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SHORT REPORT

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Serological biomarkers in triage of FIT-positive subjects?

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ABSTRACT

FIT-based colorectal cancer screening has been implemented in many countries including Denmark, where 916 colorectal cancer and 4468 high- or medium-risk adenoma patients were identified within April–December 2014, among 16,806 subjects with a positive FIT test. Screening increases the overall requirements for colonoscopy, which may challenge the current capacity. Some countries have increased their initial FIT cut-off level in order to comply with lack of colonoscopy capacity. Many patients with neoplasia will not be detected, however, by using increased FIT cut-off levels. The number of patients with neoplastic lesions missed by increased cut-off levels appears to be much higher than expected. Therefore, tests that identify those patients missed by increased FIT cut-off levels must be developed. Preliminary results of determination of one of several biomarker entities currently under investigation show that nucleosome blood tests may be one option for identifying some of these patients. Implementation of a triage test consisting of FIT, blood-based biomarkers and plus/minus colonoscopy is suggested to identify subjects with FIT levels between the initial and the increased cut-off level that must be offered colonoscopy. In addition, triage may reduce the frequency of unnecessary colonoscopies by 25%.

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Report

As with a number of European countries, Denmark has initiated population screening for colorectal cancer (CRC) among the average risk population of 50–74 years of age. The Danish model is based on Fecal Immunochemical Testing (FIT) using an out-reach procedure with subsequent offer of colonoscopy to all subjects with a positive FIT result (cut-off: 100 ng/ml). Requests to screening were launched by March 2014, with invitations being sent to approximately a 25% of the screen relevant population annually including all Danish citizens with birth dates occurring during three different months at Year 1, during three other months at Year 2, etc. The entire screen relevant population is estimated to be invited before the end of December 2017.

Reports are available for the year 2014; overall the screening has been an overwhelming success. In total 390,509 subjects were invited in that period, the compliance rate was 64% and 16,806 subjects (6.8%) had a positive FIT result that led to subsequent referral to colonoscopy that was completed within 14 days. Colonoscopy referral was accepted by 89% and led to detection of 916 patients with CRC, and 4418 with high-risk (HRA) or medium-risk (MRA) adenoma (Table 1). The CRC stage [Union for International Cancer Control (UICC)] distribution was: Stage I: 28% (12%), Stage II: 36% (35%), Stage III: 26% (34%) and Stage IV: 10% (19%) (stage distribution among non-screen diagnosed patients).

It is well-recognised that CRC screening leads to substantially increased numbers of colonoscopy referrals in addition to those required for routine diagnostics and surveillance. Calculations by the Danish CRC screening board estimated that screening would require an additional 18,000 colonoscopy procedures annually during the 4 years taken to offer a first FIT screening of all screen relevant Danish citizens. To meet the increased demand, some countries, including Denmark, have launched training programmes for nurses to perform some of the colonoscopy procedures, and the present Danish colonoscopy capacity appears to meet demands. By 2018, however, the screening invitation interval will be reduced from every fourth year to every second year which, by current estimates, will require ~34,500 colonoscopies annually in addition to those required for routine diagnostics and surveillance. This is likely to lead both to an immediate challenge to the colonoscopy capacity through FIT-positive referrals, as well as an increase in surveillance colonoscopy procedures required for patients with HRA and MRA (Table 1).

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Insufficient colonoscopy capacity may be one of the factors that have led to increased cut-off levels used in FIT screening in some European countries, including Sweden, Scotland and the Netherlands. However, increased cut-off levels may result in a number of CRC and HRA/MRA patients not being detected. Consequently, the value of CRC screening may be jeopardised substantially, even if this is temporary. Thereby, the populations may develop a distrust of the value of CRC screening.

We recently completed the accrual of Part 1 of the Endoscopy III validation study on blood-based biomarkers for early detection of neoplastic lesions, including CRC [1]. The primary aim of the study, which followed the REMARK guidelines [2], was to collect blood samples and data from >8000 subjects screened positive with FIT; analyses of various biomarkers can be used to develop and validate a blood test for screening of CRC either as single testing or in combination with FIT [1]. The final inclusion comprised blood samples, demographic data, occult blood (FIT) concentration ($100 \leq$ FIT <1000 ng/ml) and subsequent colonoscopy data from 8415 subjects with a positive FIT result and in addition blood samples and demographic data from 5118 subjects with a negative FIT result [$35 <$ FIT <100 ng/ml (35 ng/ml is the detection limit)]; subjects with negative FIT results did not undergo colonoscopy. All included subjects were participants of the Danish screening programme. The files are presently audited on-site or by access to national databases, including colonoscopy and pathology reports. The results of the first 1977 of the 8415 included FIT-positive subjects are finalised and presented with the findings at colonoscopy grouped by FIT thresholds. Indeed, the 1977 subjects were accrued in the same period as the reported data from the Danish screening programme (2014). Table 2 shows the number and percentage of colonoscopies that could be avoided in the study population by increasing the FIT cut-off levels.

Efforts to reduce the number of screen colonoscopies in Denmark from 2018 may lead to similar considerations to increase the FIT cut-off level as in other European countries. It must be considered however, that although increased FIT cut-off levels reduce the numbers of screen colonoscopies substantially, such changes will lead to considerable number of diseased subjects, who would not be detected by screening as illustrated in Table 2. In the event that the future cut-off is increased from 100 to 200 ng/ml, screen colonoscopies could be reduced by 32.6% (Table 2), but 11 CRCs, 52 HRAs and 90 MRAs would not have been detected among the 644 FIT-screened subjects. Therefore, tests to identify those subjects at risk of having neoplastic bowel lesions, but not identified by current screening programmes using increased FIT cut-off levels, must be developed, validated and implemented. Such test options may consider serological biomarkers including proteomics, genomics, epigenomics, transcriptomics, metabolomics, etc., which may be used as single markers or in various combinations to improve the sensitivity of the testing. Ideally, a triage concept may be developed and clinically validated in studies, which also focus on cost-benefit issues, for implementation in future screening programmes. For instance, such programmes using FIT testing with a cut-off level at $\geq$200 ng/ml may offer a blood test to subjects with FIT between 100 ng/ml and the increased level (Table 2). Thereby, the subjects that need a colonoscopy could be identified from those, who do not need a colonoscopy.

At present, analyses of various cell-free, circulating nucleosomes and histone modifications, including 5-methylcytosine-methylated DNA, histone variant H2AZ, histone 3 variant H2AX, histone variant mH2A1.1 and total nucleosomes, have been performed in serum samples from the 1977 subjects with a positive FIT test [3,4]. Application of these results would have identified a number of subjects in need of colonoscopy due to increased risk of having neoplastic lesions (Table 2). The statistical model to differentiate between subjects, who need and subjects, who do not need colonoscopy is based on logistic regression using a threshold corresponding to 75% sensitivity for those with FIT concentration between 100 ng/ml and the increased level shown in Table 2.

These preliminary results show that inclusion of triage (FIT/blood test/colonoscopy) could be advantageous compared to increased, fixed FIT cut-off levels to reduce the numbers of screen colonoscopies. The Screening Boards should consider recommendation of an increase of the FIT cut-off level for instance to 200 ng/ml; in addition, it may be considered offering subjects with FIT levels $100 \leq$ FIT <200 ng/ml a subsequent blood test. Present calculations show that the total number of unnecessary colonoscopies could be reduced by 25% by FIT/blood test screening using

Table 1. Present Danish criteria for histological diagnosis of the various adenoma categories.

HRA	Size $\geq$20 mm	Lesions $\geq$5	Resected by piece-meal technique	
MRA	10 $\leq$ size $<$20 mm	3 $\leq$ lesions $<$5	High-grade neoplasia	Villous elements
LRA	Size $<$10 mm	Lesions $<$3	Low-grade neoplasia	Tubular lesions

LRA: low-risk adenoma.

Table 2. Shows fixed, increased FIT cut-off levels and number (%) of unnecessary colonoscopies defined by the levels among 1977 subjects with positive FIT result.

FIT cut-off level (ng/ml)	Spared co-lonoscopies, *n* (%)	Missed CRC, *n* (%)	Missed HRA, *n* (%)	Missed MRA, *n* (%)	Identified CRC, *n* (%)[a]	Identified HRA, *n* (%)[a]	Identified MRA, *n* (%)[a]
200	644 (32)	11 (9)	52 (20)	90 (25)	5 (45)	15 (28)	41 (45)
250	868 (44)	27 (23)	78 (30)	122 (45)	10 (37)	21 (27)	50 (41)
300	1005 (51)	33 (28)	95 (36)	145 (41)	14 (42)	26 (27)	54 (37)
400	1193 (60)	43(36)	120 (46)	183 (52)	18 (42)	33 (27)	65 (35)
500	1311 (66)	47 (40)	142 (55)	204 (58)	19 (40)	44 (31)	70 (34)
1000	1977 (100)	118 (100)	260 (100)	350 (100)	52 (44)	92 (35)	123 (35)

Numbers (%) of neoplastic lesions missed at the various cut-off levels and numbers (%) of neoplastic lesions identified by analyses of serum nucleosomes.
[a]Percentage of the number missed by increased FIT cut-off levels.

the FIT cut-off level at 200 ng/ml. That calculation includes the number of subjects with FIT result between 100 and 200 ng/ml minus those subjects recommended for colonoscopy due to the result of the blood testing. This preliminary and still not sufficient predictor would correctly identify 5 (45%) with CRC, 15 (28%) with HRA and 41 (45%) with MRA.

The next steps in further evaluating triage in FIT screening will include analyses of all 13,533 Endoscopy III Part 1 samples, interpretation of the results to settle the optimal FIT cut-off level, inclusion of additional biomarker entities such as proteins, DNA methylations, miRNA, metabolomes, etc. [1,5–12] in various combinations, focus on logistics including considerations of on-site or regional analyses and the timeframe between FIT result and blood collection, interpretation of the analysis results at the end-user level, and in particular cost/benefit issues. Indeed, based on the present, preliminary results an on-line algorithm will be developed and applied on results of the remaining samples. Such on-line tools may be valuable in future screening performance and acceptability. Finally, a clinically controlled study must be initiated to validate the triage concept in detail.

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Appendix

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