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Three-year risk of colorectal cancer by faecal haemoglobin concentration in subjects with a negative FIT

Donella Puliti[1], Giuseppe Gorini[1], Adele Caldarella[1], Teresa Intrieri[1], Zhanna Tumanova[2], Gianfranco Manneschi[1], Carmen Beatriz Visioli[1], Beatrice Mallardi[1], Francesca Battisti[1], Simonetta Bisanzi[1], Cristina Sani[1], Giovanna Masala[1], Marco Zappa[1] and Paola Mantellini[1]

[1]*Istituto per lo Studio, la Prevenzione e la Rete Oncologica (ISPRO), Florence, Italy*
[2]*Dipartimento di Sanità Pubblica, Università degli Studi di Firenze, Florence, Italy*

Abstract

Introduction and aim. To evaluate whether a risk-stratified colorectal cancer (CRC) screening approach based on a single faecal immunochemical test (FIT) performed better than one using two consecutive tests.

Methods. Approximately 420,000 negative FIT results (positivity cut-off: 20 µg faecal haemoglobin, f-Hb/g) were collected between 2000 and 2015 within the Florence screening program.

Results. The 3-year CRC risk was 15.9‰ in individuals with 15-20 µg/g on a single test, and 9.36‰ for those with a cumulative f-Hb concentration of 20-40 µg/g across two tests.

Discussion. The two-tests approach showed lower discriminatory capacity in identifying the highest-risk subgroup compared to a single test. Screenees with 15-20 µg/g accounted for <1%, but faced a significantly increased 3-year CRC risk.

Conclusion. While increasing screening intensity would require more colonoscopies, extending the screening interval for individuals <60 years with undetectable f-Hb, could allow for the reallocation of colonoscopy resources.

Key words

- colorectal cancer
- faecal haemoglobin concentration
- faecal immunochemical test (FIT)

INTRODUCTION

Colorectal cancer (CRC) is the second most leading cause of cancer-related mortality worldwide, accounting for approximately 900,000 deaths in 2022 [1]. Population-based CRC screening programs using faecal immunochemical tests (FIT) significantly reduce both CRC incidence and mortality and are now widely implemented across Europe [2]. In Italy, as in many other European countries, the positivity cut-off is set at 20 µg of faecal haemoglobin per gram of faeces (µg f-Hb/g). Current screening programmes follow similar protocols: f-Hb concentrations at or above the established threshold are considered positive and warrant a referral for total colonoscopy (TC). Conversely, concentrations below the cut-off are classified as negative, with individuals re-screened every two years in most European countries, or annually in the United States and a few other regions [2].

Accumulating evidence indicates that individuals with a negative FIT but an f-Hb value just below the threshold exhibit increased CRC detection rates in subsequent rounds, and a higher probability of developing interval cancers (ICs) compared to those with no detectable f-Hb [3-10]. These studies pursued varied objectives, such as estimating IC risk following a negative FIT in single or consecutive screening rounds [4, 7, 10], developing risk-scoring models that integrate f-Hb concentrations from a single FIT with other CRC risk factors [8], or evaluating the predictive value of the first two consecutive FIT rounds [3, 9]. Furthermore, cross-study comparisons remain challenging due to substantial heterogeneity in f-Hb stratification and positivity threshold: for instance, the cut-off was set at ≥47 µg/g in the Netherlands [7, 9], ≥20 µg/g in Denmark and Italy [3, 10], and ≥80 µg/g in Scotland [8]. To address this, a recent systematic review and dose-response meta-analysis synthesized these data, demonstrating that individuals with sub-threshold f-Hb concentrations of 5, 10, and 15 µg/g had a 4-, 6-, and 8-fold higher risk of CRC, respectively, at the subsequent screening round compared to individuals with undetectable f-Hb [11].

These findings suggest that risk-stratified screening strategies – tailoring protocols to individual risk levels – could enhance detection rates, optimize the benefit-

Address for correspondence: Giuseppe Gorini, Istituto per lo Studio, la Prevenzione e la Rete Oncologica, Villa delle Rose, Via Cosimo il Vecchio 2, 50139 Florence, Italy. E-mail: g.gorini@ispro.toscana.it.

to-harm ratio, and improve the efficiency of endoscopy resource allocation. Conversely, implementing risk-based screening introduces significant organizational challenges and requires robust information technology and communication frameworks. The 2022 Council of the European Union Recommendation affirmed that quantitative FIT is the preferred tool for colonoscopy referral and endorsed its use in risk-tailored strategies, suggesting the introduction of thresholds tailored to sex, age and previous screening history [12].

However, the optimal strategy for designing such a risk-based approach remains poorly defined. Specifically, it is unclear whether a single FIT performs better than two consecutive tests in assessing subsequent CRC risk. Notably, a single-test strategy offers substantial organizational advantages, given that screening adherence fluctuates and many individuals do not consistently participate in every consecutive round.

The objective of this study is to evaluate, within a retrospective cohort from the ongoing FIT-based screening program (20 µg/g cut-off) in the province of Florence, Italy, whether a risk-stratification approach based on a single FIT outperforms one based on two consecutive tests in discriminating the cumulative three-year CRC risk. Discriminatory capacity will be assessed by the ability to differentiate – relative to the average risk of all FIT-negative subjects – a higher-risk subgroup, that warrants a more intensive protocol from lower-risk subgroup eligible for a de-escalated screening interval.

MATERIALS AND METHODS

Screening program and study population

The Florence district provides a population-based, biennial colorectal cancer (CRC) screening program using faecal immunochemical test (FIT) for individuals aged 50-70. Established in the 1980s, the program utilized the OC-Hemodia (Eiken) method from 2000 to 2003, transitioning to the OC-Sensor (Eiken) method in 2004. The positivity threshold is set at 20 µg/g of faeces (equivalent to 100 ng/ml). Negative FIT results are mailed to participants with a recommendation to repeat the test in two years, with no mention of their specific f-Hb concentration. Individuals with a positive FIT are contacted by screening staff and offered a total colonoscopy (TC) at dedicated endoscopy reference centres. All screening services are free of charge and funded by the Regional Health System Program performance is routinely monitored using standard quality indicators [13]; target population participation rates fluctuated between 48.0% and 57.0% during the 2000-2015 study period [14-16]. This analysis was conducted as part of the routine monitoring of program performance. The study protocol was approved by the local Ethics Committee of Florence area (Comitato Etico Regionale per la Sperimentazione Clinica della Regione Toscana, Sezione Area Vasta Centro; reference number: 23723_oss).

We evaluated all negative FIT (index FIT) obtained between January 1, 2000 and December 31, 2015, from individuals aged 50-65 years, for each negative FIT, data on f-Hb level, sex, and age were retrieved. Linkage with the screening database allowed classification of each test as either a first or subsequent screening round. To compare the 3-year cumulative CRC risk based on a single negative FIT with the predictive value of cumulative f-Hb levels across two consecutive tests, we selected a subset of index FITs that had a preceding test recorded 18 to 60 months prior. Following the categories proposed by Senore *et al*. [3], f-Hb concentrations for a single FIT were stratified into five groups: 0 µg/g or undetectable f-Hb; 0-4 µg/g (≤4 µg/g); 4-10 µg/g (from 4.1 to 9.9 µg/g); 10-15 µg/g (from 10 to 14.9 µg/g); and 15-20 µg/g (from 15 to 19.9 µg/g). Cumulative f-Hb concentrations across two consecutive FITs were stratified into six groups: 0 µg/g; 0-4 µg/g; 4-10 µg/g; 10-15 µg/g; 15-20 µg/g; and 20-40 µg/g (from 20 to 39.9 µg/g).

Outcome assessment and data linkage

CRC incidence was tracked via linkage with the regional cancer registry, which was updated through December 31, 2020. Adenocarcinomas of the colon and rectum (ICD-10 codes C18, C19 and C20) [17] diagnosed within 3 years of a negative FIT were included. Disease stage was determined using histology reports or clinical records according to the 7th edition of the TNM classification [18], with Stages III and IV classified as advanced CRC.

CRCs were categorized into: (i) screen-detected (SD) CRCs: cancers diagnosed within 6 months of a positive FIT (including individuals who underwent a TC and non-compliant participants who refused the screening TC); (ii) interval cancers (ICs): CRCs diagnosed between 6 and 36 months following a negative FIT. Cancers diagnosed more than 6 months after a positive FIT underwent individual clinical review to ensure accurate classification as either an SD CRC or an IC.

Statistical analysis

The statistical unit of analysis was the individual test, and differences in proportions were evaluated using the Chi-square test. Person-time was calculated from the date of the index negative FIT to the earliest of the following events: a subsequent negative FIT, a CRC diagnosis, or the end of the 3-year follow-up period. Consequently, a subject diagnosed with CRC following a subsequent positive FIT exited the cohort at the time of diagnosis, whereas a subject with a subsequent positive FIT but no CRC diagnosis remained under observation for the full 3 years.

The association between f-Hb concentration levels and the risk of total or advanced CRC was estimated using multivariate Cox regression models, adjusted for age-group (50-54, 55-59, 60-65 years), sex, and screening history (first *vs* subsequent test). All statistical tests were two-sided, with significance defined as $p<0.05$.

RESULTS

From January 2000 to December 2015, 419,822 negative FIT results were obtained from 168,437 individuals aged 50-65 years within the Florence CRC screening program (average 2.5 FITs per person). First examinations accounted for 39.8% of the tests, while 60.2% were subsequent screenings. Faecal haemoglo-

bin (f-Hb) was undetectable (0 µg/g) in 48.9% of the negative FITs, and exceeded 15 µg/g in only 0.9%. Individuals with higher f-Hb levels were predominantly male and older (*Table S1 available online as Supplementary Material*).

During the 3-year follow-up period after a negative FIT, 554 CRCs were diagnosed, corresponding to an overall risk of 1.32‰ (95% CI: 1.21‰-1.43‰). Of these, 53.8% (n=298) were diagnosed at an early stage (stages I-II), 38.1% (n=211) at an advanced stage (stages III-IV), and 8.1% (n=45) had an unknown stage (*Table 1a*). Tumour stage distribution did not significantly differ across f-Hb categories (p=0.950). Under the single-test strategy, the 3-year CRC risk increased progressively from 0.65‰ among individuals with undetectable f-Hb, to 15.9‰ among those with f-Hb level of 15-20 µg/g. After adjusting for age group, sex, and screening history, the hazard of a CRC diagnosis ranged from 60% higher in the <4 µg/g group (adjusted Hazard Ratio – aHR: 1.61; 95% CI: 1.29-2.02), to 21-fold higher in the 15-20 µg/g group (aHR: 21.00; 95% CI: 15.35-28.74) compared to the undetectable f-Hb reference group. Advanced CRC risk followed an identical trend; notably, 10.4% of advanced cancers (22/211) were diagnosed within 3 years among the 0.9% of individuals (3,595/419,822) in the highest sub-threshold f-Hb tier (*Table 1a*; *Figure 1a*).

A subset of 241,243 negative FITs (57.5%) had a preceding test recorded within an 18-60 month interval. In this cohort, 318 CRCs were diagnosed within

Table 1
Crude three-years risk (‰) of CRC and advanced CRC by f-Hb at a single FIT (a) and by cumulative f-Hb at two consecutive FIT (b) and adjusted hazard ratios (aHR)

a)		**CRC**		**Advanced CRC**	
f-Hb (µg/g)	**N**	**N**	**aHR***	**N**	**aHR***
	FIT (%)	**‰**	**(95% CI)**	**‰**	**(95% CI)**
0	205,365	134	*ref*	52	*ref*
	(48.9%)	0.65‰		0.25‰	
(0-4)	172,312	180	1.61	67	1.52
	(41.0%)	1.04‰	(1.29-2.02)	0.39‰	(1.06-2.19)
[4-10)	31,648	125	5.76	46	5.42
	(7.5%)	3.95‰	(4.51-7.37)	1.45‰	(3.64-8.09)
[10-15)	6,902	58	11.47	24	12.26
	(1.6%)	8.40‰	(8.41-15.65)	3.48‰	(7.52-20.00)
[15-20)	3,595	57	21.00	22	20.91
	(0.9%)	15.9‰	(15.35-28.74)	6.12‰	(12.66-34.55)
Total	419,822	**554**	-	**211**	-
	(100%)	**1.32‰**		**0.50‰**	
b)		**CRC**		**Advanced CRC**	
Cumulative f-Hb (µg/g)	**N**	**N**	**aHR****	**N**	**aHR****
	FIT (%)	**‰**	**(95% CI)**	**‰**	**(95% CI)**
0	73,203	43	*ref*	14	*ref*
	(30.3%)	0.59‰		0.19‰	
(0-4)	119,685	96	1.35	42	1.82
	(49.6%)	0.80‰	(0.94-1.94)	0.35‰	(0.99-3.34)
[4-10)	35,250	82	3.84	27	3.91
	(14.6%)	2.33‰	(2.65-5.58)	0.77‰	(2.04-7.51)
[10-15)	7,497	47	9.97	19	12.42
	(3.1%)	6.27‰	(6.60-15.08)	2.53‰	(6.24-24.71)
[15-20)	4,113	36	13.70	15	17.55
	(1.7%)	8.75‰	(8.79-21.34)	3.65‰	(8.47-36.38)
[20-40)	1,496	14	13.57	6	17.91
	(0.6%)	9.36‰	(7.39-24.91)	4.01‰	(6.84-46.86)
Total	**241,243**	**318**	**-**	**123**	-
	(100%)	**1.32‰**		**0.51‰**	

*Adjusted for sex, age and previous screening test; **Adjusted for sex and age; CRC: colorectal cancer; FIT: faecal immunochemical test; f-Hb: faecal haemoglobin; CI: confidence interval.

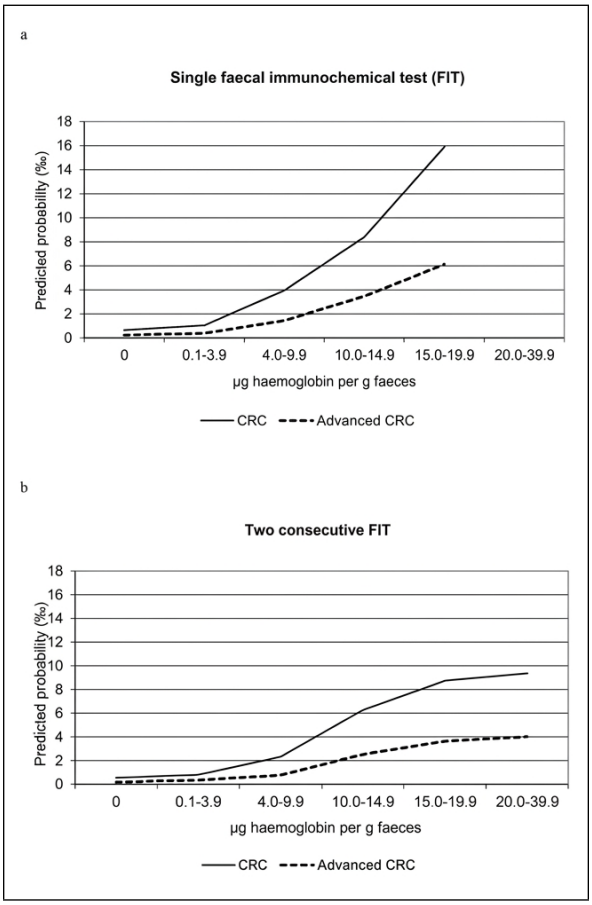

Figure 1
Crude 3-year risk (95% CI) of CRC by f-Hb concentration at a single or a two consecutive FIT.
CRC: colorectal cancer; f-Hb: faecal haemoglobin; FIT: faecal immunochemical test.

Table 2
Predictors of the 3-years CRC and advanced CRC risk (n=241,243)

	CRC		Advanced CRC	
Predictors	**aHR**	**95% CI**	**aHR**	**95% CI**
f-Hb (µg/g) at the index FIT				
0	*ref*		*ref*	
(0-4)	1.69	1.26-2.27	1.66	1.03-2.68
[4-10)	5.22	3.73-7.32	4.22	2.38-7.50
[10-15)	9.64	6.18-15.03	12.07	6.14-23.74
[15-20)	18.65	12.03-28.91	22.21	11.37-43.39
f-Hb (µg/g) at the previous FIT				
0	*ref*		*ref*	
(0-4)	1.18	0.92-1.51	1.26	0.84-1.88
[4-10)	2.14	1.52-3.02	2.06	1.17-3.65
[10-15)	2.43	1.39-4.25	1.30	0.41-4.13
[15-20)	1.91	0.83-4.38	2.38	0.72-7.89
Sex				
M	*ref*		*ref*	
F	0.80	0.64-1.00	0.74	0.51-1.05
Age class				
50-54	*ref*		*ref*	
55-59	0.95	0.65-1.41	1.02	0.56-1.88
60-65	1.46	1.00-2.12	1.53	0.84-2.77

CRC: colorectal cancer; aHR: adjusted hazard ratios; f-Hb: faecal haemoglobin; FIT: faecal immunochemical test; CI: confidence interval.

3 years, yielding an overall risk of 1.32‰ (95% CI: 1.18‰-1.47‰). Early-stage tumours comprised 52.8% (n=168) of cases, advanced-stage tumours accounted for 38.7% (n=123), and 8.5% (n=27) were of unknown stage, with no significant differences across f-Hb categories (p=0.278) (data not shown). The 3-year CRC risk using the two-test cumulative f-Hb modality ranged from 0.59‰ to 9.36‰ (*Table 1b*). The 3-year CRC risk was significantly higher in the ≥15 µg/g category of the single-test approach than in the ≥20 µg/g category of the two-test approach – 15.9‰ (57/3,595) *vs* 9.36‰ (14/1,496), p<0.001. Furthermore, the single-test approach identified a significantly higher percentage of high-risk individuals than the two-test modality – 0.86% (3,595/419,822) *vs* 0.62% (1,496/241,243), p=0.001. Conversely, both methods demonstrated comparable performance in identifying the lowest-risk group (0.65 for single FIT *vs* 0.59% for two consecutive tests, ‰ p=0.549). For the two-test strategy, the adjusted hazard of CRC ranged from a non-significant 35% increase for f-Hb <4 µg/g (aHR:1.35; 95% CI: 0.94-1.94), to 14-fold increase for cumulative levels of 20-40 µg/g (aHR: 13.60; 95% CI: 7.40-24.91). Advanced CRC risk mirrored these trends, with 4.9% of advanced cancers (6/123) occurring within the 0.6% of tests representing the highest cumulative tier (*Table 1b*; *Figure 1b*).

Table 2 presents the multivariate aHRs for f-Hb levels of the index and previous FITs, sex, and age-group within the two-test subset (n=241,243). The index (most recent) FIT f-Hb categories carried the strongest predictive weight, with the aHR escalating from 1.69 to 18.65 relative to the reference group. In contrast, f-Hb levels from the previous screening round significantly increased risk only above 4 µg/g, exhibiting a plateau thereafter; the aHR for the highest previous f-Hb level did not reach statistical significance (aHR: 1.91; 95% CI: 0.83-4.38). Females demonstrated a non-significantly lower risk than males, while risk increased progressively with age.

For the single FIT approach (*Table 3a*), 191 interval cancers (ICs) were diagnosed within 24 months of a negative test (0.45‰; 95% CI: 0.39‰-0.52‰). The risk gradient across f-Hb categories was more pronounced than for overall 3-year incidence: individuals in the >15 µg/g class exhibited an approximate 30-fold higher risk than those with undetectable f-Hb (aHR: 29.49; 95% CI: 17.41-49.96; data not shown). Between 24 and 36 months, 53 ICs were diagnosed (0.13‰; 95% CI: 0.10‰-0.17‰), showing an attenuated risk gradient (aHR for

>15 µg/g: 12.13; 95% CI: 4.06-36.30). Within 3 years of the index test, 82.2% of individuals (345,176/419,822) underwent a subsequent FIT yielding an overall positivity rate of 3.3% (*Table 3a*). The subsequent positivity rate scaled from 2.3% for individuals with undetectable index f-Hb to 15.8% for those exceeding 15 µg/g.

Under the two-test strategy (*Table 3b*), 117 ICs were diagnosed within 24 months (0.49‰), displaying a steeper risk gradient than the overall cohort (aHR for ≥15 µg/g: 22.48; 95% CI: 8.38-60.32; data not shown). An additional 21 ICs were diagnosed between 24 and 36 months (0.09‰), with a risk gradient mirroring the overall incidence. Within 3 years, 88.6% of participants (213,733/241,243) completed a subsequent FIT, with an overall positivity rate of 3.3% (*Table 3b*); subsequent round positivity ranged from 2.0% (undetectable cumulative f-Hb) to 19.5% (cumulative f-Hb ≥15 µg/g).

When stratified by age using a single FIT, all age cohorts with an f-Hb ≥15 µg/g experienced a 3-year risk exceeding 10‰, whereas all age groups with undetectable f-Hb maintained a risk below 1‰. Furthermore, the 50-54 and 55-59 age groups retained a risk profile below 1‰ if their f-Hb was <4 µg/g (*Table S2a available online as Supplementary Material*). Conversely, using the two-test cumulative approach, no age group within the cumulative >15-20 µg/g tier exceeded a 3-year risk of

Table 3
Interval cancers and screen-detected (SD) cancers at subsequent round (within 36 months after the index FIT) by f-Hb level at a single FIT (a) and by cumulative f-Hb at two consecutive FIT (b)

a)		Interval cancers		Next screening round	
		0-24 months	24-36 months	Subsequent FIT	SD cancer^
f-Hb (µg/g)	N	N	N	N	N
		‰	‰	*(% FIT+)*	‰
0	205,365	39	16	169,654	79
		0.19‰	0.08‰	*(2.3%)*	0.47‰
(0-4)	172,312	52	18	141,708	110
		0.30‰	0.10‰	*(3.3%)*	0.78‰
[4-10)	31,648	51	14	25,566	60
		1.61‰	0.44‰	*(7.1%)*	2.35‰
[10-15)	6,902	27	1	5,405	30
		3.91‰	0.14‰	*(11.6%)*	5.55‰
[15-20)	3,595	22	4	2,843	31
		6.12‰	1.11‰	*(15.8%)*	10.9‰
Total	**419,822**	**191**	**53**	**345,176**	**310**
		0.45‰	**0.13‰**	***(3.3%)***	**0.90‰**
b)					
f-Hb (µg/g)	**N**	**N**	**N**	**N**	**N**
		‰	**‰**	***(% FIT+)***	**‰**
0	73,203	12	3	65,675	28
		0.16‰	0.04‰	*(2.0%)*	0.43‰
(0-4)	119,684	30	7	105,883	59
		0.25‰	0.06‰	*(2.7%)*	0.56‰
[4-10)	35,250	31	6	30,901	45
		0.88‰	0.17‰	*(5.0%)*	1.46‰
[10-15)	7,497	22	1	6,472	24
		2.93‰	0.13‰	*(8.9%)*	3.71‰
[15-20)	4,113	16	3	3,540	17
		3.89‰	0.73‰	*(10.7%)*	4.80‰
[20-40)	1,496	6	1	1,263	7
		4.01‰	0.67‰	*(19.5%)*	5.54‰
Total	**241,243**	**117**	**21**	**213,733**	**180**
		0.49‰	**0.09‰**	***(3,3%)***	**0.84 ‰**

^Two cancers diagnosed respectively 10 and 12 months after a FIT positive test, were considered to be screen-detected after evaluation of their screening history.
f-Hb: faecal haemoglobin; FIT: faecal immunochemical test.

10‰, while all age cohorts with cumulative f-Hb levels <4 µg/g remained below 1‰. The 50-54 and 55-59 age cohorts consistently demonstrated the lowest risks, except within the highest f-Hb tier (*Table S2b available online as Supplementary Material*).

DISCUSSION

Quantitative faecal haemoglobin (f-Hb) levels in individuals with negative faecal immunochemical test (FIT) results strongly correlate with the subsequent risk of colorectal cancer (CRC) diagnosis [2-11]. Our findings confirm that among screening participants with a negative FIT, those with sub-threshold f-Hb levels exceeding 15 µg/g on a single test, 20 µg/g across two consecutive tests, represent less than 1% of the population yet face a substantially elevated risk of CRC within three years. These results support the implementation of risk-stratified screening strategies [3-5, 19], such as: (i) extending screening intervals for individuals with consistently undetectable f-Hb (0 µg/g), given that CRC yield remains stable at 2- *vs* 3-year intervals, (ii) intensifying screening (i.e., shortening intervals to 6-12 months or directly referring to total colonoscopy – TC) for individuals in the highest sub-threshold f-Hb tiers. The resulting increase in endoscopy TC demand could be balanced across subsequent rounds by extending intervals for the low-risk cohort, potentially optimizing resource allocation and reducing interval cancer rates.

The primary novelty of this study lies in determining the optimal approach for identifying elevated f-Hb values among FIT-negative individuals, specifically comparing a single-test strategy against a two-test cumulative approach. Our data indicate that a single test offers superior predictive ability and discriminatory capacity for identifying the highest-risk subgroup compared to two consecutive tests (3-year risk: 15.9% *vs* 9.36%), while maintaining comparable accuracy for the lowest-risk subgroup (0.65‰ *vs* 0.59‰). Furthermore, the single-test modality identified a larger absolute number of high-risk individuals (3,595 *vs* 1,496), whereas f-Hb levels from the preceding screening round did not add robust predictive value. Relying on a single test also provides significant organizational advantages. In the Florentine cohort, approximately 20% of individuals who completed an initial test defaulted from the subsequent round. Capturing these individuals is clinically vital, as those with a first sub-threshold f-Hb ≥15 µg/g, were more likely to present with either an interval cancer or a screen-detected CRC in the following round.

Intensifying screening for individuals with f-Hb levels between 15 and 20 µg/g inevitably demands additional endoscopy capacity. In our screening program, recommending immediate TC for all individuals with an f-Hb ≥15 µg/g on any FIT would increase the baseline positivity rate from 5.0% to 5.9%. However, because roughly 20% of these individuals went on to test positive within the subsequent five years anyway, this initial increase in TC volume would balance out over a 5-year period. Endoscopic resources could be further reclaimed by extending the screening interval (e.g., to 2.5 years) for individuals under 60 years of age with undetectable f-Hb levels. Prior literature confirms that lowering the FIT positivity threshold increases sensitivity for advanced adenomas in population-based screenings at the expense of specificity, ultimately reducing CRC incidence in subsequent rounds [20-22]. Moreover, baseline sub-threshold f-Hb concentrations have been strongly tied to long-term CRC risk, with an eightfold risk increase observed at an 8-year follow-up among individuals with baseline levels of 8-10 µg/g compared to those with undetectable f-Hb [5].

Relying on cumulative results from two consecutive negative tests present distinct limitations. Within-subject correlation between consecutive f-Hb measurements was weak, and the predictive value of cumulative f-Hb remained similar, regardless of the test sequence [4, 5]. Furthermore, repeated FIT screening can miss a subset of adenomas and CRCs due to systematic false-negative results, typically driven by non-bleeding lesions [23].

From a behavioural perspective, risk-based screening may enhance compliance among high-risk groups, but risk decreasing participation among low-risk individuals due to a false sense of reassurance [24, 25]. Alternatively, low-risk individuals – particularly those of higher socioeconomic status – might reject reduced access to standard services and independently seek more intensive screening elsewhere.

Balancing the precision of a detailed risk classification with real-world implementation remains a challenge. Insights from two ongoing randomized controlled trials evaluating tailored invitation intervals based on single or consecutive FIT results will provide crucial evidence on the scalability of these strategies [26, 27].

Integrating additional risk factors such as age and sex could further refine these models. Some programs already employ sex-specific FIT thresholds or sex-specific starting ages for screening colonoscopy [24]. Similarly, a risk-stratified age for screening cessation – where additional screening is offered to elderly cohorts based exclusively on the f-Hb levels of their last routine round – merits consideration, especially since guidelines generally recommend screening cessation in older populations unless life expectancy exceeds 10 years [28].

Regarding target population expansion, three national and international references indicate that the optimal target population for colorectal cancer screening should be between 50 and 74 years of age, thereby adding individuals aged 70-74 to the standard target population (people aged 50-69 years): the Recommendations of the Council of the European Union (2022/0290 NLE) [12]; the National Italian Prevention Plan 2020-2025 [29]; the National Italian Oncology Plan 2023-2027 (PON) [30].

Our study has certain limitations. Advanced adenomas were not captured because the regional cancer registry tracks malignant neoplasms exclusively. Additionally, lifestyle, socioeconomic variables, anthropometric measures, and family history were unavailable, though such parameters are rarely accessible in routine, population-based screening databases. Finally, historical variations in specimen collection methods, or buffer formulations might have subtly influenced f-Hb concentration distributions [19, 31].

Conversely, key strengths of this work include the

longitudinal analysis across multiple screening rounds, stratification by precise f-Hb levels, and a true population-based setting governed by a highly consistent screening protocol.

CONCLUSION

Among screenees with a negative FIT, those with f-Hb levels ≥15 µg/g represent less than1% of the population but face a significantly higher risk of a subsequent CRC diagnosis. A single-test approach demonstrates superior predictive ability compared to a two-test cumulative approach. While intensifying screening for individuals with elevated sub-threshold f-Hb requires greater colonoscopy capacity, these resources can be successfully reclaimed by extending screening intervals for individuals under 60 years of age with undetectable f-Hb. Future prospective studies and simulation models are warranted to refine the precise logistical and clinical parameters of these risk-stratified strategies.

Data availability

The data have been archived in the Zenodo repository and are available upon request from the corresponding author; doi: 10.5281/zenodo.17788549.

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Conflict of interest statement

None to declare.

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