Peer Review File

Reviewer A

Major suggestions

Comment 1. The study baseline and follow-up period need to be clearly identified.

Reply: Thank you for the suggestion. We have rewritten the description of baseline (section 3.2 Comparison of baseline between patency and dysfunction group). The following sentences have been included in the revised result section:

"The clinical and demographic characteristics of the entire study cohort are summarized in Table 1. … There was no significant difference for other factors. (Table 1)." (Page 7, line 139-143).

Because some baseline data were not normally distributed, we re-perform Mann Whitney U test and revised the results. The results are shown in table 1.

The data about follow-up period also has been included in the result section:

"the follow-up ranged from 5 to 39 months with a mean of 20.9±10.1 months. … There was no statistically significant difference between the patency and dysfunction group (P = 0.191)" (Page 7-8 table 1 and Page 6-7 Line 131-136).

Comment 2. It would be great if the timing or intervals of biochemical measurements could be clarified. The authors indicated the blood tests were performed before dialysis. However, they also mentioned the labs were repeated ≥3 month after dialysis sessions.

Reply: We apologize for the confusion. We modified the description in the research methods section (Page 5 line 99-101).

The average AVF maturation time is 8-12 weeks. So the blood test results is relatively

stable from 3 months after initial dialysis.

Comment 3. Please be more specific and list all variables included in the multivariate Cox model. In the multivariable analysis, please state how the variables were removed. For example, if a backward elimination method was used, what is the threshold?

Reply: Thank you for the suggestion. The approach we adopted was "enter" of COX model. All variables were included in the regression equation at beginning, and the variables would be removed if P-value ≥0.05.

By performing Log rank test to analyze the difference of the AVF survival curves, we demonstrated that Phosphorus, TC, LDL and gender are associated with AVF patency with $p < 0.05$ (Figure1 to 4). In addition, the literatures have shown that, HbA1c and age (1,2) can affect the patency of AVFs. Therefore, the above six variables were included in the Cox model. We have rewritten the section in the text and listed the results in table 2.

(Page 8-9 Line 150-155 and Page 9 table 2)

These sentences were added in the text:

"Figures 1 to 4 shows the AVF patency survival curves with respect to P, TC, LDL, and gender, respectively. …… multivariate cox proportional hazard regression,"

[1] Wärme A, Hadimeri H, Nasic S, et al. The association of erythropoietin-stimulating agents and increased risk for AV-fistula dysfunction in hemodialysis patients. A retrospective analysis. BMC Nephrol. 2021;22(1):30. Published 2021 Jan 18.

[2] Woo K, Lok CE. New Insights into dialysis vascular access: What is the optimal vascular access type and timing of access creation in CKD and dialysis patients? Clin J Am Soc Nephrol 2016;11(8):1487-1494.

Comment 4. T-test is not the correct statistical method to compare continuous variables if they are not normally distributed.

Reply: We totally agree with the reviewer. For the non-normally distributed, we adopted the Mann Whitney U test in nonparametric tests and revised the statistical results. (See

Page 7-8 table 1 and Page 6 Line 113-115)

The following sentences were added in the "2.2.4 Statistical analyses" section of the text:

"Comparisons between groups were performed by independent t-tests if normal distribution is conformed. Mann Whitney U test was used if normal distribution was not conformed."

Comment 5. This is a single-center study with a small sample size. Please include power as a limitation in the Discussion section. Please also discuss selection bias and missing as limitations.

Reply: Thank you for the suggestions.

We performed a power test of the research by PASS software. The result shows that the power of the data in the COX model was 0.92 (>0.8). Therefore, differential variables in log rank test can be included into the Cox model. We have added relevant sections in the text.

"We acknowledge that our study analyzes data from a single center … The prospective cohort with larger sample size may be used to validate the risk factors, such as phosphorus and TC, for AVF dysfunction in future."

(Page 13-14 Line 248-256)

Comment 6. The manuscript indicated the conflicting result of association of total lipid with AVF patency was caused by sample size. Are there other studies showing hypercholesterolemia is associated with AVF dysfunction?

Reply: Thank you for the suggestion. In this study, we demonstrated that TC was an independent risk factor for AVF latency, but TT was not. Cui TL et al. from Hua xi Hospital, China also reported that TC is associated with early AVF failure (1). In addition, Gagliardi, G. M. (2) also suggested that patients with AVF stenosis have higher level of TC than patients without AVF stenosis. Therefore, TC was associated

with AVF dysfunction.

[1] T Cui, R Zhang, F Liu, et al. Effect of diabetes mellitus on early dysfunction of arteriovenous fistula in patients with end-stage renal disease. Journal of Sichuan University (Medical Edition) 2012,43 (03): 438-441. China

[2] Gagliardi GM, Rossi S, Condino F, et al. Malnutrition, infection and arteriovenous fistula failure: is there a link? J Vasc Access. 2011;12(1):57-62.

Minor edits

Comment 1. Title: Please capitalize

Reply: We have modified the title as suggested. (See Page 1 Line 1)

Comment 2. Introduction: please add a couple of sentences to describe the issue of AVF dysfunction and patient outcomes related to it.

Reply: Thank you for the suggestion. We have added the following two sentences in the introduction section.

"The patient had to undergo surgery again if the AVF could not meet the requirement for dialysis. This will not only increase the costs, but also lead to the waste of vascular resources."

(See Page 3, Line 53-55)

Comment 3. Page 3, line 51, please spell out MHD, even it was spelled in the Abstract, it is needed for main text.

Reply: As suggested by the reviewer, we have spelled out the full name of MHD on its first use in the main text. (See Page 3, Line 50)

Comment 4. Line 76-80, suggest rewriting this paragraph, for example, "Patients who could not finish telephone follow-up including those who were deaf…. were excluded. Patients who were diagnosed with … and those who died and received transplant…"

Reply: Thank you for the suggestion. We have rewritten this paragraph. (See Page 4, Line 73-76):

Comment 5 Page 5, line 85-86, this sentence is confusing "…we mainly calculated the proportion of women in each group"

Reply: We apologize for the confusion. Theoretically, the male to female ratio should be calculated separately. But this sentence is somewhat unnecessary, so we removed it.

Comment 6. Line 87, please keep only one decimal place for numbers and percentages, same in tables.

Reply: We have modified the numbers and percentages as suggested.

Comment 7. Line 98, maybe could write like "A functional AVF must be absence of …".

Reply: Thank you for the suggestion. We have modified our text. (See Page 5 Line 96)

Comment 8. Page 6, line 107, change "index" to "variables"

Reply: We have changed "index" to "variables" as suggested. (See Page 5 Line 103)

Line 113-114, please define AVF patency

Reply: Thank you for the suggestion. We have added the definition of AVF patency as follows:

"At the same time, AVF patency (Blood flow $\geq$ 250 ml / min) was also recorded". (See Page 5 Line 110)

Line 116, please add what is descriptive analysis for categorical variables

Reply: As suggested by the reviewer, we have added the following sentence about the

descriptive analysis for categorical variables:

"Categorical variables were reported using frequency counts and percentages". (See Page 6 Line 114-115).

Line 116, please move "Statistical analyses were performed by SPSS 19" to the end of section

Reply: We have modified the text as suggested. (See Page 6 Line 121-122)

Page 7, line 125-130, description of patient enrollment was presented in both Methods and Results section.

Please round all numbers and percentages to one decimal place.

Reply: Thank you for the suggestion. We have rewritten the two sections and rounded all numbers and percentages to one decimal place. (See Page 4 Line 78-83 and Page 6-7 Line 125-137)

2.1.4 Grouping of patients (Page 4 Line 78-83**)**

77 patients who met the inclusion criteria were included in the study. … was approved by the Ethics Committee of Xuanwu Hospital (No. L YS[2020]018) with a waiver of informed consent.

3.1 Follow-up results and overall patency of fistula (Page 6-7 Line 125-137)

Generally, 121 patients with ESRD were initially assessed in this study. … There was no statistically significant difference between the patency and dysfunction group (P = 0.191). During the observation period, 30 (39.0%) patients developed AVF dysfunction whereas 47 (61.0%) patients did not.

Page 8, line 152, this sentence has grammar error

Reply: We have rewritten the sentence and corrected the grammar error.

(See Page 10 Line 176-178)

Line 154, "reported on" should be "reported"

Reply: We have corrected it. (See Page 6 Line 115)

Table 1. Capitalize headings, remove x square and keep numbers and percentages in one decimal place. Change x±μ to mean±SD. The footnotes should be edited, and the grammar errors should be removed.

Reply: We have modified Table 1 following reviewer's suggestions. (See Page 7-8 Table 1)

Table 2. Please listed RR, 95%CI and p values for all the variables in the Cox model without beta. Please keep two decimals for RR and 95%CI and three decimals for p values.

Reply: Thank you for the suggestion. We have modified Table 2. (See Page 9 table 2)

Page 9, line 164-165, not sure why the authors mentioned "confounded by weak immune system", which seems irreverent to AVF.
Please remove RR, HR, 95% CI, and significant values in the Discussion section.

Reply: Thank you for the suggestion. This sentence seems to be unnecessary, so we removed it. Meanwhile, we have removed RR, HR, 95% CI, and significant values in the Discussion section as suggested.

Comment 1: There are multiple grammatical errors that need to be corrected throughout the document. The grammatical errors are too numerous to list. Please utilize available online software or consultant services to assist with proofing

Reply: Thank you for the suggestion. We have asked consulting services for help in revising the manuscript.

Comment 2: There are spelling errors that need to be corrected.

Reply: We have corrected the spelling mistakes carefully.

Comment 3: All abbreviations should be spelled out fully immediately before use of the abbreviation (this includes tables and figures). Please double-check the following abbreviations to make sure they are completely written out in the manuscript before use: CRP, CREA, GB, HB. Many of these are not standard abbreviations.

Reply: Thank you for your suggestion. We have spelled out each of the abbreviations fully before using it for the first time. Incorrect abbreviations were corrected as much as possible.

Comment 4: There needs to be consistency throughout the manuscript with the nomenclature used to describe the two groups of the study. A few examples of how the groups were described includes:
• Methods section – "development" vs. "lack of vascular access dysfunction"
• Results section – "AVF dysfunctional" vs. "non-dysfunction group"
• Grouping of patients section – "patency" vs "dysfunction group"
• Results section 3.2 – "patency" and "non-patency group"

Reply: We apologize for the confusion. We have renamed the two groups as "patency group" and "dysfunction group", respectively.

Comment 5: Please double-check the use of "enrollment" of patients into a

retrospective study.

Reply: Thank you for your advice gratefully. We have replaced "enrollment" by "included".

Major

Comment 1: In this study, I do not think the authors considered the most important factor, the surgical skill of the operator. It is better that authors consider factors representing surgical skill, such as number of years of experience in AVF-development.

In addition, it is better that authors show the data about method of surgical operation. For example, side-to-end or side-to-side anastomosis, anastomotic diameter, or others. Are cholesterol and phosphate still the independent factors after considering above things?

Reply: Thank you for the suggestion. We have included the information about surgery in the revised article. (Page 4-5 Line 86-92).

The following sentences is what we add in the text:

"First, the necessity and safety of operation were carefully evaluated. … . The operation was performed by the same doctor."

The operations of all patients were performed by doctor Tao Luo with the assistance of other doctors. He is the chief surgery of the vascular specialty group of general surgery, Xuanwu Hospital of Capital Medical University. In addition, cholesterol and phosphate were indeed the independent factors after considering the surgical factor. The P value of P and TC were 0.032 and 0.043 respectively.

Comment 2: When the patients were divided into the Patency group and the Dysfunction group, did the observation periods be the same? For example, it would be unfair if a patient who was observed for only 1 year and whose AVF did not become obstructed was allocated in the Patency group, while patients who was observed for 10 years and whose AVF was obstructed after 8 years was allocated in the Dysfunction group.

Reply: We totally understand the reviewer's concern. We added the data about observation periods (follow-up time), the following sentences were added in the text:

"the follow-up ranged from 5 to 39 months with a mean (±SD) of 20.9±10.1 months. … There was no statistically significant difference between the patency and dysfunction group (P = 0.191).". (See Page 6-7 Line 131-136)

Comment 3: Was there a significant difference between the 2 groups in Fig 1 and Fig 2? How about comparing with Log-rank test?

Reply: Thank you for the suggestion. We compared the two groups in Fig 1 and Fig 2 with Log-rank test and got the p value of 0.007 and 0.001, respectively. Thus, increased blood phosphorus and TC are significantly associated with AVF dysfunction. In addition, we also analyzed the AVF patency survival curves for LDL and Gender because the results of univariate analysis for both variables were also significantly associated with AVF dysfunction with log rank test P value of 0.012 and 0.013, respectively (Fig 3 and Fig 4). The results have been shown in the text. (See Page 8-9 Line 150-155). The following sentences were added in the text:

"Figures 1 to 4 shows the AVF patency survival curves …… Therefore, P, TC, LDL, Gender, HbA1c and age were included in the multivariate cox proportional hazard regression"

Comment 4: From the result of this study, it is better that authors mention how we should change our practice behavior. Do authors think we need to lower serum Phosphate and LDL-C levels to prevent AVF dysfunction?

Reply: Thank you for the constructive suggestion. We do need to lower serum phosphorus and LDL-C levels. The relevant content has been included in the revised article (See Page 13 Line 237-246).

The following sentences is what we add in the text:

"Currently, there are many measures to correct hypercholesterolemia, … which may provide a new clue for the treatment of AVF dysfunction."

Comment 5: Authors need to describe the Limitations of this study. For example, a)

this is a single-center study, b) the small sample size might have generated type-1 and/or type-2 error. c) this is an exploratory study with no use of any validation cohort. It has not been verified whether the provided regression model (shown in Table 2) fits to different cohort.

Reply: Thank you for the suggestion. The present study does have the limitations mentioned by the reviewer. We have added relevant content in the article. (See Page 13-14 Line 248-256).

"We acknowledge that our study analyzes data … may be used to validate the risk factors, such as phosphorus and TC, for AVF dysfunction in future."

Meanwhile, Power test of the research was also performed by PASS software. The result shows that the power of the data in the COX model was 0.92 (>0.8). Therefore, differential variables in log rank test can be included into the Cox model. The results have been added in the text.

Minor

Comment 1: In abstract section, it is better to delete the part of "and how it influences survival and prognosis of patients.", since the authors have not investigated survival and prognosis as outcomes in this study. (Line 22-23)

Reply: Thank you for the suggestion. We have deleted these sentences.

Comment 2: Line 82-89 and Line 125-130 overlap. It is better to summarize in either one.

Reply: Thank you for the suggestion. We have rewritten the two sections.

2.1.4 Grouping of patients (Page 4 Line 78-83)

77 patients who met the inclusion criteria were included in the study. … by the Ethics Committee of Xuanwu Hospital (No. L YS[2020]018) with a waiver of informed consent.

3.1 Follow-up results and overall patency of fistula (Page 6-7 Line 125-137)

"Generally, 121 patients with ESRD were initially assessed in this study. …… During the observation period, 30 (39.0%) patients developed AVF dysfunction whereas 47 (61.0%) patients did not."

Comment 3: In main text (method section), authors should describe that there was no missing data.

Reply: Thank you for the suggestion. There are some missing data in this study. Of the 121 patients, 23 were lost to follow-up, 10 received a kidney transplant, and 11 died. For the remaining 77 patients included in this study, there was no missing data. The above information has been included in the method section:

"Of the 121 patients, 23 were lost to follow-up, 10 received a kidney transplant, and 11 died." (Page 6 Line 125-126)

Meanwhile, it also has been mentioned in the discussion section.

(See Page 13-14 Line 250-251)

Comment 4: Authors need to spell out the first abbreviations (CVC, GB, AVG, MHD, etc.).

Reply 4: Thank you for your advice gratefully. We have spelled out each abbreviation fully before using it for the first time.