Pergamon

European Journal of Cancer, Vol. 35, No. 6, pp. 973–977, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(99)00062-3

Original Paper

Reducing Colorectal Cancer Mortality by Repeated Faecal Occult Blood Test: a Nested Case–control Study

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Randomised trials have shown the efficacy of faecal occult blood testing (FOBT) in reducing colorectal cancer mortality, but observational studies are needed to monitor such efficacy in population programmes. We conducted a nested case–control study on a cohort of 21 879 subjects who participated in a colorectal screening programme from 1978 to 1995, undergoing at least one FOBT test. 95 fatal cases of colorectal cancer were eligible for the study. For each fatal case, 5 non-fatal matched controls were randomly selected from the cohort. FOBT screening history was less common among cases than controls. The odds ratio of colorectal cancer mortality among 'attenders' (defined as those who underwent a second FOBT within 2 years of study entry) with respect to 'non-attenders' was 0.64 (95% confidence interval 0.36–1.15). We also computed odds ratios defining exposure as one or more tests in the detectable preclinical period, hypothesising various lengths for the latter, which, however, yielded an efficacy estimate biased towards the null. A strong inverse relationship was observed between mortality and the number of tests, but this phenomenon is interpretable as 'healthy screenee bias'. The results suggest that the potential efficacy in preventing colorectal cancer mortality through annual FOBT screening may be of the order of one third. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: colorectal cancer, faecal occult blood testing, screening, nested case–control study
Eur J Cancer, Vol. 35, No. 6, pp. 973–977, 1999

INTRODUCTION

COLORECTAL CANCER is the second leading cause of death from cancer in most Western countries [1], and over 50% of patients with colorectal cancer die of the disease within 5 years of diagnosis [2]. There has been a growing interest in the use of screening for the early detection of colorectal cancer, in particular through faecal occult blood testing (FOBT). A randomised controlled trial design is preferable for the evaluation of screening efficacy and a consistent reduction in fatal colorectal cancer has been seen in such studies [3–6]. Once the efficacy of a screening test has been proven, however, population programmes should be monitored to assess whether the potential efficacy is actually achieved.

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Received 23 Sep. 1998; revised 16 Feb. 1999; accepted 17 Feb. 1999.

The case–control approach has been suggested as an efficient alternative design for the quantification of the efficacy of a screening programme and it may be suitable to monitor ongoing programmes. The efficacy of screening for faecal occult blood has been evaluated in several case–control studies [7–10], showing a mortality reduction ranging from 20 to 60%. The analysis of such studies, however, may be severely flawed by the so-called 'healthy screen bias' [11, 12]. In this study, we investigated the efficacy of a colorectal cancer screening programme carried out from 1978 to 1995 in Milan (Italy), using nested case–control methodology.

PATIENTS AND METHODS

Between 1978 and 1995 a free colorectal cancer screening programme was offered to the population of Milan. The test was proposed for individuals who were 40 years of age or

over, as part of a cancer prevention programme supported by the Italian League Against Cancer. This study was based on the cohort of 21 879 individuals who agreed to participate, undergoing one or more screening tests. The screening protocol foresaw the use of Hemoccult using the standard procedure (two samples for 3 consecutive days) and a restrictive diet. A chemical test for faecal occult blood without rehydration was used from 1978 to 1983. In 1984 Hemoccult with rehydration was introduced and used until the end of the study. The screening protocol included the recommendation of an annual repetition of FOBT and a diagnostic clinical examination in subjects who were positive so as to determine the possible source of bleeding. Nevertheless, no systematic procedure was undertaken for the follow-up of non-compliers.

Follow-up procedures

To evaluate the efficacy of the programme, a systematic follow-up was initiated in December 1996, with the aim of ascertaining the vital status and the cause of death of participants. Both active (by telephone) and passive (by checking the life status through the regional file of the resident population) follow-up was carried out. An additional source of information was the programme itself where, in the case of medical problems, the participants often asked for intervention and counselling. For all subjects who were known to have died, death certificates were obtained through the municipality archives. For all subjects who died due to colorectal cancer, or where the suspicion of such an event arose (i.e. liver metastases or gastrointestinal cancer as the cause of death), medical records were obtained. Information on diagnosis, such as anatomical location and stage of cancer, was obtained by reviewing the medical records.

Definition and identification of cases and controls

Formally, screening is a medical procedure addressed to healthy subjects. Therefore, subjects who presented with symptoms at the first screening test and those who were diagnosed as having colorectal cancer within 12 months of the first test were excluded. We also excluded subjects presenting with other diseases of the large bowel (e.g. chronic inflammatory bowel disease, diverticulitis), because they required a greater medical surveillance than healthy subjects. In this study, a colorectal cancer death was defined as a 'case'. For each case, 5 controls were randomly selected from the cohort of subjects who participated in the screening programme between 1976 and 1995. Controls were selected as being alive at the time of the case diagnosis (index date). Controls were matched with cases on an individual basis according to age quinquennium, gender, area of birth (northern, central or southern Italy), calendar year of admission into the screening programme (± 1 year) and to the fact that they were alive and free from colorectal cancer at the time of the index date of the corresponding case. As previously stated, all cases and controls underwent one screening test upon entry into the study.

Definition of exposure

Screening history for both cases and controls was determined. All tests performed before diagnosis (index date) were considered for cases. The test leading to the diagnosis of screen-detected cases was included, but not tests performed between diagnosis and death. FOBTs carried out as part of the diagnostic examination of symptomatic patients were excluded. For controls, only tests performed before the index date of the matched case were considered [13].

As stressed in a recent review on quantification of exposure in case–control studies of cancer screening [12], 'ever screened' and 'never screened' in a given time period are the recommended exposure categories for estimating screening efficacy. A comparison of cases and controls by number of screening examinations, on the contrary, would be affected by the so-called 'healthy screenee bias' [12, 14]. Whenever the sensitivity of the screening test is over zero, in fact, cases cannot have been screened as frequently as controls in a given period before diagnosis (the detectable preclinical period, DPCP), because sooner or later their cancer would have been detected, thus preventing any further screening.

As screening cannot be effective before the onset of a lesion potentially detectable with the test (i.e. the onset of DPCP), it has also been recommended to limit the definition of exposure to a (variable) number of years before the diagnosis [14, 15]. Too short a period (shorter than the DPCP), however, would underestimate the frequency of screening among controls. In fact, while cases detected by screening would be classified as exposed at whatever point in their DPCP they have been screened, their matched controls would be classified as exposed only if screened in a shorter period. We call this phenomenon the DPCP shrinkage bias. Its dramatic effect on the odds ratio has been shown by Etzioni and Weiss [15] simulating various DPCP lengths. A further bias causing an underestimation of the effect may derive from the misclassification of tests performed in response to cancer symptoms as screening tests [16].

To avoid the 'healthy screenee bias' [12, 14], the 'DPCP shrinkage bias' and the bias due to symptoms leading to testing, we first considered as 'exposed' or 'attenders' only those subjects who underwent a second FOBT within 2 years of study admission. We are well aware that this second test may not have had any preventive potential *per se* if performed before the onset of the DPCP. Nevertheless, a second test within 2 years is an indicator of the tendency to accept the recommendation of periodic screening, which does have preventive potential.

As a second approach, however, we also analysed our data set by considering as exposure the occurrence of one or more tests during a variable time window prior to the index date (including the test that led to the diagnosis) [15]. This is equivalent to defining exposure as screening in the DPCP and assuming average DPCP lengths of increasing duration. The median sojourn time of colorectal neoplasms in the DPCP has been estimated between 2.1 and 2.5 years [17, 18], but its distribution is not known. One may expect that assuming shorter DPCP durations for the analysis would underestimate efficacy, while assuming longer DPCP durations would decrease the bias up to a point after which a further increase would attenuate the estimates because of the inclusion of too many tests that cannot be preventive because they were performed before the onset of the DPCP [15].

Statistical methods

Multivariate conditional logistic regression was used to analyse the data. Maximum likelihood estimates of odds ratios and 95% confidence intervals were calculated. Confidence intervals were computed using the standard errors of the estimated logistic regression coefficients [19]. We

Table 1. Odds ratios (OR) and corresponding 95% confidence interval (CI) from conditional logistic regression for developing fatal colorectal cancer by attendance to screening

	Case subjects		Control subjects		Matched pair analysis	
					Crude	Adjusted§
	n	%	*n*	%	OR (95% CI)	OR (95% CI)
All colorectal cancers						
Screening non-attenders	79	83	366	77	1†	1†
Screening attenders*	16	17	109	23	0.68 (0.38–1.21)	0.64 (0.36–1.15)
Only colon cancer sets‡						
Screening non-attenders	50	79	246	78	1†	1†
Screening attenders*	13	21	69	22	0.93 (0.45–1.88)	0.85 (0.43–1.70)
Only rectal cancer sets‡						
Screening non-attenders	30	94	134	84	1†	1†
Screening attenders*	2	6	26	16	0.34 (0.05–1.62)	0.32 (0.07–1.46)

*Subjects who underwent a second faecal occult blood test within 2 years from entry into the study. †Reference category. ‡Cancer case and corresponding set of controls. §Conditional logistic regression analysis including terms for family history for colorectal cancer and history of colorectal adenomas.

adjusted the odds ratios for age, sex, family history of colorectal cancer and history of adenomatous polyps as potential confounders which were collected at the time of enrolment.

RESULTS

Of the 21 879 subjects included in the cohort, 1315 (6%) were lost to follow-up. 8115 underwent a minimum of 10 years' observation. The median observation was 7 years. 156 deaths from colorectal cancer occurred during the study period. Of these, 42 cases presented with symptoms at the first screening and 19 were diagnosed within 12 months of the first FOBT, hence they were excluded from the analysis. For each case, 5 controls were randomly selected from the cohort set, resulting in a final study group of 95 cases and 475 controls who were considered for the following analysis.

The fatal cancers considered in our study included 16 of the right colon, four transverse, nine left colon, 28 sigmoid and 27 rectum. For 11 subjects this information was missing.

A higher proportion (25.3%; 24/95) of cases had a family history of colorectal cancer compared with the control group (17.3%; 82/475). 3 cases and 9 controls had a personal history of colorectal adenomas.

History of repeated FOBT screening was less common among case subjects than controls. Table 1 shows the odds ratios of colorectal cancer mortality according to screening attendance. As outlined in the Patients and Methods, to obtain a reasonable estimate of the efficacy of screening, we considered as potentially protected those who had at least a second screening test during the first 2 years after entry into the cohort. Our assumption was that these subjects can be considered as 'attenders' to the screening procedure (i.e. the recommendation to repeat the FOBT every year). The mortality advantage for these subjects was 32% (crude) and 36% after adjustment for family history of colorectal cancer and history of colorectal adenomas. Table 1 also shows a separate evaluation of screening benefit for the colon and for the rectum. The odds ratio for 'screening attenders' was 0.85 (95% confidence interval = 0.43–1.70) for colon cancer cases and 0.32 (95% confidence interval = 0.07–1.46) for rectal cancer. The difference between these sites was not statistically significant.

As recently suggested by Etzoni and Weiss [15], we also analysed our data by considering as potentially preventive only the tests performed in the DPCP and assuming average DPCP lengths of increasing duration (Table 2). As anticipated, short DPCP lengths (1–2 years) overestimated the odds ratio while less biased estimates were produced when a relatively long DPCP was selected for analysis. The lowest odds ratio (0.78, 95% confidence interval = 0.43–1.52) was obtained setting the time window at 5 years. This is likely to be the least biased estimate with this procedure [15], but still

Table 2. Odds ratios (OR) and corresponding 95% confidence interval (CI) from conditional logistic regression analysis for developing fatal colorectal cancer by considering the occurrence if one or more tests were conducted in increasing time windows from the index date

	Case subjects		Control subjects		Matched pair analysis	
					Crude	Adjusted*
Test occurred	Yes	No	Yes	No	OR (95% CI)	OR (95% CI)
Time from index date (years)						
≤ 1	8	13	21	84	2.46 (0.90–6.61)	2.32 (0.85–6.35)
≤ 2	10	26	46	134	1.12 (0.50–2.49)	1.09 (0.48–2.44)
≤ 3	11	35	59	171	0.91 (0.44–1.91)	0.88 (0.42–1.85)
≤ 4	12	43	69	206	0.83 (0.42–1.67)	0.80 (0.39–1.61)
≤ 5	13	50	77	238	0.80 (0.41–1.56)	0.78 (0.40–1.52)
≤ 6	15	56	86	269	0.84 (0.45–1.56)	0.81 (0.43–1.52)

*Conditional logistic regression analysis including terms for family history for colorectal cancer and history of colorectal adenomas.

Table 3. Odds ratios (OR) and corresponding 95% confidence interval (CI) from conditional logistic regression analysis for developing fatal colorectal cancer by number of screening examinations

	Case subjects		Control subjects		Matched pair analysis	
					Crude	Adjusted‡
	n	%	*n*	%	OR (95% CI)	OR (95% CI)
Number of FOBT						
Only 1*	54	57	209	44	1†	1†
2–3	33	35	175	37	0.73 (0.45–1.18)	0.71 (0.44–1.14)
4 or more	8	8	91	19	0.34 (0.16–0.75)	0.34 (0.16–0.75)

*Inclusion criterion in the study cohort. †Reference category. ‡Conditional logistic regression analysis including terms for family history for colorectal cancer and history of colorectal adenomas. FOBT, faecal occult blood test.

it is considerably higher than the odds ratio obtained with the previous procedure.

While the results were analysed for the number of screening examinations (Table 3), a continuous decrease in risk was associated with an increasing number of tests.

DISCUSSION

FOBT has been widely recommended as a screening procedure for colorectal cancer. Randomised trials are a well-accepted means of screening evaluation. The efficacy of long-term annual screening was first established by the Minnesota Colon Cancer Control Study, which found that annual screening with FOBT decreases the 13-year cumulative mortality due to colorectal cancer by 33% [5]. Other large-scale controlled trials were conducted in the U.S.A. [6], U.K. [3] and Denmark [4]. The efficacy of screening for faecal occult blood has been evaluated in several recent case–control studies [7–10]. The results obtained contrasting ever and never exposed in some time period before diagnosis suggest an approximate 25% reduction in mortality due to colorectal cancer for subjects who undergo annual or 2-yearly FOBT screening. The confidence intervals of these estimates, however, were wide ranging.

Case–control study results showing increasing benefits with increasing number of tests, on the contrary, suffer from the 'healthy screenee bias'. In ours, as in other studies, a continuous risk decrease was associated with the increasing number of tests performed. Those undergoing two or three tests had a protection of 29% (odds ratio = 0.71). Those who underwent four or more tests showed an implausible protection of 66% (Table 3). There were no cases, but only controls who underwent nine or more screening tests, i.e. an apparently complete protection from colorectal cancer mortality for these subjects. To illustrate the 'healthy screen bias' phenomenon, let us hypothesise a test with a 100% sensitivity. No cancer case could be screened twice in its DPCP because the first screening test would detect the illness and prevent further screening. As no such restriction exists for healthy controls, the odds ratio associated with two or more tests in the DPCP would be zero. If the test had less than 100% sensitivity, the odds ratio for a negative screening test performed during the DPCP would not be zero. It would not be an indicator of efficacy, but simply an indicator of the false-negative rate. The odds ratio for subjects screened twice with negative results during the DPCP would be a function of the square of the false-negative rate. This kind of analysis may be useful, therefore, to estimate sensitivity.

The analytical strategy based on choosing a given time period before diagnosis, or increasing time periods before diagnosis, and ignoring the number of tests, avoids the 'healthy screen bias', but is likely to give odds ratio estimates biased towards the null [15]. In the simulation carried out by Etzioni and Weiss, in which the unbiased odds ratio should have been 0.67, such a procedure gave results in the range 0.70–0.80, depending on the assumption on the DPCP length distribution and the frequency of screening.

So as to account for these problems, we did not consider as evidence of efficacy a dose–response relationship between protection and the number of tests carried out, and we preferred not to rely on exposure estimates in the periods preceding diagnosis. We chose instead to assess potential screening benefit comparing 'screening attenders' with 'non-attenders'. We considered the repetition of the screening test within 2 years of the first as an indicator of 'attendance' and we used this criterion as screening exposure. Such a strategy, which classifies study subjects on the basis of their attitude towards screening repetition instead of their actual potentially preventive exposure, is also likely to misclassify the relevant exposure and, consequently, to overestimate the odds ratio. The actual estimate, however, seems less biased than that obtained with variable time windows before diagnosis (0.64 versus 0.78).

In conclusion, our data, in agreement with the results of previous trials and case–control studies on screening efficacy, confirm the efficacy of FOBT in the reduction of mortality due to colorectal cancer. They also suggest that the size of the effect may be greater with respect to previous case–control estimates based on ever/never definitions of exposure, but lower than the estimates derived defining exposure on the basis of the number of tests.

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