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. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	A Nationwide Danish Cohort Study challenging the Categorization into Right Sided and Left Sided Colon Cancer
AUTHORS	Jess, Per; Hansen, Iben; Gamborg, Michael; Jess, Tine

VERSION 1 - REVIEW

REVIEWER	Jennifer M. Weiss, MD MS Assistant Professor Division of Gastroenterology and Hepatogy Department of Medicine University of Wisconsin School of Medicine and Public Health - Madison, WI USA I have no competing interests to disclose.
REVIEW RETURNED	01-Feb-2013

THE STUDY	Overall, I think the authors are investigating a very interesting question that supports the idea that colon cancer is more complex than was previously thought. I do, however, have some minor comments including additional statistical analyses should be conducted: (1) The description of the outcome measures in the abstract is not clear. After reading the paper, this reviewer assumes that the main outcome is 5-year overall survival, the main explanatory variable is tumor location, and the remainder of the variables listed are essentially control variables. This is not how the "outcome measures" section of the abstract reads. This section should be revised. It might also help to have a section in the manuscript with a more complete description of how each of these variables were defined. (2) The description of the statistical analysis is a little confusing. From the description, this reviewer cannot tell if each of the variables (ASA score, number of lymph nodes harvested, number of lymph nodes with metastases, presence of distant mets, and UICC stage) were added sequentially to the model so that previously added variables were being controlled for, or if six separate models were run. This reviewer recommends running and reporting the results of a global model that includes all of the variables, including an indicator for gender. Interactions can then be added to this global model. The impact of each of the abovementioned variables on overall 5-year survival can then be determined by seeing how the hazards ratio changes with that particular variable in and out of the model. (3) Is there any way to control for treatment such as chemotherapy? We know for AJCC stage III and IV colon cancer, there is a definite

	impact on survival with chemotherapy. (4) A statistical comparison should be done for the different groups in Table 2 with a p-value reported for each row.
RESULTS & CONCLUSIONS	This reviewer has a few suggested revisions to the presentation of the results and conclusion: (1) There are some typos in Table 1 - the median age for RCC and LCC should have a decimal point and not a comma. (2) The column headings for Table 2 need to be lined up and there should be p-values presented comparing the characteristics by different subsites of colon cancer. (3) In Table 3, it would be helpful to know how many patients are in the sample for "years 1-2" and "years 3-5". Based on the descriptive statistics of the sample, not everyone was followed for a full 5 years. (4) Formatting in Table 4 needs to be improved. (5) This reviewer recommends presenting the results of one global model for overall 5-year survival by RCC vs LCC in Table 3 and a similar global model for the more discrete colon cancer subsites in Table 5. Including all of the variables in one model would show the impact of colon cancer subsite on overall 5-year survival while controlling for all other variables at the same time. (6) The second to last paragraph in the discussion section needs to be expanded. This reviewer would like more discussion about how MSI, K-ras, and BRAF mutations differ along colonic subsites.

REVIEWER	Alain Demers Senior Epidemiologist Public Health Agency of Canada Canada I have no conflict of interest to declare.
REVIEW RETURNED	27-Feb-2013

THE STUDY	I strongly encourage the authors to use relative survival to compare the survival between the different groups and the mathematical models that go with it to compare the groups. The manuscript needs major edition regarding the English and the structure of many sentences.
REPORTING & ETHICS	The authors do not say if their study has been approved by an ethics board.
GENERAL COMMENTS	The information that the authors of the present paper bring is helpful in better understanding the complexity of the different segments of the colon and supports the change in paradigm that is arising and challenges the dichotomous division of the colon. Abstract • Design: this is not a cohort study per se. It is a study using data from a population-based cancer registry containing longitudinal data on cancer-related procedures and patient outcomes. • Outcomes and measures: the beginning of the second sentence is missing "… as well as survival…".

	• Results: the part of the sentence “the proportion of women increased the more proximal the tumor” is not clear and need to be reformulated. Methods • I would like to authors to talk about the synchronous/overlapping site CCs and what rules are used by the Registrars to allocate them to one site or the other. What percentage of CC is synchronous in their Registry? This is potentially a limitation that should be taken into account in the discussion. • The authors wrote “Non-parametric statistics were used for description of patient material”, what test exactly and what is the meaning of “patient material”? • In their survival analysis, it is not clear if the authors used death from any cause or if it is death due to CC (cause specific death) – please specify. • Since the authors are dealing with national data, I strongly suggest using relative survival and the modelization that goes with it. • The authors say that their ethics boards “have approved the use of the DCCG database for scientific purposes”, but they don’t say if their study was approved by these ethics boards. Results • Table 1 and 2 should be combined in one table as Figure 1 and 2 should. • Table notes should be added to the tables where acronyms need to be defined. • Table 3: add a table note explaining how the variables are categorized. • The authors wrote “the proportion of women increased with proximal tumor localization, except for hepatic flexure cancer, where women were not in excess” this sentence is not clear and need to be reformulated. • There is no need to repeat the confidence intervals of the HR in the text since they are available in the tables. Discussion • I would like the authors to explore why sigmoid cancers seem to be different than most of the other ones in term of morality risk. • Do the authors have an explanation for the limited 2-yr time period of increased mortality following a CC diagnosis. References Reference #20: rectal is misspelled. Other comments I would like to see the paper Yamauchi M et al. Colorectal cancer: A tale of two sides or a continuum? Gut 2012. 61(6):794-797 included in the discussion.

REVIEWER	Lai, Mao-de Zhejiang University
REVIEW RETURNED	27-Feb-2013

REPORTING & ETHICS	In this manuscript, the authors investigated and compared the clinical characteristics and survival information in the colon cancer patients with different anatomical subsites. Different distributions on age, gender, ASA scores, and mortality were found between RCC

	and LCC, and among different colon subsites. These findings challenge the simple categorization of CC into RCC and LCC and have additional potential strengths in categorization on colon cancer. Major concerns: 1. The differences between RCC and LCC were well established in the previous studies; therefore, the results in this study should focus on the differences in epidemiology, pathology and prognosis between anatomical subsites. It seems Table 1, 3 and 4 were not very important in result part. 2. In table 2, statistical significances should be examined among patients with different subsites. Discrete trends (Standard deviation or Interquartile/range) should be described for age and Number of lymph nodes harvested. 3. Besides HR (hazard ratio), survival curve and mean survival time are suggested to compare the differences among different subsites and modified/stratified age, gender, clinical stage, and periods of diagnosis, etc. To control/minimized the effect of diagnostic delay, clinical stage, and periods of diagnosis should be stratified in the analysis. 4. The conclusion should be drawn cautiously. The differences might attribute to the diagnostic delay, diagnostic accuracy and compliance of colonoscopy examination. Overall, the evidence presented in this study seems not very persuasive enough to support the conclusion. 5. The organization of table 4 was not correct and the analysis was confusing. 6. There are some mistakes on spelling, grammar and format in the manuscript. For example, Line 17 page 6, “RCC and LLC” should be “RCC and LCC”. It should be checked thoroughly. Decision: major revision

VERSION 1 – AUTHOR RESPONSE

Reviewer: Jennifer M. Weiss, MD MS
Assistant Professor
Division of Gastroenterology and Hepatogy Department of Medicine
University of Wisconsin School of Medicine and Public Health -
Madison, WI USA

I have no competing interests to disclose.

Overall, I think the authors are investigating a very interesting question that supports the idea that colon cancer is more complex than was previously thought. I do, however, have some minor comments including additional statistical analyses should be conducted:

(1) The description of the outcome measures in the abstract is not clear. After reading the paper, this reviewer assumes that the main outcome is 5-year overall survival, the main explanatory variable is tumor location, and the remainder of the variables listed are essentially control variables. This is not how the "outcome measures" section of the abstract reads. This section should be revised. It might also help to have
a section in the manuscript with a more complete description of how each of these variables were defined.

We agree that the “Outcome measures” section is not clear and it has now been corrected, reading as follows: “Outcome measures: Overall survival (Kaplan Meier plots) and mortality (hazard ratios [HR] from Cox proportional hazards regression analysis) according to CC localization. For adjustment and

stratification, we used age, sex, ASA score (the American Society of Anaesthesiologists score), tumor location and stage, number of lymph nodes harvested at operation, number of lymph nodes with metastases, and presence of distant metastases"

(2) The description of the statistical analysis is a little confusing. From the description, this reviewer cannot tell if each of the variables (ASA score, number of lymph nodes harvested, number of lymph nodes with metastases, presence of distant mets, and UICC stage) were added sequentially to the model so that previously added variables were being controlled for, or if six separate models were run. This reviewer recommends running and reporting the results of a global model that includes all of the variables, including an indicator for gender. Interactions can then be added to this global model. The impact of each of the abovementioned variables on overall 5-year survival can then be determined by seeing how the hazards ratio changes with that particular variable in and out of the model.

We agree that the statistical analyses could be better explained. The reviewer correctly understood that we have run six separate models for the listed variables. The reason for not running a global model was the loss of a great number of patients (especially applying to those patients who had died) if we included only those with all variables recorded (overall we lost 2,857 of 11,963 women and 2,841 of 11,515 men and these represented 1/3 of all cases of death). To illustrate this and to meet the concern of the reviewer, we have now added sensitivity analyses for 1-2 year survival and 3-5 year survival in a global model of the reduced patient material. The Results section now reads: "In a sensitivity analysis of survival among 9,106 women and 8,674 men with information on all confounders (ASA score, number of lymph nodes harvested, number of lymph nodes with metastases, presence of distant metastases, and UICC stage), we found the 1-2 year HR to be 1.29 (95% CI, 1.19-1.40) in a crude model and 1.28 (95% CI, 1.18-1.39) in a full model including all these variables. In men the crude 1-2 year HR was 1.34 (95% CI, 1.24-1.45) and the fully adjusted HR was 1.36 (95% CI, 1.25-1.47). The 3-5 year HR was 0.87 (95% CI, 0.77-0.97) for women and 0.97 (95% CI, 0.86-1.09) for men in the crude analysis, whereas the fully adjusted 3-5 years HR was 0.93 (95% CI, 0.82-1.06) for women and 1.06 (95% CI, 0.94-1.19) for men". As it appears, the removal of 1/3 of patients who had died influence the overall crude HR markedly more (please see HRs from the complete dataset in Table 2) than does the adjustment for all 6 variables, potentially due to the introduction of selection bias. This has been added to the Discussion.

(3) Is there any way to control for treatment such as chemotherapy? We know for AJCC stage III and IV colon cancer, there is a definite impact on survival with chemotherapy.

We agree that controlling for chemotherapy would have been of interest, but this variable was unfortunately not available. We have now added the following to the Discussion "Lack of info on chemotherapy is also a potential limitation to the study, since chemotherapy may influence survival. However, the highest frequency of stage III and IV cancers, normally leading to chemotherapy, was seen in splenic flexure cancers, and these cancers still had the highest relative mortality compared to other sites".

(4) A statistical comparison should be done for the different groups in Table 2 with a p-value reported for each row.

We agree and this has now been done.

This reviewer has a few suggested revisions to the presentation of the results and conclusion:

(1) There are some typos in Table 1 - the median age for RCC and LCC should have a decimal point and not a comma.

We agree and this has now been corrected and Table 1 and 2 have been combined.

(2) The column headings for Table 2 need to be lined up and there should be p-values presented comparing the characteristics by different subsites of colon cancer.

We agree and this has now been corrected and p-values have been added.

(3) In Table 3, it would be helpful to know how many patients are in the sample for "years 1-2" and "years 3-5". Based on the descriptive statistics of the sample, not everyone was followed for a full 5 years.

We agree and numbers have been added.

(4) Formatting in Table 4 needs to be improved.

We agree and this has been done.

(5) This reviewer recommends presenting the results of one global model for overall 5-year survival by RCC vs LCC in Table 3 and a similar global model for the more discrete colon cancer subsites in Table 5. Including all of the variables in one model would show the impact of colon cancer subsite on overall 5-year survival while controlling for all other variables at the same time.

Please see answer to question (2) under first comments.

(6) The second to last paragraph in the discussion section needs to be expanded. This reviewer would like more discussion about how MSI, K-ras, and BRAF mutations differ along colonic subsites.

We agree and this has now been done.

Reviewer: Alain Demers
Senior Epidemiologist
Public Health Agency of Canada
Canada

I have no conflict of interest to declare.

I strongly encourage the authors to use relative survival to compare the survival between the different groups and the mathematical models that go with it to compare the groups.

We agree that if the purpose of the study had been to examine survival in patients with CC as compared to individuals in the general population, we should have used relative survival, using the general Danish population of comparable age and gender for comparison. However, we find that the Cox proportional hazards model is the best suited model for assessment of mortality within different CC groups, which was the purpose of the present study.

The authors do not say if their study has been approved by an ethics board.

We agree that this need to be clearly stated and the sentence now reads as follows:
“The Danish National Committee on Biomedical Research Ethics and the Danish Data Protection Agency have approved the use of the DCCG database for scientific purposes and for the present study, also.”

The manuscript needs major edition regarding the English and the structure of many sentences.

This has now been done.

The information that the authors of the present paper bring is helpful in better understanding the complexity of the different segments of the colon and supports the change in paradigm that is arising and challenges the dichotomous division of the colon.

Abstract
* Design: this is not a cohort study per se. It is a study using data from a population-based cancer registry containing longitudinal data on cancer-related procedures and patient outcomes.

We find the use of the term ‘cohort study’ to be in accordance with the latest edition of ”A Dictionary of Epidemiology” stating that “The term ‘cohort’ describes any designated group of persons who are followed or traced over a period of time, as in a cohort study”. We study a well-defined national cohort of patients with CC over time using person-specific longitudinal national data on survival.

* Outcomes and measures: the beginning of the second sentence is missing "... as well as survival...".

We agree and this has now been corrected.

* Results: the part of the sentence "the proportion of women increased the more proximal the tumor" is not clear and need to be reformulated.

We agree and the sentence now reads “Overall, the proportion of patients who were women increased the closer the tumour site was to the small intestine”.

Methods
* I would like to authors to talk about the synchronous/overlapping site CCs and what rules are used by the Registrars to allocate them to one site or the other. What percentage of CC is synchronous in their Registry? This is potentially a limitation that should be taken into account in the discussion.

We agree and have now added the following to the Discussion: “It may also be seen as a limitation to the study that patients with synchronous or overlapping site CCs have been categorized according to the localization of the cancer with the highest UICC stage. However, only approximately 5% of CCs are synchronous [13]. Potentially we may oversee mild LCCs in patients with higher stage synchronous RCCs (or vice versa) but this is judged to have little impact on results and interpretation of these”.

* The authors wrote "Non-parametric statistics were used for description of patient material", what test exactly and what is the meaning of "patient material"?

We agree that this is not clear and the sentence now reads: “Non-parametric statistics (median, range, chi-square test, and Mann-Whitney test) were used for description of the demographic, clinical, and pathological characteristics of the patient population”.
* In their survival analysis, it is not clear if the authors used death from any cause or if it is death due to CC (cause specific death) - please specify.

We agree and it has now been stated clearly that we used death from any cause.

* Since the authors are dealing with national data, I strongly suggest using relative survival and the

modelization that goes with it.

Please see our answer to the first comment.

* The authors say that their ethics boards "have approved the use of the DCCG database for scientific purposes", but they don't say if their study was approved by these ethics boards.

We agree that this is not clear and it has been corrected as stated above.

Results
* Table 1 and 2 should be combined in one table as Figure 1 and 2 should.

We agree and both tables and figures have now been combined.

* Table notes should be added to the tables where acronyms need to be defined.

We agree and this has been done.

* Table 3: add a table note explaining how the variables are categorized.

We agree and this has now been done.

* The authors wrote "the proportion of women increased with proximal tumor localization, except for hepatic flexure cancer, where women were not in excess" this sentence is not clear and need to be reformulated.

We agree and the sentence now reads: "…the proportion of patients who were women increased the closer the tumor site was to the small intestine. However, among patients with hepatic flexure cancers, women were not in excess".

* There is no need to repeat the confidence intervals of the HR in the text since they are available in the tables.

We respectfully disagree as we find the CIs very important to report in relation to HRs for correct interpretation of these.

* Do the authors have an explanation for the limited 2-yr time period of increased mortality following a CC diagnosis.

This may be due to the fact that recurrence of cancer often is seen in the first years after diagnosis, what has now been added to Discussion.

References
Reference #20: rectal is misspelled.

This has been corrected.

Other comments
I would like to see the paper Yamauchi M et al. Colorectal cancer: A tale of two sides or a continuum?

Gut 2012. 61(6):794-797 included in the discussion.

We agree that this is a relevant paper, which has now been included.

Reviewer: Mao-de Lai
Zhejiang University

In this manuscript, the authors investigated and compared the clinical characteristics and survival information in the colon cancer patients with different anatomical subsites. Different distributions on age, gender, ASA scores, and mortality were found between RCC and LCC, and among different colon subsites. These findings challenge the simple categorization of CC into RCC and LCC and have additional potential strengths in categorization on colon cancer.

Major concerns:
1. The differences between RCC and LCC were well established in the previous studies; therefore, the results in this study should focus on the differences in epidemiology, pathology and prognosis between anatomical subsites. It seems Table 1, 3 and 4 were not very important in result part.

We agree that the focus of this paper is to question the categorization into RCC and LCC by illustrating differences at the colon subsite level. However, we find that the latter findings need to be shown in context of the RCC/LCC findings to illustrate the problems with the conservative classification.

2. In table 2, statistical significances should be examined among patients with different subsites. Discrete trends (Standard deviation or Interquartile/range) should be described for age and Number of lymph nodes harvested.

We agree and p-values have now been added for differences across subsites. Also, numbers and ranges have been added.

3. Besides HR (hazard ratio), survival curve and mean survival time are suggested to compare the differences among different subsites and modified/stratified age, gender, clinical stage, and periods of diagnosis, etc. To control/minimized the effect of diagnostic delay, clinical stage, and periods of diagnosis should be stratified in the analysis.

We thank you for these comments. We have already presented survival curves in addition to HRs . We do not find it reasonable to calculate mean survival times in a dataset with censoring, whereas the median survival time appears from survival curves. We have already adjusted for clinical stage in analysis, whereas we are uncertain what the reviewer means by period of diagnosis? All cancers were diagnosed within the same time period (i.e. within the same decade).

4. The conclusion should be drawn cautiously. The differences might attribute to the diagnostic delay, diagnostic accuracy and compliance of colonoscopy examination. Overall, the evidence presented in this study seems not very persuasive enough to support the conclusion.

We agree and have modified the wording of the conclusion.

5. The organization of table 4 was not correct and the analysis was confusing.

We agree and have now corrected Table 4 and analyses have been described under the table.

6. There are some mistakes on spelling, grammar and format in the manuscript. For example, Line 17

page 6, "RCC and LLC" should be "RCC and LCC". It should be checked thoroughly.

We agree and the manuscript has now been checked thoroughly.
We thank you again for the useful comments and hope that you will find the manuscript improved and suitable for publication in BMJ Open.

VERSION 2 – REVIEW

REVIEWER	Jennifer M. Weiss, MD MS Assistant Professor Division of Gastroenterology and Hepatology Department of Medicine University of Wisconsin School of Medicine and Public Health - Madison, WI USA I have no competing interests to disclose.
REVIEW RETURNED	21-Apr-2013

THE STUDY	In the Article Summary I would make two minor changes: (1) In the first sentence, I recommend using the word "often" instead of "normally" (2) Under the strengths and limitations, I recommend adding the lack of information on chemotherapy as a potential limitation
GENERAL COMMENTS	In the Statistical Analysis section, there are a few grammatical errors: (1) Page 9, line 22 - "mortality of any course" should be "mortality fom any cause" or "all-cause mortality" (2) Page 9, lines 39-41 - I recommend changing the sentence to say "with the survival among patients with sigmoid colon cancer" or "using patients with sigmoid colon cancer as the reference group" Overall, the authors were very responsive to the reviewers' comments and I only have very minor suggestions.

REVIEWER	Maode Lai Professor Department of Pathology, School of Medicine, Zhejiang University, China I have no competing interests to disclose
REVIEW RETURNED	14-Apr-2013

GENERAL COMMENTS	The authors have addressed all the questions raised before.