PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Androgen Deprivation Therapy and the Risk of Iron-Deficiency Anaemia among Patients with Prostate Cancer: A Population-based Cohort Study
AUTHORS	Wu, Fang-Jen; Li, I-Hsun; Chien, Wu-Chien; Shih, Jui-Hu; Lin, Yi-Chun; Chuang, Chin-Min; Cheng, Yih-Dih; Kao, Li-Ting

VERSION 1 – REVIEW

REVIEWER	Blanaid Hicks Queen's University Belfast, UK
REVIEW RETURNED	17-Oct-2019

GENERAL COMMENTS	For the Authors This is a population-based cohort study, using the Taiwan National Health Insurance Research Dataset, investigating the risk of iron-deficiency anaemia associated with ADT in men with prostate cancer. Followed for a period of three years, ADT users are propensity score matched to non-users. The authors report that ADT use was associated with a 61% increased risk of IDA. This is the first study to look at this in an Asian population. The authors have applied appropriate methods to answer this question however I have a number of concerns that should be addressed before I can recommend this manuscript for publication, including potentially important confounders including metastases, the potential for immortal time bias, and the choice of exposure definition. Major comments; • The authors make no mention about how they deal with stage of prostate cancer. Do the authors have information on stage, grade and or a code for the presence of metastases at diagnosis? Furthermore, metastases associated with the bone is associated with anaemia. Therefore, if available, patients diagnosed with metastatic prostate cancer should be excluded from the cohort, either in the main or in sensitivity analyses. Similarly, chemotherapy, radiotherapy and surgery also influence anaemia, therefore models could include these potential confounders if the data is available. In line with this, it appears the authors did not make any attempt to account for a previous diagnoses of any cancer. The authors should consider whether patients with a previous cancer should be included in the cohort (notably blood cancers) and/or adjust for a history of cancer in their model. • ADT is indicated in advanced and metastatic prostate cancer patients (although rates of use in localised disease are increasing).

	Use of ADT in this study seems quite high (20,265 patients; 82%), from which ADT users were selected. It appears that this is use of ADT anytime during the follow-up period and not within a certain period from diagnosis? Could the authors provide an average time to ADT use (T0 for ADT users) from prostate cancer diagnosis? The authors state that T0 for ADT users was the date of ADT administration while for non-users this was the first outpatient visit in which patients are diagnosed. It seems possible, given that ADT use can be anytime during the 3 year follow-up period (and the authors have not used a time varying approach), that some patients may start ADT a substantial time after diagnosis. Given, this difference in T0 for users and non-users it is possible that immortal time bias may have been introduced. • The authors seem to apply an intention to treat approach for their ADT exposure definition. Therefore, once a patient has been exposed to ADT they are considered continuously exposed. However I would question the use of this exposure definition for an acute outcome such an anaemia. If a patient started ADT but quit within 6 months, and later developed anaemia (e.g. 2 years later), one could question whether it is biologically plausible that their anaemia was 'caused' by ADT. Could the authors also conduct analyses using an as treated exposure definition? • The author's definition if IDA is based on ICD-9 codes. However there are different types of anaemia, including various severities (mild, normocytic and severe) which are based on various haemoglobin level ranges. Is it possible that by using clinical codes, rather than blood results, this study is only capturing severe anaemia? Are haemoglobin measures available in this data? If so, the authors could also use these for their outcome definition. In line with this, the authors also make no mention of treatments for anaemia such as iron supplementation, transfusions etc. The authors could consider, if this data is available, to also include these in their outcome definition as a sensitivity analyses to deal with outcome misclassification. • The authors state that they excluded 681 patients who had never received a diagnosis of IDA. Is this correct? (the number for one seems quite low) If so can the authors justify why they would only include those patients with a history of iron deficiency anaemia? It seems to me that this should be opposite, patients with a history of anaemia (who therefore have a higher baseline risk) should be excluded? In line with this, and with the comment above relating to the lack of blood measures, another limitation of this study is that the authors may be including patients who already have mild or normocytic anaemia but this may not be captured via ICD codes. If this is differential between ADT users and non-users, this may introduce confounding. • The authors only consider ADT overall. It would also be interesting to investigate risk by individual ADT types. Also is dose or number of prescriptions etc available? A dose response analyses would also be beneficial. • It is not totally clear how ADT is defined? Is this from clinical information i.e. secondary care? And how is this coded (ATC

	codes etc). Is all ADT use handled in secondary care or is this managed by primary care in Taiwan, as is the case in the UK? Minor comments; • The authors adjust for a number of confounders however, there are a number of conditions that influence the risk of anaemia that are missing from the model including for e.g. Chron's disease, GI bleeds, surgery. • There is a typo on line 14 page 6 vary to varied • The authors reference the study by Hicks et al, criticising studies for short follow-up and small numbers. It seems this refers to a number of studies. Could the authors include references here for all studies to which they are referring. • Could the authors provide more explanation on their propensity score method? Was a caliper used with or without trimming? • Unless journal policy could, the authors remove the stars in table for p values. This adds little value to the readership. • Footnotes on table 2 'a' is used twice and is not depicted in the table

REVIEWER	Dr Patrick J Owen Deakin University, Australia
REVIEW RETURNED	20-Nov-2019

GENERAL COMMENTS	The authors present a retrospective cohort study examining anaemia in prostate cancer (PCa) patients treated with androgen deprivation therapy (ADT). The study is strengthened by the use of a database purported to represent >99% of the Taiwanese population. The sample size of this study is impressive and the authors should be commended for working with such a large dataset. Notably, the paper was difficult to read given the lack of English language proficiency – I urge the authors to have this manuscript reviewed by a native English language speaker before resubmission. Specific comments are as follows: MAJOR 1. Written English language needs attention. The current sate of the manuscript is not grammatically sound. I started to provide feedback in minor comments regarding disjointed sentences, but stopped after paragraph 2 of the introduction due to the clear need for amendment 2. The introduction does not contend why this question is important. ADT-induced anaemia is an established phenomenon, and the importance and rationale for examining this in an East Asian population is lacking. Currently, the novelty (and inferred importance) is the geographical location of the population group, rather than a clinically relevant reason (e.g. is this population at greater risk of anaemia for other reasons?) 3. Given the opportunity to select the sample from the database, why was a 2:1 matching performed? Why not 1:1? 4. Methods, Statistical Analysis: What is meant by patients who died were censored? How was this dealt with statistically? 5. Discussion, Para 2: This is just a list of findings from other studies. The results of the current study need to be discussed in context with these findings. Currently, this reads more like a literature review than a discussion 6. The discussion, as it stands, is too brief regarding comparisons to prior studies and the clinical implications of the findings in the current study. The mechanistic paragraph is nice however.

	7. Adequate statistical is mentioned in the discussion, but a power calculation is not provided, nor is additional data to support this statement 8. Conclusion: 'comorbid medical disorders' cannot be said to have been adjusted for entirely, especially in a concluding remark, as this was a noted limitation of the current study. MINOR 1. Intro, para 1, line 1: 293,000 deaths globally per year? 2. Suggest abbreviating prostate cancer to PCa, rather than PC 3. Intro, para 1, line 6: Sentence structure is disjointed 4. Intro, para 1: Refs 7-10 include some that are >10yr old, suggest to use more recently published literature (e.g. Owen PJ, Daly RM, Livingston PM, Fraser SF. Prostate Cancer and Prostatic Diseases. 2017;20(2):137; Nguyen PL, Alibhai SM, Basaria S, D'Amico AV, Kantoff PW, Keating NL, Penson DF, Rosario DJ, Tombal B, Smith MR. European Urology. 2015;67(5):825-36) 5. Intro, para 2, line 1: Disjointed sentence (many instances of this throughout) 6. Methods, Study Sample: Why were patients <40yr excluded?

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1
Reviewer Name: Blanaid Hicks
Institution and Country: Queen's University Belfast, UK
Please state any competing interests or state 'None declared': None declared
Please leave your comments for the authors below
For the Authors
This is a population-based cohort study, using the Taiwan National Health Insurance Research Dataset, investigating the risk of iron-deficiency anaemia associated with ADT in men with prostate cancer. Followed for a period of three years, ADT users are propensity score matched to non-users. The authors report that ADT use was associated with a 61% increased risk of IDA. This is the first study to look at this in an Asian population. The authors have applied appropriate methods to answer this question however I have a number of concerns that should be addressed before I can recommend this manuscript for publication, including potentially important confounders including metastases, the potential for immortal time bias, and the choice of exposure definition.

Response: Thank you for all your comments. As suggested, we have considered more confounders in the regression models. However, several factors were not available in our database. We have addressed the relevant limitations in Discussion. We also provide clear statements about the methodology for ADT exposure definition and avoiding bias. Additionally, we have classified the time of ADT use into two groups and conducted the relevant analysis once again. Thank you so much!

Major comments;
• The authors make no mention about how they deal with stage of prostate cancer. Do the authors have information on stage, grade and or a code for the presence of metastases at diagnosis? Furthermore, metastases associated with the bone is associated with anaemia. Therefore, if available, patients diagnosed with metastatic prostate cancer should be excluded from the cohort,

either in the main or in sensitivity analyses. Similarly, chemotherapy, radiotherapy and surgery also influence anaemia, therefore models could include these potential confounders if the data is available.

Response: Thank you for your comments. We totally agreed that the stage, grade for the presence of metastases and detail chemotherapy, radiotherapy and surgery might play roles in the relationship between ADT and anaemia. However, we are so sorry that the data was not available for this study. We have addressed these Limitations in Discussion as follows: “Third, information on the family history of anaemia, cancer stage and grade for the presence of metastases, chemotherapy, radiotherapy and surgery was unavailable this study.” (1st paragraph, page 17) Nevertheless, even though this study had some limitations, we considered that the relevant findings could alert the connection between ADT and anaemia and provide a good starting point for the further research. We really appreciate your understanding. Thank you so much!

In line with this, it appears the authors did not make any attempt to account for a previous diagnoses of any cancer. The authors should consider whether patients with a previous cancer should be included in the cohort (notably blood cancers) and/or adjust for a history of cancer in their model.

Response: Thank you for your valuable comments. As suggested, we have considered the previous medical history of cancer in the regression models to estimate the potential association between ADT and anaemia among patients with prostate cancer. The relevant findings were displayed in Table 2. After adjusting previous diagnoses of any cancer, the ADT was sill associated with anaemia (adjusted HR=1.61; 95% CI=1.28-2.03). We have addressed relevant statements in Results as follows: “After adjusting for patients’ age, monthly income, geographic location, residential urbanisation level and the incidence of hyperlipidemia, diabetes, hypertension, coronary heart disease, inflammatory bowel disease, other cancers and gastrointestinal bleeding, the HR for the study group compared with the control group was 1.61 (95% CI = 1.28–2.03; p ≤ 0.001).” (page 12- page 13)

• ADT is indicated in advanced and metastatic prostate cancer patients (although rates of use in localised disease are increasing). Use of ADT in this study seems quite high (20,265 patients; 82%), from which ADT users were selected. It appears that this is use of ADT anytime during the follow-up period and not within a certain period from diagnosis?

Response: Thank you for the suggestions. In Taiwan, NHI system provided comprehensive and affordable medical services for 99% of residents. The waiting time is very short in Taiwan. We have checked the average time to ADT use from prostate cancer diagnosis (T0 for ADT users). An average time to ADT use (T0 for ADT users) from prostate cancer diagnosis was about 111.17 days. Accordingly, most PCa patients will receive ADT within 3 months.

Could the authors provide an average time to ADT use (T0 for ADT users) from prostate cancer diagnosis? The authors state that T0 for ADT users was the date of ADT administration while for non-users this was the first outpatient visit in which patients are diagnosed. It seems possible, given that ADT use can be anytime during the 3 year follow-up period (and the authors have not used a time varying approach), that some patients may start ADT a substantial time after diagnosis. Given, this difference in T0 for users and non-users it is possible that immortal time bias may have been introduced.

Response: Thank you for your comments. The average time to ADT use from prostate cancer diagnosis was about 111.17 days in our study (T0 for ADT users). We have addressed relevant statements in Methods as follows: “The average time from PCa diagnosis was approximately 111.17 days in our study.” (1st paragraph, page 9) Because the waiting time is very short in Taiwan and this study randomly assigned the date of an outpatient visit as the index date in which patients received

the diagnosis of PCa who did not receive ADT as the matched controls. Therefore, the immortal time bias might be eliminated in this study.

• The authors seem to apply an intention to treat approach for their ADT exposure definition. Therefore, once a patient has been exposed to ADT they are considered continuously exposed. However I would question the use of this exposure definition for an acute outcome such an anaemia. If a patient started ADT but quit within 6 months, and later developed anaemia (e.g. 2 years later), one could question whether it is biologically plausible that their anaemia was 'caused' by ADT. Could the authors also conduct analyses using an as treated exposure definition?

Response: Thank you for the comments. In our study, the average time of ADT exposure was about 549.25 days. Most patients would receive approximately 2-year ADT exposure. Therefore, those patients started ADT but quit within 6 months, and later developed anaemia might not occur in this study. However, in order to eliminate the potential bias due to short-time exposure, we have classified the ADT users into two groups: 'long-term ADT exposure' and 'short-term exposure' Then, we have conducted all relevant analysis once again. We found that both short-term and long-term use of ADT may increase the risk of anaemia. The relevant statements were addressed in Results as follows: "This study further classified patients in the study group as short-term and long-term ADT users based on the median duration of use. Data presented in Table 3 reveal that both short-term and long-term and ADT use can increase the risk of anaemia. Compared with the findings in the control group, the adjusted HRs for long-term and short-term ADT use in the study group were 1.65 (95% CI = 1.28–2.13) and 1.56 (95% CI = 1.19–2.04), respectively." (page 13)

• The author's definition if IDA is based on ICD-9 codes. However there are different types of anaemia, including various severities (mild, normocytic and severe) which are based on various haemoglobin level ranges. Is it possible that by using clinical codes, rather than blood results, this study is only capturing severe anaemia?
Are haemoglobin measures available in this data? If so, the authors could also use these for their outcome definition.
In line with this, the authors also make no mention of treatments for anaemia such as iron supplementation, transfusions etc. The authors could consider, if this data is available, to also include these in their outcome definition as a sensitivity analyses to deal with outcome misclassification.

Response: Thank you for the comments. We totally agreed that lack of blood measures (haemoglobin levels) was a major limitation in this study. We are so sorry that the relevant data were not available in this study. Therefore, several patients may not be captured via ICD codes in this study, since several patients who had mild or normocytic anaemia may not look for the medical service immediately. Nevertheless, this study identified ADT users as cases and PCa patients (without ADT) as controls. In general, physicians in Taiwan would provide a complete blood count (CBC) test for the PCa patients in order to provide suitable treatments. Therefore, we considered that these factors might not affect the findings in this study. However, we still highlighted this Limitations as follows: "Second, it is plausible that the database did not include all patients with PCa and IDA in Taiwan because some patients might have sought alternative medicines not recorded by the NHI programme and some patients with mild or normocytic anaemia may not have immediately sought medical treatment. Therefore, these patients might not have been captured via diagnosis codes."; "Finally, this study lacked information regarding blood variables. Nevertheless, this study identified patients with PCa who used ADT as cases and patients with PCa patients who did not use ADT as controls. In general, physicians in Taiwan perform complete blood counts for patients with PCa to identify suitable treatments. Consequently, we considered that these factors may not have affected the findings in this study." (2nd paragraph, page 17)

Additionally, as suggested, we have attempted to consider the iron supplementations in the regression models. However, the findings were hard to explain! In Taiwan, most patients would be diagnosed with IDA for receiving the iron supplementations (for NHI premium subsidies). These two factors (X: iron supplementations; Y: IDA) were highly correlated. Thus, if we considered the iron supplementations in the regression models, the relevant findings would indicate that increasing use of iron supplementations may elevate the risk of anaemia. Consequently, this study did not consider iron supplementations in the logistic regression models finally.

• The authors state that they excluded 681 patients who had never received a diagnosis of IDA. Is this correct? (the number for one seems quite low) If so can the authors justify why they would only include those patients with a history of iron deficiency anaemia? It seems to me that this should be opposite, patients with a history of anaemia (who therefore have a higher baseline risk) should be excluded?
In line with this, and with the comment above relating to the lack of blood measures, another limitation of this study is that the authors may be including patients who already have mild or normocytic anaemia but this may not be captured via ICD codes. If this is differential between ADT users and non-users, this may introduce confounding.

Response: Thank you for the reminders. There was a typo in relevant sentences. We have corrected the relevant statements as follows: “Meanwhile, 681 patients who had received a diagnosis of IDA (ICD-9-CM codes 280, 280.0, 280.1, 280.8 and 280.9) and 2730 patients who had undergone orchiectomy prior to their index date were excluded.” (1st paragraph, page 9)

Additionally, we totally realized that lack of blood measures was a major limitation in this study. In addition, patients who had mild or normocytic anaemia may not look for the medical service immediately. Thus, these patients may not be captured via ICD codes. Nevertheless, this study identified ADT users as cases and PCa patients (without ADT) as controls. In general, physicians in Taiwan would provide a complete blood count (CBC) test for the PCa patients in order to provide suitable treatments. Therefore, we considered that these factors might not affect the findings in this study. However, we still highlighted this Limitations as follows: “Second, it is plausible that the database did not include all patients with PCa and IDA in Taiwan because some patients might have sought alternative medicines not recorded by the NHI programme and some patients with mild or normocytic anaemia may not have immediately sought medical treatment. Therefore, these patients might not have been captured via diagnosis codes.”; “Finally, this study lacked information regarding blood variables. Nevertheless, this study identified patients with PCa who used ADT as cases and patients with PCa patients who did not use ADT as controls. In general, physicians in Taiwan perform complete blood counts for patients with PCa to identify suitable treatments. Consequently, we considered that these factors may not have affected the findings in this study.” (2nd paragraph, page 17)

• The authors only consider ADT overall. It would also be interesting to investigate risk by individual ADT types. Also is dose or number of prescriptions etc available? A dose response analyses would also be beneficial.

Response: Thank you for the comments. We have provided the information regarding the individual ADT types in this study and the relevant statements were stated as follows: “Specifically, 49.50, 17.98, 15.74, and 16.78% of the ADT prescriptions were for cyproterone, bicalutamide, flutamide and other types of medications, respectively.” (1st paragraph, page 9)

We also considered the length of ADT use in the study and conducted the dose-dependent analyses. The findings were displayed in Table 3. We found that both patients receiving long-term and short-term ADT had higher risk of IDA. We have addressed relevant statements in Results as follows: “Data

presented in Table 3 reveal that both short-term and long-term and ADT use can increase the risk of anaemia. Compared with the findings in the control group, the adjusted HRs for long-term and short-term ADT use in the study group were 1.65 (95% CI = 1.28–2.13) and 1.56 (95% CI = 1.19–2.04), respectively." (page 13)

• It is not totally clear how ADT is defined? Is this from clinical information i.e. secondary care? And how is this coded (ATC codes etc). Is all ADT use handled in secondary care or is this managed by primary care in Taiwan, as is the case in the UK?

Response: Thank you for the suggestions. The ADT were defined by using ATC codes in this study and we have addressed relevant definitions in Methods as follows: "The date of ADT administration (including gonadotropin-releasing hormone agonists, anti-androgens, ketoconazole and estrogens; ATC codes L02AE03, L02AE02, L02AE04, L02BB03, G03HA01, L02BB01, J02AB02 and L02AA) was assigned as the index date for patients with PCa who subsequently received ADT treatment." (1st paragraph, page 9) Additionally, in Taiwan, ADT use need to be handled by the oncologists or urologists. This may manage by either secondary care or primary care in Taiwan, because the General practice in Taiwan is still developing.

Minor comments;
• The authors adjust for a number of confounders however, there are a number of conditions that influence the risk of anaemia that are missing from the model including for e.g. Chron's disease, GI bleeds, surgery.

Response: Thank you for your comments. As suggested, we have considered the confounders, including inflammatory bowel disease and gastrointestinal bleeding in the regression model and conducted the relevant analysis once again. The relevant findings were displayed in Table 2. After considering these factors in the models, ADT was still associated with anaemia (adjusted HR=1.61; 95% CI=1.28-2.03). We have addressed the relevant statements in Results as follows: "After adjusting for patients' age, monthly income, geographic location, residential urbanisation level and the incidence of hyperlipidemia, diabetes, hypertension, coronary heart disease, inflammatory bowel disease, other cancers and gastrointestinal bleeding, the HR for the study group compared with the control group was 1.61 (95% CI = 1.28–2.03; $p \leq 0.001$)." (page 12- page 13)

• There is a typo on line 14 page 6 vary to varied

Response: Thank you for the reminders. We have corrected the relevant sentences as follows: "However, to date, few studies have been conducted in East Asian populations even though the hereditary risk of PCa substantially varies by ethnicity and geography." (page 7)

• The authors reference the study by Hicks et al, criticising studies for short follow-up and small numbers. It seems this refers to a number of studies. Could the authors include references here for all studies to which they are referring.

Response: Thank you for the reminders. We have included references here for all studies and revised the relevant sentences as follows: "All of these studies investigated the association of ADT with haemoglobin levels, which is vital given that haemoglobin levels are important for identifying anaemia. However, these clinical studies included small sample sizes and featured a short duration.22 23 30 31 Separately, Hicks et al. performed a cohort study using the United Kingdom Clinical Practice Research Database linked to the Hospital Episode Statistics repository. Their findings revealed that patients with non-metastatic PCa who received ADT had a nearly 3-fold greater risk of anaemia than non-users (HR = 2.90, 95% CI = 2.67–3.16).24 However, this study was performed in a Western

population, and generalisation of its findings to other ethnic groups is not possible." (1st paragraph, page 15)

• Could the authors provide more explanation on their propensity score method? Was a caliper used with or without trimming?

Response: Thank you for the comments. This study used the propensity score matching with the Mahalanobis metric (caliper of 0.25 standard deviation of the propensity score).
As suggested, we have provided more explanation on their propensity score method and the relevant statements were addressed as follows: "Propensity score matching was used to identify 8474 patients with PCa who received ADT. This methodology could eliminate the differences attributable to differences in patient demographics and medical history between the groups. We used propensity score matching using the Mahalanobis metric (caliper of 0.25 standard deviations of the propensity score). The matching variables included age, monthly income, geographic location, residential urbanisation level (divided into five levels, with 1 being the most urbanised and 5 being the least urbanised),29 hyperlipidaemia, diabetes, hypertension, coronary heart disease and inflammatory bowel disease. We performed a 2:1 matching study, because increasing sample size of cases or controls could increase the statistic power of the findings." (2nd paragraph, page 9 & 1st paragraph, page 10)

• Unless journal policy could, the authors remove the stars in table for p values. This adds little value to the readership.

Response: Thank you for the comments. As suggested, we have removed the stars in table for p values.

• Footnotes on table 2 'a' is used twice and is not depicted in the table

Response: Thank you for the reminders. We have revised the footnotes on Table 2. Thank you again for offering so many valuable comments and guidance. They are very helpful for us on revising this manuscript.

Reviewer: 2
Reviewer Name: Dr Patrick J Owen
Institution and Country: Deakin University, Australia
Please state any competing interests or state 'None declared': None declared
Please leave your comments for the authors below
The authors present a retrospective cohort study examining anaemia in prostate cancer (PCa) patients treated with androgen deprivation therapy (ADT). The study is strengthened by the use of a database purported to represent >99% of the Taiwanese population. The sample size of this study is impressive and the authors should be commended for working with such a large dataset. Notably, the paper was difficult to read given the lack of English language proficiency – I urge the authors to have this manuscript reviewed by a native English language speaker before resubmission. Specific comments are as follows:
MAJOR
1. Written English language needs attention. The current sate of the manuscript is not grammatically sound. I started to provide feedback in minor comments regarding disjointed sentences, but stopped after paragraph 2 of the introduction due to the clear need for amendment

Response: Thank you for the suggestions. This manuscript has been thoroughly copyedited by a professional copyediting service (We have provided the English Editing Certificate).

2. The introduction does not contend why this question is important. ADT-induced anaemia is an established phenomenon, and the importance and rationale for examining this in an East Asian population is lacking. Currently, the novelty (and inferred importance) is the geographical location of the population group, rather than a clinically relevant reason (e.g. is this population at greater risk of anaemia for other reasons?)

Response: Thank you for the comments. As suggested, we have highlighted the importance and rationale for examining this in an East Asian population in this study. The relevant statements were addressed in Introduction as follows: “Accordingly, the association between ADT and anaemia has been reported in several Western studies using Caucasian populations.22-24 However, to date, few studies have been conducted in East Asian populations even though the hereditary risk of PCa substantially varies by ethnicity and geography.25 26 In addition, the haemoglobin concentration, which is a critical indicator of anaemia, is recognised to vary by race/ethnicity, lifestyle, demographics and other variables.27 Furthermore, a recent study of 32 patients receiving ADT demonstrated that haematologic toxicities such as anaemia were more frequent in Chinese patients with PCa than in their Western counterparts.28 Consequently, this study examined whether ADT is associated with a subsequent risk of anaemia in patients with PCa by employing a propensity score matching strategy using an Asian population-based dataset in Taiwan.” (page 7)

3. Given the opportunity to select the sample from the database, why was a 2:1 matching performed? Why not 1:1?

Response: Thank you for the suggestions. In general, increasing the number of subjects can give a more precise estimate of differences.1,2 In other words, increasing in sample size for both case and control may lead to increase in statistical power. Therefore, we have performed a 2:1 matching study rather than 1:1 matching to increase to the statistic power of the findings. We have addressed the relevant statements in Methods as follows: “We performed a 2:1 matching study, because increasing sample size of cases or controls could increase the statistic power of the findings.” (1st paragraph, page 10)

References:
1. Hennessy, S., W. B. Bilker, J. A. Berlin, and B. L. Strom. Factors Influencing the Optimal Control-to-Case Ratio in Matched Case-Control Studies.” American Journal of Epidemiology 149, 2: 195–97.
2. Jones, S. R., S. Carley, and M. Harrison. “An Introduction to Power and Sample Size Estimation.” Emergency Medicine Journal 20, 5: 453–58.

4. Methods, Statistical Analysis: What is meant by patients who died were censored? How was this dealt with statistically?

Response: Thank you for the comments. In order to provide clear information, we have revised the relevant statements in Methods as follows: “Additionally, Cox proportional hazard regression analysis was conducted to examine the relationship between ADT use and IDA risk during the 3-year study period. Data for patients who died or who were lost to follow-up during the study period were censored in the Cox regression. In total, 2716 patients died during the 3-year study period (2020 ADT users and 796 non-users).” (2nd paragraph, page 10)

5. Discussion, Para 2: This is just a list of findings from other studies. The results of the current study need to be discussed in context with these findings. Currently, this reads more like a literature review than a discussion

Response: Thank you for the suggestions. In order to discuss the similarity and differences between previous studies and our research, we have revised the relevant statements in Discussion as follows:

“The findings were consistent with those of prior Western studies. For instance, in the United States, Strum et al. demonstrated that the haemoglobin levels of patients who received ADT for PCa declined from a mean of 149 g/L at baseline to 139, 132 and 131 g/L after 1, 2 and 3 months, respectively.30 In Canada, Timilshina et al. performed an observational study of 250 patients with non-metastatic PCa and found that ADT was independently associated with a reduction of haemoglobin levels over 12 months.23 Among 110 patients with PCa who received ADT, one cohort study reported a decline in haemoglobin levels from 14.8 g/dL at baseline to 12.9 g/dL at evaluation.22 Another study of 72 patients with non-metastatic PCa who received adjuvant radiotherapy plus ADT reported that the haemoglobin level had significant declined after 2 years of androgen suppression.31 All of these studies investigated the association of ADT with haemoglobin levels, which is vital given that haemoglobin levels are important for identifying anaemia. However, these clinical studies included small sample sizes and featured a short duration.22 23 30 31 Separately, Hicks et al. performed a cohort study using the United Kingdom Clinical Practice Research Database linked to the Hospital Episode Statistics repository. Their findings revealed that patients with non-metastatic PCa who received ADT had a nearly 3-fold greater risk of anaemia than non-users (HR = 2.90, 95% CI = 2.67–3.16).24 However, this study was performed in a Western population, and generalisation of its findings to other ethnic groups is not possible.” (2nd paragraph, page 14 & 1st paragraph, page 15)

6. The discussion, as it stands, is too brief regarding comparisons to prior studies and the clinical implications of the findings in the current study. The mechanistic paragraph is nice however.

Response: Thank you for your suggestions. We have provided more information regarding the prior studies and revised the relevant statements in Discussion as follows: “The findings were consistent with those of prior Western studies. For instance, in the United States, Strum et al. demonstrated that the haemoglobin levels of patients who received ADT for PCa declined from a mean of 149 g/L at baseline to 139, 132 and 131 g/L after 1, 2 and 3 months, respectively.30 In Canada, Timilshina et al. performed an observational study of 250 patients with non-metastatic PCa and found that ADT was independently associated with a reduction of haemoglobin levels over 12 months.23 Among 110 patients with PCa who received ADT, one cohort study reported a decline in haemoglobin levels from 14.8 g/dL at baseline to 12.9 g/dL at evaluation.22 Another study of 72 patients with non-metastatic PCa who received adjuvant radiotherapy plus ADT reported that the haemoglobin level had significant declined after 2 years of androgen suppression.31 All of these studies investigated the association of ADT with haemoglobin levels, which is vital given that haemoglobin levels are important for identifying anaemia. However, these clinical studies included small sample sizes and featured a short duration.22 23 30 31 Separately, Hicks et al. performed a cohort study using the United Kingdom Clinical Practice Research Database linked to the Hospital Episode Statistics repository. Their findings revealed that patients with non-metastatic PCa who received ADT had a nearly 3-fold greater risk of anaemia than non-users (HR = 2.90, 95% CI = 2.67–3.16).24 However, this study was performed in a Western population, and generalisation of its findings to other ethnic groups is not possible.” (2nd paragraph, page 14 & 1st paragraph, page 15)

7. Adequate statistical is mentioned in the discussion, but a power calculation is not provided, nor is additional data to support this statement

Response: Thank you for the comments. As suggested, we have conducted a power analysis to evaluate the reliability of the results in this study. The statistical power (1-β) is over 0.95 in this study. Therefore, we considered that the statistical power in this study is enough to detect a statistical significance of the final outcomes. We have added the power and sample size calculations for this study into the Methods: “The power for this study was adequate (power = 0.987).” (1st paragraph, page 10)

8. Conclusion: ‘comorbid medical disorders’ cannot be said to have been adjusted for entirely, especially in a concluding remark, as this was a noted limitation of the current study.

Response: Thank you for the suggestions. In order to avoid misleading, we have revised the relevant statements in Conclusion as follows: “In conclusion, the present study detected an increased IDA risk during a 3-year follow-up period among patients with PCa who received ADT. Medical professionals are recommended to be aware of the risk of anaemia following ADT. Clinicians and pharmacists need to consider the possible risk of IDA among patients with PCa who received ADT and assess the efficacy of preventative and treatment modalities for anaemia.” (page17-page18)

MINOR

1. Intro, para 1, line 1: 293,000 deaths globally per year?

Response: Thank you for the comments. We have revised the relevant statements as follows: “Prostate cancer (PCa) is the leading cause of cancer in males, and it accounted for 293,000 deaths globally in 2013.” (1st paragraph, page 6)

2. Suggest abbreviating prostate cancer to PCa, rather than PC

Response: Thank you for the comments. As suggested, we have abbreviated ‘prostate cancer’ to ‘PCa’. We have revised the words throughout out all manuscript.

3. Intro, para 1, line 6: Sentence structure is disjointed

Response: Thank you for the suggestions. We have revised the relevant statements as follows: “Androgen deprivation therapy (ADT), also known as hormone therapy, has been a fundamental component of metastatic PCa management for more than half a century.4 This therapy can improve long-term survival for many patients.5 6 However, the decreased serum levels of endogenous androgen associated with ADT might result in some adverse effects, including decreased muscle mass, increased insulin resistance and fall risk, among patients with PCa.7-12” (1st paragraph, page 6) In addition, this manuscript has been thoroughly copyedited by a professional copyediting service. Thank you!

4. Intro, para 1: Refs 7-10 include some that are >10yr old, suggest to use more recently published literature (e.g. Owen PJ, Daly RM, Livingston PM, Fraser SF. Prostate Cancer and Prostatic Diseases. 2017;20(2):137; Nguyen PL, Alibhai SM, Basaria S, D’Amico AV, Kantoff PW, Keating NL, Penson DF, Rosario DJ, Tombal B, Smith MR. European Urology. 2015;67(5):825-36)

Response: Thank you for the comments. As suggested, we have added more recently published studies (including both Prostate Cancer Prostatic Dis. 2017;20(2):137-145. and Eur Urol. 2015;67(5):825-836.)

5. Intro, para 2, line 1: Disjointed sentence (many instances of this throughout)

Response: Thank you for the comments. As suggested, this manuscript has been thoroughly copyedited by a professional copyediting service. We have revised the relevant statements as follows: “Iron-deficiency anaemia (IDA) is a common disease that affects approximately 5% of females and 2% of males in the United States.13” (2nd paragraph, page 6)

6. Methods, Study Sample: Why were patients <40yr excluded?

Response: Thank you for the comments. This study excluded patients aged less than 40 years, because the prevalence of prostate cancer was low in patients <40 years old. Therefore, we have added clear statements in Methods as follows: “In total, 454 patients younger than 40 years were excluded because the prevalence of PCa is extremely low in this age strata.” (2nd paragraph, page 8) Thank you again for offering so many valuable comments. They are very helpful for us on revising this manuscript.

VERSION 2 – REVIEW

REVIEWER	Blánaid Hicks Queen's University Belfast, UK
REVIEW RETURNED	16-Jan-2020

GENERAL COMMENTS	The authors have made a number of changes to their manuscript in response to the reviewer’s comments. In particular, the quality of English throughout the manuscript has greatly improved. The authors have also provided further discussion on the limitations of their study, including certain data that is not available (metastases etc), have provided further details on the statistical approach and justification for conducting this study, given that this is not a novel question but rather replication in a different population group. The authors have also conducted further analyses. They have adjusted for further factors (co-morbidities) have attempted to deal with the potential for immortal time bias and secondary analyses on duration of ADT use. However I have a few queries that should be addressed before recommending for publication. Comments; 1. The authors have applied a new approach at identifying T0 for users/non-users. As requested the authors report that the average time to ADT use was 111.17 days. The authors now applied a randomly assigned date of an outpatient visit at the T0 of Pca patients who did not received ADT. This is the prescription time-distribution matching approach. Key to this approach for the elimination of immortal time bias is that the number of days to treatment for users is assessed (as the authors have done) and for non-users a number at random is selected from this set so that the overall distribution of time is matched. In line with this can the authors provide the distribution of time to T0 for both non-users and users? 2. Also it is unclear. Have the authors excluded patients who had a diagnosis of anaemia before their T0 for both non-users and users? Non-users who had an event before the assigned T0 should be excluded from the analysis (http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.573.4443&rep=rep1&type=pdf). Yet the number of patients (non-users) in the new analysis matched that of the primary submission (not using randomly selected controls) (all fields are identical in Table 1 across manuscript versions), which would seem unlikely using a different method for identifying controls. 3. The authors have conducted additional analyses by duration of use. I still have a few issues regarding this. Firstly, the authors have helpfully provided us with an average time of ADT use, of 549.25 days. This is somewhat reassuring however the authors have not provided a standard deviation or range measure, and notably they report the median of about 1 year (353

	days). It is still possible that there are those users who have quite some time between their anaemia diagnosis and ADT use. Anaemia is an acute outcome, on which drugs are likely to have an acute effect (possibly with a short wash out period, where the medications may still exert an effect on anaemia). Indeed in previous analyses conducted by my colleagues and I the risk of anaemia was reversed upon discontinuation (Hicks et al 2017). While the authors have conducted the analyses by duration, while a helpful analyses to investigate duration/ dose response this does in fact not deal with "recency" of use. It is still possible in this analyses that users(in particular short-term use) may have anaemia diagnosed some time after ADT discontinuation. A more appropriate analyses would include an exposure definition using an as treated approach (e.g. current use, past, no or censoring). Although the follow-up is short I would still recommend that the authors consider an analyses such as this as a sensitivity analysis. 3. Could the authors also be specific for the readership how exactly they categorised short and long term durations based on the median? Are these <353 and >353? 4. I apologise to the authors for the confusion regarding iron supplementation. The authors are correct that to include iron supplementation in their regression model would lead to variables highly correlated and would be inappropriate. What I meant by my suggestion was that the authors could use receipt of iron supplementation as an additional measure of the outcome of anaemia I.e. in a sensitivity analysis anaemia could be considered as either a diagnosis of anaemia OR a claim for iron supplementation (or blood transfusion also a possible end point). This type of sensitivity analysis would highlight any potential outcome misclassification.

REVIEWER	Dr Patrick J Owen Deakin University, Australia
REVIEW RETURNED	30-Dec-2019

GENERAL COMMENTS	The authors should be commended for their thorough responses to my initial queries. I thank the authors for integrating these suggestions and believe the manuscript is now suitable for publication. Notably, the written English language has substantially improved and I would now deem the manuscript grammatically sound.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 2

Reviewer Name: Dr Patrick J Owen

Institution and Country: Deakin University, Australia

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

The authors should be commended for their thorough responses to my initial queries. I thank the authors for integrating these suggestions and believe the manuscript is now suitable for publication. Notably, the written English language has substantially improved and I would now deem the manuscript grammatically sound.

Response: Thank you so much! Thank you again for offering so many valuable comments and guidance. They are very helpful for us on revising this manuscript!

Reviewer: 1

Reviewer Name: Blánaid Hicks

Institution and Country: Queen's University Belfast, UK

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

The authors have made a number of changes to their manuscript in response to the reviewer’s comments. In particular, the quality of English throughout the manuscript has greatly improved. The authors have also provided further discussion on the limitations of their study, including certain data that is not available (metastases etc), have provided further details on the statistical approach and justification for conducting this study, given that this is not a novel question but rather replication in a different population group. The authors have also conducted further analyses. They have adjusted for further factors (co-morbidities) have attempted to deal with the potential for immortal time bias and secondary analyses on duration of ADT use. However I have a few queries that should be addressed before recommending for publication.

Comments;

1. The authors have applied a new approach at identifying T0 for users/non-users. As requested the authors report that the average time to ADT use was 111.17 days. The authors now applied a randomly assigned date of an outpatient visit at the T0 of Pca patients who did not received ADT. This is the prescription time-distribution matching approach. Key to this approach for the elimination of immortal time bias is that the number of days to treatment for users is assessed (as the authors have done) and for non-users a number at random is selected from this set so that the overall distribution of time is matched.

In line with this can the authors provide the distribution of time to T0 for both non-users and users?

Response: Thank you for the comments. As suggested, we have checked the T0 for users and non-users in this study. Due to the reviewer comments, we have excluded those patients who had a diagnosis of anaemia before their T0 for both non-users and users. We then performed the propensity score matching once again and considering year of the entry date, age, monthly income, geographical location, residential urbanisation level and medical comorbidities in the matching model. Thus, the selected patients were different from previous version of manuscript. However, relevant findings in new manuscript were consistent with previous version. Among the new selected cohorts, the distribution of time to T0 for both non-users and users were 758.4 days and 796.9 days, respectively. We have addressed the relevant statements in Methods as follows: “The average time from PCa

diagnosis date to patient entry date for both non-users and users were 758.4 days and 796.9 days, respectively."

2. Also it is unclear. Have the authors excluded patients who had a diagnosis of anaemia before their T0 for both non-users and users? Non-users who had an event before the assigned T0 should be excluded from the analysis (http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.573.4443&rep=rep1&type=pdf). Yet the number of patients (non-users) in the new analysis matched that of the primary submission (not using randomly selected controls) (all fields are identical in Table 1 across manuscript versions), which would seem unlikely using a different method for identifying controls.

Response: Thank you for your valuable comments. In previous version, we did not exclude patients who had a diagnosis of anaemia before their T0 for both non-users and users. We totally agreed that the anaemia event before the assigned T0 should be excluded from all analyses to avoid some potential bias. Therefore, we have excluded the patients who had a diagnosis of anaemia before their T0 for both non-users and users. We then performed the PSM matching (considering year of the entry date, age, monthly income, geographical location, residential urbanisation level and medical comorbidities in the model) and all relevant analyses once again. We have revised the relevant statements and Tables throughout all manuscript. Thank you so much!

Additionally, we apologize for the typo in the primary submission. In this study, we initially identified the index date for the controls by using the randomly assigned date to avoid the immortal time bias (we never used the first PC date to identify the controls). Therefore, all fields are identical in Table 1 across prior manuscript versions and we have revised the typo error in following submission. Thank you for your reminders! They are very helpful for us to avoid mistake in this manuscript.

3. The authors have conducted additional analyses by duration of use. I still have a few issues regarding this.

Firstly, the authors have helpfully provided us with an average time of ADT use, of 549.25 days. This is somewhat reassuring however the authors have not provided a standard deviation or range measure, and notably they report the median of about 1 year (353 days). It is still possible that there are those users who have quite some time between their anaemia diagnosis and ADT use. Anaemia is an acute outcome, on which drugs are likely to have an acute effect (possibly with a short wash out period, where the medications may still exert an effect on anaemia). Indeed in previous analyses conducted by my colleagues and I the risk of anaemia was reversed upon discontinuation (Hicks et al 2017).

While the authors have conducted the analyses by duration, while a helpful analyses to investigate duration/ dose response this does in fact not deal with "recency" of use. It is still possible in this analyses that users(in particular short-term use) may have anaemia diagnosed some time after ADT discontinuation. A more appropriate analyses would include an exposure definition using an as treated approach (e.g. current use, past, no or censoring). Although the follow-up is short I would still recommend that the authors consider an analyses such as this as a sensitivity analysis.

current use

Response: Thank you for the comments. As suggested, we have classified ADT users into current users (patients who had received ADT prior to the outcome date within 1 month) and past users

(other remaining users) by using the new identified study cohorts. In addition, we have carried out the relevant sensitivity analyses. The findings were displayed in Supplementary Table 1. The results show that the anaemia is actually an acute outcome. Current ADT users had a much higher risk of anemia (HR= 81.52, 95% CI: 60.58-109.69) than the nonusers in this study. However, we considered that more well-designed studies were still required to estimate the time issue about the connection between ADT and anemia.

3. Could the authors also be specific for the readership how exactly they categorised short and long term durations based on the median? Are these <353 and >353?

Response: Thank you for the suggestions. We have revised the relevant statements as follows: “We categorised the patients receiving ADT into two levels according to the median duration of ADT use (median = 144 days). Those patients who received ADT <144 days were identified as short-term ADT users. Moreover, patients receiving ADT ≥ 144 days were defined as long-term ADT users.”

4. I apologise to the authors for the confusion regarding iron supplementation. The authors are correct that to include iron supplementation in their regression model would lead to variables highly correlated and would be inappropriate. What I meant by my suggestion was that the authors could use receipt of iron supplementation as an additional measure of the outcome of anaemia I.e. in a sensitivity analysis anaemia could be considered as either a diagnosis of anaemia OR a claim for iron supplementation (or blood transfusion also a possible end point). This type of sensitivity analysis would highlight any potential outcome misclassification.

Response: Thank you for the comments. As suggested, we have used prescriptions of iron supplementation as an additional measure of the outcome of anaemia and conducted the relevant sensitivity analyses to avoid the potential outcome misclassification. The findings were displayed in Supplementary Table 2. If we identified anemia cases by using both anaemia diagnosis and receiving iron supplementation, ADT users had much higher risk of anemia (HR= 19.14, 95% CI: 8.50-43.10) than the nonusers in this study. Thank you again for offering so many valuable comments and guidance. They are very helpful for us on revising this manuscript!

VERSION 3 – REVIEW

REVIEWER	BLANAID HICKS Queen's University Belfast , Northern Ireland
REVIEW RETURNED	03-Mar-2020

GENERAL COMMENTS	I thank the authors for conducting the additional analysis and answering my queries.