PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to the GUT but declined for publication following peer review. The authors addressed the reviewers’ comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

ARTICLE DETAILS

| TITLE (PROVISIONAL) | Impact of Faecal Haemoglobin Concentration on Colorectal Cancer Mortality and All Cause Death |
|---------------------|-----------------------------------------------------------------------------------------|
| AUTHORS             | Chen, Tony Hsiu-Hsi; Chen, Li-Sheng; Yen, Amy Ming-Fang; Fraser, Callum; Chiu, Sherry Yueh-Hsia; Fann, Jean Ching-Yuan; Wang, Po-En; Lin, Sheng-Che; Liao, Chao-Sheng; Lee, Yi-Chia; Chiu, Han-Mo |

VERSION 1 - REVIEW

| REVIEWER | van Leerdam, M. Erasmus University Medical Centre, Gastroenterology and Hepatology |
|----------|----------------------------------------------------------------------------------|
| REVIEW RETURNED | 11-Jul-2013 |

GENERAL COMMENTS

This is a large observational study of cohorts over time based on two population based CRC screening programs in Taiwan including a total of about 200 000 individuals. A total of 217 prevalent cases and 1016 incident cases were found. FU time was 3.5 years. An increased risk of CRC death was seen with rising f-Hb value. This is not very surprising. There seems to be a relation between the more advanced tumors (stage III and IV) and the level of f-Hb. The question is of f-Hb levels will replace pathological stage/ TNM or will be additional to pathological stage/ TNM for predicting prognoses. This part of the study results have very limited implications. The other part of the results showed a similar, but less marked trend for all-cause mortality related to f-Hb level. This is an interesting finding and the question is of course what the reason for this association might be. The study is original. The paper is well written.

Main comments
1. Pathological stage was not put into the multivariate regression model (neither as confounder, nor was checked for interaction). I do expect that stage is the strongest predictor of CRC mortality. Why is the f-Hb level still important if you have stage for all your CRC cases? What does it add? Why did you not use the coefficient of variation between stage and f-hb?
2. Pathological stage was only available for 44% of the cohort, but is very important information when CRC mortality is the outcome of your study.

Minor comments
How might it impact ….
Pg 5; may have value as an indicator for predicting CRC mortality and also life expectancy. When you want to use this in daily practice
you need information about the accuracy (sensitivity/ specificity/ PPV etc). I do believe that the f-Hb is a proxy for the pathology stage, but probably not more than that. At the moment, the f-Hb level is not a supplemental predictor for prognosis.

Introduction:
Pg 7, Line 12; this intermediate effect ..... facilitating early therapy and improves staging strategies. What do the authors mean by this? Why would you postpone therapy of CRC pt with a lower level of f-hb? Why should it improve staging strategies? How well can you rely on such a level?

Materials and Methods
Pg 8; can you add information about the selection procedure? Is it a population based screening program or were individuals select via the GP?
Pg 10; what happens with a individual with a positive test, without any findings on the subsequent colonoscopy. Does this patient again receive an invitation for f-Hb test the next round?
Pg 12 line 1; normally all factors that have a p-value below 0.10 are included in the multivariate model, why did you choose for 0.05?
Pg 12, line 4; I do not understand why pathology stage was not put into the model as a confounder. Did you check for interaction? Or do you want to prove that the f-Hb is a proxy for pathology stage? Why didn't you test for coefficient of variance (R), which would be a better analysis to determine the relation between stage and f-hb with regard to outcome.

Results
Pg 13, line 10; the prevalent rate of CRC is low (0.12). How do you explain this? (young age, selection of more healthy individuals?)
Pg 13, line 46; what happens when also pathology stage is put into the model. Stage is a well known riskfactor for CRC mortality (as shown in table xxx). It seems logic that CRC in an advanced stage are in general larger CRC and tend to bleed more (higher f-hb lever). Why is this important?
Pg 14, line 25; …. Indicating that the risk for both advanced CRC and advanced adenoma increased….. Why do you not give information on the non-advanced CRC, which seems to be as important as the other two groups.
Pg 14, line 50; 85 cases were stage 0; this is currently classified as high grade dysplasia and is not regarded as invasive carcinoma. This group should be included at the adenoma group (Vienna classification). Stage was missing in 547/1233 cases, which is a large proportion of data missing with important information regarding prognosis. So the multi-variate regression model is only performed on 44% of the study population.
Pg 15, line 11; …. May be partially explained by the intermediate effect of f-hb on the severity of CRC. I do think that this the main observation of your paper and this should be stressed. What is the coefficient of variance R?
Pg 15, line 24; this is new information, not previously reported by others. F-Hb is associated with all cause mortality.

Discussion
Pg 16, line 25; … can be used to stratify the underlying population into different risk groups ...... In table 2 and table you showed that only for f-Hb levels over 100 ng Hb/ml, there is a significant association with CRC death in the univariate and multivariate analyses. So what do you mean by this sentence. How would you stratify the population into different groups? All individuals with a f-Hb level over 100 will already be advised to undergo colonoscopy. In table 3 you show the HR for risk of advanced adenoma or CRC stage III and IV. The HR is significantly increased for the group with
hb levels between 50-99 (HR 1.84). Would you than suggest to lower the cut-off level to 50 or decrease the interval?

Pg 16, line 30; .... Subjects with higher f-hb but not yet classified as worthy of investigation for colorectal neoplasia at first screen....What do you mean? Discussions about the right cut-off level should be based on cost-effectiveness analyses.

Pg 16, line 35; .... On the other hand, the interval between repeated FIT screens could be extended to avoid false positive cases for those with a lower f-Hb. However we know that FIT has a low sensitivity for advanced adenoma and CRC, maybe because of the intermittent bleeding pattern of the neoplasia. Therefore, the test should be repeated within a certain time interval. How do you explain this sentence?

Pg 16, line 50 and pg 17 line 3; .... Maybe a reliable indicator for the prognosis of CRC. However, for all CRC cases in clinical practice we work with a c/pTNM stage. What does the f-Hb level add to this information regarding CRC death? .... And possible added to TNM stage as a novel supplemental predictor ... However, when pathology stage is inserted to the model f-Hb is no longer associated with CRC death. Why do you suspect additional information to the TNM stage?

Pg 17, line 40; I do agree with the authors that it is interesting to note the impact of f-Hb on all cause mortality.

Pg 18, line 20. Other explanations may be other cancers in the GI tract (esophageal, stomach, small bowel cancer), cardiovascular disease with use of anticoagulants.

References
The references are up to date
Tables and figures
Table 3, pg 29. The CI in the last row is missing for HR advanced adenoma (107.77 – xxx). Can you draw a vertical line between the adenoma and CRC group, this will make the table easier to read
Figures; I do miss the legend of the figures. Did you use the baseline level of f-hb, or the latest or all hb-results of all individuals? This is not clear, what is the N?.

The manuscript received two reviews at The GUT but the other reviewer have declined to make the reviews public. Please contact BMJ Open editorial office for any further information.

VERSION 1 – AUTHOR RESPONSE

Main comments

1 (Q) Pathological stage was not put into the multivariate regression model (neither as confounder, nor was checked for interaction). I do expect that stage is the strongest predictor of CRC mortality. Why is the f-Hb level still important if you have stage for all your CRC cases? What does it add? Why did you not use the coefficient of variation between stage and f-hb?

(A) Thank you for the reviewer suggestion. The original Table 3 has been already considered pathological stage as a strong confounder when the effect of f-Hb level on CRC deaths was investigated. Pathological stage is still the strongest predictor but the effect of f-Hb on CRC death was not statistically significant after controlling for pathological stage. The finding is not contradictory to the
reviewer expectation. 
Note that the original idea on this part was to demonstrate whether the relationship between an incremental increase in f-Hb and CRC mortality can be explained through the intermediate effect of f-Hb on more advanced CRC (defined by pathological stage III or higher). We then consider f-Hb as a surrogate endpoint for the prognosis of CRC. However, based on the two referees' comments, we, one second thought, are determined to drop this section regarding the evidence that f-Hb was regarded as a surrogate for the severity of colorectal neoplasia. In the revised manuscript, we only focused on the relationship of f-Hb to CRC mortality and all-cause mortality. The revised manuscript has therefore excluded this part.

2 (Q) Pathological stage was only available for 44% of the cohort, but is very important information when CRC mortality is the outcome of your study.
(A) First of all, we hope the reviewer is aware of the fact that pathological stage was available for 56% rather than 44% of the cohort. Regarding unavailable information on pathological stage, it should be noted that pathological information on tumour staging has only started since 2000 in Taiwan. This can account for why complete information on pathological stage was only 56% (44% was not available). We try to improve the quality of information on pathological stage year by year. As our dataset was still in early period of enacting the involved hospitals to report pathological stage, 56% with complete information is the best we can do. However, this part has been deleted form the revised manuscript as explained in the Q (1).

Minor comments
How might it impact ….

3 (Q) Pg 5; may have value as an indicator for predicting CRC mortality and also life expectancy. When you want to use this in daily practice you need information about the accuracy (sensitivity/ specificity/ PPV etc). I do believe that the f-Hb is a proxy for the pathology stage, but probably not more than that. At the moment, the f-Hb level is not a supplemental predictor for prognosis.
(A) Again, this part has been deleted in the revised manuscript.

Introduction:

4 (Q) Pg 7, Line 12; this intermediate effect ….. facilitating early therapy and improves staging strategies. What do the authors mean by this? Why would you postpone therapy of CRC pt with a lower level of f-hb? Why should it improve staging strategies? How well can you rely on such a level?
(A) With the similar reason, this sentence has been removed.

Materials and Methods

5 (Q) Pg 8; can you add information about the selection procedure? Is it a population based screening program or were individuals select via the GP?
(A) The FIT screening program from the two cohorts is an organized population-based screening program, not opportunistic screening program. The details of this program have been elucidated in Materials and Methods section. (Page 10, Line 36; Page 11, Line 24)

6 (Q) g 10; what happens with a individual with a positive test, without any findings on the subsequent colonoscopy. Does this patient again receive an invitation for f-Hb test the next round?
(A) For those subjects with false positive finding from colonoscopy, they are returned to next screen in the next five years.

7 (Q) (7)Pg 12 line 1; normally all factors that have a p-value below 0.10 are
included in the multivariate model, why did you choose for 0.05?
(A) The procedure of selecting significant factors in our model is not based on the conventional model selection like forward, backward, stepwise method. We just applied the entry method to include the possible significant factors (P < 0.05) identified from univariate analysis when the multi-variable regression model was performed. From statistical viewpoint, we try to be conservative about the selection of significant factors and therefore chose strict criteria (the lower the significance level chosen, the stronger the evidence required from statistical point of view). However, we have changed the criteria of setting 0.10 the results remained similar.

8 (Q) Pg 12, line 4; I do not understand why pathology stage was not put into the model as a confounder. Did you check for interaction? Or do you want to prove that the f-Hb is a proxy for pathology stage? Why didn’t you test for coefficient of variance (R), which would be a better analysis to determine the relation between stage and f-hb with regard to outcome.
(A) See the Q 1 of major comments.

Results

9 (Q) Pg 13, line 10; the prevalent rate of CRC is low (0.12). How do you explain this? (young age, selection of more healthy individuals?)
(A) Our Asian countries have still lower background incidence rate of CRC compared with Western countries. Our detection rate of colorectal neoplasia among those with positive iFOBT subjects aged 40-69 years was 157 per 1000 (=1246+217)/2031) at prevalent screen. If study participants limited to aged 50-69 years, it became 175 per 1000 (=1123+185)/7445). This is lower than 350-500 per 1000 reported in literatures, most of which are from Western countries. The lower background incidence rate of CRC compared with western countries accounts for this lower figure.

10 (Q) Pg 13, line 46; what happens when also pathology stage is put into the model. Stage is a well known riskfactor for CRC mortality (as shown in table xxx). It seems logic that CRC in an advanced stage are in general larger CRC and tend to bleed more (higher f-hb lever). Why is this important?
(A) see the Q 1 of major comments.

11 (Q) Pg 14, line 25; …. Indicating that the risk for both advanced CRC and advanced adenoma increased….. Why do you not give information on the non-advanced CRC, which seems to be as important as the other two groups.
(A) This part has been deleted.

12 (Q) g 14, line 50; 85 cases were stage 0; this is currently classified as high grade dysplasia and is not regarded as invasive carcinoma. This group should be included at the adenoma group (Vienna classification). Stage was missing in 547/1233 cases, which is a large proportion of data missing with important information regarding prognosis. So the multi-variate regression model is only performed on 44% of the study population.
(A) See the Q (2) of major comments.

13 (Q) Pg 15, line 11; …. May be partially explained by the intermediate effect of f-hb on the severity of CRC. I do think that this the main observation of
your paper and this should be stressed. What is the coefficient of variance R?
(A) In response to Q(1) from the reviewers 1 and 2, this part has been deleted.

14 (Q) Pg 15, line 24; this is new information, not previously reported by others.
F-Hb is associated with all cause mortality.
(A) Yes, this is a new finding, which has been addressed in the text.

Discussion
15 (Q) Pg 16, line 25; … can be used to stratify the underlying population into different risk groups …… In table 2 and table you showed that only for f-Hb levels over 100 ng Hb/ml, there is a significant association with CRC death in the univariate and multivariate analyses. So what do you mean by this sentence. How would you stratify the population into different groups?
All individuals with a f-Hb level over 100 will already be advised to undergo colonoscopy. In table 3 you show the HR for risk of advanced adenoma or CRC stage III and IV. The HR is significantly increased for the group with hb levels between 50-99 (HR 1.84). Would you than suggest to lower the cut-off level to 50 or decrease the interval?
(A) As the original Table 3 has been deleted, we are therefore very conservative to elaborate how to use our finding to suggest inter-screening interval but just give an implication for individually-tailored screening for CRC with FIT.

16 (Q) Pg 16, line 30 … Subjects with higher f-hb but not yet classified as worthy of investigation for colorectal neoplasia at first screen….What do you mean? Discussions about the right cut-off level should be based on cost-effectiveness analyses.
(A) For those subjects with levels of f-Hb but not yet classified as worthy of investigation for colorectal neoplasia at first screen may need a shorter inter-screening interval with FIT. As most of results and interpretation on the relationship between f-Hb and advanced CRC have been deleted we are also conservative about the elaboration of the optimal cutoff. However, we have added one sentence like the following: “The optimal cutoff for individually-tailored screening should be considered on the basis of cost-effectiveness analysis”. (Page 19, Line 42)

17 (Q) Pg 16, line 35; …. On the other hand, the interval between repeated FIT screens could be extended to avoid false positive cases for those with a lower f-Hb. However we know that FIT has a low sensitivity for advanced adenoma and CRC, maybe because of the intermittent bleeding pattern of the neoplasia. Therefore, the test should be repeated within a certain time interval. How do you explain this sentence?
(A) The reviewer concern is most related to false negative cases that have been addressed antecedent to this sentence like the following: “To avoid the former, subjects with higher f-Hb but not yet classified as worthy of investigation for colorectal neoplasia at first screen may need a shorter inter-screening interval with FIT or any further assessment of risk for CRC. On the other hand, the interval between repeated FIT screens could be extended to avoid false positive cases for those with
a lower f-Hb, particularly 50 ng Hb/mL or below. This has been added to the sentence in the discussion part (Page 19, Line 30-42).

18 (Q) Pg 16, line 50 and pg 17 line 3; …. Maybe a reliable indicator for the prognosis of CRC. However, for all CRC cases in clinical practice we work with a c/pTNM stage. What does the f-Hb level add to this information regarding CRC death? …. And possible added to TNM stage as a novel supplemental predictor … However, when pathology stage is inserted to the model f-Hb is no longer associated with CRC death. Why do you suspect additional information to the TNM stage?
(A) This part has been pruned from the text in the revised manuscript.

19 (Q) Pg 17, line 40; I do agree with the authors that it is interesting to note the impact of f-Hb on all cause mortality.
(A) Agreed.

20 (Q) Pg 18, line 20. Other explanations may be other cancers in the GI tract (esophageal, stomach, small bowel cancer), cardiovascular disease with use of anticoagulants.
(A) Indeed, FIT may be also useful for detecting bleeding resulting from the conditions proposed by the reviewer. However, the relationship between f-Hb and all-cause death is an exploratory finding we try to be conservative for the explanation about this part. Moreover, some studies have reveals the cancer detection in up GI is poor from FIT (Chiang et al). We will keep this comment in mind and hope to be clarified in the future research.

Chiang TH, Lee YC, Tu CH, Chiu HM, Wu MS. Performance of the immunochemical fecal occult blood test in predicting lesions in the lower gastrointestinal tract. CMAJ. 2011;183(13):1474-81.

References
Q(21) The references are up to date
(A) Thank you for your comment.

Tables and figures
21 (Q) Table 3, pg 29. The CI in the last row is missing for HR advanced adenoma (107.77 – xxx). Can you draw a vertical line between the adenoma and CRC group, this will make the table easier to read
(A) The original Table 3 has been abridged.

22 (Q) Figures; I do miss the legend of the figures. Did you use the baseline level of f-hb, or the latest or all hb-results of all individuals? This is not clear, what is the N?.
(A) The baseline f-HB levels were used to present in Figures. This has been indicated in the legend of the figures (see new Figure 1 & Figure 2).