PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (http://bmjopen.bmj.com/site/about/resources/checklist.pdf) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

| TITLE (PROVISIONAL) | Assessment of sex-related differences and outcome in patients who underwent cryoballoon pulmonary vein isolation for atrial fibrillation: an observational cohort study |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AUTHORS             | Ekrami, Neda Khalilian; Magni, Federico; Dayalani, Vijay; van Gelder, Isabelle; Groenveld, Hessel F.; Tieleman, Robert; Wiesfeld, Ans; Tan, Yong; Rienstra, Michiel; Blaauw, Yuri; Mulder, Bart |

VERSION 1 – REVIEW

| REVIEWER           | Brasca, Francesco |
|--------------------|-------------------|
| REVIEW RETURNED    | 10-May-2022       |

GENERAL COMMENTS

The paper by Mulder et al is well written and understandable, investigating an interesting issue: sex-related differences in CB-PVI outcome and safety, as I previously wrote in a review for another journal. The large population is the main strength point in this single center, non-randomized, retrospective cohort study.

As, I previously observed, some issues should be considered: My previous major revisions unsolved are:

1) The article is really similar to the one by Ricciardi et al, cited as reference 12. I think that authors should underline the differences found in their results and try to interpretate these differences in the discussion.

2) Population study: there are significant differences in population study compared with the studies cited in references. It could be advisable to discuss this point. Similarly, a focus on sex related differences in risk factors and BMI could be of interest.

I don't think that a criticism of paper by Ricciardi is useful. There are different follow up and different results, but also differences in population. Please, try to justify it.

Please, remove the sentence: “To overcome this issue we have limited the results to a 1 year follow-up” It makes no sense.

3) In the results: “No differences were observed in the duration of time from first AF diagnosis until time of first cryoballoon ablation (median 3.8 years, interquartile range 1.8-7.2 years). Prior to PVI, women were more often treated with AADs (73% vs. 64%; P=0.02).” A comment on time from first AF diagnosis and AAD use could be advisable, because it seems that there is no delay in women treatment (good job) but why have they failed a larger number of AAD treatments? Could the authors discuss this point?

I think that the response should be included in the discussion, not as an answer to the reviewer.
4) The authors found that complications were associated with LAVi, but this data is not available in Table 1. Then, discussing this point, the authors reported a reference (21) but in a wrong way. The article by Kuhne assessed that LA dimension is not a predictor of phrenic nerve palsy! Maybe, it could be discussed that Kuhne tested only LA diameter and not LAVi.

Please, discuss your result not only as an answer to the reviewer.

Minor issues
In the "catheter ablation procedure" section:
- the authors assessed that venous access was not guided by ultrasound (that it is useful to discuss vascular complication). Further information could be of interest: was haemostasis obtained by compressive medication or other strategy (i.e. "Figure-of-Eight" suture technique)?

Could I suggest to focus on this point in the discussion?

In the discussion:
- (pag 8 line 49) "Some studies suggest a significantly lower rate of freedom from AF/AT recurrence in women (please, provide references)". The author has previously answered: "The paper by Kuck, et al. (Impact of female sex on clinical outcomes in the FIRE AND ICE trial of catheter ablation for atrial fibrillation. Circ Arrhythm Electrophysiol. 2018;11(5):e006204) was already referred here. We did not add additional papers." Thus, change the sentence: A study suggests...

- the ref 20 was used to support this sentence. "Underlying mechanisms that play a role in the observed sex difference in the occurrence of phrenic nerve palsy are unknown" I could not find this assessment in the study cited. The previous answer to my observation on this point did not justify the use of this reference.

REVIEWER
Muresan, Lucian
Hôpital Emile Muller

REVIEW RETURNED
23-Jun-2022

GENERAL COMMENTS
Compared to the initial manuscript, this second variant is better, since the authors complied with the reviewers' requests. However I still have one more comment:

Page 25, line 26: "3. Page 10, Discussion section, line 10. The authors state that: "In our study LAVI was found to be associated with periprocedural complications in women. An enlarged left atria could result in increased risk given that cryoballoon ablation in these atria occurs distally and deeper within the pulmonary veins resulting in lesions closer to the course of the phrenic nerve." However, table 1 shows that women actually had a smaller LA size and volume. So the explanation provided by the authors is not good. Please provide a reasonable explanation for this finding.

Reply: Thank you for this comments. The reason this conclusion was drawn was due to the finding of the multivariate model. In this analysis LAVI was the only variable associated with the occurrence
of complications. We agree that LAVI was not different at baseline but this does not mean it can be associated with the outcome by doing a form of regression analysis.”

This is not true. LAVI was different at baseline, as can be seen in table 1: 64 ± 19 ml for women vs 71 ± 18 ml for men, p = 0.016 (the authors chose a cut-off of 0.05 for the p value, as stated in the Methods section, last paragraph). I agree that in the regression analysis LAVI was associated with the outcome. However, you need to correctly interpret this association. What does this mean (that the left atrial volume index was associated with the outcome = complications)? The authors’ explanation, that “An enlarged left atria could result in increased risk given that cryoballoon ablation in these atria occurs distally and deeper within the pulmonary veins resulting in lesions closer to the course of the phrenic nerve” is not good, because women had actually a smaller LA volume. This does not change the fact that LAVI was associated with the outcome (complications) in the regression analysis. It simply means that having a smaller LA volume, they did not have a larger LA volume, that could allow the cryoballoon to reach deeper in the pulmonary veins. Please provide another explanation for the fact that the LAVI was associated with more complications in women.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Francesco Brasca

Comments to the Author:
The paper by Mulder et al is well written and understandable, investigating an interesting issue: sex-related differences in CB-PVI outcome and safety, as I previously wrote in a review for another journal. The large population is the main strength point in this single center, non-randomized, retrospective cohort study.

Response: many thanks for the nice comment and the time you took to review our paper.

As, I previously observed, some issues should be considered:
My previous major revisions unsolved are:

1. The article is really similar to the one by Ricciardi et al, cited as reference 12. I think that authors should underline the differences found in their results and try to interpretate these differences in the discussion.

Response: Despite the very similar aim of the two studies, the study by Ricciardi et al. and ours show different results on multiple occasions. First, the population under investigation. In our study, the female group was on average older, had a higher body mass index (BMI), and a smaller left atrium than men. Women had a higher prevalence of diabetes mellitus, hypertension, and chronic kidney disease, and prior to PVI, women were more often treated with AADs. This highlights a slightly more “diseased” female group as compared to the male group, in our study. In the study by Ricciardi et al., female patients were also more likely to be older and were more often treated with AADs compared to men. However, males were more likely to have ischemic cardiomyopathy, persistent AF, and larger left atrial dimensions. Second, the association with AF recurrence. In our study, variables significantly associated with an increased AF/AT recurrence in multivariate analysis were type of AF, number of failed AADs, chronic kidney disease and LVEF. In the study by Ricciardi et al., female sex, AF type, and age were confirmed as independent predictors of AF recurrence upon multivariable model
Lastly, Ricciardi et al. observed no difference in rate of periprocedural complications between the two groups and no association with known risk factors. In our study, female patients had a higher incidence of periprocedural complications, especially groin hematomas and phrenic nerve palsy, and LAVI was found to be associated with incidence of complications.

The differences observed by the two studies are likely related to methodological differences. As mentioned in our discussion, the study by Ricciardi et al. enrolled patients from 47 different centers while our population was treated exclusively in our center. Multicentricity has been indicated by the authors themselves as a possible limitation to their study, opening to possible selection and treatment variability and biases. This variability associated with multicentricity may partly explain why the study by Ricciardi et al. found a significant association between sex and AF recurrence, which was not confirmed in ours.

2. Population study: there are significant differences in population study compared with the studies cited in references. It could be advisable to discuss this point. Similarly, a focus on sex related differences in risk factors and BMI could be of interest.

Response: We added on page 12:

“Sex-based differences in risk factors
In our study, women were on average older, had a higher BMI, and a smaller left atrium than men. Women had a higher prevalence of diabetes mellitus, hypertension, and chronic kidney disease, and prior to PVI, women were more often treated with AADs. This is in line with findings from previous studies which also showed that women undergoing catheter ablation of AF are on average older with increased comorbidities compared with men.(3-5, 12) In the ORBIT-AF trial, women were shown to be less likely to undergo an electrical cardioversion (26.7% vs. 32.4%, P < 0.001) and to be referred for AF ablation (4.9% vs. 5.9%, P = 0.04).13 In our study no differences were observed in the duration of time from first AF diagnosis until time of first cryoballoon ablation (median 3.8 years, interquartile range 1.8-7.2 years), suggesting no delay in ablation treatment.

3. Kloosterman M, Crijns HJGM, Mulder BA, et al. Sex-related differences in risk factors, outcome, and quality of life in patients with permanent atrial fibrillation: Results from the RACE II study. Europace. 2020;22(11):1619-1627.

4. Kloosterman M, Chua W, Fabritz L, et al. Sex differences in catheter ablation of atrial fibrillation: Results from AXAFA-AFNET 5. Europace. 2020;22(7):1026-1035.

5. Linde C, Bongiorni MG, Birgersdotter-Green U, et al. Sex differences in cardiac arrhythmia: A consensus document of the European heart rhythm association, endorsed by the heart rhythm society and Asia pacific heart rhythm society. Europace. 2018;20(10):1565-1565ao.

12. Ricciardi D, Arena G, Verlato R, et al. Sex effect on efficacy of pulmonary vein cryoablation in patients with atrial fibrillation: Data from the multicenter real-world 1STOP project. J Interv Card Electrophysiol. 2019;56(1):9-18.

13. Piccini, P., et al. "Differences in clinical and functional outcomes of atrial fibrillation in women and men: two-year results from the ORBIT-AF registry." JAMA cardiology 1.3 (2016): 282-291.

- I don't think that a criticism of paper by Ricciardi is useful. There are different follow up and different results, but also differences in population. Please, try to justify it. Please, remove the sentence: "To overcome this issue we have limited the results to a 1 year follow-up" It makes no sense.
Response: thank you for this suggestion, the sentence was removed accordingly. As you clearly highlight, our study and that by Ricciardi et al. differ under several methodological choices and factors: study design (single center vs. multicenter), follow-up (12 months vs 24 and 36 months), and baseline characteristics of the included population (specifically, differences in cardiovascular risk factors between male and female patients). Our choice of leveraging data from a single center was made to limit biases, at the expense of sample size. Duration of follow-up was also limited to 12 months to retain scientific and statistical validity on our smaller patient population.

3. In the results: "No differences were observed in the duration of time from first AF diagnosis until time of first cryoballoon ablation (median 3.8 years, interquartile range 1.8-7.2 years). Prior to PVI, women were more often treated with AADs (73% vs. 64%; P=0.02)." A comment on time from first AF diagnosis and AAD use could be advisable, because it seems that there is no delay in women treatment (good job) but why have they failed a larger number of AAD treatments? Could the authors discuss this point?

Response: In our study we observed comparable times from first AF diagnosis to ablation between male and female patients. This is very much in contrast with the findings of previous studies, showing that women are on average referred for AF ablation later and with a more complex clinical pre-operative presentation compared to men.(1-3)

With regards to AAD use, as shown in a post-hoc analysis of the RACE I trial, a trial conducted in the Netherlands, the higher prevalence of failed AADs among women was partly explained by the higher incidence of AAD-related adverse effects.(4)

1. Forleo Giovanni B, Tondo Claudio, De Luca Lucia, Dello Russo Antonio, Casella Michela, De Sanctis Valerio, Clementi Fabrizio, Fagundes Rafael Lopes, Leo Roberto, Romeo Francesco, Mantica Massimo. Gender-related differences in catheter ablation of atrial fibrillation. Europace. 2007 Aug;9 (8):613–20.
2. Dagres Nikolaos, Clague Jonathan R, Breithardt Günter, Borggrefe Martin. Significant gender-related differences in radiofrequency catheter ablation therapy. J. Am. Coll. Cardiol. 2003 Sep 17;42 (6):1103–7.
3. Roten Laurent, Rimoldi Stefano F, Schwick Nicola, Sakata Takao, Heimgartner Chris, Fuhrer Juerg, Delacrétaz Etienne, Tanner Hildegard. Gender differences in patients referred for atrial fibrillation management to a tertiary center. Pacing Clin Electrophysiol. 2009 May;32 (5):622–6.
4. Rienstra M, Van Veldhuisen DJ, Hagens VE, et al. Gender-related differences in rhythm control treatment in persistent atrial fibrillation: data of the Rate Control Versus Electrical Cardioversion (RACE) study. J Am Coll Cardiol. 2005;46(7):1298-1306. doi:10.1016/j.jacc.2005.05.078

I think that the response should be included in the discussion, not as an answer to the reviewer.

Manuscript change: We added on page 12: “With regards to AAD use, as shown in a post-hoc analysis of the RACE II trial by Rienstra et al., conducted at our center, the higher prevalence of failed AADs among women was partly explained by the higher incidence of AAD-related adverse effects.14

4. The authors found that complications were associated with LAVi, but this data is not available in Table 1. Then, discussing this point, the authors reported a reference (21) but in a wrong way. The article by Kuhne assessed that LA dimension is not a predictor of phrenic nerve palsy! Maybe, it could be discussed that Kuhne tested only LA diameter and not LAVi.

Response: Thank you for pointing this out. We removed the reference. Upon further investigation in the literature, associations have been found between incidence of PNP and angle of PV with respect
to left atrial wall, of which LAVI could be a surrogate. Also, a study has shown a relation between incidence of PNP and female sex and lower BMI.

1. Ströker, Erwin, et al. "Anatomic predictors of phrenic nerve injury in the setting of pulmonary vein isolation using the 28-mm second-generation cryoballoon." Heart Rhythm 13.2 (2016): 342-351.
2. Pott, Alexander, et al. "Predicting Phrenic Nerve Palsy in Patients Undergoing Atrial Fibrillation Ablation With the Cryoballoon—Does Sex Matter?." Frontiers in cardiovascular medicine 8 (2021).

Please, discuss your result not only as an answer to the reviewer

Response: the response is now included in the discussion.

Manuscript change: we have added (including the before mentioned references): Other explanations could be that the angle between of the pulmonary vein and the left atrial wall in some patients might increase the risk for phrenic nerve palsy. Furthermore, a relation between the incidence of phrenic nerve injury, females sex and lower BMI has been reported before.[23, 24]

- Minor issues

In the "catheter ablation procedure" section: the authors assessed that venous access was not guided by ultrasound (that it is useful to discuss vascular complication). Further information could be of interest: was haemostasis obtained by compressive medication or other strategy (i.e. "Figure-of-Eight" suture technique)? Could I suggest to focus on this point in the discussion?

Response: Thank you for this comment. At this point in time, we did not routinely use vascular ultrasound and the figure of eight stitch was only introduced a couple of years ago. However, as shown in the ULTRA-FAST trial, investigating the efficacy and safety of ultrasound-guided venipuncture of femoral veins in patients undergoing catheter ablation for AF, rates of major vascular complications (haematoma, arteriovenous fistula, or pseudoaneurysm) were not reduced.

Yamagata, Kenichiro, et al. "Ultrasound-guided versus conventional femoral venipuncture for catheter ablation of atrial fibrillation: a multicentre randomized efficacy and safety trial (ULTRA-FAST trial)." EP Europace 20.7 (2018): 1107-1114.

we have added on page 14: “At the time of this study, we did not routinely use ultrasound guided vascular access and the figure of eight stitch was only introduced a couple of years ago. This may have affected the rate of complications registered in our population. However, as shown in the ULTRA-FAST trial, investigating the efficacy and safety of ultrasound-guided venipuncture of femoral veins in patients undergoing catheter ablation for AF, rates of major vascular complications were not reduced using ultrasound to guide femoral access."

- In the discussion:
  o (pag 8 line 49) "Some studies suggest a significantly lower rate of freedom from AF/AT recurrence in women (please, provide references)". The author has previously answered: "The paper by Kuck, et al. (Impact of female sex on clinical outcomes in the FIRE AND ICE trial of catheter ablation for atrial fibrillation. Circ Arrhythm Electrophysiol. 2018;11(5):e006204) was already referred here. We did not add additional papers." Thus, change the sentence: A study suggests ...

Response: thank you, this was changed accordingly.

- the ref 20 was used to support this sentence. "Underlying mechanisms that play a role in the
observed sex difference in the occurrence of phrenic nerve palsy are unknown” I could not find this assessment in the study cited. The previous answer to my observation on this point did not justify the use of this reference.

Response: as mentioned in the response to one of your other comments, we have added: Other explanations could be that the angle between of the pulmonary vein and the left atrial wall in some patients might increase the risk for phrenic nerve palsy. Furthermore, a relation between the incidence of phrenic nerve injury, females sex and lower BMI has been reported before.[23, 24]

Including references:
1. Ströker, Erwin, et al. "Anatomic predictors of phrenic nerve injury in the setting of pulmonary vein isolation using the 28-mm second-generation cryoballoon.” Heart Rhythm 13.2 (2016): 342-351.
2. Pott, Alexander, et al. "Predicting Phrenic Nerve Palsy in Patients Undergoing Atrial Fibrillation Ablation With the Cryoballoon—Does Sex Matter?.” Frontiers in cardiovascular medicine 8 (2021).

Reviewer: 2
Dr. Lucian Muresan, Hôpital Emile Muller Comments to the Author:
Compared to the initial manuscript, this second variant is better, since the authors complied with the reviewers’ requests. However I still have one more comment:

Response: thank you for the nice comments an taking the time to review the paper.

Page 25, line 26: “3. Page 10, Discussion section, line 10. The authors state that: “In our study LAVI was found to be associated with periprocedural complications in women. An enlarged left atria could result in increased risk given that cryoballoon ablation in these atria occurs distally and deeper within the pulmonary veins resulting in lesions closer to the course of the phrenic nerve.” However, table 1 shows that women actually had a smaller LA size and volume. So the explanation provided by the authors is not good. Please provide a reasonable explanation for this finding. Reply: Thank you for this comments. The reason this conclusion was drawn was due to the finding of the multivariate model. In this analysis LAVI was the only variable associated with the occurrence of complications. We agree that LAVI was not different at baseline but this does not mean it can be associated with the outcome by doing a form of regression analysis”. This is not true. LAVI was different at baseline, as can be seen in table 1: 64 ± 19 ml for women vs 71 ± 18 ml for men, p = 0.016 (the authors chose a cut-off of 0.05 for the p value, as stated in the Methods section, last paragraph). I agree that in the regression analysis LAVI was associated with the outcome. However, you need to correctly interpret this association. What does this mean (that the left atrial volume index was associated with the outcome = complications)? The authors’ explanation, that “An enlarged left atria could result in increased risk given that cryoballoon ablation in these atria occurs distally and deeper within the pulmonary veins resulting in lesions closer to the course of the phrenic nerve” is not good, because women had actually a smaller LA volume. This does not change the fact that LAVI was associated with the outcome (complications) in the regression analysis. It simply means that having a smaller LA volume, they did not have a larger LA volume, that could allow the cryoballoon to reach deeper in the pulmonary veins. Please provide another explanation for the fact that the LAVI was associated with more complications in women.

Response: Thank you for pointing this out, which was also raised by reviewer 1. We have added: Other explanations could be that the angle between of the pulmonary vein and the left atrial wall in some patients might increase the risk for phrenic nerve palsy. Furthermore, a relation between the incidence of phrenic nerve injury, females sex and lower BMI has been reported before.[23, 24]
Including references:
1. Ströker, Erwin, et al. "Anatomic predictors of phrenic nerve injury in the setting of pulmonary vein isolation using the 28-mm second-generation cryoballoon." Heart Rhythm 13.2 (2016): 342-351.
2. Pott, Alexander, et al. "Predicting Phrenic Nerve Palsy in Patients Undergoing Atrial Fibrillation Ablation With the Cryoballoon—Does Sex Matter?." Frontiers in cardiovascular medicine 8 (2021).