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EVALUATING PERFORMANCE OF COMBINED HEMOGLOBIN AND TRANSFERRIN DETECTION IN FECAL IMMUNOCHEMICAL TESTING FOR COLORECTAL NEOPLASIA

Background. Evidence-based screening strategies can substantially reduce colorectal cancer (CRC) mortality. While colonoscopy is the gold standard, its invasiveness renders it less preferable as an initial screening tool. A two-step approach using a non-invasive fecal immunochemical test (FIT) followed by a confirmatory colonoscopy is gaining favor. A novel FIT that simultaneously detects fecal hemoglobin (F-Hb) and fecal transferrin (F-Tf) demonstrates variable diagnostic performance. **Aim.** This study compared the diagnostic performance of four screening strategies using three FITs with different cutoffs for F-Hb and F-Tf to detect neoplastic lesions in patients with suspected CRC. **Materials and Methods.** We conducted a cross-sectional study involving suspected CRC patients aged ≥ 18 at Hasan Sadikin Hospital, Bandung, from March 2023 to August 2023. The study included 72 clinically suspected CRC patients who underwent colonoscopy. We compared four CRC screening strategies using FITs designated as FIT-I (F-Hb ≥ 10 ng/mL), FIT-II (F-Hb ≥ 50 ng/mL), FIT-IIIa (F-Hb ≥ ≥ 100 ng/mL or F-Tf ≥ 40 ng/mL), and FIT-IIIb (F-Hb ≥ 100 ng/mL and F-Tf ≥ 40 ng/mL). **Results.** The FIT-IIIb strategy, which requires positive results for both markers, yielded the highest diagnostic performance for detecting neoplastic lesions, with 60.0% sensitivity, 96.6% specificity, a 93.8% positive predictive value, and a 73.7% negative predictive value. **Conclusion.** A dual-marker FIT detecting both F-Hb and F-Tf is a promising and effective screening tool for CRC. Future research should explore its implementation in broader populations and potential impacts on screening guidelines.

Keywords: colorectal cancer; fecal immunochemical test; hemoglobin; transferrin.

Colorectal cancer (CRC) is a major public health concern, being one of the leading causes of cancer-related morbidity and mortality worldwide [1, 2]. Early detection through effective screening strategies is crucial for improving survival rates [3]. Current guidelines recommend colonoscopy as the gold standard for CRC screening [4—6]. However, non-invasive screening tests such as fecal immunochemical

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tests (FIT) have gained traction due to their practicality and acceptability among patients [7]. Numerous studies have established that FIT is effective in detecting CRC and advanced adenomas, offering a viable alternative to invasive procedures [8—11].

The dual approach to screening, which combines non-invasive fecal tests with follow-up colonoscopy for positive results, has shown potential in increasing screening rates and delivering timely interventions [12, 13]. This study investigates the efficacy of fecal hemoglobin (F-Hb) and fecal transferrin (F-Tf) as biomarkers for neoplastic lesions, a key indicator of CRC. Previous research has highlighted Tf as a promising marker, particularly in the context of gastrointestinal pathology [14, 15]. By comparing the performance of F-Hb and F-Tf detection, this study aims to help optimize non-invasive CRC screening strategies and improve the early detection of neoplastic lesions.

Materials and Methods

Study design and subjects. This study was conducted at Hasan Sadikin Hospital, Bandung, Indonesia, from March 2023 to August 2023. The participant selection process is illustrated in Fig. 1. Participants included patients aged 18 years or older with clinical suspicion of CRC who were scheduled for colonoscopy. Exclusion criteria consisted of individuals with a history of other malignancies, loss to follow-up, inadequate stool sample, and incomplete or failed colonoscopy. All participants were informed of the study details and provided informed consent before inclusion. They were given a stool collection kit to collect their stool sample the day before the colonoscopy. Stool samples with visible blood were excluded from the study.

During the colonoscopy, the entire colon was examined. If deemed necessary, a tissue biopsy was performed for histopathological examination. Findings were categorized into two groups: neoplastic lesions (dysplasia, adenoma, and adenocarcinoma) and non-neoplastic lesions (non-dysplastic inflammation).

Four interpretive strategies were evaluated using three different fecal immunochemical tests (FIT) with varying cutoffs:

1. FIT I, defined as a positive result for F-Hb at 10 ng/mL or more using dBEST^{TM} One Step Occult Blood Test (AMETEK, Inc., USA).

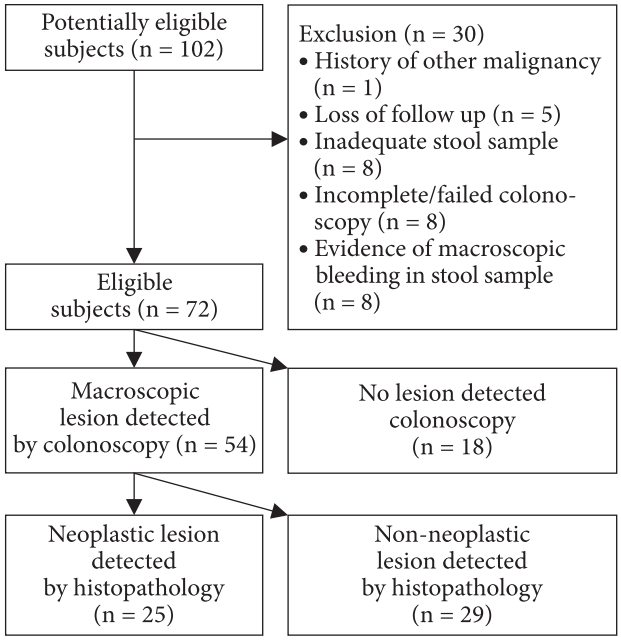

Fig. 1. Diagnostic evaluation of fecal immunochemical tests (FIT)

2. FIT II, defined as a positive result for F-Hb at 50 ng/mL or more using Rapid Response^{TM} Fecal Immunochemical Test (BTNX, Inc., Canada).

3. FIT IIIa, defined as a positive result for either F-Hb (≥100 ng/mL) or F-Tf (≥40 ng/mL) using Oncoprobe^{TM} Fecal Occult Blood + Transferrin (Oncoprobe, Inc., Indonesia).

4. FIT IIIb, defined as a positive result for both F-Hb (≥100 ng/mL) and F-Tf (≥40 ng/mL) using Oncoprobe^{TM} Fecal Occult Blood + Transferrin (Oncoprobe, Inc., Indonesia).

We conducted a diagnostic study analysis to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) for each method. The diagnostic accuracy of each FIT strategy was then compared.

Statistical analysis. Descriptive statistics were employed to analyze and present participant demographic data. The diagnostic accuracy of each FIT method for detecting neoplastic lesions in CRC was calculated using Microsoft Excel software (Microsoft Corporation, USA).

Results

Demographic and clinical characteristics of study subjects. The demographic and clinical characteristics of the study participants are summarized in Table 1. Among patients with neoplastic lesions,

Table 1. **Demographic and clinical characteristics of study subjects**

Characteristics	Neoplastic lesion (n = 25)	Non-neoplastic lesion (n = 29)	Total (n = 54)
	n (%)	n (%)	n (%)
Gender			
Male	15 (60.0)	11 (37.9)	26 (48.1)
Female	10 (40.0)	18 (62.1)	28 (51.9)
Age (years)			
18—25	2 (8.0)	6 (20.7)	8 (14.8)
26—35	3 (12.0)	1 (3.5)	4 (7.4)
36—45	4 (16.0)	3 (10.3)	7 (13.0)
46—55	8 (32.0)	7 (24.1)	15 (27.8)
56—65	7 (28.0)	7 (24.1)	14 (25.9)
>65	1 (4.0)	5 (17.3)	6 (11.1)
BMI*			
Underweight	8 (32.0)	6 (20.7)	14 (25.9)
Normal	14 (56.0)	17 (58.6)	31 (57.4)
Overweight	3 (12.0)	4 (13.8)	7 (13.0)
Obesity	0 (0.0)	2 (6.9)	2 (3.7)
Smoking			
Yes	10 (40.0)	5 (17.2)	15 (27.8)
No	15 (60.0)	24 (82.8)	39 (72.2)

Note: * BMI — body mass index.

Table 2. **Evaluation of FIT and histopathological findings**

Characteristics	Neoplastic lesion (n = 25)	Non-neoplastic lesion (n = 29)	Total (n = 54)
	n (%)	n (%)	n (%)
FIT I*			
Positive	15 (60.0)	7 (24.1)	22 (40.7)
Negative	10 (40.0)	22 (75.9)	32 (59.3)
FIT II**			
Positive	15 (60.0)	5 (17.2)	20 (37.0)
Negative	10 (40.0)	24 (82.8)	34 (63.0)
FIT IIIa***			
Positive	15 (60.0)	6 (20.7)	21 (38.9)
Negative	10 (40.0)	23 (79.3)	33 (61.1)
FIT IIIb***			
Positive	15 (60.0)	1 (3.4)	16 (29.6)
Negative	10 (40.0)	28 (96.6)	38 (70.4)

Notes: * Detected using dBEST^{TM} One Step Occult Blood Test (AMETEK, Inc.).

** Detected using the Rapid Response™ Fecal Immunochemical Test (BTNX, Inc.).

*** Detected using the Oncoprobe™ Fecal Occult Blood + + Transferrin Test (Oncoprobe, Inc.).

the majority were male (60.0%) and within the 46—55-year age group (32.0%). Additionally, most subjects with neoplastic lesions exhibited a normal BMI. Subjects with a smoking history of at least five years were grouped under «Yes.» The majority of the participants reported no history of smoking (72.2%); however, the proportion of subjects with a smoking history was higher among those with neoplastic lesions (40.0%) compared to those with non-neoplastic lesions.

Evaluation of FIT and histopathological findings. The stool samples of the participants were tested using three different FITs, interpreted with four different strategies, and then compared against colonoscopy results based on histopathological findings, as shown in Table 2. All four FIT strategies yielded the same number of true positive (n = 15) and false negative (n = 10) results when compared to histopathology. FIT I (F-Hb ≥ 10 ng/mL) exhibited the highest positivity rate (40.7%) but also had a 24.1% false positive rate. FIT IIIb had the lowest false positive rate (3.4%), whereas the rates for FIT II, FIT IIIa, and FIT I were 17.2%, 20.7%, and 24.1%, respectively.

Diagnostic performance of each FIT strategy. The data from Table 2 were further analyzed to determine sensitivity, specificity, PPV, NPV, LR+, and LR–. The diagnostic performance of each FIT for detecting neoplastic lesions is summarized in Table 3. All FIT strategies demonstrated a sensitivity of 60% in detecting neoplastic lesions, with varying specificity. FIT IIIb exhibited the highest specificity (96.6%) and PPV (93.8%), while FIT I had the lowest specificity (75.9%) and NPV (68.2%). Furthermore, FIT IIIb showed the highest LR+ (17.4%).

Discussion

Screening for CRC plays a pivotal role in managing this prevalent disease. The impact of earlier intervention on survival rates is profound, especially in cases detected at earlier stages. Among the screening methodologies, the fecal immunochemical test (FIT) has emerged as a valuable preliminary step, providing a less invasive alternative to colonoscopy, the gold standard in CRC diagnostics [16].

Male participants constituted 60% of the patients with neoplastic lesions, a finding consistent with existing research identifying gender as a significant risk factor for CRC. Men often display higher rates

of red meat and alcohol consumption, alongside a greater propensity for smoking—habits that correlate with increased cancer risk [1, 17, 18]. Meanwhile, the rising incidence of CRC among women prompts further investigation into previously underestimated risk factors within this demography [19—21].

Current guidelines recommend initiating CRC screening at age 50 and continuing until age 75 [22]. However, our findings challenge these conventional recommendations, revealing cases of CRC in individuals considerably younger than 50. The reasons for this demographic shift are under investigation, but the findings suggest that current screening protocols may require re-evaluation [23—25].

Interestingly, while obesity is widely acknowledged as a risk factor for CRC, our findings revealed that the majority of the subjects in our study maintained a normal body mass index (BMI). Previous research indicates that obesity during early adulthood signifies a correlation with increased risks of CRC development, as every five-unit increase in BMI accounts for a 20% escalation in risk [26, 27]. The underlying mechanisms appear to involve disturbances in metabolic processes that adversely affect the gastrointestinal mucosa, fostering long-term carcinogenic effects [28].

Moreover, we scrutinized the implications of smoking on CRC risk. Although smoking is a well-documented risk factor for various malignancies, we found that fewer participants with positive colonoscopy findings were smokers. This observation is notable because while smoking is a known contributor to early-onset CRC, the majority of our participants (72.2%) reported no active smoking history [29]. This situation highlights the complications associated with passive smoking; Gram et al. [30] reported that even individuals without active smoking histories may face elevated risks, particularly relating to CRC.

Delving into the biological mechanisms linking cigarette exposure to CRC development reveals significant insights. Tobacco smoke is associated with mutations in key oncogenes, particularly in the p53 and *BRAF* genes [29, 31, 32]. The potential for alterations in gut microbiota due to tobacco use further complicates this situation—this dysbiosis may activate pathophysiological processes in the colonic epithelium that foster carcinogenic developments [29, 31, 33, 34].

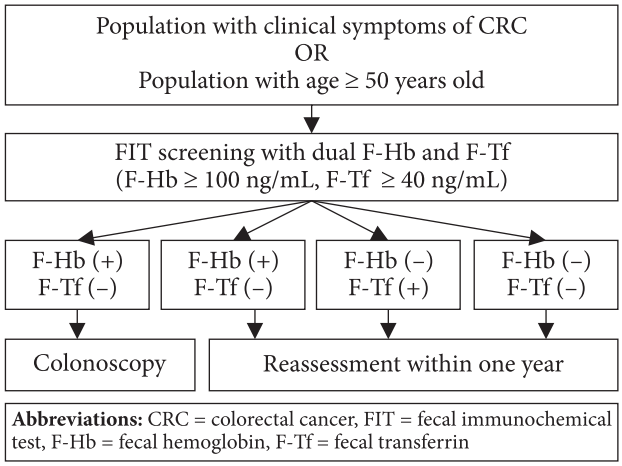

Fig. 2. Proposed screening algorithm

In constructing an effective screening method, several critical criteria must be met: cost-efficiency, ease of use, and patient comfort are essential, and the results should be both reproducible and valid for the early detection of disease. Stool examinations have emerged as a highly effective initial screening tool, effectively meeting most of these criteria [9].

Our investigation centered on the efficacy of F-Hb (fecal hemoglobin) and F-Tf (fecal transferrin) detection when employed alongside traditional colonoscopy and histopathological evaluations for those suspected of having CRC. We observed that some subjects with negative colonoscopy findings did not progress to histopathological assessment, emphasizing the importance of comprehensive testing. The results showcased a notable variance in diagnostic performance between FIT and colonoscopy, with both methods demonstrating a sensitivity of 60% and negative predictive values

Table 3. **Diagnostic performance of each FIT for neoplastic lesions**

Methods	SN (%)	SP (%)	PPV (%)	NPV (%)	LR+	LR–
FIT I*	60.0	75.9	68.2	68.8	2.5	0.5
FIT II**	60.0	82.8	75.0	70.6	3.5	0.5
FIT IIIa***	60.0	79.3	71.4	69.7	2.9	0.5
FIT IIIb***	60.0	96.6	93.8	73.7	17.4	0.4

Notes: * Detected using dBEST™ One Step Occult Blood Test (AMETEK, Inc.).

** Detected using the Rapid Response™ Fecal Immunochemical Test (BTNX, Inc.).

*** Detected using the Oncoprobe™ Fecal Occult Blood + Transferrin Test (Oncoprobe, Inc.).

ranging from 68.8% to 73.7% to detect colorectal neoplastic lesions.

In this study, FIT IIIb was the most effective strategy, using cutoffs of ≥100 ng/ml for F-Hb and ≥40 ng/ml for F-Tf. These parameters yielded a sensitivity of 60.0%, with a specificity reaching 96.6%. The positive predictive value was impressive at 93.8%, and the negative predictive value stood up at 73.68%. In contrast, FIT II, which employed an F-Hb cutoff of ≥50 ng/mL, presented slightly inferior results. The superior performance of FIT IIIb compared to FIT II can primarily be attributed to the inclusion of F-Tf analysis, as F-Hb detection in both methodologies yielded identical outcomes.

Although F-Tf might be expected to offer greater sensitivity, our findings indicate that its inclusion primarily enhanced specificity. This can be attributed to the unique stability of transferrin compared to hemoglobin, which is resistant to degradation from digestive enzymes and bacteria [35]. Importantly, F-Tf is secreted primarily during episodes of gastrointestinal bleeding, limiting its sensitivity in screening applications [35]. The differing performances of F-Hb and F-Tf have been corroborated by previous research; for instance, Hirata et al. reported F-Hb sensitivity at 67.3% and specificity at 90.6%, further supporting the need for well-rounded diagnostic approaches [14].

A successful screening tool must strike a balance between sensitivity and specificity. High sensitivity is critical to ensure that positive test results are indeed indicative of disease presence, while high specificity ensures that negative results accurately reflect the absence of disease. This balance holds even greater significance in screening for curable conditions in the preclinical stages [6, 36, 37]. Our findings advocate for a refined and thoughtful screening strategy that can guide clinicians in identifying individuals at medium or high risk for colorectal cancer. By implementing an initial screening with FIT, healthcare providers can better determine when further diagnostic evaluations are needed. We propose a clear pathway for this screening process, illustrated in Fig. 2.

Our study demonstrates that the fecal immunochemical test, which detects both F-Hb and F-Tf, shows promise as an effective screening tool for CRC. While the insights garnered from this study are significant, we acknowledge certain limitations. The classification of neoplastic lesions was not as detailed as in some previous research, which was a consequence of the small sample size. Future research with larger sample sizes is warranted to refine classifications and deepen our understanding of CRC risk factors.

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Conflict of Interest

The authors declare no competing interests relevant to the content of this article.

Ethical Statement

The study protocols comply with the ethical principles of the Declaration of Helsinki. Approval was also obtained from the Ethics Committee of Hasan Sadikin Hospital, Bandung, Indonesia (approval number LB.02.01/X.6.5/79/2023).

Data Availability Statement

Data are available upon request.

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ОЦІНКА ДІАГНОСТИЧНОГО ЗНАЧЕННЯ СУМІСНОГО ВИЯВЛЕННЯ ГЕМОГЛОБІНУ І ТРАНСФЕРИНУ В КАЛІ ДЛЯ ІМУНОХІМІЧНОГО ТЕСТУВАННЯ КОЛОРЕКТАЛЬНОГО РАКУ

Стан питання. Обґрунтовані стратегії скринінгу дозволяють суттєво знизити смертність від колоректального раку (КРР). Хоча колоноскопія є золотим стандартом, цей метод дослідження є інвазивним, що робить його менш бажаним засобом первинного скринінгу. Все більшу увагу привертає неінвазивний фекальний імунохімічний тест (ФІТ) з наступною колоноскопією для підтвердження. Нові тест-системи ФІТ, що дозволяють одночасно виявляти гемоглобін і трансферин у фекаліях (ф-гб і ф-тф), різняться своїми характеристиками. **Мета** роботи полягала у порівнянні діагностичних характеристик трьох тест-систем з різними відсікаючими значеннями для ф-гб і ф-тф і можливостей їх застосування для виявлення неопластичних утворень у хворих із підозрою на КРР. **Матеріали та методи.** Проведено дослідження за перехресною схемою у хворих старших за 18 років з підозрою на КРР з березня по серпень 2023 р. Дослідження охоплювало 72 хворих із клінічною підозрою на КРР, яким проводили колоноскопію. Порівняно чотири варіанти скринінгу із застосуванням тест-систем ФІТ, з наступними параметрами: ФІТ-I (ф-гб ≥ 10 нг/мл), ФІТ-II (ф-гб ≥ 50 нг/мл), ФІТ-IIIа (ф-гб ≥ 100 нг/мл або ф-тф ≥ 40 нг/мл), ФІТ-IIIб (ф-гб ≥ 100 нг/мл та ф-тф ≥ 40 нг/мл). **Результати.** Найкращі результати показав варіант ФІТ-IIIб, за умовами якого вимагалося, щоб позитивними були результати виявлення обох маркерів. Із застосуванням такого варіанту чутливість складала 60,0%, специфічність 96,6%, позитивна прогностична цінність 93,8%, негативна прогностична цінність 73,7%. **Висновки.** Визначення обох маркерів ф-гб і ф-тф за допомогою тест-систем ФІТ є ефективним засобом скринінгу на КРР. Наступні дослідження мають показати його дієвість на більш широких популяціях з можливим уточненням настанов для проведення скринінгу.

Ключові слова: колоректальний рак, фекальний імунохімічний тест, гемоглобін, трансферин.