BJC

Serum sCD26 for colorectal cancer screening in family-risk individuals: comparison with faecal immunochemical test

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Background: The development of specific screening programs for individuals with a family history of colorectal cancer (CRC) is a priority. This study evaluates the diagnostic performance of serum soluble CD26 (sCD26) in family-risk individuals and compares this marker with the faecal immunochemical test for the detection of advanced neoplasia (AN) (CRC or advanced adenomas; AA).

Methods: Five hundred and sixteen asymptomatic individuals with at least one first-degree relative with CRC were included. Serum sCD26 was measured in all the individuals who also underwent a colonoscopy (53 AA and four cancer cases were found) and a faecal immunochemical test.

Results: Setting specificity to 90% and 95%, respectively, sCD26 showed a sensitivity of 39.6% and 28.3% for AA, and of 42.1% and 28.1% for AN. The combination of sCD26 and the faecal test detected AA and AN with a 52.8% and 56.1% sensitivity, corresponding to 93.5% specificity.

Conclusions: The combination of serum sCD26 and the faecal blood test could result a valuable strategy for detecting AN in familial-risk CRC screening.

Colorectal cancer (CRC) is the third most common cancer and the fourth cause of cancer-related death worldwide (http://globocan.iarc.fr). Early-stage diagnosis and adenoma removal contribute to the reduction of CRC mortality and incidence (Zauber et al, 2012; von Karsa et al, 2013).

Several epidemiological risk factors have been established for CRC, including age and sex, as well as other CRC-specific factors like family history of CRC and inflammatory bowel disease (reviewed in Brenner et al, 2014). Individuals with first-degree relatives (FDR) with CRC, especially with multiple affected relatives or relatives diagnosed at young ages, have a two- to fourfold higher risk of developing CRC (Butterworth et al, 2006; Sulz et al, 2014).

Although clinical guidelines suggest more intensive strategies for these individuals at increased risk, no consensus exists for the best screening approach. Colonoscopy is empirically recommended as the first screening strategy (Winawer et al, 2003; Burt et al, 2010), though this procedure is accepted by < 40% of the risk population (Gimeno-García et al, 2009). Therefore, in order to increase the participation rate in screening programs, non-invasive tests like the faecal occult blood test (FOBT) have been evaluated. The guaiac-FOBT showed reduced utility for detecting cancer and adenomas in FDR of CRC patients (Houlston et al, 1990; Kristinsson et al, 2001); however, faecal immunochemical test (FIT) demonstrated high accuracy to detect cancer in familial CRC screening (Terhaar sive Droste et al, 2012; Castro et al, 2014).

Nevertheless, inappropriate performance to detect advanced adenomas (AA) mainly due to the intermittent and infrequent bleeding of lesions is a limitation of FIT (Terhaar sive Droste et al, 2012; Chiu et al, 2013; Ng et al, 2013), including its dependence on localisation, resulting more useful for distal lesions compared with proximal ones (Morikawa et al, 2005; Ciatto et al, 2007; Haug et al, 2011; Khalid-de Bakker et al, 2011). Moreover, as bleeding from the lower intestinal tract is a symptom related to conditions like diverticular disease, colitis, Crohn's disease and anorectal disorders, false-positive results may be increased (Barnert and Messmann, 2009). Therefore, there is an imperative need for the identification of non-invasive, blood-based markers that can help in the detection of cancer and AA.

The protease CD26, also known as dipeptidyl peptidase IV, is a transmembrane glycoprotein expressed in a variety of cell types and also present in plasma, serum and other biological fluids in a form named soluble CD26 (sCD26) (Gorrell et al, 2001). This multifunctional protein has been extensively related to cancer. On one hand, its regulatory role for degrading cytokines and chemokines has been associated to neoplastic transformation and progression, whereas on the other hand, its ability to bind to extracellular matrix proteins also suggested a role in tumour growth, migration and metastasis (Cordero et al, 2009; Yu et al, 2010).

Our group demonstrated that serum sCD26 has high diagnostic efficiency in a cohort of CRC patients and healthy controls, including early-stage tumours (Cordero et al, 2000). A second study revealed that sCD26 was also related to high-grade dysplasia and AA in symptomatic individuals who underwent a colonoscopy (De Chiara et al, 2010). Therefore, the aim of this study was to evaluate the diagnostic performance of sCD26 in a cohort of asymptomatic individuals with at least one FDR with CRC, and to compare this marker with FIT for the detection of CRC and AA.

MATERIALS AND METHODS

Study population. We designed a prospective, controlled, double-blinded study that included asymptomatic individuals with at least one FDR with histologically confirmed CRC. These individuals, recruited from Complexo Hospitalario Universitario de Ourense, were referred to perform a colonoscopy as a CRC-screening method. Patients with a personal history of CRC, adenomas or inflammatory bowel disease, hereditary CRC or colonoscopy examination within the past 5 years were excluded. A colonoscopy examination and a FIT were performed to all the individuals, as well as a blood extraction to obtain a serum sample. The study followed the clinical-ethical practices of the Spanish Government and the Helsinki Declaration, and was approved by the Galician Ethical Committee for Clinical Research. An informed consent was obtained from each individual and anonymity was warranted.

Colonoscopy. Colonoscopy was performed by experienced endoscopists (>200 colonoscopies per year), following the recommendations from the Spanish guidelines on quality of colonoscopy in CRC screening (Jover et al, 2012). Individuals were classified according to the most advanced lesion. Polyps were categorised as non-neoplastic (inflammatory and hyperplastic) or neoplastic (non-advanced adenomas (NAA) and AA). AA included adenomas ⩾10 mm, with tubulovillous or villous histology, high-grade dysplasia or intramucosal carcinoma. Cancer was classified according to the AJCC staging system (Edge and Compton, 2010). Advanced neoplasia (AN) was defined as AA or invasive cancer. The location of the lesions diagnosed in the individuals was classified as 'proximal' when located only proximal to the splenic flexure of the colon, and 'distal' when lesions were found only in the distal colon or in both distal and proximal colon.

Blood samples and sCD26 measurement. Blood was extracted 1 week before the endoscopic procedure. Samples were coagulated at room temperature for 20 min, and centrifuged at 2000 g for 15 min. Sera were stored at −20 °C. The sCD26 concentration was measured in duplicate with the Human sCD26 platinum ELISA kit (eBioscience; Vienna, Austria) according to the manufacturer's instructions. Colorimetric quantification was performed with a microplate reader (model 550; Bio-Rad; Hercules, CA, USA) at 450/570 nm.

Stool samples and FIT. A stool sample was collected the week before the colonoscopy without specific diet or medication restrictions. The faecal occult blood (ng haemoglobin ml^{-1}) was measured using a quantitative immunological test for the automated OC-Sensor (Eiken Chemical, Tokyo, Japan).

Data analysis. The data were included in a specifically designed database (http://www.coloncruzer.org/). Comparisons of sCD26 or FIT levels for two (Mann–Whitney U-test) or multiple groups (Kruskal–Wallis test) were performed. The ability of sCD26 or FIT to separate healthy from diseased patients was studied by receiver-operating characteristic (ROC) curves. Two cutoffs were selected for sCD26-setting specificity values close to 90% and 95%, respectively, as typically required in a screening setting. In the case of FIT, the cutoff at 100 ng ml^{-1} was used, which is the default setting defined by the manufacturer and is the standard used in many studies (van Rossum et al, 2008; Rozen et al, 2010; reviewed in Halloran et al, 2012). The criteria used to combine sCD26 and FIT was based on their individual cutoffs: a test was considered positive when at least one of the markers was positive (sCD26 and/or FIT), whereas a test was negative when both markers resulted negative. The performance characteristics (sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios) were calculated using MedCalc (v. 12.7.8). McNemar test was used to compare the sensitivities of sCD26 or sCD26 combined with FIT in relation to only FIT for the detection of AN or AA. The statistical analyses were performed with the SPSS software (v.20.0). P-values ⩽0.05 were considered statistically significant.

RESULTS

Baseline characteristics of the study population. The study included 516 asymptomatic individuals with at least one FDR with CRC, who completed a FIT, underwent a colonoscopy and had a serum sample. Individuals consisted of 212 men (41.1%) and 304 women (58.9%) with ages ranging from 28 to 84 years. According to the colonoscopy findings, patients were classified as follows: 338 cases with no neoplasia (65.5%, comprising no colorectal pathologies (n = 174) and benign pathologies that included haemorrhoids (n = 68), diverticula (n = 46), inflammatory (n = 5) and hyperplastic polyps (n = 39), and other minor findings (n = 6)), 121 cases with NAA (23.4%), 53 cases with AA (10.3%) and 4 CRC cases (0.8%; 2 stage I cases, 1 stage II case and 1 stage III case). Regarding the location of AA, 39 lesions (73.6%) were classified as distal, whereas 14 (26.4%) were proximal. The four CRC cases had a distal location.

Serum sCD26 levels in the study population. Soluble CD26 levels were analysed in relation to gender and age of the individuals (Table 1). We found no statistically significant differences regarding age (P-value = 0.126) or gender (P-value = 0.331). Differences were not observed (P-value = 0.479) in relation to the number or age of FDR with CRC.

Serum sCD26 levels according to the colonoscopy findings are presented in Table 2. Within the no neoplasia group, no statistically significant differences were found among the subgroups included in

Table 1. Serum sCD26 concentration according to the demographic characteristics of the study population

Characteristics	N (%)	Mean ± s.d. (ng ml^{-1})	Median (ng ml^{-1})	Range (ng ml^{-1})	P-value
Age (years)					
⩽49	179 (34.7)	517.9 ± 178.7	511.0	127–1167	
50–59	173 (33.5)	567.9 ± 221.1	535.0	102–1332	0.126[a]
⩾60	164 (31.8)	539.8 ± 200.0	514.0	97–1072	
Gender					
Male	212 (41.1)	531.4 ± 201.7	512.0	97–1167	0.331[b]
Female	304 (58.9)	548.8 ± 200.8	532.0	102–1332	
Familial risk					
1 FDR ⩾60 years	343 (66.5)	544.9 ± 202.1	526.0	102–1332	
1 FDR <60 years	119 (23.1)	522.0 ± 198.2	503.0	97–1120	0.479[a]
⩾2 FDR	54 (10.5)	564.1 ± 201.9	543.0	173–1093	

Abbreviation: FDR = first-degree relative.
[a] P-value for Kruskal–Wallis test.
[b] P-value for Mann–Whitney U-test.

Table 2. Serum sCD26 concentration according to the colonoscopy findings

Colonoscopy findings	N	Mean ± s.d. (ng ml^{-1})	Median (ng ml^{-1})	Range (ng ml^{-1})	P-value
No neoplasia	338	553.4 ± 192.0	535.0	102–1332	—
Non-advanced adenoma	121	573.3 ± 198.0	549.0	117–1167	0.333[a]
Advanced neoplasia	57	404.3 ± 210.1	341.0	97–1120	<0.001[a,b]
Advanced adenoma	53	401.5 ± 205.5	360.0	97–1120	<0.001[a]
Cancer	4	441.0 ± 301.0	302.5	268–891	0.133[a]

P-value for Mann–Whitney U-test for comparisons with the no neoplasia group (a) and the non-advanced adenoma group (b).

this collective (no colorectal pathologies: $546.7 \pm 196.0 \, \text{ng ml}^{-1}$, haemorrhoids: $569.2 \pm 205.3 \, \text{ng ml}^{-1}$, diverticula: $610.5 \pm 192.6 \, \text{ng ml}^{-1}$, inflammatory polyps: $572.0 \pm 127.4 \, \text{ng ml}^{-1}$, hyperplastic polyps: $495.3 \pm 146.6 \, \text{ng ml}^{-1}$, and other minor findings: $495.2 \pm 115.5 \, \text{ng ml}^{-1}$; Kruskal–Wallis test, P-value = 0.083).

When the sCD26 concentration was compared according to the diagnostic subgroups no neoplasia, NAA, AA and CRC, statistical significant differences were found (Kruskal–Wallis test, P-value <0.001). The no neoplasia and the NAA groups showed similar sCD26 levels (P-value = 0.333), whereas decreased concentrations were found in AA and CRC, with significant differences between AA and no neoplasia (P-value <0.001). Regarding CRC, no differences were found (P-value = 0.133), although the reduced number of individuals makes this comparison unreliable. As the goal of CRC screening is not only to detect cancer but rather precancerous lesions, CRC and AA were considered together as AN. Compared with no neoplasia and NAA, we found a statistically significant decrease in the sCD26 concentration (P-values <0.001) in the AN group.

FIT in the study population. The faecal haemoglobin concentration (mean ± s.d., median and range) according to the colonoscopy findings was as follows: no neoplasia (15.0 ± 132.8; 0; $0–2311 \, \text{ng ml}^{-1}$), NAA ($23.7 \pm 124.6$; 0; $0–1256 \, \text{ng ml}^{-1}$), AA ($330.6 \pm 741.6$; 9; $0–3737 \, \text{ng ml}^{-1}$), CRC ($556.0 \pm 561.4$; 338; $168–1380 \, \text{ng ml}^{-1}$), and the AN group ($346.4 \pm 728.7$; 13; $0–3737 \, \text{ng ml}^{-1}$). Statistically significant differences were found when FIT was compared among the groups no neoplasia, NAA, AA and CRC (Kruskal–Wallis test, P-value <0.001), as well as the comparison between AN and the rest of the groups (Mann–Whitney U-test, P-values <0.001).

Diagnostic accuracy of sCD26, FIT and their combination for the detection of AN. To evaluate the diagnostic utility of sCD26 and FIT to discriminate patients with AN, ROC curves were drawn. Soluble CD26 showed an area under curve (AUC) of 0.748 (95% CI: 0.708–0.785), whereas for FIT, this resulted slightly

inferior (0.719, 95% CI: 0.678–0.758). Table 3 summarises the performance characteristics for each marker and their combination to detect AN. Two different cutoffs were selected for sCD26-setting specificity close to 90.0% and 95.0%, as required for screening tests. The first cutoff ($\leqslant 330 \, \text{ng ml}^{-1}$) showed a sensitivity of 42.1% with a specificity of 90.2%, whereas the second cutoff ($\leqslant 280 \, \text{ng ml}^{-1}$) resulted in a sensitivity of 28.1% and a specificity of 95.2%. For FIT, the reference standard cutoff $100 \, \text{ng ml}^{-1}$ resulted in a sensitivity and specificity of 36.8% and 98.3%, respectively.

The diagnostic parameters for the combined markers were analysed for both the 330 and $280 \, \text{ng ml}^{-1}$ sCD26 cutoffs. With the first, a considerable 64.9% sensitivity was reached for detecting AN, with a specificity close to 90.0%. For the $280 \, \text{ng ml}^{-1}$ cutoff, specificity increased (93.5%), whereas sensitivity moderately diminished to 56.1%. When sensitivities for the detection of AN were compared in relation to FIT, statistically significant differences were observed for both sCD26 cutoffs combined with FIT (McNemar test, P-values <0.001).

Performance of sCD26, FIT and their combination for the detection of AA. The performance of the markers for detecting AA is shown in Table 4. For the $330 \, \text{ng ml}^{-1}$ cutoff, sCD26 showed a sensitivity of 39.6% (90.2% specificity), whereas for the $280 \, \text{ng ml}^{-1}$ cutoff, this resulted in 28.3% (95.2% specificity). The combination of sCD26 and FIT resulted in a considerably superior sensitivity when using any of the two cutoffs (62.3% and 52.8%, respectively) compared with FIT (32.1%), as statistically corroborated (McNemar test, P-values ⩽0.001).

Table 4 also includes the detection rates according to the distal or proximal location of AA. FIT showed considerable differences for detecting distal and proximal lesions (41.0% distal vs 7.1% proximal). However, sCD26 seems to detect similarly distal and proximal AA for both cutoffs (~40% and 28%, respectively). This tendency for comparable distal and proximal detection rates was also evidenced when sCD26 and FIT were combined; however, the

Table 3. Performance characteristics of sCD26, FIT and their combination for the detection of advanced neoplasia

Cutoff point	Individuals with a positive result	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	P-value[a]
sCD26								
$\leqslant 330$ ng ml^{-1}[b]	69 (13.4%)	42.1 (29.1–55.9)	90.2 (87.1–92.8)	34.8 (23.7–47.2)	92.6 (89.8–94.9)	4.3 (2.8–6.5)	0.6 (0.5–0.8)	0.710
$\leqslant 280$ ng ml^{-1}[c]	38 (7.4%)	28.1 (17.0–41.5)	95.2 (92.8–97.0)	42.1 (26.3–59.2)	91.4 (88.5–93.8)	5.9 (3.3–10.5)	0.8 (0.6–0.9)	0.441
FIT								
$\geqslant 100$ ng ml^{-1}	30 (5.8%)	36.8 (24.4–50.7)	98.3 (96.6–99.2)	72.4 (52.8–87.3)	92.6 (89.9–94.8)	21.1 (9.8–45.5)	0.6 (0.5–0.8)	—
Combination of sCD26 and FIT								
sCD26 ($\leqslant 330$ ng ml^{-1})[b] and/or FIT ($\geqslant 100$ ng ml^{-1})	90 (17.4%)	64.9 (51.1–77.1)	88.5 (85.2–91.2)	41.1 (30.8–52.0)	95.3 (92.8–97.1)	5.6 (4.1–7.7)	0.4 (0.3–0.6)	<0.001
sCD26 ($\leqslant 280$ ng ml^{-1})[c] and/or FIT ($\geqslant 100$ ng ml^{-1})	62 (12.0%)	56.1 (42.4–69.3)	93.5 (90.8–95.5)	51.6 (38.6–64.5)	94.5 (92.0–96.4)	8.6 (5.7–13.0)	0.5 (0.3–0.6)	<0.001

Abbreviations: CI = confidence interval; FIT = faecal immunochemical test.
[a]P-value for McNemar test comparing the proportion of advanced neoplasia detected (sensitivity) with sCD26 or its combination with FIT in relation to only FIT.
The sCD26 cutoffs were selected setting specificity close to
[b]90.0% and
[c]95.0%.

Table 4. Performance of sCD26, FIT and their combination for the detection of advanced adenomas

	sCD26 ($\leqslant 330$ ng ml^{-1})[a]	sCD26 ($\leqslant 280$ ng ml^{-1})[b]	FIT ($\geqslant 100$ ng ml^{-1})	sCD26 ($\leqslant 330$ ng ml^{-1})[a] and/or FIT ($\geqslant 100$ ng ml^{-1})	sCD26 ($\leqslant 280$ ng ml^{-1})[b] and/or FIT ($\geqslant 100$ ng ml^{-1})
All locations (n = 53)					
Specificity % (95% CI)	90.2 (87.1–92.8)	95.2 (92.8–97.0)	98.0 (96.3–99.1)	88.5 (85.2–91.2)	93.5 (90.8–95.5)
Sensitivity % (95% CI)	39.6 (26.5–54.0)	28.3 (16.8–42.3)	32.1 (19.9–46.3)	62.3 (47.9–75.2)	52.8 (38.6–66.7)
P-value[c]	0.572	0.839	—	<0.001	0.001
Distal (n = 39)					
No. of cases detected	15/39 (38.5%)	11/39 (28.2%)	16/39 (41.0%)	26/39 (66.7%)	23/39 (59.0%)
Proximal (n = 14)					
No. of cases detected	6/14 (42.9%)	4/14 (28.6%)	1/14 (7.1%)	7/14 (50.0%)	5/14 (35.7%)

Abbreviations: CI = confidence interval; FIT = faecal immunochemical test; sCD26 = soluble CD26. The sCD26 cutoffs were selected setting specificity close to
[a]90.0%.
[b]95.0%.
[c]P-value for McNemar test comparing the proportion of advanced adenomas detected (sensitivity) with sCD26 or its combination with FIT in relation to only FIT.

reduced number of lesions, especially the proximal AA, prevents the statistical confirmation of these results.

DISCUSSION

Nowadays, the screening options for individuals with FDR with CRC are centred on colonoscopy, although its low compliance encourages the identification of alternative non-invasive tests for the detection of CRC and AA.

To our knowledge, our study is the first that analyses the diagnostic performance of serum sCD26 to detect AN in a cohort of asymptomatic individuals with at least one FDR with CRC, and additionally compares and combines its use with that of FIT.

The utility of serum sCD26 for the detection of CRC and AA suggested in our preceding studies (Cordero et al, 2000; De Chiara et al, 2010) was also found in this cohort of FDR of CRC patients, particularly in relation to AA. In the case of CRC, although a trend to decreased sCD26 levels was evidenced as well, the reduced number of CRC cases in this cohort restricts any further conclusion in relation to this group of patients for any of the markers analysed.

The cutoffs for sCD26 were selected taking into consideration the requirement of an elevated specificity for a screening test. The 330 ng ml^{-1} cutoff showed additionally a considerable sensitivity for the detection of AN, superior to that for faecal blood. Regarding FIT, as our intention is not to determine its diagnostic accuracy, but instead compare and combine this test with our experimental marker, the standard cutoff at 100 ng ml^{-1} was used (van Rossum et al, 2008; Rozen et al, 2010; reviewed in Halloran et al, 2012),

rendering a somewhat superior specificity compared with sCD26. Though individually these markers do not show the optimal performance expected for a screening test, their combination increased the sensitivity for the detection of AN to 64.9% (330 ng ml^{-1} cutoff) or 56.1% (280 ng ml^{-1} cutoff) compared with the single tests. The increased sensitivity evidenced for both cutoffs justify the convenience for combining sCD26 and FIT. In relation to specificity, though this parameter was affected to some extent when markers were combined, a value close to the 90.0–95.0% was reached for the combination, lying within the range typically required in a screening setting.

Very limited data are available on the performance of FIT in cohorts of individuals with FDR with CRC. Rozen et al (2010) reported a sensitivity (31.5%) and specificity (96.4%) for AN comparable to our results for the 100 ng ml^{-1} cutoff, whereas Terhaar sive Droste et al (2012) found a lower sensitivity and similar specificity using the 50 ng ml^{-1} cutoff. Recently, Stegeman et al (2014) proposed a risk prediction model that included besides FIT other clinical variables (AUC: 0.76). In Spain, only two studies have analysed the performance of FIT for familial-risk CRC screening. The first was a pilot study (Gimeno-García et al, 2009) that showed a sensitivity of 83% for detecting AA (no CRC cases were reported) with 91% specificity, based on the 50 ng ml^{-1} cutoff. The second included all the individuals from our cohort and others recruited from San Sebastián (País Vasco), and reported for the 100 ng ml^{-1} cutoff a similar sensitivity (40.6%) for the detection of AN, with the same specificity we found (98.0%) (Castro et al, 2014).

Although a large number of candidate biomarkers have been described for CRC and in some cases also for AA, serum sCD26 seems one of the most promising. Shimwell et al (2010) indicated that the combination of sCD26, MMP9 and DR70 improved the detection of CRC, showing a sensitivity of 61.3% with 90% specificity in a symptomatic cohort of CRC cases and non-cancer controls. Soluble CD26 was also included in a multiplex serum protein chip together with other eight proteins in a case–control study, resulting in the fourth best marker with an AUC of 0.639 for identifying CRC (Bünger et al, 2012). Tao et al (2012) measured serum sCD26 and other inflammatory markers in individuals that derived from both a screening and a clinical setting, reporting no differences in sCD26 levels between AA and no neoplasia.

In our study, we report a potentially interesting finding that deals with the detection of distal and proximal lesions. Although FIT detected better distal AA compared with proximal ones, as reported in several studies (Morikawa et al, 2005; Ciatto et al, 2007; Haug et al, 2011; Khalid-de Bakker et al, 2011), sCD26 performed similarly for detecting distal and proximal AA, suggesting a possible lack of association with the location of lesions. This trend was also evidenced when sCD26 was combined with FIT. However, these results should be cautiously interpreted and considered preliminary due to the reduced number of lesions, especially the proximal ones (14 cases).

On the basis of the suitable diagnostic performance for AN achieved when sCD26 and FIT were simultaneously combined, in addition to the potentially high compliance expected for the non-invasive serum sCD26 test, besides its immune-related origin independent of the bleeding of lesions, we propose the use of sCD26 to complement FIT for the screening of individuals with FDR with CRC. In other words, our proposal basically consists of testing serum sCD26 among FIT-negative individuals. In Figure 1, we present an algorithm summarising the outcome for the sequential use of both markers (strategy 2 and 3) compared with the single-testing with FIT (strategy 1) based on our study cohort. Two sequential approaches are shown starting with FIT, and testing serum sCD26 only among FIT-negative individuals. For FIT, we considered the $100 \, ng \, ml^{-1}$ cutoff, whereas for sCD26, the $330 \, ng \, ml^{-1}$ cutoff is used for strategy 2 and the $280 \, ng \, ml^{-1}$ cutoff for strategy 3.

According to FIT, 30 individuals would be selected for colonoscopy examination, detecting all CRC cases and 17 AA (32.1% of the total). If the screening strategy were limited to FIT (strategy 1), the rest of the AA (67.9%) would be missed, as typically only FIT-positive individuals undergo a colonoscopy in a screening initiative. In the second stage of our screening proposal, sCD26 is measured in the 486 FIT-negative individuals. Depending on the cutoff used, another 60 (strategy 2) or 32 (strategy 3) individuals would be selected for colonoscopy. In the first case, 16 cases with AA would be additionally diagnosed, whereas strategy 3 would result in 11 more AA cases compared with only FIT. As shown in Table B from Figure 1, the differences between strategy 2 and 3 rely on the following: whereas the latter implies 28 less colonoscopies, the former diagnoses five more AA cases despite 23 additional individuals are misclassified. On the basis of this data, the choice for the strategy to be implemented for the screening of individuals with FDR with CRC will depend on the requirements of the approach in terms of specificity or sensitivity. In any case, the strategy proposed by using sequentially both markers (first FIT followed by sCD26 among FIT negatives) would result in the detection of the four CRC cases and $>52\%$ of the total AA from our study cohort.

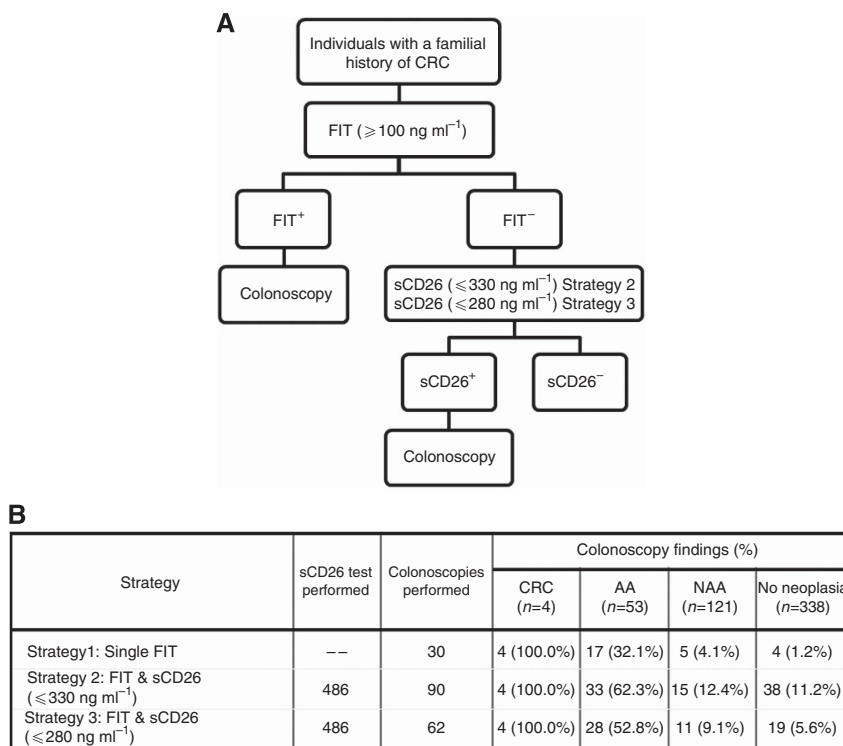| Strategy	sCD26 test performed	Colonoscopies performed	Colonoscopy findings (%)			
			CRC (n=4)	AA (n=53)	NAA (n=121)	No neoplasia (n=338)
Strategy1: Single FIT	––	30	4 (100.0%)	17 (32.1%)	5 (4.1%)	4 (1.2%)
Strategy 2: FIT & sCD26 ($\leqslant 330 \, ng \, ml^{-1}$)	486	90	4 (100.0%)	33 (62.3%)	15 (12.4%)	38 (11.2%)
Strategy 3: FIT & sCD26 ($\leqslant 280 \, ng \, ml^{-1}$)	486	62	4 (100.0%)	28 (52.8%)	11 (9.1%)	19 (5.6%)

Figure 1. Two-stage algorithm based on FIT and serum sCD26 tests for individuals having FDR with CRC. The step-by-step algorithm (A) comprises a sequential testing, starting with FIT and followed by serum sCD26. FIT positive (FIT+) individuals would be selected for colonoscopy, whereas FIT− individuals would be tested using sCD26 (with the $330 \, ng \, ml^{-1}$ cutoff for Strategy 2 or the $280 \, ng \, ml^{-1}$ cutoff for Strategy 3). sCD26-positive (sCD26+) individuals would be referred also to colonoscopy. The table (B) shows the outcome for only FIT (Strategy 1) or for FIT and sCD26 (Strategy 2 and 3) based on the study cohort (n = 516 individuals with a familial history of CRC). The percentage of colonoscopy findings regarding CRC (colorectal cancer), AA (advanced adenomas), NAA (non-advanced adenomas) and no neoplasia are related to the number of cases from the pathological group in the study cohort.

Our study has four main strengths: (i) unlike the majority of studies that analyse the performance of a new biomarker, we have included individuals with colonoscopy, FIT and serum sCD26, which allows the direct comparison of performance characteristics of our proposed experimental marker with the so far most valuable non-invasive FIT test; (ii) as our study was conducted in a true screening setting, the findings are not biased like many studies in which NAA and other benign colorectal pathologies are excluded; (iii) the comparisons carried out in our work are not biased by the inclusion of only FIT-positive individuals; (iv) the timing between the FIT test, colonoscopy and blood extraction were carefully established in the study design to avoid interference with the result of each test due to sedation or bowel preparation for colonoscopy.

The limitations of the study should also be mentioned, starting with the modest sample size, particularly the reduced number of cancer cases. The low prevalence of CRC cases (0.8%) found in our cohort complicated the estimation of the diagnostic performance of both markers to detect cancer. However, cancer prevalence of 0.65–0.7% has been described by others in familial-risk-screening scenarios (Del Vecchio Blanco et al, 2013; Ng et al, 2013). This difficulty is frequently reported in high-risk cohorts evaluating performance of FIT, with no >6/595–20/1682 CRC cases detected (Rozen et al, 2010; Castro et al, 2014). As stated previously, the reduced number of distal-proximal AA also limited the interpretation of our results regarding the detection related to the anatomical site. In addition, as our cohort mainly consists of individuals with one FDR with CRC, we should also include individuals with higher CRC risk (more than one FDR). Another limitation that should also be mentioned is that serum sCD26 may potentially appear altered in individuals with pathologies like oral squamous cell carcinoma, hepatocellular carcinoma and other liver pathologies, Crohn's disease, and certain autoimmune diseases (reviewed in Cordero et al, 2009), although the symptomatology of these pathologies will possibly conduct clinicians to perform specific procedures.

The identification of a non-invasive-screening test, with an outstanding diagnostic performance, that can achieve high patient compliance and that is cost-effective is an extraordinary challenge. In our study, we found that serum sCD26 can contribute to improve the performance of FIT for detecting AN in FDR of CRC patients, complementing its capacity when offered to FIT-negative individuals. This strategy could result a valuable screening approach to be validated in a large, multi-centric cohort.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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