PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Progression of disease preceding lower extremity amputation in Denmark: A longitudinal registry study of diagnoses, use of medication and healthcare services 14 years prior to amputation
AUTHORS	Jensen, Pia; Petersen, Janne; Kirketerp-Møller, Klaus; Poulsen, Ingrid; Andersen, Ove

VERSION 1 - REVIEW

REVIEWER	Misty Humphries University of California Davis Medical Center
REVIEW RETURNED	24-Feb-2017

GENERAL COMMENTS	The authors have taken an extremely rich data source and retrospectively reviewed what care patients that ultimately require amputation get in the years prior. While this type of data is useful, the flaw of this work is the lack of patients that did not require amputation to garner some type of association of treatment and to find ways to change care to prevent amputation. My comments for specific portions of the work are below. Introduction Line 98-99. The ranges your give overlap so much that this needs to be revised. To say that 50-90% are due to PAD and then 20-80% are due to diabetes is so wide spread that it misleading. There are population based studies that have looked at the breakdown of amputations. Traumatic amputations in these studies have typically been higher than 10% and more in line with 30% of all amputations that are performed. Line 103- Is cardiovascular disease a risk factor or is it just the same disease process located in a different area? I would argue the later. It is a predictor of PAD, but not truly a risk factor. Line 106- There is definitely more than one study that has looked at disease treatment prior to amputation. I can think of two off the top of my head and in both of those cases they found similar results that many patients are not on appropriate medical therapy prior to amputation and there is a delay in diagnosis and referral. They may not have used the same methods as you, but they did the same work as you. Change the wording a bit. Line 108: When you are describing global prevalence, you need one approximate number. If you want to discuss prevalence in developed countries, you can use can use a range, but this range is just confusing to readers. Especially if it is 90% and it's supposed to increase by 55%, then everyone is going to have it. Simplify the

	numbers and make them more logical. I realize this can be hard because the numbers ARE all over the map, but take the time to assimilate the data you found in all those studies rather than just reporting everything. Line 115: “To ensure early identification 115 of patients at risk of amputation, we need more knowledge about the progression of LEA-related diseases” and line 118-119. This is not what your study is doing so you need to change the wording. You are not looking at the progression of all patients with PAD and DM to identify which patients progress. You are looking at patient characteristics for patients that had an amputation. There is a huge difference here. I realize you acknowledge the lack of a control group as a limitation, but you cannot claim that you are examining the progression of LEA related disease. I would refer you to the following paper: Looking Forward, Looking Back: Assessing Variations in Hospital Resource Use and Outcomes for Elderly Patients with Heart Failure Line 120: I don’t quite understand what an unselected population is, but I am hoping you will explain it later in the methods. Methods: Line 150-157 What about patients that underwent amputation for cancer? This is a population that may undergo hip disarticulation. The use of disarticulation for atherosclerotic disease is rare. You may want to consider removing these patients or at least reporting how many patients had a diagnosis of malignancy such as sarcoma or other advanced malignancy. Line 187: According to the document I received it was table 3 that described your ACT codes not Table 2. Line 194 A visit to a GP was defined as a show- up at the GP clinic and visits to outpatient clinics included only clinics at the hospitals. What do you mean by clinics at the hospital? Are there not other outpatient clinics that are not associated with the hospital. This may be an American nuance that cause me not to understand this comment, but I work in an outpatient clinic that is not at the hospital as do many of my partners. Line 204: You already defined major and minor amputation, you don’t need to do it twice. Results: Figure 1: Reading this figure I became concerned about how you defined your cohort. When you say foot and ankle amputation I think you need to clarify this. If you are saying the foot was removed at the level of the ankle, then that should be considered a major amputation. If however, you are speaking of a forefoot amputation such as a transmetatarsal amputation, or a ray amputation then that can be considered a minor amputation. I just want to make sure it is clear. The word foot or ankle amputation makes me think the amputation was through the ankle, in which case the foot is removed and the patient has had a major amputation. Table 1: there are a very low number of patients that had an angioplasty or a bypass procedure. Why do you think this is? Do you not have a homogenous cohort? Do you possibly have patients for

	whom the amputation was done for other reasons besides atherosclerotic disease and DM. That would explain why you have low use of cholesterol lower agents, hypertensive medications, or DM medications. Or does Denmark not to a lot of these types of procedures? Figures: The labeling can be a bit better on Figure 2. The legend wording is small and it is difficult to tell what disease is being shown in the individual graphs. Figure 3: Same here with the labeling. I had to go to the foot note to understand what the difference is between a and b. It would be nice to have it in the figure itself. Discussion: Paragraph 2 of the discussion: I can't follow what the theme of this paragraph is. It is all over the place. Is it about the increased prevalence of atherosclerosis or is it about a lack of recognition by the GP? Both are valid points, but the paragraph is not really flushing them out. The idea that GPs do not recognize the symptoms is real, and you should discuss that more. You go back to Opioids in the third paragraph and not recognizing pain as PAD. This is a very good point, but you need to focus an entire paragraph on it by itself not in two different areas.

REVIEWER	Thomas Almdal, Consultant Endocrinologist DMSc Department of Endocrinology Rigshospitalet DENMARK
REVIEW RETURNED	13-Apr-2017

GENERAL COMMENTS	The purpose of the present study was to examine medical history, use of drugs, and contact with the health care system in a cohort of app 2000 patient who undergoes lower extremity amputations. The study is based on informations obtained from a number of registers used in the Danish health care system. In principle the study is retrospective as the patient are identified at the time of amputation and register information is obtained from the up to 14 years prior to amputation. Moreover no attempt to identify a control group have been made Although this is not clearly stated among the aim it appears that the authors wants to study to which extend the patient have been less than optimal treated and although this not stated to characterized patients which was undiagnosed in relation to arteriosclerotic diseases at the time of amputation. According to the authors there is a lack of knowledge in the area. The study is relevant and can provide informations based on which hypothesis in relation to more optimal treatment can be based. Below is some comments and suggestion, which may improve the study In relation to etiology: 1. It would be informative with a figure or a table which provided information in relation to the proportion of patient which had been diagnosed with I: Any arteriosclerotic disease (Cardiac, cerebral or other including peripheral arteriosclosis. II: Diabetes, suggested that use of glucose lowering drugs is used as criteria for this, III: I+II, ie

	patients with a known risk factor for LEA and IV: none of these 2. Group IV could be further characterized in relation to gender, social characteristics, contact to GP, alcohol abuse ect to examined whether it possible to identifed this gruop better 3. If possible it would be relevant to know why the patients was amputated i.e to which extend was it due to acute ischemia ie an embolus, and to which extend was it rather due to a progressive atherosclerosis. If the proportion with acute ischemia is noteworthy it would be relevant to examine to which extend these patient had been diagnosed with atrial fibrillation. 4. If possible it is relevant with information of whether those in group IV suffered from arteriosclerosis – information from pathology register following operation ? 5. Among those with diabetes it is relevant with information in relation to previous foot ulcers In relation to possible suboptimal treatment 1. Please discuss in in the introduction whether treatment with cholesterol lowering drugs is beneficial in relation to prevention of LEA ? 2. Report used of cholesterollowering drugs in groups I – III mentioned above as guidelines advice use of these in all three groups, thus anything less than 95 % suggest suboptimal treatment 3. If possible information of to which extend the patient had been examined for peripheral arteriosclerosis before LEA, information from NPR in relation to procedures.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Misty Humphries
Institution and Country: University of California Davis Medical Center Please state any competing interests or state ‘None declared': None

The authors have taken an extremely rich data source and retrospectively reviewed what care patients that ultimately require amputation get in the years prior. While this type of data is useful, the flaw of this work is the lack of patients that did not require amputation to garner some type of association of treatment and to find ways to change care to prevent amputation. My comments for specific portions of the work are below.

Introduction
Line 98-99. The ranges your give overlap so much that this needs to be revised. To say that 50-90% are due to PAD and then 20-80% are due to diabetes is so wide spread that it misleading. There are population-based studies that have looked at the breakdown of amputations. Traumatic amputations in these studies have typically been higher than 10% and more in line with 30% of all amputations that are performed.

Response: Thank you for pointing this out, we agree that the prevalence's overlap can be miss leading, we have therefore now changed the prevalence's based on newer national wide studies. The original text "Among all major amputations, approximately 50-90% are related to peripheral artery disease (PAD), 20-80% are related to diabetes, and 10% to trauma (13)." has been changed to :

"Studies have reported prevalence's of diabetes to be between 52%- 64% (3,5,14) and approximately 80 % of the patients with LEA are either diagnosed with diabetes or PAD (12).
We have moved the following text up from line 139-142: "In a cohort of patients with diabetes, 18% had a cardiovascular disease with PAD being most prevalent (27). Among patients diagnosed with both diabetes and PAD, the risk of amputation is 1.5 times higher than in patients diagnosed with PAD alone and five times higher than in patients only diagnosed with diabetes (13)."
We have added this to start the next sections "The global prevalence of diabetes and PAD among patients with LEA varies among populations due to factors such as ethnicity and socioeconomic e.g.(4,15)" please see line 85-92, marked copy line 100-107

Line 103- Is cardiovascular disease a risk factor or is it just the same disease process located in a different area? I would argue the later. It is a predictor of PAD, but not truly a risk factor.

Response: We agree with the reviewer and have changed the original text from: "The risk factors for PAD are age, smoking, history of cardiovascular diseases, diabetes, hypertension, dyslipidaemia, and obesity (15)." to: "The risk factors for PAD are age, smoking, diabetes, hypertension, dyslipidaemia, and obesity" , please see line 96-97, marked copy line 114

Line 106- There is definitely more than one study that has looked at disease treatment prior to amputation. I can think of two off the top of my head and in both of those cases they found similar results that many patients are not on appropriate medical therapy prior to amputation and there is a delay in diagnosis and referral. They may not have used the same methods as you, but they did the same work as you. Change the wording a bit.

Response: We agree that there are some cross-sectional and a few follow-up studies that have investigated the delay in diagnosis, treatment pathway and referral before amputation. We have therefore changed the wording from: "To our knowledge, only one previous study has investigated the progression of LEA-related diseases by examining the use of medication over a seven-year period prior to amputation among patients diagnosed with diabetes (16). Buckley et al. recommended an earlier referral to a medical specialist to prevent LEA" to:
"To our knowledge, only a few studies have investigated the progression of diseases and use of health-care services before amputation using historical longitudinal data. One case-control study including data collected seven years before amputation recommended early referral to a medical specialist to prevent LEA among patients with diabetes. A population-based study found that repeated visit to the hospital did not lower the risk of amputation among patients with diabetes/PAD (23). Other studies have also shown delayed referral to revascularization to prevent loss of an extremity, and inadequate treatment of cholesterol-lowing drug (24,25)", please see line 102- 109, marked copy line 120-134.

Line 108: When you are describing global prevalence, you need one approximate number. If you want to discuss prevalence in developed countries, you can use can use a range, but this range is just confusing to readers. Especially if it is 90% and it's supposed to increase by 55%, then everyone is going to have it. Simplify the numbers and make them more logical. I realize this can be hard because the numbers ARE all over the map, but take the time to assimilate the data you found in all those studies rather than just reporting everything.

Response: We agree that the wording can be confusing. We have changed the text to make it more logical for the reader. Further, we have moved this section up in the introduction to the prevalence of PAD. The original text stated: "Currently, the estimated global prevalence of diabetes is 9% and 90% is characterised as type 2 diabetes (17). Furthermore, the prevalence of diabetes is estimated to increase by 55% over the next twenty years, which represents 10% of the global population". This has

been changed to: "Currently, the global prevalence of diabetes is estimated to 9% of which 90% is characterised as type 2 diabetes (16) and is expected to continue to increase over the next twenty years to 10%.", We have further moved this from line 131-134, please see line 93-95, marked copy line 109-111.

Line 115: "To ensure early identification of patients at risk of amputation, we need more knowledge about the progression of LEA-related diseases" and line 118-119. This is not what your study is doing so you need to change the wording. You are not looking at the progression of all patients with PAD and DM to identify which patients progress. You are looking at patient characteristics for patients that had an amputation. There is a huge difference here. I realize you acknowledge the lack of a control group as a limitation, but you cannot claim that you are examining the progression of LEA related disease. I would refer you to the following paper: Looking Forward, Looking Back: Assessing Variations in Hospital Resource Use and Outcomes for Elderly Patients with Heart Failure

Response: Thank you for pointing this out, we agree and are fully aware of the difference in modelling forward and backwards, and the limitation regarding predicting when conditing on the future. We have selected our design because we believe that it can be imperative to get knowledge of the disease developments, treatment and heath care services before an event/illness/operation in a unselected population (e.g. not selected diseases indication for amputation, or agreed to participate in studies). This study needs to be confirmed and followed by a prospection follow-up study in populations identified in this study, which in this study could be a study of patients receiving opioids at GP's over a longer period. To stress that we are aware that we condition on amputation in future, we have rewritten to: "The first step to improving the early identification is to acquire more knowledge on the characteristics of patients, variation and progression of diseases and use of health care services prior to amputations.", please see line 111 -113, marked copy line 139-142.

Line 120: I don't quite understand what an unselected population is, but I am hoping you will explain it later in the methods.

Response: We agree the word "unselected" can be ambiguous and we have therefore changed the wording from: "We examined the use of medication and the number of contacts with health care services during the 14 years leading up to LEAs, in an unselected population of all Danish patients that underwent LEAs", to "We examined the use of medication and the number of contacts with health care services during the 14 years leading up to LEA, among all Danish patients that underwent LEAs in 2010 or 2011", please see line 114-116, marked copy line 144-150.

Methods:

Line 150-157 What about patients that underwent amputation for cancer? This is a population that may undergo hip disarticulation. The use of disarticulation for atherosclerotic disease is rare. You may want to consider removing these patients or at least reporting how many patients had a diagnosis of malignancy such as sarcoma or other advanced malignancy.

Response: We agree that patients undergoing LEA due to cancer are a special group and in Table 2 listed are the numbers of patients with metastatic cancer and bone cancer listed. Further, there is a group of patients where the indication for amputation is not due to neither diabetes nor PAD. A description of these patients is now included in our manuscript: "A subgroup analysis of characteristics, comorbidities and medical treatment among patients diagnosed with either cardiovascular diseases (CVD) including arteriosclerosis, diabetes or neither are presented in Table 4. A total of 2350 (82%) patients were diagnosed with CVD of which 1185 had CVD without diabetes, and 1451 patients were diagnosed with diabetes of which 286 were not diagnosed with CVD. " See line 220-224, marked copy lines 265-269.

Line 187: According to the document I received it was table 3 that described your ACT codes not Table 2.
Response: Reviewer is correct. Further, we have deleted this reference as the table do not list the ACT codes, and we have referred to table 3 in the result section, please see line 117, marked copy line 220

Line 194 A visit to a GP was defined as a show- up at the GP clinic and visits to outpatient clinics included only clinics at the hospitals. What do you mean by clinics at the hospital? Are there not other outpatient clinics that are not associated with the hospital. This may be an American nuance that causes me not to understand this comment, but I work in an outpatient clinic that is not at the hospital as do many of my partners.

Response: In Denmark, the departments at the hospitals have their out-patients clinics at the hospitals, but we also have private clinics with medical specialist who treats patients who had been referred by their GP. We have illustrated this to the updated figure 4 to include patients' visits to medical specialist in private clinics. We have also changed the wording in the methods section to: "A visit to a GP was defined as a show-up at the GP clinic, visits to outpatient clinics included only clinics at the hospitals while a visit to a medical specialist only includes private clinics. ", please see line 184-186, marked copy line 227-230.
Line 204: You already defined major and minor amputation, you don't need to do it twice.

Response: Yes, that is correct, and this has been erased, see line 240

Results:
Figure 1: Reading this figure I became concerned about how you defined your cohort. When you say foot and ankle amputation, I think you need to clarify this. If you are saying the foot was removed at the level of the ankle, then that should be considered a major amputation. If however, you are speaking of a forefoot amputation such as a trans metatarsal amputation, or a ray amputation then that can be considered a minor amputation. I just want to make sure it is clear. The word foot or ankle amputation makes me think the amputation was through the ankle, in which case the foot is removed and the patient has had a major amputation.

Response: Thank you for pointing this out and we agree this can be misleading. Minor amputation was all trans metatarsal amputation or at a more distal level. We have changed "Ankle/foot amputation" to "Foot amputation" in both Figure 1 and the corresponding text.

Table 1: There are a very low number of patients that had an angioplasty or a bypass procedure. Why do you think this is? Do you not have a homogenous cohort? Do you possibly have patients for whom the amputation was done for other reasons besides atherosclerotic disease and DM. That would explain why you have low use of cholesterol lower agents, hypertensive medications, or DM medications. Or does Denmark not to a lot of these types of procedures?

Response: This is an excellent question, and we do not have a good explanation. The prevalence that we have found is low but not very different from another population-based study (Moxey et al. 2010). However, we do not believe that the low number is due to a non –homogenous cohort, but more likely to be caused by the study design being conditioned on amputation and not on a specific disease like diabetes. Further, we only have included people who ended up with an amputation and not patients who had limb saving revascularization performed which may explain the low prevalence to a certain extent. Moreover, this study only includes index amputations all though only 352 patients were excluded on this indication. Further, in this cohort of patients with index amputations we found that 89 % of the major amputation had not previous been amputated, and for this group, the frequency of

examinations with distal blood pressure could have shared some light on this matter. Unfortunately, we do not have these data. We have included this in our limitation; please see line 384-385, marked copy line 481-483.

Figures: The labelling can be a bit better on Figure 2. The legend wording is small, and it is difficult to tell what disease is being shown in the individual graphs.

Response: We agree and figure 2 has been updated with a heading for each of the three figures so it should be easy to identify the diseases that are depicted. We have increased the front size in the legend wording.

Figure 3: Same here with the labeling. I had to go to the foot note to understand what the difference is between a and b. It would be nice to have it in the figure itself.

Response: Same changes have been made for Figure 3. Further, due to a request from reviewer2, we have added table 4 and therefore combined figure 3 and 4 to comply with the guidelines.

Discussion:
Paragraph 2 of the discussion: I can't follow what the theme of this paragraph is. It is all over the place. Is it about the increased prevalence of atherosclerosis or is it about a lack of recognition by the GP? Both are valid points, but the paragraph is not really flushing them out. The idea that GPs do not recognize the symptoms is real, and you should discuss that more. You go back to Opioids in the third paragraph and not recognizing pain as PAD. This is a very good point, but you need to focus an entire paragraph on it by itself not in two different areas.

Response: Thank you for pointing this out, we agree and have rewritten paragraph 2 and 3; please see line 320 - 362, marked copy line 378 – 423.

Reviewer: 2
Reviewer Name: Thomas Almdal, Consultant Endocrinologist DMSc Institution and Country: Department of Endocrinology, Rigshospitalet, DENMARK Please state any competing interests or state ‘None declared': None declared

The purpose of the present study was to examine medical history, use of drugs, and contact with the health care system in a cohort of app 2000 patient who undergoes lower extremity amputations. The study is based on informations obtained from a number of registers used in the Danish health care system. In principle the study is retrospective as the patient are identified at the time of amputation and register information is obtained from the up to 14 years prior to amputation.

Moreover no attempt to identify a control group have been made Although this is not clearly stated among the aim it appears that the authors wants to study to which extend the patient have been less than optimal treated and although this not stated to characterized patients which was undiagnosed in relation to arteriosclerotic diseases at the time of amputation.

According to the authors there is a lack of knowledge in the area. The study is relevant and can provide informations based on which hypothesis in relation to more optimal treatment can be based.

Below is some comments and suggestion, which may improve the study

In relation to etiology:
It would be informative with a figure or a table which provided information in relation to the proportion of patient which had been diagnosed with I: Any arteriosclerotic disease (Cardiac, cerebral or other

including peripheral arteriosclerosis. II: Diabetes, suggested that use of glucose lowering drugs is used as criteria for this, III: I+II, ie patients with a known risk factor for LEA and IV: none of these

Response: We agree that this is an important point made by the reviewer. We have added a Table 4 with characteristics, comorbidities and perceived medication. The coding for this subgroup analysis is added to the supplementary material.

2. Group IV could be further characterised in relation to gender, social characteristics, contact to GP, alcohol abuse ect to examined whether it possible to identifed this group better

Response: we agree this is an important group and a section have now been added, see response to reviewer 1 (please see line 220-224, marked copy lines 265-269).

3. If possible it would be relevant to know why the patients were amputated i.e., to which extent was it due to acute ischemia ie an embolus, and to which extend was it rather due to a progressive atherosclerosis. If the proportion with acute ischemia is noteworthy it would be relevant to examine to which extend these patient had been diagnosed with atrial fibrillation.

Response: We agree this information could be very informative. Unfortunately, we only have information on the diagnosis but not which of these that was the indication for the amputation.

4. If possible it is relevant with information of whether those in group IV suffered from arteriosclerosis – information from pathology register following operation ?

Response: Again we agree, this information could give new insights into the progression of arteriosclerosis, though no national registers in Denmark exist for this purpose.

5. Among those with diabetes, it is relevant with information in relation to previous foot ulcers
Response: We agree this is relevant information and is reported in Table 1.

In relation to possible suboptimal treatment
Please discuss in in the introduction whether treatment with cholesterol lowering drugs is beneficial in relation to prevention of LEA ?

Response: This is a good point and we have added the following to the introduction section "The NICE guidelines for lower limb peripheral arterial disease states that there is substantial evidence establishing benefits for lowering cholesterol drugs for patients with PAD and the use of limb-saving procedure are also recommended (20). The benefits of cholesterol lowering drugs have shown significant reduction in the risk of major amputation (21,22); please see line 97- 101, marked copy line 114-118.

Report used of cholesterol-lowering drugs in groups I – III mentioned above as guidelines advice use of these in all three groups, thus anything less than 95 % suggest suboptimal treatment

Response: This information is listed in Table 4 but as this is one of the main results of this study we have also reported this in the section of Comorbidities and medical treatment. We have added the following text:" Among patients diagnosed with cardiovascular diseases (CVD) and patients diagnosed with diabetes, had 46% (543/1185) and 65% (940/1451) received cholesterol-lowering before the amputation, see Table 4. please see line 229 -231, marked copy line 274-276.

3. If possible information of to which extend the patient had been examined for peripheral arteriosclerosis before LEA, information from NPR in relation to procedures.

Response: We agree with the reviewer. These numbers would be important and nice to have. Unfortunately, we have no access to these data. We have now included this in our limitation.

VERSION 2 – REVIEW

REVIEWER	Thomas Almdal Dept of Endocrinology Rigshospitalet Copenhagen DENMARK
REVIEW RETURNED	18-Jun-2017

GENERAL COMMENTS	The author have answered all questions adequtely, and the MS have improved a lot and merits publication as it contains very usefull information and analysis.