PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Association of vascular access flow with short- and long-term mortality in chronic hemodialysis patients—a retrospective cohort study
AUTHORS	Wu, Chung-Kuan; Wu, Chia-Lin; Lin, Chia-Hsun; Leu, Jyh-Gang; Kor, Chew-Teng; Tarng, DC

VERSION 1 – REVIEW

REVIEWER	Amanda Valliant, MD Volunteer Faculty, Nephrology University of Kansas School of Medicine Wichita Nephrology Group, PA USA
REVIEW RETURNED	06-Apr-2017

GENERAL COMMENTS	This is a well written study addressing a very relevant clinical question for all clinical nephrologists, and particularly for interventional nephrologists. I would be interested to see the data broken down by AVG versus AVF, as the "critical" Qa for access dysfunction is typically quite different between the groups and I would expect there to be a different Qa/mortality relationship between the two groups. Multi-center studies looking at this question, as well as further evaluation of the cohort of patients with Qa > 2000 mL/min would be useful.

REVIEWER	Nikolai Krivitski Transonic Systems Inc. USA
REVIEW RETURNED	I am inventor of technology used to collect patient data (shunt flow and cardiac output)

GENERAL COMMENTS	1. It is not clear why authors present correlation graph CI vs. Access flow with log of CI? Why not just CI or CO? 2. Did p value in Table 1 belong to more/ less 1000 ml/min? (there are 4 columns) 3. Discussion about the age (more /less 65 years)and CI>4 l/min/m2 on page 16 is confusing, not clear. These are not clear from results section. Same about sex related differences. 4. Some of the parameter relationships not always make sense, like high CI in some cases related to high mortality, it will be nice if others come up with speculations on the issue.

REVIEWER	Andrea Remuzzi Italy
REVIEW RETURNED	22-May-2017

GENERAL COMMENTS	This investigation was aimed at establishing the effect of access blood flow on patient vascular and all-cause mortality. Despite the issue is interesting, the data presented and analyzed do not show definite conclusions that can add valuable information to what is already known. I have the following comments for Authors consideration. 1) My main concern regards study design, significance of the results and their interpretation. As stated in the introduction, low Qa may be associated with poor dialysis, while high Qa (>2000mL/min) was prevoiusly reported to be associated with heart failure. It is also well known that for adequate dialysis procedure Qa must be above 500-600mL/min. It is not clear why the Authors divided patients in four groups (with Qa threshold 500, 1000 and 1500 mL/min). It seems that there were no patients with flow higher than 2000 mL/min, but probably they would have been the most interesting group. 2) On the basis of the arbitrary choice to divide patents with Qa higher or lower than 1000 mL/min, it is concluded that this is the cutoff or mortality risk. However that data show that there is no difference in mortality between these two groups for more than 500 weeks (about 9 years). I do not think the conclusion of the Authors has a meaningful clinical value. In addition, the Authors also adjusted this threshold to 1020 mL/min, which is a non-sense as this small difference of 20mL/min is probably even lower than the mean accuracy in Qa measurement with Transonic. 3) Another major concern I have is with the classification of patients on the basis of a single Qa measurements. In average patient followup was more than five years. I suspect that these vascular access do not last so long and patients have multiple accesses of different type and location. If I am correct in this consideration, I do not see how the single Qa measurement can be indicative of patient mortality. To me the study design is not adequate. 4) In line with the previous comment, patients groups are not homogeneous, in particular as regards age, diabetes and hypertension, that are independent risk factors for mortality as well as for vascular access disfunction. This might have affected results of the study and it is not clear from the analysis provided if this aspect has been adequately taken into account. 5) Another important issue is the measurement of Qa using transonic. While this instrument is commonly used to see recirculation and turbulence, its use for Qa measurement is still controversial, authors only reported one study as reference and most of clinicians and researchers worldwide now use doppler ultrasound to measure the flow in vascular access. This methodology should have been previously validated in the clinical center for reproducibility and accuracy. 6) In Results section, authors repeated several time that Qa <1000ml/min was a risk factor and in discussion they stated this is "reasonable", but they did not elaborate at all on this conclusion.

	They did not explain why it should be reasonable and the mechanisms underling the relation between Qa<1000mL/min and mortality. 7) As a minor comment, Authors took into account both AVF and AVG, which have different characteristics, patency, flow rates and are created in patients with very different vascular characteristics. Therefore, discriminating between these two vascular accesses and providing different analysis each group might be a more reasonable conception of the study.

VERSION 1 – AUTHOR RESPONSE

Response to reviewer #1:

Comment: This is a well written study addressing a very relevant clinical question for all clinical nephrologists, and particularly for interventional nephrologists. I would be interested to see the data broken down by AVG versus AVF, as the "critical" Qa for access dysfunction is typically quite different between the groups and I would expect there to be a different Qa/mortality relationship between the two groups. Multi-center studies looking at this question, as well as further evaluation of the cohort of patients with Qa > 2000 mL/min would be useful.

Responses:
We thank the reviewer for reminding us this important issue.
1) The data was broken into patients with an arteriovenous fistula (AVF) and those with an arteriovenous graft (AVG). Time-dependent receiver operating characteristic curve analysis was used to determine the optimal access flow rate for discriminating between patients with mortality and those without mortality. The optimal access flow rate for patients with an AVF and an AVG in this study were 1020 ml/min and 970 ml/min, respectively (Supplementary Figure S3). However, further studies to determine the association between the access flow rate of AVG and mortality are warranted because of the small number of patients with an AVG (n=57) in our cohort study. We have revised a sentence in the Discussion section:
Moreover, the optimal cutoff access flow level in patients with an AVF was similar to that in patients with an AVG in our study cohort. (Supplementary Figure S3). (Page 15, line 17)
2) Many thanks for the reviewer's suggestions and we're going to investigate into the associations between cardiovascular outcomes and high access flow rates, especially access flow >2000 mL/min in chronic hemodialysis patients.

Response to reviewer #2:
Comment 1: It is not clear why authors present correlation graph CI vs. Access flow with log of CI ? Why not just CI or CO ?

Response:
We thank the reviewer for allowing us to clarify the vagueness in our manuscript. In our study, the data of cardiac index (CI) and cardiac out (CO) were not normally-distributed (Kolmogorov-Smirnov test, $p<0.05$; shown below). Thus, the data of CI or CO had to be log-transformed for the Pearson's correlation analysis.
Kolmogorov-Smirnov test
Statistic df Sig.
Cardiac index .085 378 0.00
Cardiac output .075 378 0.00
log (Cardiac index) .039 378 0.20

We added “(e.g. CI)” into the sentence as follows:
Non-normally distributed variables were either log-transformed (e.g. CI) for the correlation analysis or analyzed using nonparametric tests. (Page 8, line 17)

Comment 2: Did p value in Table 1 belong to more/ less 1000 ml/min? (there are 4 columns)

Response:
P values in all variables of Table 1 referred to differences among 4 groups (access flow < 500 ml/min, 500 to 999.9 ml/min, 1000 to 1499.9 ml/min, and 1500 to 1999.9 ml/min). Multiple comparisons among the four groups were performed using the Kruskal–Wallis test for continuous variables or the Pearson chi-squared test for categorical variables or the Fisher’s exact test for categorical variables if the number less than 5 in each cell. Please see the footnotes “a”, “b”, “c” at the bottom of Table 1. Moreover, we have added the sentence stating that “or the Fisher’s exact test for categorical variables if the number was less than 5 in each cell”:
Multiple comparisons among the four groups were performed using the Kruskal–Wallis test for continuous variables or the Pearson chi-squared test for categorical variables or the Fisher’s exact test for categorical variables if the number was less than five in each cell. (Page 9, line 1)

Comment 3: Discussion about the age (more /less 65 years) and CI>4 l/min/m2 on page 16 is confusing, not clear. These are not clear from results section. Same about sex related differences.

Response:
We thank the reviewer for the excellent comment but they are not confusing. Subgroup analysis was performed to determine the effect modification and to compare the effect of low Qa on aHRs of all-cause mortality among different patient groups. In this analysis, Qa significantly interacted with age, sex, and CI in terms of the risk of all-cause death (P for interactions <0.05). That is, Qa had significant differential effects on all-cause mortality in patients with age<65 years, male sex, and with CI>4 l/min/m2 compared with those with age□65 years, female sex, and CI□4 l/min/m2. However, we found a sentence which may lead to some perplexity and have revised it as follows:
…..”suggesting that chronic HD patients with lower Qa levels have a significantly higher risk of all-cause mortality, particularly in male patients aged <65 years with a CI of >4 L/min/m2those are male, those aged <65 years, or those with a CI of >4 L/min/m2.” (Page 17, line 8)
In the discussion section, we have proposed our speculations about how age, sex, and cardiac function interact with Qa and affect the risk of all-cause mortality. After all, more studies are required to confirm the effect of Qa on mortality in these subpopulations. We sincerely hope that the reviewer could approve our explanation.

Comment 4: Some of the parameter relationships not always make sense, like high CI in some cases related to high mortality, it will be nice if others come up with speculations on the issue.

Response:
Many thanks for the reviewer’s suggestions and the inspiration. We’re going to investigate into the associations between cardiovascular outcomes and high access flow rates, especially access flow >2000 mL/min in chronic hemodialysis patients.

Response to reviewer #3:

Comment 1: My main concern regards study design, significance of the results and their interpretation. As stated in the introduction, low Qa may be associated with poor dialysis, while high Qa (>2000mL/min) was prevoiusly reported to be associated with heart failure. It is also well known that for adequate dialysis procedure Qa must be above 500-600mL/min. It is not clear why the Authors divided patients in four groups (with Qa threshold 500, 1000 and 1500 mL/min). It seems that there were no patients with flow higher than 2000 mL/min, but probably they would have been the most interesting group.

Response: Our cohort patients were divided into four groups (with three Qa thresholds: 500, 1000, and 1500 mL/min) in Table 1 to compare the clinical characteristics among these groups. We referred to a previous article “Relation between access blood flow and mortality in chronic hemodialysis patients”.(1) Besides, patients with a high Qa (>2000 mL/min) was excluded because of the small number (n=16) in our cohort. We totally agreed with the reviewer’s comment that patients with Qa >2000mL/min would have been the most interesting group. Thus, we reanalyzed our cohort patients after including the patients with a Qa >2000 mL/min. However, the optimal access flow and the association between Qa<1000 mL/min and mortality remained unchanged. Further studies for clarifying the associations between cardiovascular outcomes and high access flow in chronic hemodialysis patients with an access flow rate >2000 mL/min are needed.
(1) Al-Ghonaim M, Manns BJ, Hirsch DJ, et al. Relation between access blood flow and mortality in chronic hemodialysis patients. Clin J Am Soc Nephrol 2008;3(2):387-91. doi: 10.2215/CJN.03000707
Time-dependent receiver operating characteristic (ROC) curve of Qa for all-cause mortality after inclusion of patients with a Qa >2000 ml/min.

Risks of all-cause and vascular mortality among the access flow groups during the follow-up period after inclusion of patients with an access flow rate >2000 ml/min

Analysis	All-Cause Death		Cardiovascular or Cerebrovascular Death[a]	
	Hazard ratio (CI95)	P Value	Hazard ratio (CI95)	P Value
Univariate				
Access flow 1000 mL/min	1 (reference)		1 (reference)	
Access flow < 1000 mL/min	1.83 (1.36–2.47)	<0.001	1.85 (1.21–2.84)	0.005
Multivariate[b]				
Access flow 1000 mL/min	1 (reference)		1 (reference)	
Access flow < 1000 mL/min	1.39 (1.004–1.91)	0.047	1.39 (0.87–2.21)	0.167

Abbreviations: CAD, coronary artery disease; CHF, congestive heart failure; CI95, 95% confidence interval; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; ESA, erythropoiesis-stimulating agent; ESRD, end-stage renal disease.
aCompeting risks with cause-specific hazards.
bAdjusted for age; sex; diabetes mellitus; CAD; CHF; CVA; COPD; liver cirrhosis; malignancy; systolic blood pressure; diastolic blood pressure; hemodialysis vintage; vascular access type; ESRD cause; cardiac index; hemoglobin, albumin, cholesterol, ferritin, sodium, potassium, ionized calcium, phosphate, and parathyroid hormone levels; Kt/V; and ESA dosage.

Comment 2: On the basis of the arbitrary choice to divide patents with Qa higher or lower than 1000 mL/min, it is concluded that this is the cutoff or mortality risk. However, that data show that there is no difference in mortality between these two groups for more than 500 weeks (about 9 years). I do not think the conclusion of the Authors has a meaningful clinical value. In addition, the Authors also adjusted this threshold to 1020 mL/min, which is a non-sense as this small difference of 20mL/min is probably even lower than the mean accuracy in Qa measurement with Transonic.

Response:
We thank the reviewer for this important issue. First, Figure 2 implies that the cumulative incidence of all-cause mortality was higher in the lower-Qa groups (500–999.9 and <500 mL/min) than in the higher-Qa groups (1000–1499.9 and 1500–1999.9 ml/min). Thus, we compared the cumulative incidence between the lower- and higher-Qa groups by using the log-rank test. The result showed a significant difference in all-cause mortality among these groups (<500 and 500–999.9 vs. 1000–1499.9 and 1500–1999.9) during the follow-up of more than 500 weeks (P=0.001; Figure 2). But it is still arbitrary to conclude that Qa of 1000 ml/min is the cutoff value of Qa for mortality risk. Thus, we determined the most discriminatory value of baseline Qa for all-cause mortality in our study cohort by using time-dependent receiver operating characteristic curve analysis and the optimal cutoff value of Qa was 1020 mL/min. However, according to the FDA's documentation (available at: https://www.accessdata.fda.gov/cdrh_docs/pdf/K960817.pdf), the accuracy of Transonic HD01plus system is □15% of access flow (or □50 ml/min), similar to that of color Doppler duplex sonography (□14% of , but operator-dependent). Therefore, Qa level of 1000 mL/min regarded as a cutoff value for all cause-mortality would be more practical in clinical settings. We sincerely hope that the reviewer could approve our explanation.

Comment 3: Another major concern I have is with the classification of patients on the basis of a single Qa measurements. In average patient follow up was more than five years. I suspect that these vascular access do not last so long and patients have multiple accesses of different type and location. If I am correct in this consideration, I do not see how the single Qa measurement can be indicative of patient mortality. To me the study design is not adequate.

Response: We thank the reviewer for reminding us this important issue. We think it would be a limitation in this study. Therefore, we added a sentence stating that "Fourth, changes with the type and location of vascular access in patients during long-term follow up were not taken into accounts in the cohort study." (Page 19, line 5) in the limitation section. We tried to avoid the impact of rapid changes of Qa on mortality. Patients who had AVF or AVG failure within 3 months before the date of Qa measurement were excluded in this study to ensure no dramatic changes of Qa values before its measurement. Additionally, we have reviewed the longitudinal changes in Qa levels in our study cohort. We used the generalized estimating equations (GEE) to evaluate the change of Qa levels over time in our study cohort. The GEE model (as shown below) revealed that the Qa levels of our study patients were not significantly associated with the duration of observation (P=0.09). The changes in Qa levels over time in our study cohort may be relatively small. We sincerely hope that the reviewer could approve our explanation.
Analysis of the evolution of Qa levels by GEE.

Comment 4: In line with the previous comment, patients groups are not homogeneous, in particular as regards age, diabetes and hypertension, that are independent risk factors for mortality as well as for vascular access dysfunction. This might have affected results of the study and it is not clear from the analysis provided if this aspect has been adequately taken into account.

Response: We thank the reviewer for the excellent suggestions. The Kaplan-Meier method analysis estimating cumulative incidences of all-cause and vascular mortality during the follow-up and log-rank test comparing the incidences among the higher- and lower-Qa groups were not taking the confounders into account. Therefore, we performed multivariate logistic regression analyses to calculate the adjusted ORs of all-cause mortality within 1 year, and multivariate Cox's analysis to estimate the adjusted HRs of all-cause mortality during the follow-up period taking all potential confounders into account. After multivariate adjustment, a Qa level of <1000 mL/min is still an independent risk factor for both short- and long-term all-cause mortality in chronic HD patients.

Comment 5: Another important issue is the measurement of Qa using transonic. While this instrument is commonly used to see recirculation and turbulence, its use for Qa measurement is still controversial, authors only reported one study as reference and most of clinicians and researchers worldwide now use doppler ultrasound to measure the flow in vascular access. This methodology should have been previously validated in the clinical center for reproducibility and accuracy.

Response: We thank the reviewer for reminding us this important issue. The reproducibility and accuracy of this method have been evaluated and validated in clinical settings. Lopot et al. concluded that access blood flow detected by ultrasound indicator dilution (Krivitski's method) had very high reproducibility and the negligible impact changes in blood flow on the accuracy of access flow measurement and was justified as reference method for access flow evaluation after comparison of different techniques of hemodialysis vascular access flow evaluation.(1) Garland et al. concluded that access flow measurement by ultrasound indication dilution is the standard of care for access surveillance after reviewing the methods of screening for vascular access dysfunction in PTFE grafts and fistulae.(2) Lok et al. concluded that low access flow detected by using Transonic monitoring were associated with increased thrombosis but stenosis detected using duplex ultrasonography was not a strong predictor of incipient thrombosis.(3) Even in children, ultrasound indicator dilution is still a valid indicator of access flow(4) and is very sensitive to hemodynamically significant stenosis.(5)

We have added this sentence as follows:
The method was regarded as a reference method for access flow evaluation because of high reproducibility and accuracy.16-18 (Page 7, line 18 and page 24, line 1 to 9)
(1) Lopot F, Nejedly B, Sulkova S, et al. Comparison of different techniques of hemodialysis vascular access flow evaluation. Int J Artif Organs 2003;26(12):1056-63.
(2) Garland JS, Moist LM, Lindsay RM. Are hemodialysis access flow measurements by ultrasound dilution the standard of care for access surveillance? Adv Ren Replace Ther 2002;9(2):91-8.
(3) Lok CE, Bhola C, Croxford R, et al. Reducing vascular access morbidity: a comparative trial of two vascular access monitoring strategies. Nephrol Dial Transplant 2003;18(6):1174-80.
(4) Goldstein SL, Allsteadt A. Ultrasound dilution evaluation of pediatric hemodialysis vascular access. Kidney Int 2001;59(6):2357-60. doi: 10.1046/j.1523-1755.2001.00753.x
(5) Ashoor IF, Hughson EA, Somers MJ. Arteriovenous access monitoring with ultrasound dilution in a pediatric hemodialysis unit. Blood Purif 2015;39(1-3):93-8. doi: 10.1159/000368976

Comment 6: In Results section, authors repeated several time that Qa <1000ml/min was a risk factor and in discussion they stated this is "reasonable", but they did not elaborate at all on this conclusion. They did not explain why it should be reasonable and the mechanisms underling the relation between Qa<1000mL/min and mortality.

Response:
We thank the reviewer for allowing us to explain more. Patients with a low Qa level, especially lower than a critical level, are tend to have vascular stenosis or thrombosis. On the contrary, a high Qa level greater than 2000 mL/min is associated with left ventricular dilation and high-output heart failure. Our results demonstrated that Qa was significantly and positively related to cardiac index (Figure 1). That is, patients with a low Qa level might had low cardiac index status. Additionally, our findings showed that a lower Qa level (<1000 mL/min) was an independent risk factor for both short- and long-term all-cause mortality. Therefore, we suggest that Qa levels of 1000–2000 mL/min might be more reasonable for chronic HD patients and further studies are required to confirm our findings. (Page 16, line 7)

Second, the mechanism underlying the relationship between Qa<1000mL/min and mortality is far from clear because Qa can be affected by many factors including systemic hemodynamics, size and endothelial function of vessels supplying and draining the access, and presence of significant vascular stenosis. We speculated that lower Qa levels may be correlated with vascular access dysfunction, low cardiac output status and subsequent CV or cerebrovascular events. Further researches are needed to clarify whether vascular interventions increasing Qa or improvement of cardiac function while Qa <1000 mL/min could improve survival in these patients. (Page 16, line 13 to Page 17, line 5) We sincerely hope that the reviewer could approve our explanation.

Comment 7: As a minor comment, Authors took into account both AVF and AVG, which have different characteristics, patency, flow rates and are created in patients with very different vascular characteristics. Therefore, discriminating between these two vascular accesses and providing different analysis each group might be a more reasonable conception of the study.

Response: We thank the reviewer for reminding us this important issue. The data was broken into patients with an arteriovenous fistula (AVF) and those with an arteriovenous graft (AVG). Time-dependent receiver operating characteristic curve analysis was used to determine the optimal access flow for discriminating between patients with mortality and those without mortality. The optimal access flow rates for patients with an AVF and an AVG in this study were 1020 ml/min and 970 ml/min, respectively (Supplementary Figure S3; shown below). However, further studies to determine the association between the access flow rate of AVG and mortality are warranted because of the small number of patients with an AVG (n=57) in our cohort study. We have revised a sentence in the Discussion section:
Moreover, the optimal cutoff access flow level in patients with an AVF was similar to that in patients with an AVG in our study cohort. (Supplementary Figure S3). (Page 15, line 17)
Figure S3. Time-dependent receiver operating characteristic (ROC) curve of Qa for all-cause mortality regarding the type of vascular access.

Time-dependent ROC analysis identified the most discriminatory values of Qa (1020 mL/min for patients with an AVF and 970 mL/min for patients with an AVG) for all-cause mortality in chronic hemodialysis patients.

VERSION 2 – REVIEW

REVIEWER	Amanda Valliant, MD Interventional Nephrologist Wichita Nephrology Group, PA United States of America
REVIEW RETURNED	26-Jun-2017

GENERAL COMMENTS	Well written and interesting subject matter. If reproducible results, this could impact dilution surveillance guidelines and timing of interventions to protect patency of access. I would love to see a larger, multicenter study to replicate these results.

REVIEWER	Nikolai Krivitski Transonic Systems Inc. USA
REVIEW RETURNED	14-Jul-2017

GENERAL COMMENTS	I have a question in first review: Did p value in Table 1 belong to more/ less 1000 ml/min? (there are 4 columns) Do not understand the answer. If authors claim some prognostic value of particular access flow (AF) range let say from A to B, then they need to compare the data related to this particular patient group (with AF from A to B) to other groups? This will be clear for me. But authors compare parameters between all groups. Let say some data from patient group with AF between C and D is statistically different from data in group with AF from E and F, what is this to do with the purpose of the study? Not clear.

REVIEWER	Andrea Remuzzi University of Bergamo Biomedical Engineering
REVIEW RETURNED	29-Jun-2017

GENERAL COMMENTS	The revised manuscript was almost satisfactorily changed, following my comments. In details, I can make the following evaluation of the response to my comments and the changes in the manuscript. Comment #1 - Adequately addressed Comment #2 - Adequately addressed Comment #3 - This was a crucial point. Without followup measurements it is impossible to assume that “Qa level were not significantly associated with the duration of observation". Even if the study is retrospective, the patency of the VA must be considered in this extensive analysis. In my opinion, the use of the method of generalized estimating equations to derive the conclusion that “Qa level over time … may be relatively small” is not solid enough. I can not really approve Authors’ explanation. This point should be stated more clearly in the manuscript. Comment #4 - Adequately addressed Comment #5 - Adequately addressed Comment #6 - Adequately addressed Comment #7 - Adequately addressed

VERSION 2 – AUTHOR RESPONSE

Response to reviewer #1:
Comment: Well written and interesting subject matter. If reproducible results, this could impact dilution surveillance guidelines and timing of interventions to protect patency of access. I would love to see a larger, multicenter study to replicate these results.

Response: We thank the reviewer for the affirmative response to this manuscript. We fully agree with the reviewer on the importance of vascular access surveillance and timing of interventions to protect patency of access. Therefore, we also look forward to whether our results can be replicated in a large-scale, prospective 10ulticentre study.

Response to reviewer #2:
Comment: I have a question in first review: Did p value in Table 1 belong to more/ less 1000 ml/min? (there are 4 columns)
Do not understand the answer. If authors claim some prognostic value of particular access flow (AF) range let say from A to B, then they need to compare the data related to this particular patient group (with AF from A to B) to other groups? This will be clear for me. But authors compare parameters between all groups. Let say some data from patient group with AF between C and D is statistically different from data in group with AF from E and F, what is this to do with the purpose of the study? Not clear.

Response:
We thank the reviewer for allowing us to explain more. According to the study results, a Qa level of <1000 mL/min is an independent risk factor for both short- and long-term all-cause mortality in chronic HD patients. It may be not so clear to compare all parameters among 4 different groups of access flow because the purpose of the study is to emphasize that chronic HD patients with a Qa level of < 1000 mL/min had worse short- and long-term survival than those with a Qa level of > 1000 mL/min. Thus, we added a new table (Supplementary Table 3) as following to clarify the vagueness of Table 1. We sincerely hope that the reviewer could approve our explanation.
We have added a sentence in the Result section:
Last, proportions of all-cause and vascular mortality in patients stratified by a Qa level of 1000 ml/min were in consistent with those in Table 1 (Supplementary Table 3). (Page 14, line 16)

Response to reviewer # 3:

Comment: The revised manuscript was almost satisfactorily changed, following my comments. In details, I can make the following evaluation of the response to my comments and the changes in the manuscript.
Comment #3 - This was a crucial point. Without follow-up measurements it is impossible to assume that “Qa level were not significantly associated with the duration of observation". Even if the study is retrospective, the patency of the VA must be considered in this extensive analysis. In my opinion, the use of the method of generalized estimating equations to derive the conclusion that “Qa level over time … may be relatively small” is not solid enough. I can not really approve Authors’ explanation. This point should be stated more clearly in the manuscript.

Response: We thank the reviewer again for reminding us this crucial point. We agreed with the reviewer that “although longitudinal changes in the Qa levels over time may have been relatively small” may not be solid enough by just using the methods of generalized estimating equations because the patency of vascular access and radiological or surgical interventions for vascular access dysfunction could influence Qa.

Hence, we have modified the limitation section as follows:
Further studies with longitudinal measurements of Qa to clarify the independent role of baseline Qa are needed. (Page 18, line 15)
Third, a Qa level changes inevitably during the long-term follow-up because of the development of intimal hyperplasia of vascular access and the following radiological or surgical interventions. In our HD unit, patients with a Qa level of <600 mL/min in grafts and those with a Qa level of <400-500 mL/min in fistulae were routinely referred for fistulography to diagnose stenosis or thrombosis6. Further studies focusing on the impact of vascular interventions are also needed. (Page 18, line 19)

VERSION 3 – REVIEW

REVIEWER	Nikolai Krivitski Transonic Systems Inc. Ithaca, USA
REVIEW RETURNED	12-Aug-2017

GENERAL COMMENTS	The presented Table 3 in Supplementary materials addressed my last question.