PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Long-term risk of cardio- and cerebrovascular disease after removal of the colonic microbiota by colectomy: a cohort study based on the Danish National Patient Register from 1996 through 2014
AUTHORS	Jensen, Anders; Ajslev, Teresa; Brunak, Søren; Sørensen, Thorkild

VERSION 1 - REVIEW

REVIEWER	Uri Gophna Tel Aviv University
REVIEW RETURNED	04-Jul-2015

GENERAL COMMENTS	This is an intersting look at the effects of colectomy on CVD risk. However, the limitations that the authors acknowledge in the discussion, pretty much require further analysis that I suggest below. 1. "we therefore restricted the analyses to patients who had colectomy at the age of 45 years and above" While it is true that younger patients are less likely to experience CVD, that decision is the major limitation of this work. Whatever the effects of TMAO may be, cumulative exposure by the age of 45 may be already sufficient for atherscleroticprocesses, and a few years without a colon may pale in comparison. A separate analysis of patients of ages 30-45 at time of surgery with appropriate controls may show a larger effect size, even if overall CVD numbers will be much lower. An additional analysis that will be especially revealing is that of FAP patients that undergo colectomy without prior serious disease other than their polyps. 2. The paragraph in the discussion starting in "The effect of colectomy on CVD risk may have been minimized for various other reasons", is somewhat naive. There can be many obscuring reasons, but the ones pointed out by the authors are not convincing a. "admixture with beneficial microbiota" - according to many studies the microbiota of colorectal cancer patients and IBD patients is anything but "beneficial", so there is no reason to assume it is "better" CVD-wise than that of non-IBD non-cancer patients that undergo surgery, such as controls b. The pouch microbiota is much smaller (at least in terms of total microbial cells) than the colonic microbiota and its metabolic output is excpected to be much smaller as a consequence. Furthermore, several studies show it is never quite "colon-like" and so even if all colectomized patients have had a pouch this would still produce a dramatic change in microbiota and would almost certainly kill-off many "TMAO-generating" taxa. Comparing such known "TMAO-

	generating" taxa to ones observed in pouch study is clearly beyond the scope of this manuscript, so probably best to remove this argument.

REVIEWER	Zeneng Wang Assistant Professor Department of Cellular and Molecular Medicine Cleveland Clinic
REVIEW RETURNED	04-Sep-2015

GENERAL COMMENTS	This paper compared the patients after total colectomy with patients undergoing other types of surgery in risk of CVD and concluded that colectomy can not reduce risk of CVD. The clinical data collected are very precious. However, some concerns will affect the conclusion in this paper. First, this paper starts the propose that gut microbiota may be involved in the development of atherosclerotic CVD and the removal of gut microbiota from the largest gut segment, colon, should decrease patient risk for CVD. In fact, in each segment of gut, the microbiota is active in the involvement of the host-microbial symbiosis. We are not sure whether one segment is removed, the other segment will make up it. On the other hand, not all gut microbiota show bad effects leading to atherosclerosis. Second, in order to test whether colectomy will reduce the general risk of CVD, the authors used other surgery as controls in this paper. So this study in fact is used to compare the difference in risk of CVD among different surgeries. If the other sugeries will reduce CVD risk, the conclusion may not be right. So if the authors compare the CVD risk between colectomy patients and non-colectomy patients without any other surgery, the results will be even more accurate to state whether colectomy will reduce the general risk of CVD.

VERSION 1 – AUTHOR RESPONSE

> Reviewer: 1
> Reviewer Name Uri Gophna
> Institution and Country Tel Aviv University Please state any
> competing interests or state None declared: None declared
>
> Please leave your comments for the authors below

This is an intersting
> look at the effects of colectomy on CVD risk.

R: Thank you.

However, the limitations that the authors acknowledge in the discussion, pretty much require further analysis that I suggest below.

R: We have carefully considered each of the suggestions for further analysis.

>

> 1. "we therefore restricted the analyses to patients who had colectomy at the age of 45 years and above"
>
> While it is true that younger patients are less likely to experience CVD, that decision is the major limitation of this work. Whatever the effects of TMAO may be, cumulative exposure by the age of 45 may be already sufficient for athersclerotic processes, and a few years without a colon may pale in comparison. A separate analysis of patients of ages 30-45 at time of surgery with appropriate controls may show a larger effect size, even if overall CVD numbers will be much lower.

R: We can agree on the logics of the idea that the effects of colectomy may be different in the younger age group, but, unfortunately, even though we have access to all patients from the entire country of Denmark through the time period, where such data could be captured, the sample size and event rate in our study population end up being far too low for reliable estimation of a risk departing from what we have reported. Thus, in the age range of 30-44 years, we have recorded 978 patients who underwent colectomy and otherwise fulfilled the same entry criteria as reported in the paper, and only 84 (8.5%) of them experienced a CVD event during the available observation time. Using the same procedure as in the paper for identifying matched controls, we get 14670 patients among whom 1424 (9.7%) experienced a CVD event. The corresponding numbers for the age group 45+ years in our paper are 1530 colectomy patients, 394 (25.8%) among whom with a CVD event, and 22950 control patients, 6564 (28.6%) among whom with a CVD event. Since the statistical power of this type of analysis is driven mainly by number of events, it is not possible for the few additional and similarly distributed patients with CVD events among the younger colectomy and control patients to produce a different outcome of the analyses that may change our conclusions. The numbers of events are also far too low to allow the corroborating stratified analyses. Finally, experiencing a CVD event at that young age is likely to be due to a particular predisposition to CVD, usually based on the genetic profile. While clearly of clinical interest, the public health impact of the possible differences in the younger group is also limited. We would find a much longer observation time than we have available for the young patients very relevant for further elucidation of the effects of colectomy. Rather than going forward with these analyses, we have added the following arguments to the Discussion:

M: “By restricting our study group to patients who had colectomy at the age of 45 years and above, we have excluded a younger group of patients who were unlikely to experience a CVD event in the available observation time unless they were particularly predisposed. Following the same entry and follow-up criteria, there were 978 patients in the age range 30-44 years of age, who had colectomy. However, only 84 (8.5%) of them, and 1424 (9.7%) of the 14670 matched control patients experienced a CVD event. With these numbers and distribution of event we find it unlikely that they can change the conclusion, and, moreover, the numbers do not allow the corroborating stratified analyses. It would be interesting to continue follow-up of this group through the years where the CVD risk increased to the level of the current group.”

An additional analysis that will be especially revealing is that of FAP patients that undergo colectomy without prior serious disease other than their polyps.

R: We agree that the FAP group of patients would be suitable for further interrogation of the hypothesis for the reason mentioned. However, as for the younger group of patients discussed above, the number available in the national register does not allow a reliable analysis. Most of them are likely to be included in the young group mentioned above (n=21), and in the group analyzed in the paper, only 10 of the colectomy patients had the FAP diagnosis. To run analyses of this type of patients would require large-scale international cooperation or access to such data from much larger populations than the Danish one. To address this idea, we have added the following to the Discussion:

M: “In this regard, patients who had colectomy because of familial adenomatous polyposis would be of particular interest to investigate because of the likely absence of a condition influencing the CVD risk. However, the national register includes far too few of such patients for reliable analyses (10 of the colectomy patients included in the analyses and 21 of the 30-44 year old colectomy patients).”
>
> 2. The paragraph in the discussion starting in "The effect of
> colectomy on CVD risk may have been minimized for various other
> reasons", is somewhat naive. There can be many obscuring reasons, but the ones pointed out by the authors are not convincing a. "admixture with beneficial microbiota" - according to many studies the microbiota of colorectal cancer patients and IBD patients is anything but "beneficial", so there is no reason to assume it is "better" CVD-wise than that of non-IBD non-cancer patients that undergo surgery, such as controls b. The pouch microbiota is much smaller (at least in terms of total microbial cells) than the colonic microbiota and its metabolic output is excpected to be much smaller as a consequence. Furthermore, several studies show it is never quite "colon-like" and so even if all colectomized patients have had a pouch this would still produce a dramatic change in microbiota and would almost certainly kill-off many "TMAO-generating" taxa. Comparing such known "TMAO-generating" taxa to ones observed in pouch study is clearly beyond the scope of this manuscript, so probably best to remove this argument.

R: We thank the reviewer for these precise and insightful arguments about the microbiota in these patient groups, and we have followed the suggestion to remove the arguments rather than expand on them in a more speculative way, which, as mentioned, would go beyond the scope of this paper. Thus, we have replaced these sentences in the Discussion:

M: “It is possible that the intestinal microbiota in this study population is less harmful than expected because of admixture of individuals with an overall beneficial microbiota. Following colectomy, the remaining gut, especially the ileo-rectal pouch, may be overgrown by the pre-existing microbiota.”

R: With the following sentences:

M: “Thus, the patient groups may be a mixture of patients with intestinal microbiota that are either increasing or decreasing the CVD risk in such a way that the effect of colectomy in the entire group becomes a net result of interference with oppositely acting microbiota. The risk may also modified by the microbiota associated with the diseases leading to colectomy and by the alterations in the microbiota in the remaining intestine following colectomy. Furthermore, the…”

> Reviewer: 2
> Reviewer Name Zeneng Wang
> Institution and Country Assistant Professor Department of Cellular
> and Molecular Medicine Cleveland Clinic Cleveland, OH 44195 USA
> Please state any competing interests or state None declared: None
> declared
>
> Please leave your comments for the authors below

This paper compared
> the patients after total colectomy with patients undergoing other types of surgery in risk of CVD and concluded that colectomy can not reduce risk of CVD. The clinical data collected are very precious.

R: Thank you.

However, some concerns will affect the conclusion in this paper.

R: We have carefully considered these concerns.
>
> First, this paper starts the propose that gut microbiota may be involved in the development of atherosclerotic CVD and the removal of gut microbiota from the largest gut segment, colon, should decrease patient risk for CVD. In fact, in each segment of gut, the microbiota is active in the involvement of the host-microbial symbiosis. We are not sure whether one segment is removed, the other segment will make up it. On the other hand, not all gut microbiota show bad effects leading to atherosclerosis.

R: We agree that we need to make reservations about the possibilities of the changes in the microbiota from before to after colectomy. We think the reservations are covered by the changes inspired by the 2nd comments made by the former reviewer.
>
> Second, in order to test whether colectomy will reduce the general risk of CVD, the authors used other surgery as controls in this paper. So this study in fact is used to compare the difference in risk of CVD among different surgeries. If the other sugeries will reduce CVD risk, the conclusion may not be right. So if the authors compare the CVD risk between colectomy patients and non-colectomy patients without any other surgery, the results will be even more accurate to state whether colectomy will reduce the general risk of CVD.

M: While we appreciate the theoretical, experimentally oriented logic in the argument, we honestly disagree that it would be more appropriate in the current setting to compare the colectomy patients with patients not undergoing surgery. The fundamental problem is the high risk of confounding by indication derived from the clinical decision-making about colectomy, which would imply that the patients in whom it was decided to perform a major surgery such as colectomy is a subset of patients at an a priori relatively low CVD risk. To cope with this bias, we need to compare with other patients who undergo surgery and in whom the a priori CVD risk is similarly considered before deciding to offer surgery. We were pleased to observe that irrespective of which of many different types of surgery we used to create the control groups, the essential results were the same. Moreover, we find it unlikely that these several other types of surgery would have an influence on the microbiota and possibly associated CVD risk that are similar to that following colectomy, had it had such influence. In order to take advantage of the reviewers' idea we have added the following to the paragraph in the Discussion of the confounding by indication (the 3rd:

M: "In theory, it would be ideal to compare the colectomy patients to a group of patients with the same underlying diseases and with the same a priori risk of CVD, but not undergoing colectomy. However, identifying such control group in the register is not feasible; colectomy is performed on indications based on the type and severity of the underlying diseases, and comparable patients who have not had colectomy, but with the same type and severity of diseases combined with the same a priori CVD risk are unlikely to exist in this population."

R: General reply:

R: In view of the emphasis on the necessary reservations to made in the interpretation of the results of our study, we have also modified the final concluding paragraph of the Discussion to the following:

M: "While keeping the reservations in mind, we conclude that removal of the major reservoir of the gut microbiota by colectomy did not reduce the risk of CVDs in this population, except for the reduced risk of hypertensive disorders."

VERSION 2 – REVIEW

REVIEWER	Uri Gophna Tel Aviv University, Israel
REVIEW RETURNED	16-Oct-2015

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.

REVIEWER	Zeneng Wang Department of Cellular & Molecular Medicine Cleveland Clinic USA
REVIEW RETURNED	26-Oct-2015

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.