Supplementary Material

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Supplement 1: Assumptions and criteria for classifying CRCs as screen-detected CRCs and early stage CRCs in the alternative scenarios.

Screen-detected CRC:

A CRC in a person whose baseline FIT level was equal or beyond the cutoff level of the respective scenario was classified as screen-detected. This assumes that this CRC developed from a lesion that was detectable at baseline colonoscopy. The lesion may have been precancerous (i.e. not yet malignant) at baseline, but as this is not known we cautiously considered it as screen-detected rather than prevented CRC.

Early stage CRCs:

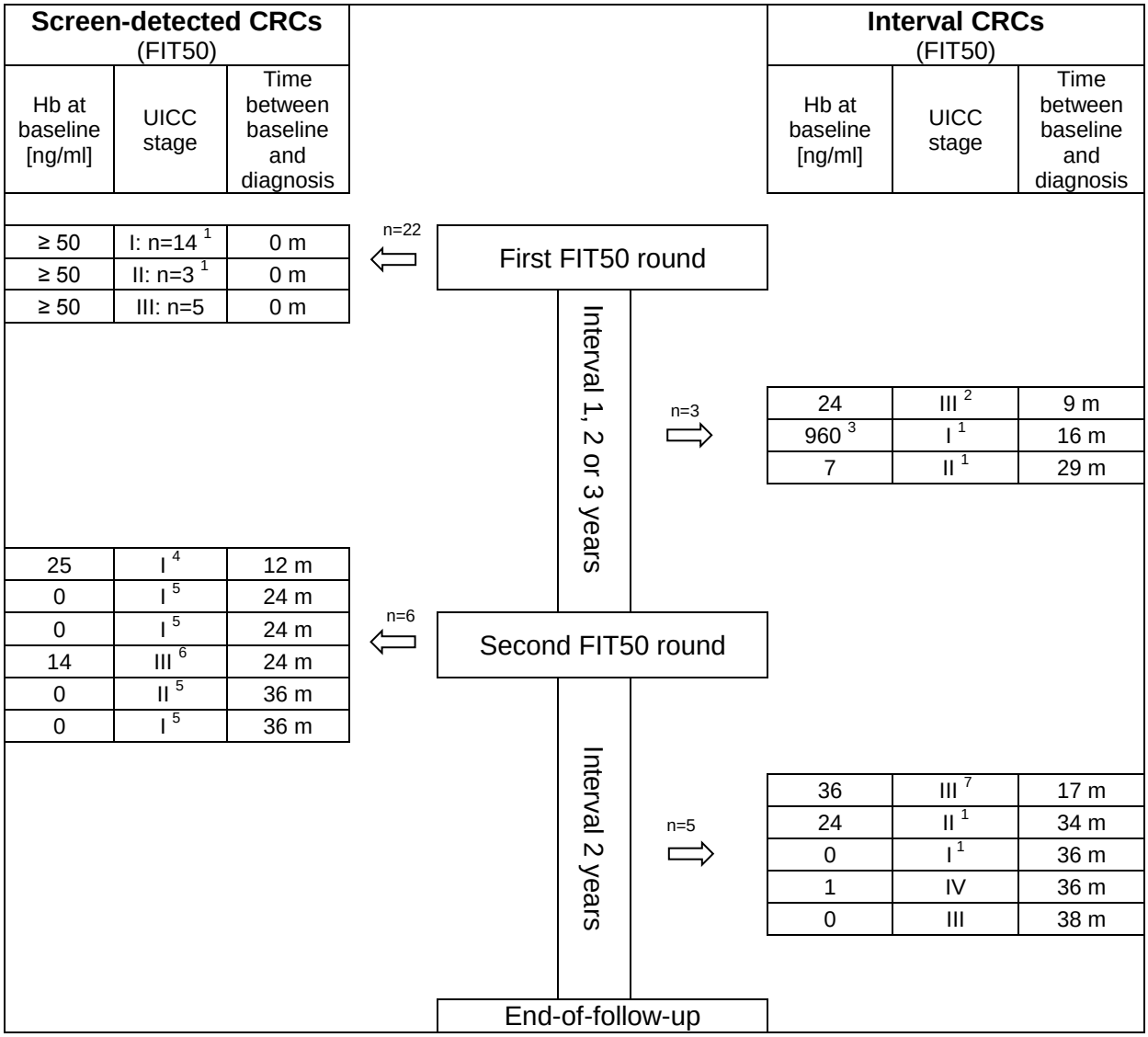
- CRC definitely detected at an early stage: This category includes CRCs that were diagnosed at an early stage at baseline or during the follow-up period and would have tested positive at the hypothetical single initial round (i.e. the baseline haemoglobin level was equal or above the cutoff level of the respective scenario). This category also includes interval CRCs diagnosed at an early stage.
- CRC possibly detected at an early stage, category A: This category includes CRCs that were diagnosed at an advanced stage during the follow-up period and would have tested positive at the hypothetical single initial round (i.e. the baseline haemoglobin level was equal or above the cutoff level of the respective scenario). If detected at baseline, they might have been at an early (or precancerous) stage.
- CRC possibly detected at an early stage, category B: This category refers to CRCs that were screen-detected at an early stage at the second FIT50 round of the standard scenario (i.e. the round that was skipped at the hypothetical scenarios), but would have tested negative at the hypothetical single initial round (i.e. the baseline haemoglobin level was below the cutoff level of the respective scenario). It is not plausible to assume that *all* of these CRCs would have been detected at an advanced stage at the alternative scenarios. They may

  i) have remained at an early stage until being screen-detected at a following round or

  ii) have been symptom-detected at an early stage. The latter is expected for 40% of CRCs given the stage distributions observed in the pre-screening era and among interval CRCs.

  To take into account the possibilities described under i) and ii), we classified *half* of these CRCs as being possibly detected at an early stage. For example, in the alternative scenario using a cutoff level of 14 ng/ml, 4 CRCs were in category B overall. We considered *half* of these CRCs (n=2) to possibly be still at an early stage if diagnosed later.

The footnotes of the following figure describe the classification of CRCs according to the different categories of (possible) early detection in the alternative scenarios.



CRC: colorectal cancer, FIT: faecal immunochemical test for haemoglobin, Hb: haemoglobin; m: months.

1 CRCs classified as being definitely detected at an early stage (by screening or as early stage interval CRC).

2 CRC classified as being possibly detected at an early stage (category A) in the alternative scenarios with cutoff levels of 11 ng/ml, 14 ng/ml and 22 ng/ml.

3 This person tested positive at the first FIT50 round but follow-up colonoscopy was negative.

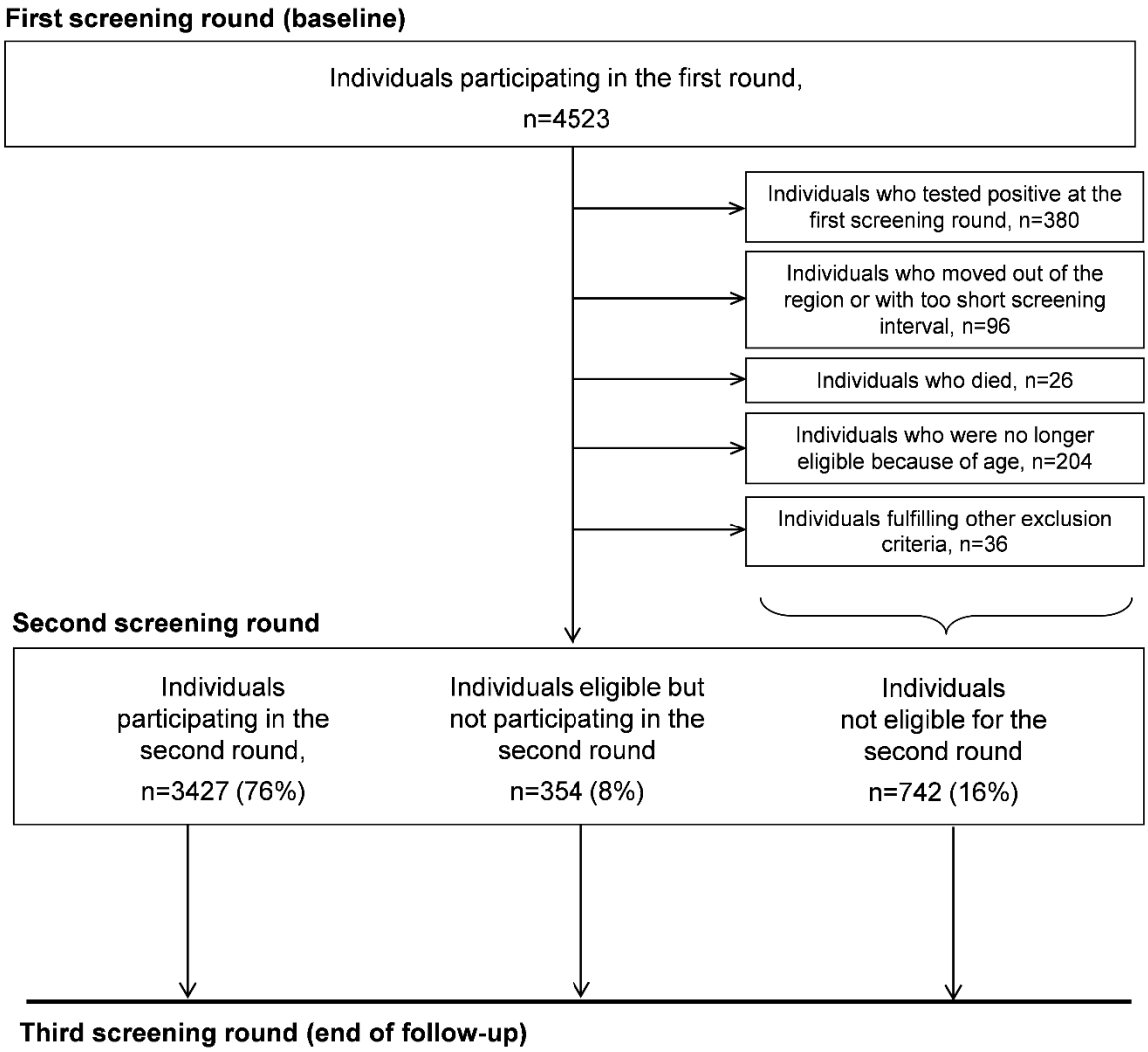
4 CRC classified as being definitely detected at an early stage (or prevented through removal at the adenoma stage) in the alternative scenarios with cutoff levels of 11 ng/ml, 14 ng/ml and 22 ng/ml. In scenarios with higher cutoff levels, it was assigned to category B.

5 CRCs assigned to category B in the alternative scenarios. Half of the CRCs in category B were classified as being possibly detected at an early stage.

6 CRC was classified as being possibly detected at an early stage (category A) in the alternative scenarios with cutoff levels of 11 ng/ml and 14 ng/ml.

7 CRC classified as being possibly detected at an early stage (category A) in the alternative scenarios with cutoff levels of 11 ng/ml, 14 ng/ml and 22 ng/ml and 36 ng/ml.

Supplement 2: Flow chart of the cohort from baseline to the end of the follow-up period. It describes screening eligibility and participation at the second FIT50 round (corresponding to the round that would have been skipped at the alternative scenarios).



Supplement 3: Overlap in subjects diagnosed with advanced adenomas between the standard and the alternative scenarios.

At the standard scenario, the number of subjects diagnosed with advanced adenomas was composed of those detected at the first and the second FIT50 round. The alternative scenarios consisted only of the first screening round, i.e., subjects with advanced adenomas could only be detected at this hypothetical first round. The number of subjects diagnosed with advanced adenomas at this hypothetical first round was higher as compared to the first round of the standard scenario due to the lower cutoff level.

To a certain extent, there was an overlap in subjects diagnosed with advanced adenomas between the standard and the alternative scenarios. First, all 126 subjects with advanced adenomas detected at the first FIT50 round would, as a matter of cause, also have tested positive at the alternative scenarios using lower cutoff levels at the first round. Second, subjects diagnosed with advanced adenomas at the second FIT50 round may have tested positive at the hypothetical first round. We found that of those 54 subjects diagnosed with advanced adenomas at the second FIT50 round, respectively, 21, 17, 12, 5, and 2 subjects would have tested positive at baseline when using a cutoff level of 11 ng/ml, 14 ng/ml, 22 ng/ml, 36 ng/ml and 45 ng/ml for the hypothetical first round.

Those subjects diagnosed with advanced adenomas at the alternative scenarios that did not overlap with the standard scenario would have come from another fraction of subjects bearing advanced adenomas at baseline.

Supplement 4: Methodological issues regarding the case-control studies cited by the European guidelines that recommend an interval of ≤3 years for FIT screening.

The European guidelines for quality assurance in CRC screening and diagnosis recommend that the screening interval for FIT should not exceed 3 years, referring to 3 case-control studies from Japan [1-3]. In these studies, odds ratios (ORs) of dying from CRC and ORs of developing CRC among subjects screened with FIT vs. non-screened subjects were determined according to the number of years (up to 5 years) within which FIT screening was performed before cancer diagnosis. In the main analyses, the proportion of subjects who had *any* FIT screening within a certain time period (e.g. 4-5 years) were compared between cases and controls. However, with respect to identifying the optimal screening interval, subgroup analyses that focused on the *most recent* screening history were relevant. For example, these subgroup analyses compared the proportion of subjects whose most recent FIT screening was 4-5 years ago between cases and controls.

These subgroup analyses had a very low sample size, which resulted in large confidence intervals, particularly for longer intervals. For example, in the study by Saito et al. the 95% confidence interval of the OR of dying from CRC ranged from 0.09-8.68 and from 0.16-9.20, respectively, for intervals of 3-4 years and 4-5 years since the most recent FIT screening (see Table III in Ref. 1). In another study by Saito et al. the 95% confidence interval of the OR of dying from CRC ranged from 0.04-9.73 and from 0.15-53.91, respectively, for intervals of 2-3 years and 3-4 years since the most recent FIT screen, while no OR for the interval 4-5 years was reported (see Table IV in Ref. 2). Nakajima et al. determined the OR of developing advanced CRC according to the number of years since the most recent FIT screening. They reported an OR of 0.58 and a 95% confidence interval of 0.22-1.52 for an interval of 3 years, i.e. the risk reduction was not statistically significant. They reported that no reduction in risk was observed after more than 3 years but no further information was provided [3].

Moreover, while the confounders age and sex were controlled for in the main analyses by matching cases and controls accordingly, it seems that these factors were not considered in the subgroup analyses that were relevant to determine the optimal screening interval. There was no control for other potential confounders either. Accordingly, the findings regarding the optimal screening interval may be biased due to uncontrolled confounding.

1) Saito H, Soma Y, Koeda J, et al. Reduction in risk of mortality from colorectal cancer by fecal occult blood screening with immunochemical hemagglutination test. A case-control study. Int J Cancer 1995;61:465-9.
2) Saito H, Soma Y, Nakajima M, et al. A case-control study evaluating occult blood screening for colorectal cancer with hemoccult test and an immunochemical hemagglutination test. Oncol Rep 2000;7:815-9.
3) Nakajima M, Saito H, Soma Y, et al. Prevention of advanced colorectal cancer by screening using the immunochemical faecal occult blood test: a case-control study. Br J Cancer 2003;89:23-8.