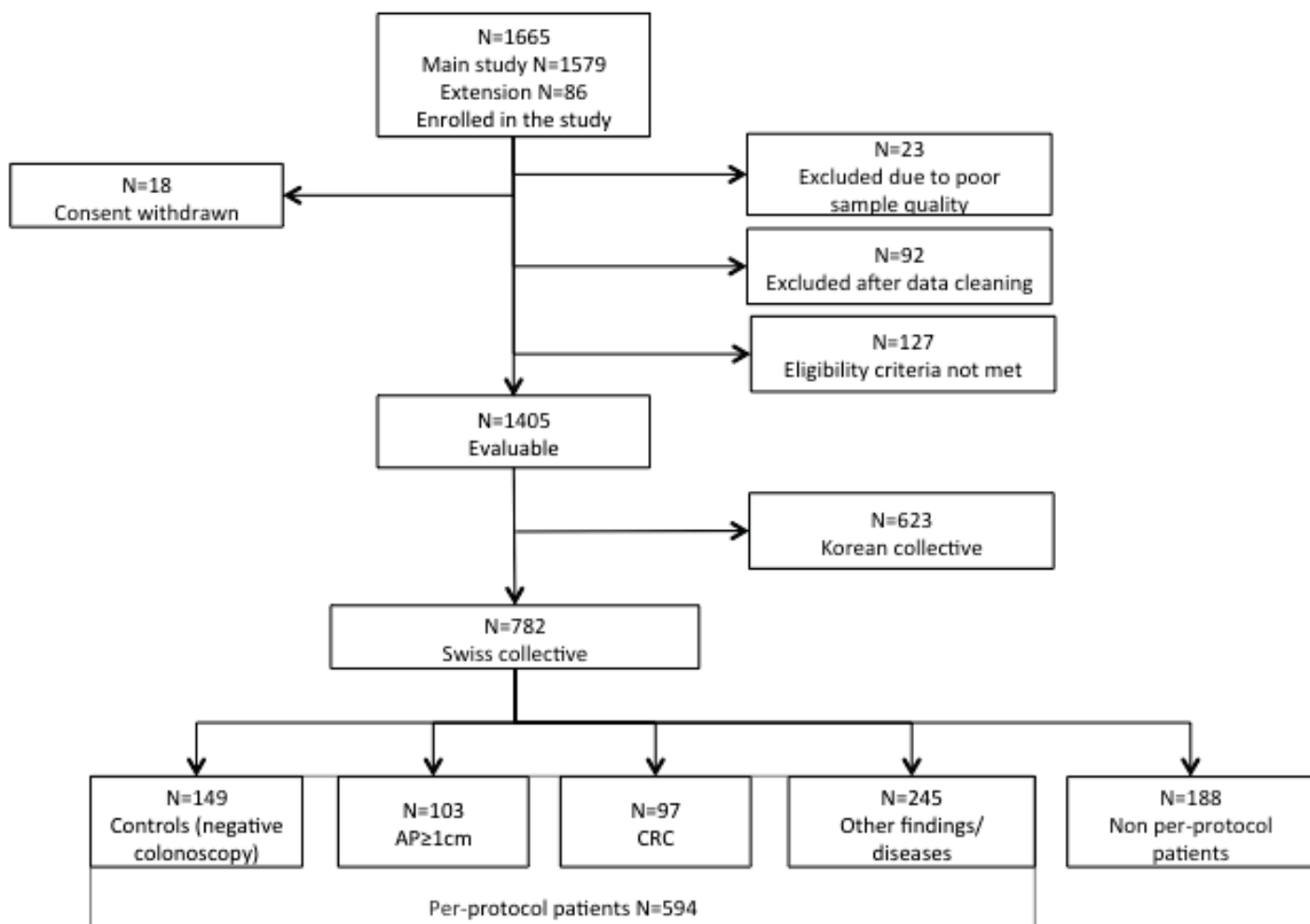


Figure 1. Flowchart showing patient disposition

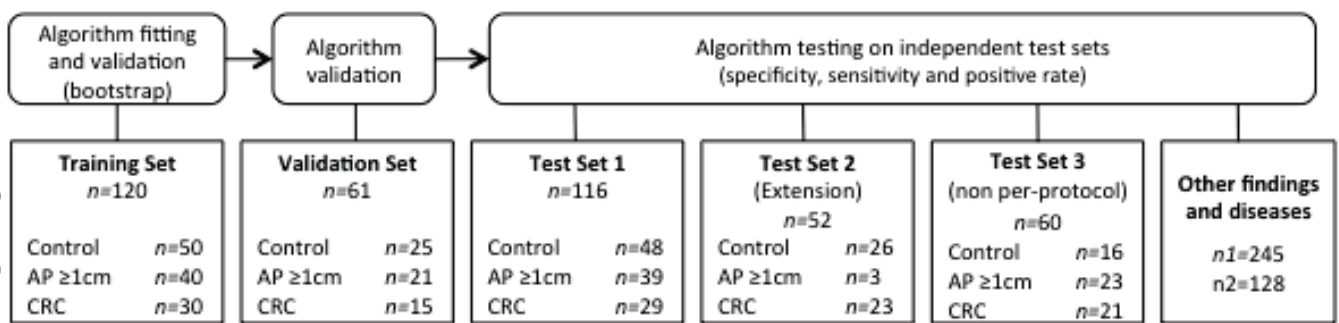


Figure 2. Study design diagram.

Table 1. Clinical and demographic characteristics of the study participants

Category	Patient (n)	Age (mean \pm SD)	Male (%)
Control	149	60.6 \pm 7.8	46
LAP	103	67.3 \pm 8.2	64.1
1-2 cm	57	67.5 \pm 8.4	66.7
> 2cm	46	67.1 \pm 8.1	60.9
CRC ^a	97	69.4 \pm 9.7	61.9
Stage I	22	71.2 \pm 9.2	59.1
Stage II	22	69.8 \pm 8.6	63.6
Stage III	31	68.1 \pm 12.3	54.8
Stage IV	22	69 \pm 7.0	72.7
Other colorectal lesions and diseases	245	-	-
AP <1cm	67	65.2 \pm 8.9	77.6
Hyperplastic polyps	61	60.4 \pm 7.6	59
IBD ^b	14	58.7 \pm 6.1	71.4
Other GI diseases ^c	19	62.7 \pm 10.3	42.1
Inflammatory diseases ^d	11	65 \pm 8.3	18.2
Hepatitis, Herpes, AIDS	10	61.8 \pm 5.9	40
Other cancers	63	67.2 \pm 8.0	71.4
Non per-protocol cases	188	65.1 \pm 10.0	56.4

^a All CRC were adenocarcinomas except one squamous carcinoma. Two were mucinous type.

^b Eight Crohn's disease and 6 ulcerative colitis

^c Including 7 gallstones and 9 diverticulitis

^d Three autoimmune hepatitis, 5 psoriasis and 3 rheumatoid arthritis

Table 2. Specificity and sensitivity of the MGMC algorithm for CRC and large AP detection

	Test Set 1		Test Set 2		Test Sets 1+2	Test Sets 1+2+3	
	Total	Sensitivity (95% CI)	Total	Sensitivity (95% CI)	Sensitivity (95% CI)	Total	Sensitivity (95% CI)
	<i>n</i>	%	<i>n</i>	%	%	<i>n</i>	%
LAP	39	51.3 (35-68)	3	100 (29-100)	54.8 (39-70)	65	55.4 (43-68)
1-2 cm	22	54.5 (32-76)	1	100	56.5	-	-
> 2cm	17	47.1 (23-72)	2	100	52.6	-	-
Low grade	30	50.0 (31-69)	0	-	50.0 (31-69)	-	-
High grade	6	50.0 (12-88)	3	100 (29-100)	66.6 (30-93)	-	-
Tubular	9	44.4 (14-78)	0	-	44.4 (14-78)	-	-
Tubulovillous	21	47.6 (26-70)	3	100 (29-100)	54.2 (33-74)	-	-
Villous	5	60.0 (15-95)	0	-	60.0 (15-95)	-	-
Serrated	4	75.0 (19-99)	0	-	75.0 (19-99)	-	-
CRC	29	75.9 (57-90)	23	73.9 (52-90)	75.0 (61-86)	73	79.5 (68-88)
Stages I-II	14	71.4 (42-92)	9	44.4 (14-78)	60.9 (39-80)	33	66.7 (48-82)
Stages III-IV	15	80.0 (52-96)	14	92.9 (66-100)	86.2 (68-96)	40	90.0 (76-97)
Stages I-II-III	22	81.8 (60-95)	19	68.4 (44-87)	75.6 (60-88)	58	79.3 (67-89)
Low grade	23	69.6 (47-87)	18	66.7 (45-89)	68.3 (63-92)	-	-
High grade	5	100 (48-100)	3	100 (29-100)	100 (63-100)	-	-
		Specificity (95% CI)		Specificity (95% CI)	Specificity (95% CI)		Specificity (95% CI)
	<i>n</i>	%	<i>n</i>	%	%	<i>n</i>	%
Controls							
(negative colonoscopy)	48	89.6 (77-97)	26	88.5 (70-98)	89.2 (80-95)	90	90.0 (82-95)

Table 3. Positive rate of the MGMC and MGMC-P algorithms for other colorectal lesions and diseases

		MGMC	MGMC-P
	Total	Positive rate	Positive rate
	<i>n</i>	%	%
AP<1cm	67	29.9	28.4
Hyperplastic polyps	61	29.5	27.9
IBD	14	42.9	28.6
Other GI diseases	19	36.8	31.6
Inflammatory diseases	11	18.2	18.2
Hepatitis, Herpes, AIDS	10	20.0	10.0

Table 4. Specificity and sensitivity of the MGMC-P algorithm for CRC and large AP detection

	Test Sets 1 + 2		Test Sets 1 + 2 + 3	
	Sensitivity		Sensitivity	
	Total	(95% CI)	Total	(95% CI)
	<i>n</i>	%	<i>n</i>	%
LAP	42	52.4 (37-69)	65	52.3 (40-65)
1-2 cm	23	52.2 (31-73)	-	-
> 2cm	19	52.6 (31-79)	-	-
CRC	52	75.0 (61-86)	73	78.1 (67-87)
Stages I-II	23	56.5 (35-77)	33	60.6 (42-75)
Stages III-IV	29	89.7 (73-98)	40	92.5 (80-98)
Stages I-II-III	41	70.7 (55-84)	58	74.1 (61-85)
		Specificity		Specificity
		(95% CI)		(95% CI)
	<i>n</i>	%	<i>n</i>	%
Controls				
(negative colonoscopy)	74	91.9 (83-97)	90	92.2 (85-97)

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