PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Sex differences in presenting symptoms of acute coronary syndrome: the EPIHeart cohort study
AUTHORS	Araújo, Carla; Laszczyńska, Olga; Viana, Marta; Melão, Filipa; Henriques, Ana; Borges, Andreia; Severo, Milton; Maciel, Maria Júlia; Moreira, Ilídio; Azevedo, Ana

VERSION 1 – REVIEW

REVIEWER	Louise Pilote McGill University Canada
REVIEW RETURNED	17-Aug-2017

GENERAL COMMENTS	This is a prospective cohort study of 873 adult patients living within the catchment area of two tertiary care hospitals in Portugal who were admitted to the cardiology department of either hospital with confirmed acute coronary syndrome (ACS). The study objective was to identify sex differences in presenting symptoms of male and female adults with ACS. The study included 227 women and 646 men. Consecutive sampling of all adult patients admitted to the cardiology departments of the two hospitals was carried out from August 2013 to December 2014. Many patients were excluded on the basis of uncertain ACS diagnosis. In-person patient interviews were conducted within 48 hours of admission to collect data on patient presenting symptoms. Subsequently, a second in-patient interview and a chart review were carried out to gather data on patient risk factors for ACS, socio-demographic information, past medical history, and in-patient clinical course. Exclusion criteria included patients without confirmed diagnosis of ACS, including those with vasospastic angina, death prior to interview, discharge/transfer of patient prior to interview, inability to comply with interview, illicit drug use and exclusion of patients who had been directly referred to hospital by a physician (rather than being admitted based on their presenting symptoms). Baseline characteristics of male and female subjects were notably different in the following categories: age (women were older than men), level of education (women had fewer years of formal education), smoking status (women were less likely to be current or former smokers), and past medical history (women were more likely to have hypertension, diabetes and obesity; men were more likely to have undergone prior PCI). Women were also more likely to have been managed conservatively and not submitted to coronary angiography in comparison to men during the study period.

	Regarding symptom presentation of patients in the study, women were less likely than men to present with pain (94.3% vs. 97.4%, p 0.028), were more likely to experience referred pain (68.7% vs. 58.2%, p 0.007), had higher pain intensity (range of 8-10 for women vs. 6-9 for men, $p < 0.001$) and were more likely to present with a primary symptom other than pain (82.8% vs. 68.9%, $p < 0.001$). In the cluster analysis, women were more likely to fit into cluster 3, the multiple symptoms cluster, with high probability of presenting with all symptoms (15.9% of women cluster 3 vs. 4.80% of men, $p < 0.001$) although it is unclear which symptoms were in that cluster. There are several significant methodological concerns with this study. Selection bias First, in its design, this study is likely to under-represent patients with atypical presenting symptoms or patient groups who are less strongly associated with ACS, such as women. The starting point of this study is a patient population diagnosed with ACS and admitted to hospital. It therefore fails to include those patients who were never admitted to hospital, or who were admitted to non-cardiology departments, in whom a diagnosis of ACS was missed. Due to lack of inclusion and follow-up of all-comers to hospital, we do not know the outcome of these other patients, and whether they were diagnosed with ACS at a later date. These patients may have been individuals with atypical presenting symptoms, which lowered clinical suspicion of an ACS diagnosis and led to lack of investigation. Alternatively, these patients may have been individuals with typical ACS symptoms, but socio-demographic characteristics that are less frequently associated with ACS, such as female sex. Indeed, women made up approximately 35% of this study population and men 65%, which does not reflect the usual proportion of ACS in men and women in other studies (for example, the 2017 Heart Disease and Stroke Statistics update noted a male predominance of 55% for coronary heart disease). This raises an important concern that we are receiving a biased representation of women in this study, and that only those women with more severe or typical ACS symptoms were likely to be diagnosed with ACS and included in the study population. Given this biased representation, the very results of the study, which seeks to identify sex differences in presenting symptoms of ACS, are called into question. Although the authors recognized that “the subjective experience of symptoms influences patients’ attitudes in seeking help and professionals’ interpretation of clinical presentations” (page 4 lines 12-14), they did not highlight how this resulted in a study population that already favored certain patient populations and classical ACS presentations. Although it was noted in their conclusion on page 16 that sex differences could lead to selection bias with a higher risk of non-inclusion of women in the study due to early death (due to worse prognosis associated with atypical presentation), they did not expand this further to consider that all women, not just those who died, and in particular women with less severe or classical presentations, could be under-represented in their study population. Recall Bias Second, there is a concern for recall bias in this study. Patients were questioned about their symptoms after admission to hospital and only after they had been given a diagnosis of ACS.

	Although study interviews were conducted within 48 hours of presentation, by that time point, this patient population would have already been interviewed by multiple medical practitioners. In those preceding interviews, symptoms that corresponded with their diagnosis of ACS, such as chest pain and shortness of breath, would have been highlighted. Thus, by the time patients were interviewed for the study, they were primed to place more emphasis on typical cardiac symptoms, which could result in decreased prominence and reporting of atypical symptoms. Misclassification of ACS Diagnosis There are several points that should be clarified in this study. Regarding the diagnosis of ACS, it would be helpful if the authors went into more detail regarding the way in which ACS was diagnosed in their study population. On page 6 it is outlined that patients with Left bundle branch block, ST elevation myocardial infarction (STEMI), non-STEMI, sub-acute myocardial infarction and unstable angina were all included in the study. In addition, the abstract specifically highlighted that patients with type I (primary spontaneous) ACS, were included in the study, and by inference, patients with type II (demand ischemia) were not included. However, it is not clear how these two groups were distinguished, and specific details regarding this important step in clinical decision making are needed. It is also unclear how the diagnosis of ACS was reconciled with the finding of normal or near normal coronary anatomy on cardiac angioplasty in a proportion of the study population (10.6% of women and 5.61% of men), and whether this group of patients should have been included in the study. Mislabeled Study Design It is also not clear that this represents a prospective cohort study, although this is how it is characterized by the authors. Given the short time-frame of follow-up, and the information available upon recruitment of study subjects (diagnosis, sex, etc.), it would be more appropriate to categorize this study as a cross-sectional study, with a retrospective component, given that patients were questioned about their presenting symptoms within 48 hours of admission. Age as a Confounding Factor The conclusions of the manuscript do not appear to coincide with the results. The descriptive statistics show differences between sexes yet the conclusion is that there are no differences. I think that the effect of age is predominant and adjustments for age lead to the misleading conclusion of no difference. The authors should divide their analysis and conclusion by age groups < 55 and 55 and over. Previous work has shown that symptom presentation is age sensitive and this study would add to the literature if results and analysis was focused on the age groups (1). This reference should be cited and results of current study compared to this previous study. Minor comment Finally, on Line 40 of page 7 the average age of women versus men was incorrectly reported at 69.1 vs. 64.0 years; however, it should read 69.1 vs. 62.2 years, based on the results in Table 1). 1)Sex differences in acute coronary syndrome symptom presentation in young patients. Khan NA, Daskalopoulou SS, Karp I, Eisenberg MJ, Pelletier R, Tsadok MA, Dasgupta K, Norris CM, Pilote L; GENESIS PRAXY Team. JAMA Intern Med. 2013 Nov 11;173(20):1863-71. doi: 10.1001/jamainternmed.2013.10149.

REVIEWER	Akira Sato University of Tsukuba, Japan
REVIEW RETURNED	24-Aug-2017

GENERAL COMMENTS	This study sought to investigate the sex differences in presenting symptoms of ACS. They concluded that while there are no differences in the frequency or location of pain between sexes, women are more likely to feel pain of higher intensity and to present with referred pain and symptoms other than pain. My comments are related to the following points: 1) First of all, the clinical implication of this manuscript is obscure because there have been several published reports about the sex differences in presenting symptoms of ACS. The purpose or hypothesis of this study is unclear to this reviewer, and therefore this reviewer did not find any novelty in this paper. 2) I agree that knowledge of these ACS presentation profiles is important for health policy decisions and clinical practice. The ACCF/AHA STEMI Guidelines recommend that hospitals providing primary PCI to patients with STEMI should treat patients within 90 minutes of contact with the medical system or admission to hospital. However, they did not demonstrate the relationship between presenting symptoms and these important parameters including the clinical outcomes. Please discuss these comments. 3) Please provide the flow chart of this study population.

REVIEWER	Giuseppe Berton Conegliano General Hospital, Cardiology Department Italy
REVIEW RETURNED	29-Aug-2017

GENERAL COMMENTS	Ms. Ref. No.: bmjopen-2017-018798 British Medical Journal Open Sex differences in presenting symptoms of acute coronary syndrome: the EPIHeart cohort study The Authors studied 873 patients with confirmed diagnosis of type 1 ACS, aiming to identify sex differences in presenting symptoms of ACS, to better define the clinical diagnosis of ACS and a faster and more efficient recognition of serious diseases. They considered direct and referred chest pain as typical, atypical or mixed basing on the location, and other classic both specific and non-highly specific symptoms. As a result, and according to literature, chest pain was demonstrated to be the main symptom but turned out to show a slight difference in its localization between women (more often atypical) and men (usually typical); moreover, women showed to report a higher intensity level of pain when compared with men, even if they usually had a better coronary arteries state. Even while dividing the pts in clusters, basing on the amount of symptoms, women showed to suffer more often the combination of multiple symptoms, experienced at a higher intensity when compared with men.

	Conclusion: the paper appears well conducted, even only partially original in subject and results, statistical analysis is appropriate. It may deserve interest.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1(Louise Pilote):
Comment #2
This is a prospective cohort study of 873 adult patients living within the catchment area of two tertiary care hospitals in Portugal who were admitted to the cardiology department of either hospital with confirmed acute coronary syndrome (ACS). The study objective was to identify sex differences in presenting symptoms of male and female adults with ACS. The study included 227 women and 646 men.
Consecutive sampling of all adult patients admitted to the cardiology departments of the two hospitals was carried out from August 2013 to December 2014. Many patients were excluded on the basis of uncertain ACS diagnosis. In-person patient interviews were conducted within 48 hours of admission to collect data on patient presenting symptoms.
Subsequently, a second in-patient interview and a chart review were carried out to gather data on patient risk factors for ACS, socio-demographic information, past medical history, and in-patient clinical course. Exclusion criteria included patients without confirmed diagnosis of ACS, including those with vasospastic angina, death prior to interview, discharge/transfer of patient prior to interview, inability to comply with interview, illicit drug use and exclusion of patients who had been directly referred to hospital by a physician (rather than being admitted based on their presenting symptoms).
Baseline characteristics of male and female subjects were notably different in the following categories: age (women were older than men), level of education (women had fewer years of formal education), smoking status (women were less likely to be current or former smokers), and past medical history (women were more likely to have hypertension, diabetes and obesity; men were more likely to have undergone prior PCI). Women were also more likely to have been managed conservatively and not submitted to coronary angiography in comparison to men during the study period. Regarding symptom presentation of patients in the study, women were less likely than men to present with pain (94.3% vs. 97.4%, p 0.028), were more likely to experience referred pain (68.7% vs. 58.2%, p 0.007), had higher pain intensity (range of 8-10 for women vs. 6-9 for men, $p < 0.001$) and were more likely to present with a primary symptom other than pain (82.8% vs. 68.9%, $p< 0.001$). In the cluster analysis, women were more likely to fit into cluster 3, the multiple symptoms cluster, with high probability of presenting with all symptoms (15.9% of women cluster 3 vs. 4.80% of men, $p < 0.001$) although it is unclear which symptoms were in that cluster.

Reply to comment #2
Cluster 3 was characterized by a high probability of presenting with all symptoms other than pain; symptoms are presented in the methods section (please see page 5, paragraph 3) and in supplementary table 3, which presents the marginal percentage of subjects with each symptom in each assigned cluster. To make more clear which were the symptoms of cluster 3, we listed them in the footnotes of tables 2, 3 and 4, and also in the footnotes of supplementary tables 1 and 2.

There are several significant methodological concerns with this study.
Selection bias
Comment #3
First, in its design, this study is likely to under-represent patients with atypical presenting symptoms or patient groups who are less strongly associated with ACS, such as women.

The starting point of this study is a patient population diagnosed with ACS and admitted to hospital. It therefore fails to include those patients who were never admitted to hospital, or who were admitted to non-cardiology departments, in whom a diagnosis of ACS was missed. Due to lack of inclusion and follow-up of all-comers to hospital, we do not know the outcome of these other patients, and whether they were diagnosed with ACS at a later date. These patients may have been individuals with atypical presenting symptoms, which lowered clinical suspicion of an ACS diagnosis and led to lack of investigation. Alternatively, these patients may have been individuals with typical ACS symptoms, but socio-demographic characteristics that are less frequently associated with ACS, such as female sex.

Reply to comment #3

We agree that the results of our study apply to a population of patients diagnosed with ACS and admitted to the cardiology departments, and are not generalizable to the whole population of patients with ACS. In the limitations section we noted that selection bias should be taken into account, but agree that this limitation can be further explored, as suggested. There were other patients with ACS not admitted to the hospital, namely those who died before being admitted, those who did not seek medical care, those who sought medical care, but were mistakenly discharged, or misdiagnosed and admitted to non-cardiology departments. In fact, death before reaching the hospital is frequent among patients with acute myocardial infarction; according to data from England which reports to the period 2002-2010, it occurred in nearly 25% of cases.1 According to results published by Pope JH et al.2 in 2000, the proportion of missed diagnosis was 2.1% among patients with acute myocardial infarction and 2.3% among patients with unstable angina. Women less than 55 years with acute coronary ischemia were less likely to be hospitalized (adjusted odds ratio for discharge 6.7, 95% confidence interval 1.4-32.5), while among patients with myocardial infarction, female sex was not associated with misdiagnosis and discharge.

In the two hospitals where this study was conducted, there are available cardiologists 24 hours/day, 7 days/week, who are frequently asked to evaluate patients in whom the diagnosis of ACS is doubtful. Probably, admission to cardiology departments with a type 1 ACS initial diagnosis and a discharge type 2 ACS diagnosis is more frequent than admission to non-cardiology departments of patients with a missed diagnosis of type 1 ACS. In fact, the diagnosis of ACS type 1 was not confirmed in 164 patients (please see figure 1), some of these patients were discharged from the cardiology departments with a type 2 ACS.

Despite the relevance of informing to whom our results apply, it should be considered that data on ACS clinical presentation in the subgroups of patients who are not admitted to hospital is gathered through analysis of medical records or mortality statistics, and not through questionnaires. Therefore, available data lack clinical detail and have limitations to clearly conclude what are the true reasons behind the lack of investigation or misdiagnosis of these patients. We admit, as suggested, that the process of sampling itself, which inevitably results in not including these patients, will probably result in the underestimation of the true prevalence of ACS atypical presentation, both in women and men. In the limitations section, we added these missed subgroups of patients, and the potential impact of the sample selection on results (please see page 19, paragraph 3).

Comment #4

Indeed, women made up approximately 35% of this study population and men 65%, which does not reflect the usual proportion of ACS in men and women in other studies (for example, the 2017 Heart Disease and Stroke Statistics update noted a male predominance of 55% for coronary heart disease). This raises an important concern that we are receiving a biased representation of women in this study, and that only those women with more severe or typical ACS symptoms were likely to be diagnosed with ACS and included in the study population. Given this biased representation, the very results of the study, which seeks to identify sex differences in presenting symptoms of ACS, are called into question.

Reply to comment #4

The male predominance of 55% for coronary heart disease presented in the 2017 Heart Disease and Stroke Statistics refers to mortality from coronary heart disease as a whole, and this report is based on hospitalization databases and mortality statistics.

In other cohort studies, proportions of females were also below 45%. In an analysis of 1260 patients hospitalized for ACS within SPUM-ACS (Special Program University Medicine-Acute Coronary Syndromes) prospective cohort study, conducted in 4 university medical centers in Switzerland, the proportion of women was 21.3%.3 In the EURHOBOP study, a population-based longitudinal cohort conducted in Finland, France, Germany, Greece, Portugal and Spain and comprising 12,231 consecutive ACS patients, and within Portugal 3009 patients from 10 hospitals, 30% percent of patients were women.4

Therefore we do not agree that the proportion of women of our study is per se an argument to support that the representation of women in this study is biased. Furthermore, the proportion of women without chest pain was 17.8% in the whole sample and among women below 65 years old 16.7%, similar to the 19.0% proportion observed in the GENESIS PRAXY study.

Comment #5

Although the authors recognized that “the subjective experience of symptoms influences patients’ attitudes in seeking help and professionals’ interpretation of clinical presentations” (page 4 lines 12-14), they did not highlight how this resulted in a study population that already favored certain patient populations and classical ACS presentations. Although it was noted in their conclusion on page 16 that sex differences could lead to selection bias with a higher risk of non-inclusion of women in the study due to early death (due to worse prognosis associated with atypical presentation), they did not expand this further to consider that all women, not just those who died, and in particular women with less severe or classical presentations, could be underrepresented in their study population.

Reply to comment #5

Information on sex, age and type of ACS was only available for patients who were eligible but not enrolled, and difference in sex proportion between participants and non-participants was only observed for deceased patients, therefore the conclusion on page 16 (now on page 20). We have mentioned that the proportion of patients with ACS presenting without typical chest pain or that of women with an atypical presentation could be even higher, considering that atypical presentation is associated with a worse prognosis (please see page 20, paragraph 1).

As previously stated in the reply to comment #3, other subgroups of patients were missed, namely those who died before being admitted, those who did not seek medical care, and those who despite seeking medical care were mistakenly discharged or misdiagnosed and admitted to non-cardiology departments. We added this information as suggested and the fact that the true prevalence of ACS atypical presentation, in both women and men could be underestimated (please see page 19, paragraph 3).

The literature 2 supports that women with acute myocardial ischemia, but not with myocardial infarction are more likely to be mistakenly discharged. The proportion of missed diagnosis is low, however we agree that it is possible that this type of studies underrepresent women, particularly women with unstable angina. We expanded further this limitation, as suggested (please see page 20, paragraph 1).

Recall Bias

Comment #6

Second, there is a concern for recall bias in this study. Patients were questioned about their symptoms after admission to hospital and only after they had been given a diagnosis of ACS. Although study interviews were conducted within 48 hours of presentation, by that time point, this patient population would have already been interviewed by multiple medical practitioners.

In those preceding interviews, symptoms that corresponded with their diagnosis of ACS, such as chest pain and shortness of breath, would have been highlighted.
Thus, by the time patients were interviewed for the study, they were primed to place more emphasis on typical cardiac symptoms, which could result in decreased prominence and reporting of atypical symptoms.

Reply to comment#6
We agree that the time elapsed between admission and the application of the questionnaire is of paramount importance and according to this, in the previous submitted version of the manuscript, this information was already included in the table 1 (please see table 1) and also in the results section (please see page 8, paragraph 4). The median time that elapsed between admission and application of the symptom questionnaire was slightly longer in women than in men, but the difference was not significant. We agree that a questionnaire applied hours after admission is not the same if it was applied immediately after admission, before other interviews and before the patient knows the diagnosis. However, feasibility limits the application of the questionnaire in the first few hours of myocardial infarction for several reasons. On the other hand, by applying the questionnaire within 48 hours after admission, we expect that answers are not so influenced by the anxious status characterizing the first few hours of myocardial infarction. As far as we know, despite the existence of studies supporting that questionnaires responses may be different according to the performance or not of preceding interviews, no data are available to confirm that preceding interviews to patients with ACS would decrease the report of atypical symptoms through questionnaires applied after, and that this consequence is different by sex.
The possibility of recall bias was further explored in the limitations section, as suggested (please see page 19, paragraph 3).

Misclassification of ACS Diagnosis
Comment#7
There are several points that should be clarified in this study. Regarding the diagnosis of ACS, it would be helpful if the authors went into more detail regarding the way in which ACS was diagnosed in their study population. On page 6 it is outlined that patients with Left bundle branch block, ST elevation myocardial infarction (STEMI), non STEMI, sub-acute myocardial infarction and unstable angina were all included in the study. In addition, the abstract specifically highlighted that patients with type I (primary spontaneous) ACS, were included in the study, and by inference, patients with type II (demand ischemia) were not included. However, it is not clear how these two groups were distinguished, and specific details regarding this important step in clinical decision making are needed. It is also unclear how the diagnosis of ACS was reconciled with the finding of normal or near normal coronary anatomy on cardiac angioplasty in a proportion of the study population (10.6% of women and 5.61% of men), and whether this group of patients should have been included in the study.

Reply to comment#7
The diagnosis of unstable angina, of type 1 myocardial infarction and its classification in different subtypes was determined by the treating cardiologist, based on symptoms and signs at presentation, electrocardiogram findings and the increase in cardiac enzyme levels (with high sensitivity troponins available in both hospitals), according to the third universal definition of myocardial infarction.5
Patients with type 2 myocardial infarction, namely those in whom a condition other than coronary artery disease itself contributed to an imbalance between myocardial oxygen supply and/or demand, were excluded. From the initial 1297 patients, the diagnosis was not confirmed in 164, some of these patients had a discharge diagnosis of type 2 myocardial infarction. Type 4 and 5 myocardial infarction (related to percutaneous coronary intervention/stent thrombosis and to coronary artery bypass grafting, respectively) were also not included.

The distinction between types 1 and 2 (and also types 4 and 5) myocardial infarction was made by treating cardiologists, and only patients with a definitive diagnosis of type 1 ACS made by them were included.

Myocardial infarction with non-obstructed coronary arteries (MINOCA)6, 7 can account for about 10% of all acute myocardial infarction cases, according to data from the Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA Guidelines (CRUSADE) registry.8 Increasingly recognized are also patients presenting with the syndrome of symptoms and signs suggesting ischemic heart disease, not myocardial infarction, but found to have no obstructed coronary disease.9, 10 Ischemia and No Obstructive Coronary Artery Disease (INOCA) was the term proposed to describe these patients´ presentation.11 Furthermore MINOCA and INOCA are more frequent among women.7, 11 The proportions of patients without significant obstructive coronary heart disease observed in our sample (10.6% of women and 5.61% of men) are in line with these data, and again these patients were included because treating cardiologists considered that the final diagnosis was a type 1 ACS.
We agree that more detail should be given regarding the process of ACS diagnosis in our cohort and this relevant information is now available in the methods section (please see page 5, paragraph 1). We have also changed the term normal or near normal coronary angiography to non-obstructive coronary artery disease in the methods section (please see page 7, paragraph 1), in the results section (please see page 8, paragraph 2) and in table 1.

Mislabeled Study Design
Comment#8
It is also not clear that this represents a prospective cohort study, although this is how it is characterized by the authors. Given the short time-frame of follow-up, and the information available upon recruitment of study subjects (diagnosis, sex, etc.), it would be more appropriate to categorize this study as a cross-sectional study, with a retrospective component, given that patients were questioned about their presenting symptoms within 48 hours of admission.

Reply to comment#8
Data for the current analysis were collected within a prospective cohort study, the EPIHeart study, with several variables collected during the follow-up, for example the 30-day mortality now included as outcome (according to comment#12). Clinical presentation was evaluated by applying a questionnaire within 48 hours after admission, therefore this was made retrospectively, as already stated in the limitations section (please see page 19, paragraph 3). To avoid this misinterpretation, we added in the abstract that “data were collected within a prospective cohort study”.

Age as a Confounding Factor
Comment#9
The conclusions of the manuscript do not appear to coincide with the results. The descriptive statistics show differences between sexes yet the conclusion is that there are no differences. I think that the effect of age is predominant and adjustments for age lead to the misleading conclusion of no difference. The authors should divide their analysis and conclusion by age groups < 55 and 55 and over. Previous work has shown that symptom presentation is age sensitive and this study would add to the literature if results and analysis was focused on the age groups (1). This reference should be cited and results of current study compared to this previous study.

Reply to comment#9
We agree that the descriptive statistics show differences between sex clinical presentation by age groups. We changed the results section to clearly strengthen that some of the differences observed, despite not significant, are important to take into account (please see page 10).

The descriptive results of our sample showed that those sex differences in several variables of clinical presentation were higher in the older age groups (please see table 2). Therefore we would lose discriminatory power by using the suggested cut-off of <55 and 55 and over.
We agree that the interaction between age and sex with clinical presentation is relevant. Considering the results of the univariate association between sex and clinical presentation by age groups of our sample, and data supporting that the interaction between age and sex with clinical presentation becomes attenuated with advancing age, mainly in those 65 years old or older, two age stratified multivariate models were performed using 65 years old as cut-off. Accordingly, information was added in the methods (please see page 7, paragraphs 2 and 3 and page 8, paragraph 1), in the results (please see page 13, paragraph 1), and in the discussion section. The reference was cited and results of the current study compared to this previous study, as requested (please see page 17, last paragraph and page 18, paragraph 1).

Comment#10
Minor comment
Finally, on Line 40 of page 7 the average age of women versus men was incorrectly reported at 69.1 vs. 64.0 years; however, it should read 69.1 vs. 62.2 years, based on the results in Table 1).

Reply to comment#10
We corrected this error and thank the reviewer for noticing it.

1) Sex differences in acute coronary syndrome symptom presentation in young patients. Khan NA, Daskalopoulou SS, Karp I, Eisenberg MJ, Pelletier R, Tsadok MA, Dasgupta K, Norris CM, Pilote L; GENESIS PRAXY Team. JAMA Intern Med. 2013 Nov 11;173(20):1863-71. doi: 10.1001/jamainternmed.2013.10149.

Reviewer 2 (A Sato):
This study sought to investigate the sex differences in presenting symptoms of ACS. They concluded that while there are no differences in the frequency or location of pain between sexes, women are more likely to feel pain of higher intensity and to present with referred pain and symptoms other than pain.

My comments are related to the following points:
Comment #11
1) First of all, the clinical implication of this manuscript is obscure because there have been several published reports about the sex differences in presenting symptoms of ACS. The purpose or hypothesis of this study is unclear to this reviewer, and therefore this reviewer did not find any novelty in this paper.

Reply to comment #11
Despite several studies about sex differences in presenting symptoms of ACS, the population of patients with atypical ACS presentation is still not well characterized. The clinical relevance of this study is, in our opinion, supported by the conflicting results available and the possible impact of clinical presentation on outcomes. Inequalities in management and outcomes between women and men with ACS persisted over time, stressing the need to further study potential determinants of these differences, including the complex clinical presentation. Consecutive sampling, detailed clinical information obtained through the questionnaire and adjustment for several confounding variables strengthen our results, compared to previous studies. In the introduction section, we added a sentence and a recent reference supporting that the population of patients with atypical ACS presentation is still not well characterized (please see page 4, paragraph 2).

Also in the introduction, in the last paragraph (main objective) instead of stating that numerous factors were taken into account, we now present these variables (age, socioeconomic data, previous history of CHD, risk factors, comorbidities, type of ACS and coronary anatomy), considering the relevance of appropriate confounder adjustment.
Presenting symptoms of ACS, by impacting health care seeking/reaching, and also diagnosis, are critical for ACS management. The introduction of information on the association between presenting symptoms and clinical outcomes, according to reply to comment #12, further supports the relevance of this study to clinical practice.

Comment #12
2) I agree that knowledge of these ACS presentation profiles is important for health policy decisions and clinical practice. The ACCF/AHA STEMI Guidelines recommend that hospitals providing primary PCI to patients with STEMI should treat patients within 90 minutes of contact with the medical system or admission to hospital. However, they did not demonstrate the relationship between presenting symptoms and these important parameters including the clinical outcomes. Please discuss these comments.

Reply to comment #12
We agree that the association between clinical presentation and outcomes is of paramount importance, and previously we have decided not to approach this step forward in the present manuscript, because of the detailed descriptive nature of our data and results, and the consequent extension of the manuscript. However, evaluating the association between presenting symptoms and clinical outcomes, as suggested, would undoubtedly improve the manuscript.
We opted to choose variables that measure severity of the episode, namely time of presentation (patient and system delays), and severity indicators at admission and during follow-up. We used the 30-day mortality outcome, adjusted for the GRACE 2.0 risk score, according to the European Society of Cardiology/Acute Cardiovascular Care Association quality indicators for the management of AMI proposal.13 Decisions on management, including performance of invasive procedures depend on several determinants, therefore to derive definitive conclusions of the analysis a more comprehensive evaluation would be necessary, constrained by the limits in length of the manuscript and by the fact that this is, by itself, a main objective.
The results of the present analysis were, in our opinion, very interesting, raising particularly the interest of further exploring the relation between symptoms other than pain presentation and clinical outcomes. We added information on the several sections of the manuscript, as this is new information (please see the abstract, methods section, results, table 4 and discussion).

Comment #13
3) Please provide the flow chart of this study population.

Reply to comment#13
The flow chart is now provided, as suggested (please see figure 1).

Reviewer 3 (Giuseppe Berton):
Comment #14
The Authors studied 873 patients with confirmed diagnosis of type 1 ACS, aiming to identify sex differences in presenting symptoms of ACS, to better define the clinical diagnosis of ACS and a faster and more efficient recognition of serious diseases.
They considered direct and referred chest pain as typical, atypical or mixed basing on the location, and other classic both specific and non-highly specific symptoms.

As a result, and according to literature, chest pain was demonstrated to be the main symptom but turned out to show a slight difference in its localization between women (more often atypical) and men (usually typical); moreover, women showed to report a higher intensity level of pain when compared with men, even if they usually had a better coronary arteries state. Even while dividing the pts in clusters, basing on the amount of symptoms, women showed to suffer more often the combination of multiple symptoms, experienced at a higher intensity when compared with men.
Conclusion: the paper appears well conducted, even only partially original in subject and results, statistical analysis is appropriate. It may deserve interest.

Reply to comment #14
We thank the reviewer for his comments.

References used in the authors´ response to the reviewers comments:
1. Smolina K, Wright FL, Rayner M, et al. Determinants of the decline in mortality from acute myocardial infarction in England between 2002 and 2010: linked national database study. BMJ 2012; 344.
2. Pope JH, Aufderheide TP, Ruthazer R, et al. Missed diagnoses of acute cardiac ischemia in the emergency department. N Engl J Med 2000; 342: 1163-70.
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4. Andre R, Bongard V, Elosua R, et al. International differences in acute coronary syndrome patients' baseline characteristics, clinical management and outcomes in Western Europe: the EURHOBOP study. Heart 2014; 100: 1201-7.
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6. Collste O, Sorensson P, Frick M, et al. Myocardial infarction with normal coronary arteries is common and associated with normal findings on cardiovascular magnetic resonance imaging: results from the Stockholm Myocardial Infarction with Normal Coronaries study. J Intern Med 2013; 273: 189-96.
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13. Schiele F, Gale CP, Bonnefoy E, et al. Quality indicators for acute myocardial infarction: A position paper of the Acute Cardiovascular Care Association. Eur Heart J Acute Cardiovasc Care 2017; 6: 34-59.

VERSION 2 – REVIEW

REVIEWER	Louise Pilote MD MPH PhD McGill University, Montreal, Quebec, Canada
REVIEW RETURNED	24-Oct-2017

GENERAL COMMENTS	In this updated draft of the EPIHeart prospective cohort study, which explored sex differences in presenting symptoms of patients with a diagnosis of ACS, the authors successfully addressed many of the concerns previously highlighted in our review. They adequately accounted for the possibility of both selection and recall biases influencing their study population and study results, respectively. Further clarification of the manner in which an ACS diagnosis was obtained was also outlined (i.e. diagnosis by treating cardiologist, based on presenting signs/symptoms, ECG and Troponin results). The inclusion of the section entitled “Clinical presentation and outcomes” was a good addition. On page 15, line 20 of this section, it would be helpful to clearly identify that the authors were relaying results of patients in the multiple systems cluster, in comparison to the other two clusters, as this is not clearly stated, and might lead to confusion. Regarding the multivariate analysis, a useful clarification of the symptoms included in cluster 3 was integrated into the draft. The main outstanding issue is the continued absence of an adequate analysis of the study results by age group, as previously recommended. Although the authors did comment on the differences between age groups seen in their descriptive statistics, a dedicated analysis by age (using age groups of < 55 and > 55), was not included and would add a great deal to the strength of this paper. The conclusions may be different according to age group.

REVIEWER	Giuseppe Berton Conegliano General Hospital, Cardiology Department
REVIEW RETURNED	03-Nov-2017

GENERAL COMMENTS	Sex differences in presenting symptoms of acute coronary syndrome: the EPIHeart cohort study I re-reviewed the paper. The changes AA made, appears appropriate and improved the paper quality. Conclusion: the paper deserves interest.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1 (Professor Louise Pilote)

Comment #1
In this updated draft of the EPIHeart prospective cohort study, which explored sex differences in presenting symptoms of patients with a diagnosis of ACS, the authors successfully addressed many of the concerns previously highlighted in our review. They adequately accounted for the possibility of both selection and recall biases influencing their study population and study results, respectively. Further clarification of the manner in which an ACS diagnosis was obtained was also outlined (i.e. diagnosis by treating cardiologist, based on presenting signs/symptoms, ECG and Troponin results). The inclusion of the section entitled “Clinical presentation and outcomes” was a good addition.

Reply to comment#1
We thank the reviewer for this comment.

Comment #2
On page 15, line 20 of this section, it would be helpful to clearly identify that the authors were relaying results of patients in the multiple systems cluster, in comparison to the other two clusters, as this is not clearly stated, and might lead to confusion.

Reply to comment #2
We agree that objective identification that results were reported to patients who presented with the multiple system cluster was missing and could lead to confusion. We changed the sentences as suggested (please see page 15, paragraph 2).

Comment #3
Regarding the multivariate analysis, a useful clarification of the symptoms included in cluster 3 was integrated into the draft.

Reply to comment #3
No specific question raised.

Comment #4
The main outstanding issue is the continued absence of an adequate analysis of the study results by age groups seen in their descriptive statistics, a dedicated analysis by age (using age groups of < 55 and > 55), was not included and would add a great deal to the strength of this paper. The conclusions may be different according to age group.

Reply to comment #4
We agree on the relevance of age for the relation between sex and clinical presentation, and presented the multivariate results taking into account the interaction between age and sex with clinical presentation in the previous revision, as suggested. The decision to do the stratified analysis with the cut-off of 65 years, instead of 55 years, was based on the fact that in our sample we had only 36 women below 55 years old, and because there are data to support the use of the 65 cut-off, as the association between sex and clinical presentation becomes attenuated with advancing age, mainly in those 65 years old or older.1
We agree that conclusions may be different according to age group, and that particularly the analysis of the sex differences in clinical presentation among a younger ACS population is relevant to clinical practice. Although being limited by the small number of women below 55 years included in our sample, we performed the age stratified multivariate models using 55 years as cut-off, as suggested. The results, although similar to those observed using the 65 years cut-off, stress the relevance of taking into account age to study the association between sex and clinical presentation. In fact, we found results that are in line with those found by the GENESIS PRAXY Team.2 Although the association was not significant, women below 55 years old in our sample were less likely than men below 55 years old to present with typical chest pain (adjusted odds ratio [OR] 0.65, 95% confidence interval [95% CI] 0.23-1.86). Furthermore we also observed a stronger association between female sex and referred pain, and intensity of pain higher than 8/10, among patients below 55 than among patients below 65 years old. Although the precision of the estimates is lower due to the smaller sample of patients below 55, compared with patients below 65, we agree that it is relevant to also present these results and added the multivariate analysis by age using age groups of < 55 and ≥55 years old in a supplementary table (please see supplementary table 4).

Accordingly, information was added in the methods (please see page 7, paragraph 3 and page 8, paragraph 1), in the results (please see the subsection “multivariate models” page 13), and in the discussion section (please see page 17, paragraph 3 and page 18, paragraph 1). The relevance of addressing the role of age for the relation between sex and clinical presentation was stressed in the discussion (please see page 18, paragraph 1) and in the conclusion (please see page 20, paragraph 2).

Reviewer: 3 (Professor Giuseppe Berton)

Comment #5
I re-reviewed the paper. The changes AA made appear appropriate and improved the paper quality. Conclusion: the paper deserves interest.

Reply to comment #5
We thank the reviewer for this comment.

1. Canto JG, Rogers WJ, Goldberg RJ, et al. Association of age and sex with myocardial infarction symptom presentation and in-hospital mortality. JAMA 2012; 307: 813-22.
2. Khan NA, Daskalopoulou SS, Karp I, et al. Sex differences in acute coronary syndrome symptom presentation in young patients. JAMA Intern Med 2013; 173: 1863-71.

VERSION 3 – REVIEW

REVIEWER	Dr Louise Pilote McGill Univeristy, Montreal, Canada
REVIEW RETURNED	29-Nov-2017

GENERAL COMMENTS	The authors have responded adequate by taking age into account in the analyses. However, the interpretation of their results according to age does not appear clearly in the discussion section. The authors should provide clear conclusions about presence or absence of sex differences in the different age groups so as to have a clear message.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 1 (Professor Louise Pilote)

Comment #1
The authors have responded adequate by taking age into account in the analyses.
However, the interpretation of their results according to age does not appear clearly in the discussion section.
The authors should provide clear conclusions about presence or absence of sex differences in the different age groups so as to have a clear message.

Reply to comment #1

We are pleased to reach the acknowledgement of the reviewer regarding the role of age.

We agree that the role of age for the relation between sex and clinical presentation is of paramount relevance, and we thank Professor Louise Pilote for this valuable suggestion.

We also agree that the interpretation of our findings could be better clarified. In the discussion section, besides the finding that, among patients below 55 years old, women were less likely to present with typical chest pain, although the difference was not significant; we added that the association between female sex and presence of referred pain, and of pain with intensity higher than 8 was stronger among the younger subgroups of patients (below 55 and 65 years old) (please see page 18, paragraph 1). We also added that age did not change the association between female sex and presentation with symptoms other than pain and with the multiple symptoms cluster (please see page 18, paragraph 2). Further conclusions on the role of age to the relation between sex and clinical presentation are limited by the small number of women below 55 included. We also added this conclusion (please see page 18, paragraph 1).