Systolic Blood Pressure Changes in Hemodialysis: Predictors and Long-Term Outcomes

SYSTOLIC BLOOD PRESSURE CHANGES IN HEMODIALYSIS: PREDICTORS AND LONG-TERM OUTCOMES

By

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I have reviewed this thesis. It represents work done by the author under my guidance/supervision.

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Overview

Hypertension is present in approximately 86% of patients with end-stage kidney disease (ESKD) receiving maintenance hemodialysis. (1) It is a key risk and mechanistic factor in the development of cardiovascular disease, which is the leading cause of mortality in this population. (2) Extremes of high or low blood pressures (BP) during HD treatment are frequently encountered in clinical practice and are associated with poor outcomes, including mortality. (1, 3) While in most patients, systolic BP (SBP) tends to decrease during dialysis, approximately 13-23% of patients experience intradialytic hypertension, characterized by an increase in SBP from pre-HD to post-HD. (4- 6) Previous evidence has suggested an association between intradialytic hypertension and adverse outcomes; (4, 6, 14-19) however, there is no consistent, unifying definition for intradialytic hypertension described in the literature. This has hindered a robust assessment of risks and potential predictors of this phenomenon, as well as limiting comparisons across prior studies. To address this gap, in Paper 1 we explored three potential definitions of intradialytic hypertension and evaluated their independent associations with mortality in a large contemporary US-based patient cohort.

The clinical management of intradialytic hypertension is complex and predicting which patients will experience a paradoxical increase in BP, versus other patterns of change in BP during HD, remains a challenge. (6-8) One of the keys to solving this issue is to gain a better understanding of the underlying pathophysiology. Although many potential etiologies have been invoked and studied (including volume overload, the use of erythropoiesis-stimulating agents (ESAs), and endothelial cell dysfunction), Endothelin-1 (ET-1) has emerged as a key potential mediator. ET-1 is a potent endothelium-derived vasoconstrictor, which has previously

been associated with essential hypertension in the non-HD population and adverse outcomes in patients receiving hemodialysis. (20) A study by Teng et al. reported that inappropriate rises in ET-1 were associated with increased peripheral resistance and resultant increases in SBP. (21) These results were further corroborated by Chou et al. in a case-control study which observed significant increases in post-HD ET-1 in participants who were “hypertension-prone” as compared to “non-hypertension prone” HD controls. (22) However, as these studies were relatively small (≤60 patients) and of limited duration, a knowledge gap remains to robustly examine the association of ET-1 with pre- SBP parameters in the maintenance HD population. In Paper 2, harnessing the wealth of repeated measures analysis and intradialytic BP recordings, we assessed the association of baseline ET-1 in outpatient HD patients with changes in SBP parameters (pre-SBP, intra-HD SBP, and post-SBP) over one year in large, contemporary, US-based cohort.

In summary the significance of this work is that as the HD population is at high risk for cardiovascular mortality and since there have been very few advances that have modified this risk, identifying novel opportunities for intervention that may modify this risk will be of great benefit.

Manuscript 1:

Association of Different Definitions of Intradialytic Hypertension with Long-term Mortality in Hemodialysis

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ABSTRACT

Background

Hypertension is common among patients receiving maintenance hemodialysis (HD), and a subset of patients experience an increase in systolic blood pressure (BP) from pre- to post-HD (intradialytic hypertension). While this phenomenon is known to be associated with adverse short and long-term outcomes, there is little consensus on an evidence-based definition.

Methods

Using a retrospective cohort of 3,198 participants HD patients, unadjusted and adjusted Cox proportional hazards regression models were fit to examine the association of various definitions of intradialytic hypertension (≥30% of baseline sessions with an increase in pre- to post-HD systolic BP of 1) ≥0 mmHg [Hyper0]; 2) ≥10 mmHg [Hyper10], or 3) ≥20 mmHg increase [Hyper20]) with all-cause mortality. Interaction terms were used to assess for effect modification according to pre-specified demographic (age, sex), HD-related (pre-HD systolic BP, ultrafiltration rate), and comorbid disease variables (diabetes, heart failure, and peripheral vascular disease [PVD]).

Results

At baseline, the mean age was 62 ±15 years, 57% were male, and 14% were Black. The average change in BP from pre- to post-HD was 13 ±16 mmHg (median 12 [3 to 22] mmHg). During the baseline period, 47% of individuals met the definition for Hyper0 and were at a 29% (HR 1.29; 95%CI 1.03 to 1.62) higher adjusted risk of death, compared with participants who experienced no SBP increase. Hyper10 was present in 21.2% of individuals and associated with a 21% higher

adjusted risk of death (HR 1.21; 95%CI 0.96 to 1.51). Hyper20 was present in 6.8% of individuals and associated with a 5% higher risk of death (HR 1.05; 95%CI 0.76 to 1.46). There was evidence for effect modification by age and PVD (P-interaction=0.02 for both), with a higher risk of death in those aged 45-70 years and those without PVD.

Conclusions

Individuals with any increase in systolic BP from pre- to post-HD experienced the highest adjusted risk of mortality, compared with other threshold-based definitions. The association of any increase in systolic BP with death (vs. not) is modified by age and PVD, with individuals aged 45-70 years and without PVD having the highest risk.

INTRODUCTION

End-stage kidney disease (ESKD) affects approximately 2 million people globally, and approximately 40% of deaths in this population can be attributed to cardiovascular (CV) disease.[1] While hypertension is a widespread comorbid condition in patients with ESKD receiving maintenance hemodialysis (HD),[1] the optimal blood pressure (BP) for such patients remains unknown, with expert opinion recommending the need for focused research and adequately powered interventional trials.[2]

Interestingly, the relationship of systolic BP before, during, and/or post-HD with adverse CV outcomes is non-linear, with the most potent associations observed at the extremes of systolic BP.[3] While the HD procedure generally encompasses ultrafiltration, providing a mechanism by which BP usually decreases from pre- to post-HD, a subset of individuals experience an increase in BP during HD (intradialytic hypertension). We and others have examined a possible role for higher serum endothelin-1 in the pathogenesis of this phenomenon, while other theories include contributions from volume overload, renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system activation, the use of erythropoiesis-stimulating agents (ESA), and abnormalities of mineral bone disease axes.[4–9] Despite focused research effort, the exact cause remains unclear.

The prevalence of intradialytic hypertension ranges from 13-23%,[10–12] depending on the definition utilized. While several studies have reported an association of intradialytic hypertension with adverse short-term and long-term outcomes,[10,12–16] the lack of a singular definition has limited comparisons across reports and hindered clinical utility. In the present analyses, we describe the independent associations of various definitions of intradialytic

hypertension with long-term mortality in patients receiving maintenance HD. Furthermore, we examined if important demographic and clinical characteristics modified these associations.

METHODS:

Study population

The study protocol was deemed exempt by Partners Institutional Review Board. We performed a retrospective cohort study of prevalent patients (receiving HD >180 days) in Satellite Healthcare dialysis facilities during 2012 (n=3,725). For the present analyses, participants not on thrice-weekly HD, those with a session length of >5 hours, and those who died before the end of the baseline exposure period were excluded. Our final analytic cohort consisted of 3,198 participants (Figure 1). Comparisons of included with excluded participants are presented in Supplementary Table 1.

Exposure

The main exposure of interest was the presence or absence of intradialytic hypertension during a baseline exposure period of 90 days. Individual HD sessions were considered to be complicated by intradialytic hypertension according to the following definitions: 1) any increase in systolic BP from pre- to post-HD (Hyper0); 2) any increase of more than 10 mmHg (Hyper10); and 3) any increase of more than 20 mmHg (Hyper20). Patients who experienced intradialytic hypertension in 30% or more HD sessions during the exposure period were considered as having baseline intradialytic hypertension (those who did not meet this criterion formed the reference group). These definitions were selected following review of the existing literature and with the goal of capturing clinically relevant thresholds in pre- to post-HD blood pressure changes. The

association of each definition of intradialytic hypertension with outcomes was assessed independently.

Two additional exposure metrics were considered in sensitivity analyses: 1) the percentage of baseline sessions (as a continuous variable, per 10 percentage points) that met the hyper0, Hyper10, and Hyper20 definitions; 2) the average change in systolic BP from pre- to post-HD during the baseline period was considered a continuous variable (where positive values represent a decline in BP from pre- to post-HD and negative values represent an increase).

Outcome

The primary outcome was the time to death from any cause. At-risk time for all analyses began at the end of the 90-day baseline exposure period. Subjects remained at risk until death, kidney transplant, transfer to an outside facility, or censoring at the end of follow-up (12 December 2013). The maximum potential follow-up time was 621 days.

Assessment of Other Covariates

All clinical and hemodialysis prescription data were collected from the electronic medical record. Demographic information, including age, race, ethnicity, sex, dialysis vintage, and comorbid conditions (including diabetes, heart failure, coronary artery disease, and peripheral vascular disease), were recorded at baseline. Laboratory, HD prescription, and hemodynamic data were averaged over the baseline exposure period. Ultrafiltration rate was calculated as: ultrafiltration volume/post-HD weight/session length in hours and expressed as mL/kg/hr.

Statistical Analysis

According to data distribution, continuous variables were summarized using means (standard deviations) or medians (25^{th}-75^{th} percentiles) and compared with t-tests, Wilcoxon rank-sum tests, analysis of variance, or Kruskal-Wallis tests. Categorical variables were summarized as percentages and compared with Chi-squared tests.

The association of the various definitions of intradialytic hypertension with all-cause mortality was assessed using Cox proportional hazards models. Initially, unadjusted models were fit. Subsequently, multivariable models were fit that adjusted for potential confounding variables, as follows: Model 1 adjusted for age, sex, race, ethnicity, pre-SBP, and nadir intra-HD SBP; Model 2 adjusted for the same variables as Model 1, as well as diabetes, heart failure, HD access, and ultrafiltration rate; Model 3 adjusted for the same variables as Model 2, as well as pre-HD serum sodium, phosphorus, creatinine, calcium, albumin, BUN, and glucose. The selection of variables for inclusion in these models was based on clinical and biological plausibility, without probabilistic selection criteria. Missing data was not imputed. Potential effect modification of the association of intradialytic hypertension with mortality was assessed via the creation of interaction terms (Model 3) for the following a priori selected variables: age, sex, pre-HD systolic BP, ultrafiltration rate, diabetes, heart failure, and peripheral vascular disease. To minimize the potential for chance findings, sub-group effect estimates were only determined if the P-value for the interaction was <0.05. The proportional hazard assumption was assessed through a test of a nonzero slope based on Schoenfeld residuals, visualization of Schoenfeld residuals, and log-log plots.

All analyses were performed at the nominal alpha level of 0.05 without correction for multiple hypothesis testing. Statistical analyses were performed using Stata MP (version 16.0, Stata Corp., College Station, Texas).

RESULTS

At baseline, the mean age was 62 ±15 years, 57.2% were male, and 13.6% were Black. Participants with ≥30% baseline sessions complicated by any increase in pre- to post-HD systolic BP (Hyper0 definition) were more likely to be older, female, non-Hispanic, to have a history of heart failure, dialyze with a catheter, to have lower UFR and pre-HD systolic BP, and to have lower pre-HD serum blood urea nitrogen, phosphorus, albumin, creatinine (Table 1), compared to those without baseline intradialytic hypertension. Comparisons of baseline characteristics according to the Hyper10 and Hyper20 definitions are presented in Supplementary Tables 2 and 3.

Intradialytic hypertension ≥0 mmHg and mortality

During the baseline period, 1,502 (47%) individuals had ≥30% of HD sessions complicated by any increase in pre- to post-HD systolic BP (Hyper0; Figure 2). Of these, 313 (20.8%) died during the follow-up period, compared with 216 of 1,696 (12.7%) individuals who did not meet criteria for baseline Hyper0, resulting in a 77% higher risk of death (HR 1.77; 95%CI 1.49 to 2.10). In the fully adjusted model, the effect estimate was attenuated but remained statistically significant (HR 1.29; 95%CI 1.03 to 1.62; Table 2).

Intradialytic hypertension ≥10 mmHg and mortality

During the baseline period, 679 (21.2%) individuals had ≥30% of HD sessions complicated by an increase of ≥10 mmHg in pre- to post-HD systolic BP (Hyper10; Figure 2). Of these, 152 (22.4%) died during the follow-up period, compared with 377 of 2,519 (15%) individuals who did not meet criteria for baseline Hyper10, resulting in a 61% higher risk of death (HR 1.61; 95%CI 1.34 to 1.95).

In the fully adjusted model, the effect estimate was attenuated (HR 1.21; 95%CI 0.96 to 1.51; Table 2).

Intradialytic hypertension ≥20 mmHg and mortality

During the baseline period, 217 (6.8%) individuals had ≥30% of HD sessions complicated by an increase of ≥20 mmHg in pre- to post-HD systolic BP (Hyper20; Figure 2). Of these, 49 (22.6%) died during the follow-up period, compared with 480 of 2,981 (16.1%) individuals who did not meet criteria for baseline Hyper20, resulting in a 49% higher risk of death (HR 1.49; 95%CI 1.11 to 2.00). In the fully adjusted model, the effect estimate was attenuated and non-significant (HR 1.05; 95%CI 0.76 to 1.46; Table 2).

Percentage of sessions with intra-dialytic hypertension and mortality

In sensitivity analyses, the mean percentage of baseline exposure sessions complicated with intra-dialytic hypertension was 32% for Hyper0, 18% for Hyper10, and 10% for Hyper20 definitions. In the main adjusted model, a higher proportion of baseline sessions with Hyper0 (per 10%) was associated with 13% higher risk of all-cause mortality (HR 1.13; 95%CI 1.07-1.20); for Hyper10 (per 10%) there was a 7% higher risk (HR 1.07; 95%CI 1.01-1.14); for Hyper20 (per 10%) there was an 8% higher risk (HR 1.08; 95%CI 1.00-1.16). Both unadjusted and adjusted effect estimates are presented in Table 3.

Systolic BP decline and mortality

In sensitivity analyses, during the baseline exposure period, the average decline in systolic BP from pre-HD to post-HD was 13 ±16 mmHg (median 12 [3 to 22] mmHg). In unadjusted analyses, each 10mmHg increment in pre-to-post systolic BP (i.e., a decline in systolic BP) was associated with a 20% lower risk of all-cause mortality (HR 0.80; 95%CI 0.76 to 0.85). This effect estimate was marginally attenuated in the main adjusted model and remained statistically significant (HR 0.82; 95%CI 0.74 to 0.91). The adjusted risk of death was higher for individuals whose BP increased from pre- to post-HD (Figure 3).

Effect modification and sub-group analyses

As Hyper0 had the most potent association with mortality, tests for interaction were performed with this exposure in the fully adjusted model (Model 3). There was no evidence of effect modification according to sex (HR 0.97, 95%CI 0.67 to 1.41; p=0.89), pre-HD systolic BP (HR 0.99, 95%CI 0.98 to 1.01; p=0.15), ultrafiltration rate (HR 1.01, 95%CI 0.96 to 1.01; p=0.71), presence of diabetes (HR 1.02, 95%CI 0.69 to 1.52; p=0.91), or presence of heart failure (HR 0.74, 95%CI 0.50 to 1.08; pP=0.12). However, the association of pre-HD to post-HD BP decline was modified by age (HR 0.98, 95%CI 0.97 to 1.0; p=0.02) and presence of PVD (HR 0.57, 95%CI 0.36 to 0.91; p=0.02), such that the association of Hyper0 with mortality was most apparent in those aged 45-70 years (Figure 4) and those without PVD at baseline (HR 1.37; 95%CI 1.06 to 1.76, versus 0.86; 95%CI 0.51 to 1.45 in those with PVD).

DISCUSSION

In a large cohort study of patients receiving maintenance HD, we examined the association of several definitions of intradialytic hypertension with all-cause mortality, finding that any increase from pre- to post-HD systolic BP had the most potent relationship with longer-term mortality. Further, we found that the association was different according to the age of patients and was predominantly in those without baseline PVD.

Despite being named intradialytic hypertension, this metric is typically calculated as an increase in systolic BP from pre- to post-HD, thereby ignoring the actual intradialytic excursions. Several hypotheses have been put forward to explain the pathophysiology underlying the development of intradialytic hypertension, with most focusing on contributions from hypervolemia and endothelial dysfunction.[3,17] In particular, several studies have examined a potential imbalance between vasoconstrictive and vasodilatory mediators, with an excess of endothelin-1 thought to play a central role.[5,6,18–20] Our prior work has also found an association of higher pre-HD endothelin-1 concentrations with higher pre-HD, intra-HD, and post-HD systolic BP. Conversely, in a smaller open-label interventional study involving 25 patients prone to develop intradialytic hypertension, treatment with carvedilol resulted in improved flow-mediated vasodilation and lower frequency of intradialytic hypertension, without major changes in endothelin concentrations.[20] Overall, this highlights the complexity of this condition and suggests the pathogenesis is likely multifactorial.

The prevalence of intradialytic hypertension is estimated to range from 13-23%, reflecting the lack of a standard definition, which has limited comparisons among and between different patient populations and studies. In our study, the prevalence of baseline intradialytic

hypertension was as high as 47% with the Hyper0 definition (any increase in systolic BP) and as low as 6.8% when defined as Hyper20 (any increase ≥20 mmHg). The proportion of patients meeting the Hyper0 definition in our study is higher than that in a large observational report by Park et al.,[14] which may partially reflect differences in demographics and comorbid disease burden in our study (e.g., higher proportion of Hispanic patients, patients with diabetes, and with PVD).

Although Park et al. did not critically examine different definitions of intradialytic hypertension, our finding that Hyper0 had the most potent association with mortality is consistent with the results of their analyses, which also found a higher risk of death with any increase in systolic BP from pre- to post-HD.[14] However, in contrast to our results, Park et al. observed a U-shaped association of both higher and lower BP decline with mortality. This discrepancy may partly relate to the fact that our models adjusted for the nadir of intradialytic SBP, while the models used by Park et al more extensively adjusted for biomarkers of malnutrition and inflammation. Furthermore, the association of more extreme definitions of intra-dialytic hypertension lost significance in our fully adjusted model, which was in contrast to the results reported by Inrig et al. in a post-hoc analysis of the CLIMB study and by Losito et al. in an observational report from an Italian cohort.[11,12] For example, in the CLIMB study of 443 patients receiving maintenance HD, a higher risk of the composite endpoint of 6-month non-access related hospitalization or death was noted with systolic BP increases ≥10 mmHg (odds ratio 2.17; 95%CI 1.13 to 4.15),[12] while similar findings were reported in the Italian study for all-cause mortality. In addition to the lower proportion of patients experiencing these more extreme definitions (and correspondingly fewer death events), our study had a higher proportion of

Hispanic patients and patients with diabetes, compared with both other reports, while the CLIMB participants were initially enrolled as part of a randomized trial. Other studies have reported a higher risk of mortality with systolic BP increases of ≥5 mmHg (vs. <5 mmHg),[16] while similar patterns of association have been noted for shorter-term outcomes[15] and in incident HD patients,[13] further highlighting the difficulty of comparing definitions across studies.

While our study is not able to determine the pathophysiology of why intradialytic hypertension might lead to mortality, one may specula whether potential mediators, such as hypervolemia and endothelin may play a role. For example, hypervolemia is associated with increased afterload and cardiac structural changes,[21] while higher serum ET-1 concentrations are associated with development of CV disease and progression of CKD, potentially through inflammatory and fibrotic pathways.[22] While we did not have endothelin measurements in this cohort, based on prior studies and the proposed pathophysiology, we examined for effect modification according to other pre-specified and biologically relevant parameters. Similar to the findings of Park et al., we did not find evidence for differences in mortality associations according to ultrafiltration rate, diabetes, or heart failure. On the other hand, we did note that the association of Hyper0 with mortality was most apparent in those aged 45-70 years and in those without PVD at baseline. Although these should be only considered as hypothesis-generating, based on the observed lack of association of Hyper0 with mortality at the higher age range, it is tempting to speculate that the pathophysiology and downstream effects of abnormal BP control may be different in this group. It is harder to draw more meaningful conclusions from the lower age group and in those with PVD, based on the relatively lower sample size and the overall paucity of events.

The present study has many strengths, such as the large sample size, duration of follow-up, and availability of detailed HD-related hemodynamic and treatment-specific data. However, there are also some limitations. Despite multivariable adjustment, this is an observational study with the potential for residual confounding and inherent inability to confer causality. Secondly, due to a lack of granularity in cause-specific mortality data, we were unable to specifically examine for associations with cardiovascular mortality. Additionally, the paucity of individuals (and events) meeting the more extreme definitions of intradialytic hypertension and in sub-groups of older age and PVD limited our power to detect significant differences. Although prespecified, the testing of multiple interactions terms increases the likelihood of detecting false positive results, necessitating caution in their interpretation. As the cohort consists of patients in the US, there may be limitations in generalizing the results to patients from other geographic areas with different healthcare systems. Further, due to a lack of medication data, data on bioimpedance, data on patient symptoms, and intradialytic fluid administration, we were unable to incorporate these into our current analyses. Finally, BPs in this study were collected in the setting of standard clinical practice – while reflective of real-world data, they lack the robustness and reproducibility of protocolized measurements.

In conclusion, in this contemporary US-based HD cohort, we evaluated the associations of three independent definitions of intradialytic hypertension with longer-term mortality. We found the most potent association was for participants who experienced any increase in systolic BP from pre- to post-HD. While effect modification according to age and PVD requires further examination, our results are important for risk stratification of current HD patients and may inform the design and recruitment of high-risk patients to future interventional studies.

Table 1. Baseline characteristics according to ≥0 mmHg increase in pre- to post-HD systolic BP (Hyper0)

	(-) Intradialytic HTN n=1,696	(+) Intradialytic HTN n=1,502	P
Pre- minus post-HD systolic BP, mmHg	24 ± 11	1 ± 9	<0.001
Age, yrs	61 ±15	64 ±16	<0.001
Male, n (%)	1019 (60.1%)	809 (53.9%)	<0.001
Black, n (%)	237 (14.0%)	197 (13.1%)	0.48
Hispanic, n (%)	574 (33.8%)	453 (30.2%)	0.03
Access, n (%)			<0.001
AVF	1136 (67.0%)	879 (58.5%)	
AVG	303 (17.9%)	273 (18.2%)	
Catheter	257 (15.2%)	350 (23.3%)	
Diabetes, n (%)	1005 (59.3%)	924 (61.5%)	0.19
Heart Failure, n (%)	368 (21.7%)	414 (27.6%)	<0.001
PVD, n (%)	205 (12.1%)	216 (14.4%)	0.06
Ultrafiltration rate, mL/kg/hr	12.4 ± 4.4	11.3 ± 4.5	<0.001
Higher DNa,[a] n (%)	362 (21.3%)	313 (20.8%)	0.73
Serum Sodium, mmol/L	137 ± 3	137 ± 3	0.30
Blood Urea Nitrogen, mg/dL	69 ± 16	64 ± 18	<0.001
Serum Phosphorus, mg/dL	5.6 ± 1.4	5.2 ± 1.3	<0.001
Serum Albumin, g/dL	3.9 ± 0.3	3.8 ± 0.4	<0.001
Serum Creatinine, mg/dL	9.1 ± 3.0	7.9 ± 2.9	<0.001
Serum Calcium, mg/dL	9.0 ± 0.7	8.9 ± 0.7	0.001
Serum glucose, mg/dL	142 ± 64	141 ± 58	0.89
Pre-HD systolic BP, mmHg	154 ± 19	143 ± 19	<0.001
Nadir intra-HD systolic BP, mmHg	107 ± 18	115 ± 21	<0.001
Post-HD systolic BP, mmHg	130 ± 15	142 ± 18	<0.001

HTN, hypertension; HD, hemodialysis; SBP, systolic blood pressure; AVF, arteriovenous fistula; AVG, arteriovenous graft; PVD, peripheral vascular disease; DNa, dialysate sodium

[a] Higher defined as > 140 mEq/L or sodium modeling

Table 2. Association of various definitions of intradialytic hypertension with all-cause mortality

	No. deaths/ No. participants		Hazard Ratio (95% CI)			
	(-) Intra-HD HTN (Reference)	(+) Intra-HD HTN	Unadjusted	Model 1	Model 2	Model 3
Hyper0	251/1,766 (14.2%)	397/1,642 (24.2%)	1.77 (1.49 to 2.10)	1.58 (1.28 to 1.95)	1.50 (1.21 to 1.86)	1.29 (1.03 to 1.62)
Hyper10	451/2,657 (17%)	197/751 (26.2%)	1.61 (1.34 to 1.95)	1.39 (1.12 to 1.72)	1.35 (1.09 to 1.68)	1.21 (0.96 to 1.51)
Hyper20	587/3,162 (18.6%)	61/245 (24.9%)	1.49 (1.11 to 2.00)	1.28 (0.94 to 1.75)	1.15 (0.84 to 1.58)	1.05 (0.76 to 1.46)

Hyper0 – 30% or more baseline sessions with no change or an increase in post-SBP from the pre-SBP measurement
Hyper10 - 30% or more baseline sessions with an increase in post-SBP from the pre-SBP measurement of 10 mmHg or greater
Hyper10 - 30% or more baseline sessions with an increase in post-SBP from the pre-SBP measurement of 20 mmHg or greater

Table 3. Association of various definitions of intradialytic hypertension with all-cause mortality per 10% of baseline sessions affected.

	Hazard Ratio (95% CI) per 10% of baseline sessions with intra-dialytic hypertension			
	Unadjusted	**Model 1**	**Model 2**	**Model 3**
Hyper0	1.15 (1.11 to 1.19)	1.16 (1.10 to 1.22)	1.14 (1.09 to 1.20)	1.13 (1.07 to 1.20)
Hyper10	1.14 (1.10 to 1.19)	1.11 (1.05 to 1.17)	1.10 (1.04 to 1.16)	1.07 (1.01 to 1.14)
Hyper20	1.17 (1.10 to 1.23)	1.12 (1.05 to 1.20)	1.11 (1.03 to 1.18)	1.08 (1.00 to 1.16)

Figure 1. Consort Diagram

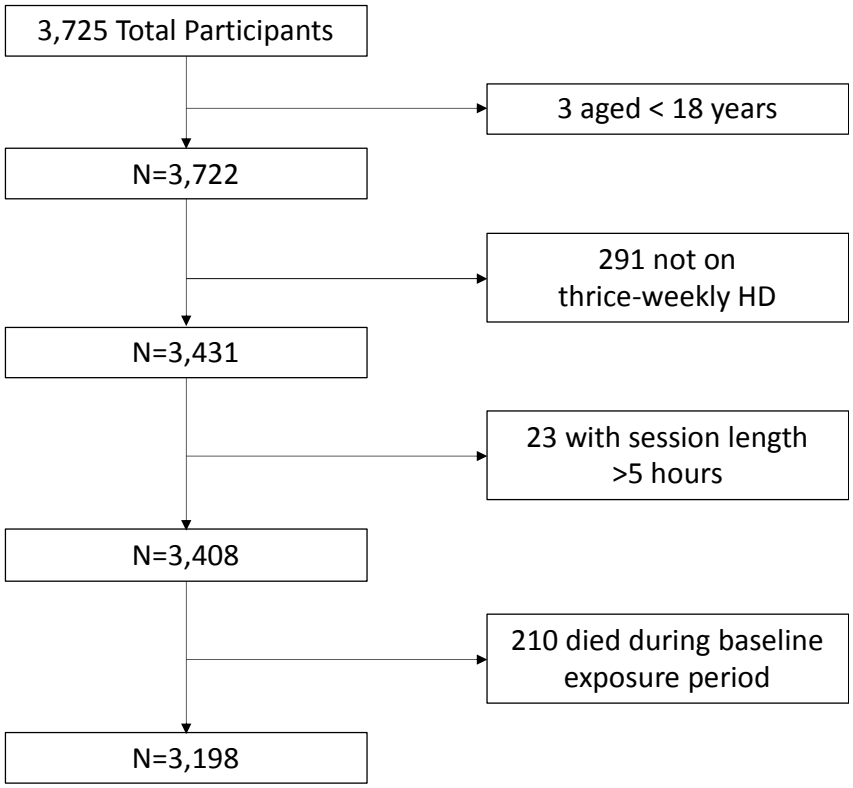
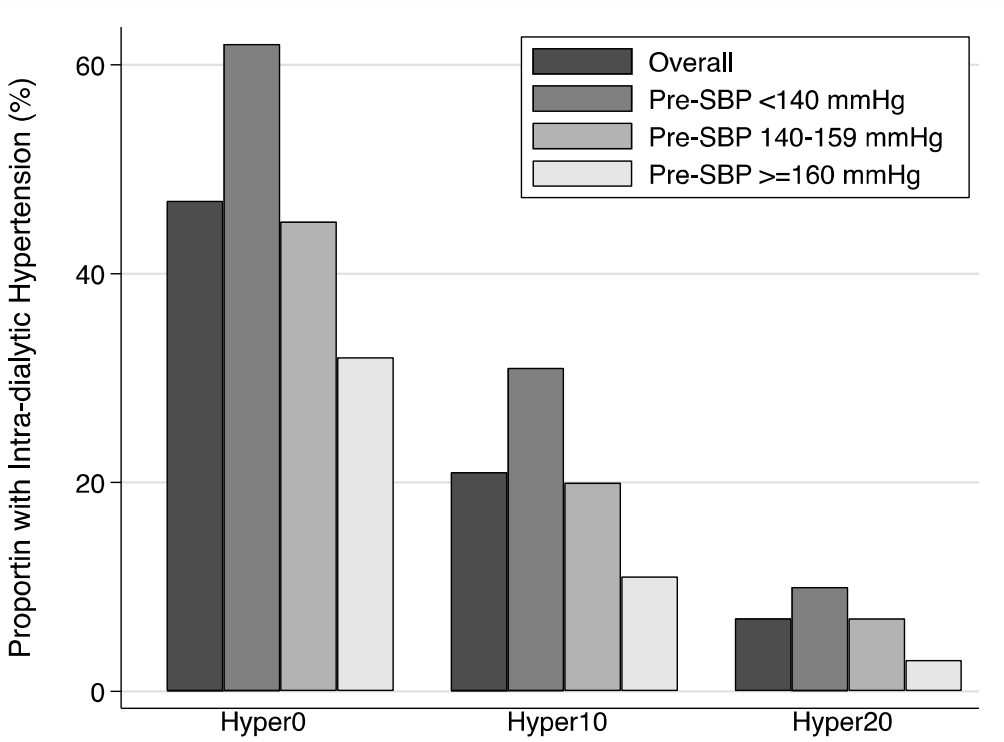


Figure 2. The proportion of patients experiencing intradialytic hypertension during the baseline period, according to various definitions and categories of pre-HD systolic blood pressure



Hyper0 – 30% or more baseline sessions with no change or an increase in post-SBP from the pre-SBP measurement

Hyper10 - 30% or more baseline sessions with an increase in post-SBP from the pre-SBP measurement of 10 mmHg or greater

Hyper10 - 30% or more baseline sessions with an increase in post-SBP from the pre-SBP measurement of 20 mmHg or greater

Figure 3. Adjusted association of change in pre- to post-HD systolic BP with mortality

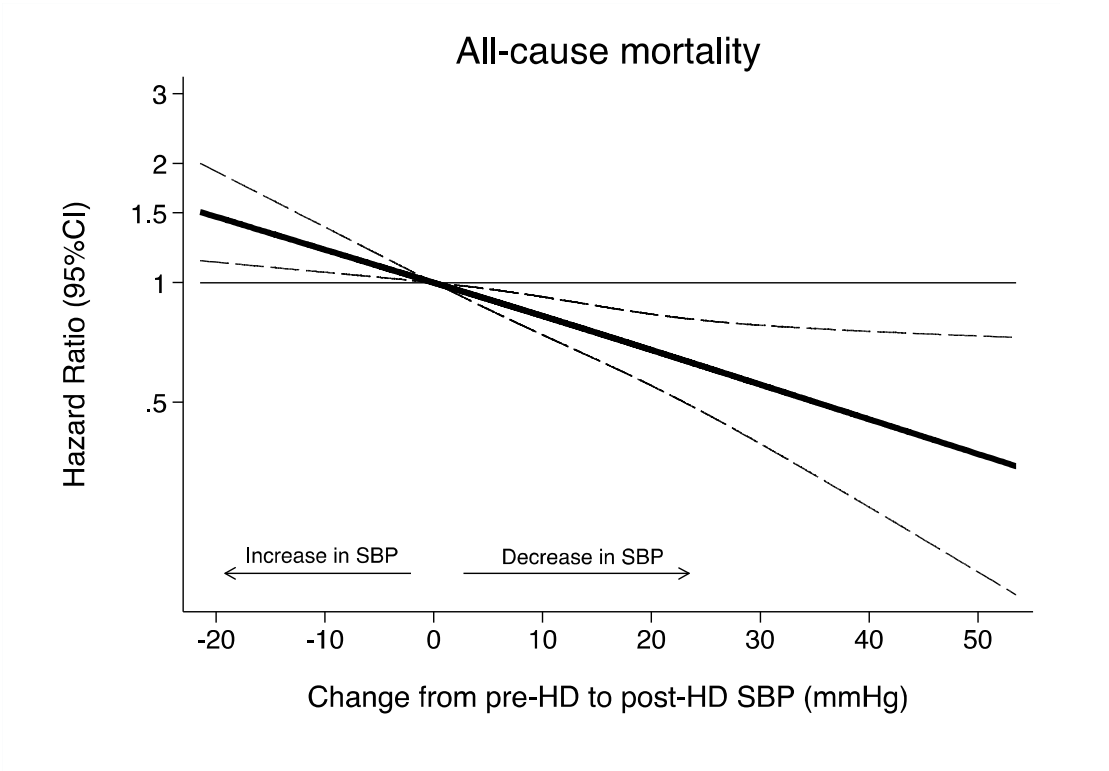
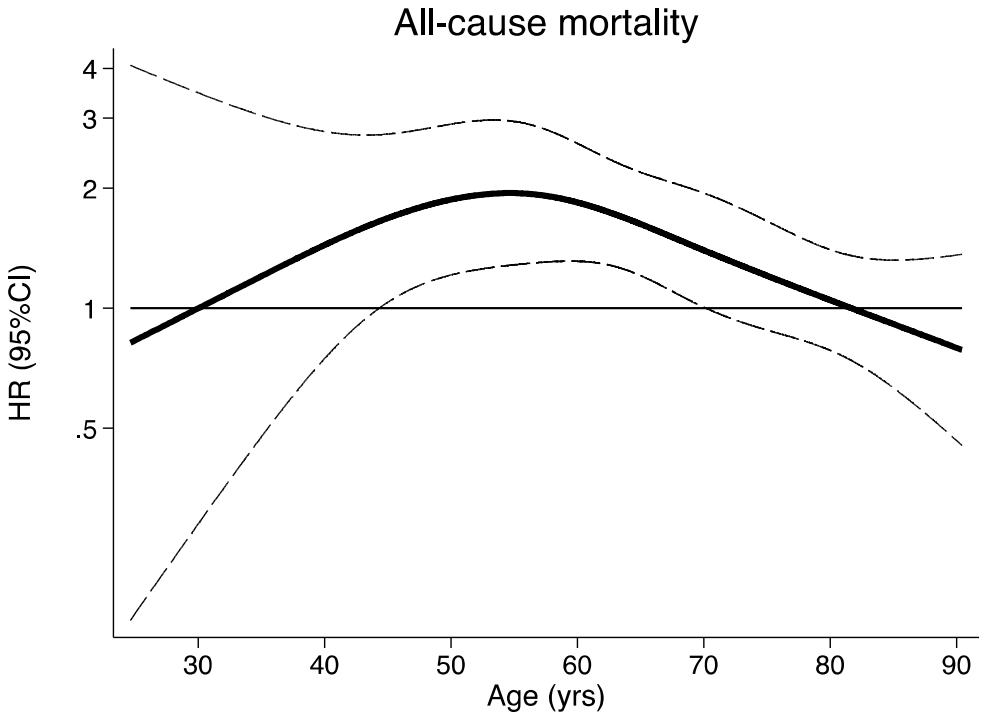


Figure 4. Association of any increase SBP from pre- to post-HD (Hyper0) with mortality, according to age



The association of pre-HD to post-HD BP decline was modified by age (HR 0.98, 95%CI 0.97 to 1.0; p=0.02)

REFERENCES

1. USRDS. United States Renal Data System Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2019. *USRDS* 2019;

2. Flythe JE, Chang TI, Gallagher MP, Lindley E, Madero M, Sarafidis PA, Unruh ML, Wang AY-M, Weiner DE, Cheung M, Jadoul M, Winkelmayer WC, Polkinghorne KR, Adragão T, Anumudu SJ, Chan CT, Cheung AK, Costanzo MR, Dasgupta I, Davenport A, Davies SJ, Dekker MJE, Dember LM, Gallego D, Gómez R, Hawley CM, Hecking M, Iseki K, Jha V, Kooman JP, et al. Blood Pressure and Volume Management in Dialysis: Conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int* 2020;**97**:861–876.

3. Assimon MM, Flythe JE. Intradialytic Blood Pressure Abnormalities: The Highs, The Lows and All That Lies Between. *American Journal of Nephrology* 2015;**42**:337–350.

4. Dubin R, Owens C, Gasper W, Ganz P, Johansen K. Associations of endothelial dysfunction and arterial stiffness with intradialytic hypotension and hypertension. *Hemodial Int* 2011;**15**:350–358.

5. Teng J, Tian J, Lv W, Zhang X, Zou J, Fang Y, Yu J, Shen B, Liu Z, Ding X. Endothelin-1 and dialytic hypertension. *Hemodial Int* 2015;**19**:279–286.

6. Raj DSC, Vincent B, Simpson K, Sato E, Jones KL, Welbourne TC, Levi M, Shah V, Blandon P, Zager P, Robbins RA. Hemodynamic changes during hemodialysis: Role of nitric oxide and endothelin. *Kidney Int* 2002;**61**:697–704.

7. Chou K-J, Lee P-T, Chen C-L, Chiou C-W, Hsu C-Y, Chung H-M, Liu C-P, Fang H-C. Physiological changes during hemodialysis in patients with intradialysis hypertension. *Kidney Int* 2006;**69**:1833–1838.

8. Krapf R, Hulter HN. Arterial Hypertension Induced by Erythropoietin and Erythropoiesis-Stimulating Agents (ESA). *Clin J Am Soc Nephro* 2009;**4**:470–480.

9. Chang TI, Abdalla S, London GM, Block GA, Correa-Rotter R, Drüeke TB, Floege J, Herzog CA, Mahaffey KW, Moe SM, Parfrey PS, Wheeler DC, Dehmel B, Goodman WG, Chertow GM. The effects of cinacalcet on blood pressure, mortality and cardiovascular endpoints in the EVOLVE trial. *J Hum Hypertens* 2016;**30**:204–209.

10. Buren PNV, Kim C, Toto RD, Inrig JK. The Prevalence of Persistent Intradialytic Hypertension in a Hemodialysis Population with Extended Follow-Up. *Int J Artif Organs* 2012;**35**:1031–1038.

11. Losito A, Vecchio LD, Rosso GD, Locatelli F. Postdialysis Hypertension: Associated Factors, Patient Profiles, and Cardiovascular Mortality. *Am J Hypertens* 2016;**29**:684–689.

12. Inrig JK, Oddone EZ, Hasselblad V, Gillespie B, Patel UD, Reddan D, Toto R, Himmelfarb J, Winchester JF, Stivelman J, Lindsay RM, Szczech LA. Association of intradialytic blood pressure changes with hospitalization and mortality rates in prevalent ESRD patients. *Kidney Int* 2007;**71**:454–461.

13. Inrig JK, Patel UD, Toto RD, Szczech LA. Association of blood pressure increases during hemodialysis with 2-year mortality in incident hemodialysis patients: a secondary analysis of the Dialysis Morbidity and Mortality Wave 2 Study. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2009;**54**:881–890.

14. Park J, Rhee CM, Sim JJ, Kim Y-L, Ricks J, Streja E, Vashistha T, Tolouian R, Kovesdy CP, Kalantar-Zadeh K. A comparative effectiveness research study of the change in blood pressure during hemodialysis treatment and survival. *Kidney international* 2013;**84**:795–802.

15. Assimon MM, Wang L, Flythe JE. Intradialytic Hypertension Frequency and Short-Term Clinical Outcomes Among Individuals Receiving Maintenance Hemodialysis. *Am J Hypertens* 2017;**31**:329–339.

16. Yang C-Y, Yang W-C, Lin Y-P. Postdialysis blood pressure rise predicts long-term outcomes in chronic hemodialysis patients: a four-year prospective observational cohort study. *Bmc Nephrol* 2012;**13**:12.

17. Buren PNV, Inrig JK. Special situations: Intradialytic hypertension/chronic hypertension and intradialytic hypotension. *Semin Dialysis* 2017;**30**:545–552.

18. Tomić M, Galešić K, Markota I. Endothelin-1 and Nitric Oxide in Patients on Chronic Hemodialysis. *Renal Failure* 2009;**30**:836–842.

19. Ottosson-Seeberger A, Ahlborg G, Hemsén A, Lundberg JM, Alvestrand A. Hemodynamic effects of endothelin-1 and big endothelin-1 in chronic hemodialysis patients. *J Am Soc Nephrol JASN* 1999;**10**:1037–1044.

20. Inrig JK, Buren PV, Kim C, Vongpatanasin W, Povsic TJ, Toto R. Probing the mechanisms of intradialytic hypertension: a pilot study targeting endothelial cell dysfunction. *Clinical journal of the American Society of Nephrology : CJASN* 1300;**7**:1300–1309.

21. Agarwal R. Hypervolemia is associated with increased mortality among hemodialysis patients. *Hypertension*. 2010;56(3):512-517. doi:10.1161/HYPERTENSIONAHA.110.154815

22. Kohan DE, Barton M. Endothelin and endothelin antagonists in chronic kidney disease. *Kidney Int*. 2014;86(5):896-904. doi:10.1038/ki.2014.143

Supplementary Table 1. Baseline characteristics of included and excluded patients

	Excluded n=527	Included n=3198	P
Pre- minus post-SBP, mmHg	11 ± 17	13 ± 15	<0.001
Age, yrs	64 ±15	62 ±15	0.02
Male, n (%)	315 (59.8%)	1828 (57.2%)	0.26
Black, n (%)	56 (10.6%)	434 (13.6%)	0.06
Hispanic, n (%)	134 (25.4%)	1027 (32.1%)	0.002
Access, n (%)			<0.001
AVF	299 (56.7%)	2003 (62.6%)	
AVG	87 (16.5%)	574 (17.9%)	
Catheter	141 (26.8%)	621 (19.4%)	
Diabetes, n (%)	314 (59.6%)	1929 (60.3%)	0.75
Heart Failure, n (%)	170 (32.3%)	782 (24.5%)	<0.001
PVD, n (%)	92 (17.5%)	421 (13.2%)	0.01
Ultrafiltration rate, mL/kg/hr	11.0 ± 5.1	11.8 ± 4.5	<0.001
Higher DNa,[a] n (%)	117 (22.2%)	687 (21.5%)	0.71
Serum Sodium, mmol/L	136 ± 3	137 ± 3	0.30
Blood Urea Nitrogen, mg/dL	62 ± 17	66 ± 17	<0.001
Serum Phosphorus, mg/dL	5.2 ± 1.5	5.4 ± 1.4	0.01
Serum Albumin, g/dL	3.7 ± 0.5	3.8 ± 0.4	<0.001
Serum Creatinine, mg/dL	7.5 ± 2.8	8.5 ± 3.0	<0.001
Serum Calcium, mg/dL	8.8 ± 0.7	8.9 ± 0.7	0.02
Serum glucose, mg/dL	149 ± 70	142 ± 62	0.02
Pre-HD SBP, mmHg	143 ± 23	149 ± 19	<0.001
Nadir intra-HD SBP, mmHg	108 ± 21	111 ± 19	<0.001
Post-HD SBP, mmHg	132 ± 19	136 ± 18	<0.001

HTN, hypertension; HD, hemodialysis; SBP, systolic blood pressure; AVF, arteriovenous fistula; AVG, arteriovenous graft; PVD, peripheral vascular disease; DNa, dialysate sodium

[a] Higher defined as > 140 mEq/L or sodium modeling

Supplementary Table 2. Baseline characteristics according to ≥10 mmHg increase in pre- to post-HD systolic BP (Hyper10)

	(-) Intradialytic HTN n=2519	(+) Intradialytic HTN n=679	P
Pre- minus post-SBP, mmHg	18 ± 13	-6 ± 8	<0.001
Age, yrs	61 ±15	65 ±14	<0.001
Male, n (%)	1477 (58.6%)	351 (51.7%)	<0.001
Black, n (%)	354 (14.1%)	80 (11.8%)	0.13
Hispanic, n (%)	820 (32.6%)	207 (30.5%)	0.31
Access, n (%)			<0.001
AVF	1628 (64.6%)	387 (57.0%)	
AVG	445 (17.7%)	131 (19.3%)	
Catheter	446 (17.7%)	161 (23.7%)	
Diabetes, n (%)	1488 (59.1%)	441 (64.9%)	0.005
Heart Failure, n (%)	593 (23.5%)	189 (27.8%)	0.02
PVD, n (%)	317 (12.6%)	104 (15.3%)	0.06
Ultrafiltration rate, mL/kg/hr	12.0 ± 4.4	11.3 ± 4.6	<0.001
Higher DNa,[a] n (%)	536 (21.3%)	139 (20.5%)	0.65
Serum Sodium, mmol/L	137 ± 3	136 ± 3	0.04
Blood Urea Nitrogen, mg/dL	68 ± 17	63 ± 17	<0.001
Serum Phosphorus, mg/dL	5.5 ± 1.4	5.2 ± 1.3	<0.001
Serum Albumin, g/dL	3.9 ± 0.3	3.7 ± 0.4	<0.001
Serum Creatinine, mg/dL	8.8 ± 3.0	7.5 ± 2.5	<0.001
Serum Calcium, mg/dL	8.9 ± 0.7	8.8 ± 0.7	0.003
Serum glucose, mg/dL	140 ± 62	145 ± 61	0.10
Pre-HD SBP, mmHg	151 ± 19	141 ± 18	<0.001
Nadir intra-HD SBP, mmHg	109 ± 19	117 ± 21	<0.001
Post-HD SBP, mmHg	133 ± 16	147 ± 18	<0.001

HTN, hypertension; HD, hemodialysis; SBP, systolic blood pressure; AVF, arteriovenous fistula; AVG, arteriovenous graft; PVD, peripheral vascular disease; DNa, dialysate sodium

[a] Higher defined as > 140 mEq/L or sodium modeling

Supplementary Table 3. Baseline characteristics according to ≥20 mmHg increase in pre- to post-HD systolic BP (Hyper20)

	(-) Intradialytic HTN n=2981	(+) Intradialytic HTN n=217	P
Pre- minus post-SBP, mmHg	15 ± 14	-14 ± 9	<0.001
Age, yrs	62 ±15	65 ±14	0.005
Male, n (%)	1724 (57.8%)	104 (47.9%)	0.004
Black, n (%)	409 (13.7%)	25 (11.5%)	0.36
Hispanic, n (%)	954 (32.0%)	73 (33.6%)	0.62
Access, n (%)			0.02
AVF	1897 (63.6%)	118 (54.4%)	
AVG	531 (17.8%)	45 (20.7%)	
Catheter	553 (18.6%)	54 (24.9%)	
Diabetes, n (%)	1770 (59.4%)	159 (73.3%)	<0.001
Heart Failure, n (%)	720 (24.2%)	62 (28.6%)	0.14
PVD, n (%)	380 (12.7%)	41 (18.9%)	0.01
Ultrafiltration rate, mL/kg/hr	11.9 ± 4.5	11.4 ± 4.8	0.13
Higher DNa,[a] n (%)	631 (21.2%)	44 (20.3%)	0.76
Serum Sodium, mmol/L	137 ± 3	136 ± 3	<0.001
Blood Urea Nitrogen, mg/dL	67 ± 17	63 ± 17	0.001
Serum Phosphorus, mg/dL	5.4 ± 1.4	5.3 ± 1.5	0.23
Serum Albumin, g/dL	3.8 ± 0.4	3.7 ± 0.4	<0.001
Serum Creatinine, mg/dL	8.6 ± 3.0	7.5 ± 2.4	<0.001
Serum Calcium, mg/dL	8.9 ± 0.7	8.8 ± 0.7	0.05
Serum glucose, mg/dL	141 ± 61	152 ± 63	0.01
Pre-HD SBP, mmHg	149 ± 19	141 ± 16	<0.001
Nadir intra-HD SBP, mmHg	110 ± 19	120 ± 20	<0.001
Post-HD SBP, mmHg	134 ± 17	154 ± 16	<0.001

HTN, hypertension; HD, hemodialysis; SBP, systolic blood pressure; AVF, arteriovenous fistula; AVG, arteriovenous graft; PVD, peripheral vascular disease; DNa, dialysate sodium

[a] Higher defined as > 140 mEq/L or sodium modeling

Manuscript 2:

Endothelin-1 and Parameters of Systolic Blood Pressure in Hemodialysis

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Running Title: Endothelin and Blood Pressure in Hemodialysis

Data Availability Statement:

The data used in these analyses were provided by DaVita Clinical Research. Requests for access to data can be made in writing to DaVita Clinical Research.

ABSTRACT

Background:

Hypertension is common in hemodialysis (HD) patients. Increased blood pressure (BP) variability, particularly higher and lower extremes, is associated with adverse outcomes. We explored the association of endothelin-1 (ET-1), a potent vasoconstrictor, with changes in BP during HD in a contemporary patient cohort.

Methods:

This study uses the DaVita Biorepository, a longitudinal prospective cohort study with quarterly collection of clinical data and biospecimens. Unadjusted and adjusted linear mixed effects regression models were fit to determine association of pre-HD ET-1 (log-transformed and quartiles) with HD-related systolic BP (SBP) parameters (pre-HD, nadir intra-HD, and post-HD). As ET-1 was measured at baseline, analyses were restricted to one-year of follow-up.

Results

Among 769 participants, the mean age was 52 years, 42% were females, and 41% were black. Mean pre-HD SBP was 152 (± 28) mmHg and mean ET-1 concentration was 2.3 (±1.2) ng/mL. In fully adjusted models, each unit increase in SD of log-transformed ET-1 was associated with a 3.2 (95% CI 2.0, 4.4) mmHg higher pre-SBP; 1.9 (95%CI 1.1, 2.6) mmHg higher nadir SBP; and 2.2 (95% CI 1.3, 3.1) mmHg higher post-SBP. Each SD increase in log-transformed ET-1 was associated with 24% higher odds of experiencing intradialytic hypertension (OR 1.24; 95%CI 1.13 to 1.37).

Conclusions

Higher baseline ET-1 levels are independently associated with higher SBP and higher odds of intradialytic hypertension. These results highlight a potential role for ET-1 in BP control in HD patients and raise the possibility of ET-1 antagonism as a therapeutic target.

Keywords: hemodialysis; hypertension, blood pressure; endothelin

INTRODUCTION

Cardiovascular (CV) disease is the leading cause of mortality in patients with end-stage kidney disease (ESKD), accounting for approximately 40% of deaths[1]. While hypertension is extremely common in patients with ESKD[2], both extremes of lower and higher pre-dialysis systolic blood pressure (SBP) are known to be associated with adverse outcomes.[3] Indeed, intradialytic hypertension, defined as an increase in SBP of ≥10mmHg from pre- to post-HD, is associated with increased morbidity and mortality in HD patients.[4,5,6]

There are a multitude of factors that contribute to BP control in HD patients, including positive sodium balance and volume overload,[7] activation of the renin-angiotensin-aldosterone (RAAS) and sympathetic nervous systems,[8] removal of antihypertensive medications by hemodialysis,[9] the use of erythropoiesis-stimulating agents (ESA),[10] and abnormalities of mineral bone disease axes.[11] Of particular note, endothelial cell dysfunction may play a central role via the synthesis and release of humoral factors such as endothelin-1 (ET-1), a potent vasoconstrictor.[12] This is particularly relevant, as pharmacologic antagonists of endothelin receptors are available and have been tested previously in patients with chronic kidney disease (CKD).[13]

Although prior studies have reported that ET-1 concentrations appear to be higher in HD patients, compared with non-HD controls, these were limited in size and duration of follow up[5,14]. Herein, we examine the association of ET-1 with HD-related BP parameters using a large contemporary and prospective patient cohort. We hypothesized that higher levels of baseline ET-1 would be associated with higher pre-dialysis SBP and development of intradialytic hypertension.

METHODS

Study Population

The current analyses were performed using a prospective cohort of anonymized samples and statistically de-identified clinical data from a biorepository assembled by DaVita Clinical Research (DCR) and made available to academic organizations through the Biospecimen Research Grant (BioReG) program. Patients who were <18 years-old, with Hgb <8.0 g/dL, pregnant, or with any physical, mental, or medical condition which limited the ability to provide written informed consent were excluded from BioReG. The present study only included patients undergoing thrice-weekly HD. The sampling protocol was approved by an Institutional Review Board (Quorum IRB, Seattle, WA, USA) and patients provided written informed consent prior to the initiation of sample collection. All clinical and hemodialysis prescription data were collected from the electronic medical record. A subset of the total cohort was provided to each of four academic institutions by DCR in a deidentified format. From a cohort of 976 participants, those not on thrice weekly hemodialysis treatment and those with ET-1 measured at baseline were excluded from our analyses, resulting in an analytic cohort of 769 participants.

Biospecimen collection and storage

Biospecimens were collected and processed according to a standardized protocol, including shipping on refrigerated packs on the same day as collection, processing, aliquoting, and storage at -80°C. Re-collection was requested for any specimen with cause for rejection (e.g., unspun tubes, insufficient volume, or thawed specimens). Specimens received > 48h from the time of collection were also rejected and re-collected. Samples were distributed frozen at -80°C across the four academic medical centers.

Exposure

The primary exposure for this study was plasma endothelin-1 (ET-1) concentration, measured at baseline (defined as first sample collection in the study). Since the distribution of ET-1 is right skewed, data were log-transformed for incorporation as a continuous variable in regression models. Effect estimates were reported per one unit increase in standard deviation (SD) to facilitate interpretability and comparison of results across the larger body of literature on this topic. Plasma ET-1 levels were measured in duplicate at Brigham and Women's Hospital using a commercially available ELISA kit (Quantikine Human ET-1 Immunoassay PDET100; R&D Systems, Minneapolis, MN). The mean inter-assay coefficient of variation from blind-split replicates was 7%.

Outcome Ascertainment

The primary outcome was defined as pre-HD treatment sitting SBP. Secondary outcomes included the nadir intradialytic SBP and post-HD SBP. We also examined the association of ET-1 with intradialytic hypertension (defined as either: 1) an increase in SBP of ≥10mmHg from pre- to post-HD; or 2) any increase in SBP from pre- to post-HD)[15] and intradialytic hypotension (defined as either an absolute intradialytic nadir SBP<90 mmHg in patients with a pre-HD SBP of <160 mmHg or nadir SBP<100 mmHg in patients with pre-HD SBP ≥160 mmHg)[16]. The primary analyses were restricted to a maximum of one year following the baseline ET-1 measurement. In sensitivity analyses, we restricted the follow-up time to 30 days.

Assessment of Other Covariates

Demographic information including age, race, sex, dialysis access and comorbid conditions including diabetes, heart failure, coronary artery disease, cerebrovascular disease, peripheral vascular disease and chronic obstructive pulmonary disease (COPD), were recorded at baseline and updated from the medical record throughout the study. Additional information such as hemodialysis prescription and vascular access, and laboratory data were measured on blood samples collected pre-HD. Dialysis session length was categorized (≤180 mins; 181-209 mins; 210-239 mins; ≥240 mins). Ultrafiltration volume was calculated by subtracting the post-dialysis weight from the pre-dialysis weight.

Statistical Analysis

All analyses were performed using R version 3.3.3 and Stata MP version 16 (StataCorp LP). Continuous variables were summarized using means (standard deviations) or medians (25^{th}-75^{th} percentiles) and compared with analysis of variance or Kruskal-Wallis tests, according to data distribution. Categorical variables were summarized as percentages and compared with Chi-squared tests. As ET-1 is non-normally distributed, ET-1 values were log-transformed for further analyses. Initially, unadjusted repeated measures regression models (to account for within person correlation) were fit to determine the association of ET-1 (first log-transformed and then in quartiles) with outcomes of interest. Subsequently, multivariable adjusted linear mixed effects models were fit, by adding a random intercept for subject-wise variability. Model 1 adjusted for age, sex, race, access, and pre-HD SBP (the latter was excluded from analyses where pre-HD SBP was the outcome of interest); Model 2 adjusted for the same variables as

model 1 with additional adjustment for categories of session length, ultrafiltration volume, diabetes, heart failure, ischemic heart disease (history of coronary artery disease, myocardial infarction, or angina), peripheral vascular disease, lung disease, and pre-HD SBP. We assessed for evidence of effect modification according to diabetes and gender[17,18] via the inclusion of cross-product terms. We also performed an exploratory analysis using Model 2 with additional adjustment for hemoglobin. The basis for the models was chosen based on clinical and biological plausibility. Analyses were performed without imputation for missing variables. A two-sided p-value <0.05 was considered to be statistically significant.

RESULTS

We examined data from 769 subjects and 110,315 HD sessions from the BioReG cohort (Figure 1). There was a higher frequency of females and a lower frequency of Black participants in those excluded, compared with those included, in the final analyses. Otherwise, baseline characteristics between these groups were similar (Supplementary Table 1). The mean age of included participants was 52 ±22 years, 42% were females, and 41% Black. Mean pre-HD SBP was 152 ±28 mmHg and mean ET-1 concentration was 2.3 ±1.2 ng/mL. Subjects in higher quartiles of baseline ET-1 tended to be younger, diabetic, have higher ultrafiltration volume, and have lower serum albumin (Table 1).

ET-1 and pre-HD systolic blood pressure

The baseline differences in HD-related blood pressure parameters according to quartiles of ET-1 are presented in Table 2. In unadjusted repeated measures analyses, each SD increase in log-

transformed ET-1 was associated with 3.4 (95%CI 2.2. to 4.6) mmHg higher pre-HD SBP. In the fully adjusted model, each SD increase in log-transformed ET-1 was associated with 3.2 (95%CI 2.0 to 4.4) mmHg higher pre-HD SBP. There was no evidence for effect modification according to gender (-0.3 [95%CI -2.7 to 2.1]; p=0.82) or diabetes (-0.7 [95%CI -1.8 to 0.5]; p=0.26). Similar patterns of associations were noted when adjusted analyses were restricted to 30-days of follow-up measurements (Supplementary Table 1).

In categorical analyses there was an increasing trend in the association of quartiles of ET-1 with pre-HD SBP (8.4 [95%CI 5.1 to 11.8] mmHg higher pre-HD SBP for Q4, compared with Q1; Figure 2).

ET-1 and nadir and post-HD systolic blood pressure

The association of ET-1 with other HD-related BP parameters was evaluated in unadjusted and adjusted repeated measures analysis (Table 3). Overall, in the fully adjusted model, each SD increase in log-transformed ET-1 was associated with 1.9 (95%CI 1.1 to 2.6) mmHg higher nadir SBP and 2.2 (95%CI 1.3 to 3.1) mmHg higher post-HD SBP. Similar patterns of associations were noted when adjusted analyses were restricted to 30-days of follow-up measurements (Supplementary Table 3).

In categorical analyses, there was a monotonic association of higher quartiles of ET-1 with higher SBP in all parameters of interest (Figure 2).

ET-1 and intradialytic hypertension and intradialytic hypotension

The proportion of sessions affected by intra-dialytic hypertension was 34.5% when defined as any increase from pre-to post-HD SBP. In adjusted analyses, each SD increase in log-transformed ET-1 was associated with 23% higher odds (OR 1.23; 95%CI 1.12 to 1.34) of experiencing this outcome. The proportion of sessions affected by intra-dialytic hypertension when defined as ≥10 mmHg increase in SBP was 20.3%. In adjusted analyses, each SD increase in log-transformed ET-1 was associated with 24% higher odds (OR 1.24; 95%CI 1.13 to 1.37) of experiencing this outcome.

The proportion of sessions affected by intra-dialytic hypotension was 13.7%. In adjusted analyses, each SD increase in log-transformed ET-1 was associated with a nominally, but statistically non-significant, lower risk of intradialytic hypotension (OR 0.91; 95%CI 0.83 to 1.01).

DISCUSSION

In this large contemporary cohort of maintenance HD patients, we observed that higher baseline ET-1 concentrations are associated with higher pre-HD SBP, nadir intradialytic SBP, and higher post-HD SBP. Similarly, higher ET-1 was associated with greater odds of developing intradialytic hypertension.

ET-1 is a 21-amino-acid peptide that was initially thought to be primarily an endothelium-derived vasoconstrictor but is now known to be produced by many other cell types[19], including podocytes[13] and glomerular endothelial cells[20]. There are two broad receptor isoforms, ET_A and ET_B. Binding to the ET_A receptor results in vasoconstriction, cell matrix accumulation and proliferation, while binding to ET_B produces the opposite counter-regulatory responses. ET-1 concentrations are elevated in several disease states and are associated with elevations in inflammatory markers, glomerular injury, and fibrosis in animal models.[13] Studies in non-CKD patients have reported elevated ET-1 levels in patients with atherosclerosis and highlight a central role in the development of hypertension via increased vascular resistance.[21] Increased levels of ET-1 have also been associated with development of CVD and progression of CKD.[22]

Not unexpectedly, ET-1 concentrations appear to be higher with more advanced stages of CKD[23, 24, 25]. In an ambulatory BP study of 27 patients with CKD, higher ET-1 was associated with higher mean BP and a lower frequency of nocturnal dipping, with some reversal of these findings following administration of an ET-1 antagonist[26]. Similar findings have been reported among patients on maintenance HD. For example, Teng et al compared 17 stable Chinese HD patients aged <75 years with intradialytic hypertension to 17 age- and sex-matched controls,

reporting that post-HD serum ET-1 concentrations were significantly higher in those who developed intradialytic hypertension (4.1 ± 2.1 vs. 2.8 ± 1.3 pg/mL, P-difference< 0.05).[27] Similar findings were reported by Chou et al from a slightly larger case-control study (n=60), where post-HD ET-1 concentrations were almost two-fold higher in those who developed intradialytic hypertension, compared with those who did not[28]. In addition, Ottosson-Seeberger et al. reported that infusions of ET-1 resulted in higher mean arterial pressure in five HD patients without a documented history of cardiac disease, supporting a central role for ET-1 in the regulation of BP in HD patients[29]. Our results from a much larger, contemporary cohort in the United States are consistent with these reports, and provide additional data related to the absolute magnitude of BP changes associated with ET-1 during the course of HD sessions. The association of higher ET-1 with hospitalization and death has been addressed by a prior analysis of the same cohort by Li et al. In fully adjusted models, they reported a 1.46-fold increased risk of death (HR 1.46, 95% CI 1.26 – 1.69) and 1.15-fold increased risk of hospitalization (HR 1.15, 95% CI 1.05 – 1.25) per unit increase in SD of log-transformed ET-1, further highlighting the potential importance of this pathway for adverse outcomes in HD patients.[30]

Our results confirm the presence of a pronounced association of ET-1 with BP control in HD patients. While some prior studies have reported higher ET-1 concentrations in normotensive[17] and hypertensive [18] Black populations, when compared with white populations, we did not find evidence for effect modification according to self-reported race in our analyses. Prior studies of have also reported higher ET-1 levels in non-ESRD patients with diabetes,[31, 32, 33] compared with non-diabetics. While data regarding differences in ET-1 in patients with diabetes

on maintenance HD is sparse, we did not find differences in baseline ET-1 according to diabetes, nor evidence of effect modification of the association with HD-related BP parameters. Our results are important, given the associations between intradialytic hypertension and adverse short term and long-term outcomes.[34,35] The relationship between intradialytic hypertension and mortality is complex and may related to downstream effects of potential predictors such as ET-1. Our results are particularly relevant given the availability of ET-1 receptors antagonists. Of note, the prior use of such agents in patients with CKD have been hampered by adverse effects related to hypervolemia[36, 37], providing the impetus for recent trials to have an enrichment period to identify those most likely to benefit and least likely to have adverse effects[37]. In theory, the risks of hypervolemia from ET receptor blockade may be lower in patients on maintenance HD therapy, who tend to be oligo-anuric and less prone to renal-mediated sodium retention.

There are several strengths to our study, including the relatively large sample size, duration of follow-up, and availability of detailed HD-related hemodynamic data. However, there are several limitations to mention. These include the use of a single baseline measurement of ET-1, non-availability of laboratory data for each HD session, limited data on the dialysate prescription, temperature, and potential for misclassification of covariables via the use of ICD-9 codes. Additionally, measures of bioimpedance, regular medications, and patient symptoms were not systematically recorded in this study. Furthermore, clinical blood pressures were used for the purposes of this study, per routine management and although these allow for a 'real-word' understanding, clinical blood pressure measurements may be unreliable. Indeed,

despite the performance of multivariable adjusted models, the potential for residual confounding remains. Furthermore, this was an observational study and therefore hypothesis generating. Finally, this represents a contemporary outpatient cohort from the US, which may not be generalizable to other cohorts.

In conclusion, we observed strong association between ET-1 and higher HD-related parameters of SBP. These results support a potential role of ET-1 in BP regulation in maintenance HD patients and provide rationale for testing ET-1 antagonists in future interventional studies.

Table 1. Baseline characteristics of participants according to quartiles of plasma endothelin-1

	Quartile 1 (n=193)	Quartile 2 (n=192)	Quartile 3 (n=192)	Quartile 4 (n=192)	P
Endothelin-1, ng/mL	1.3 [1.1, 1.4]	1.8 [1.7, 1.9]	2.3 [2.1, 2.5]	3.3 [2.9, 4.0]	<0.001
Age, yrs	56 ± 22	52 ± 22	53 ± 21	48 ± 22	<0.001
Female, n (%)	74 (38.3%)	86 (44.8%)	79 (41.1%)	80 (41.7%)	0.69
Pre-dialysis weight, kg	89.5 ± 23.8	91.1 ± 23.5	92.9 ± 24.9	90.2 ± 24.5	0.63
Ultrafiltration volume, L	1.9 ± 1.6	2.2 ± 1.6	2.0 ± 1.5	2.4 ± 1.5	0.004
Race, n (%)					0.84
White	81 (42.0%)	85 (44.3%)	80 (41.7%)	76 (39.6%)	
Black	68 (35.2%)	77 (40.1%)	85 (44.3%)	84 (43.8%)	
Other	44 (22.8%)	30 (15.6%)	27 (14.1%)	32 (16.7%)	
Access, n(%)					0.93
AVF	137 (71.0%)	124 (64.6%)	129 (67.2%)	131 (68.2%)	
AVG	24 (12.4%)	28 (14.6%)	29 (15.1%)	34 (17.7%)	
Tunneled Catheter	32 (16.6%)	40 (20.8%)	34 (17.7%)	27 (14.1%)	
Session length, mins	210 [180, 227]	210 [182, 240]	214 [188, 240]	210 [182, 240]	0.04
Diabetes, n(%)	67 (34.7%)	81 (42.2%)	101 (52.6%)	94 (49.0%)	<0.001
Hypertension, n(%)	67 (34.7%)	50 (26.0%)	68 (35.4%)	48 (25.0%)	0.18
Ischemic Heart Disease, n(%)	15 (7.8%)	12 (6.2%)	19 (9.9%)	16 (8.3%)	0.54
Serum Albumin, g/dL	3.6 ± 0.6	3.6 ± 0.5	3.5 ± 0.5	3.4 ± 0.5	0.005

Legend: Values for continuous variables are presented as mean (± standard deviation), median (25th-75th percentile). Abbreviations: HD, hemodialysis; BP, blood pressure; UF, ultrafiltration; AV, arteriovenous.

Table 2: Baseline systolic blood pressure parameters of participants according to quartiles of plasma endothelin-1

	Quartile 1 (n=193)	Quartile 2 (n=192)	Quartile 3 (n=192)	Quartile 4 (n=192)	P
Pre-dialysis SBP, mmHg	149 ± 26	151 ± 27	151 ± 28	157 ± 30	0.003
Nadir intradialytic SBP, mmHg	119 ± 25	119 ± 24	120 ± 24	123 ± 27	0.15
Post-dialysis SBP, mmHg	145 ± 27	144 ± 27	145 ± 24	150 ± 31	0.06

Legend: Values for continuous variables are presented as mean (± standard deviation), median (25th-75th percentile). Abbreviations: SBP, systolic blood pressure.

Table 3: Unadjusted and adjusted multiple linear mixed effects regression models

SBP Parameter	Model	Change in SBP per SD increase in logET-1 (mmHg)	95% CI	P
Pre-HD SBP	Unadjusted	3.4	[2.2, 4.6]	<0.001
	Model 1	3.3	[2.2, 4.5]	<0.001
	Model 2	3.2	[2.0, 4.4]	<0.001
Nadir SBP	Unadjusted	2.7	[1.7, 3.7]	<0.001
	Model 1	1.4	[0.6, 2.1]	<0.001
	Model 2	1.9	[1.1, 2.6]	<0.001
Post-HD SBP	Unadjusted	2.8	[1.7, 4.0]	<0.001
	Model 1	1.7	[0.8, 2.6]	<0.001
	Model 2	2.2	[1.3, 3.1]	<0.001

Models: unadjusted; model 1 adjusted for: age, gender, race, HD access, pre-HD systolic BP; model 2: adjusted for same as model 1 plus categories of session length, ultrafiltration volume, diabetes, congestive heart failure, ischemic heart disease, peripheral vascular disease, and lung disease

Figure 1:

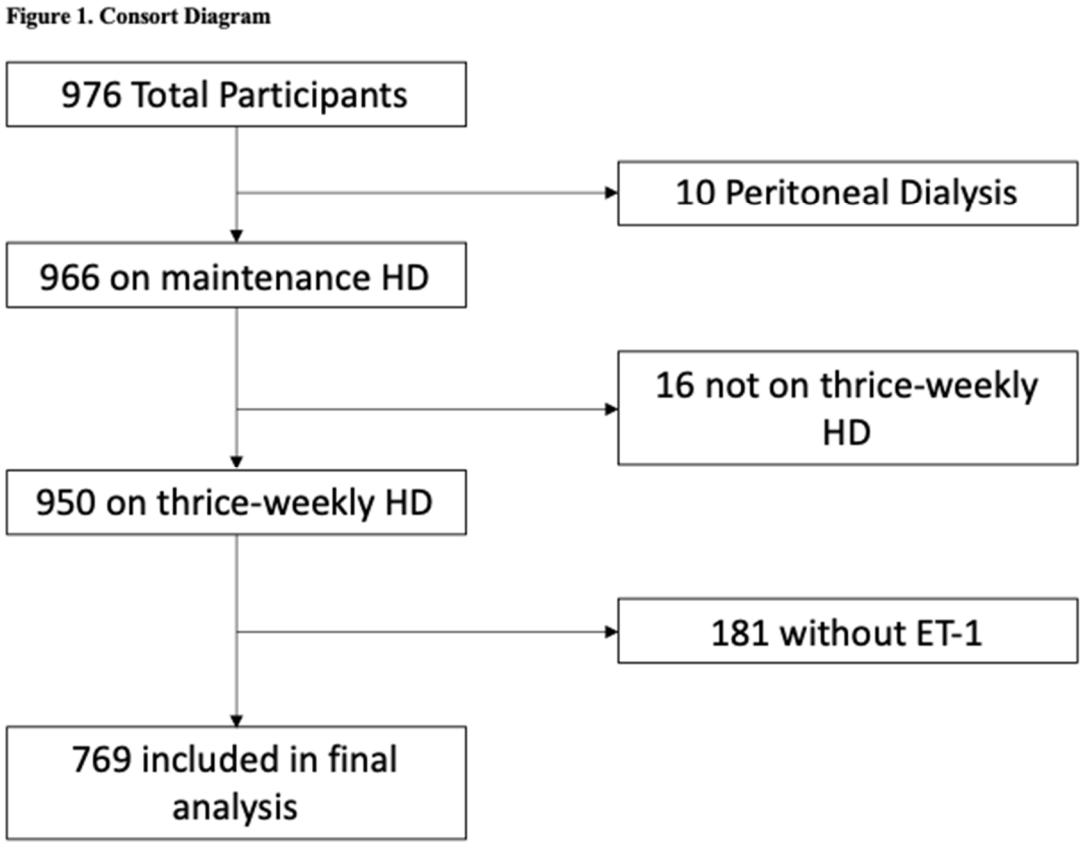
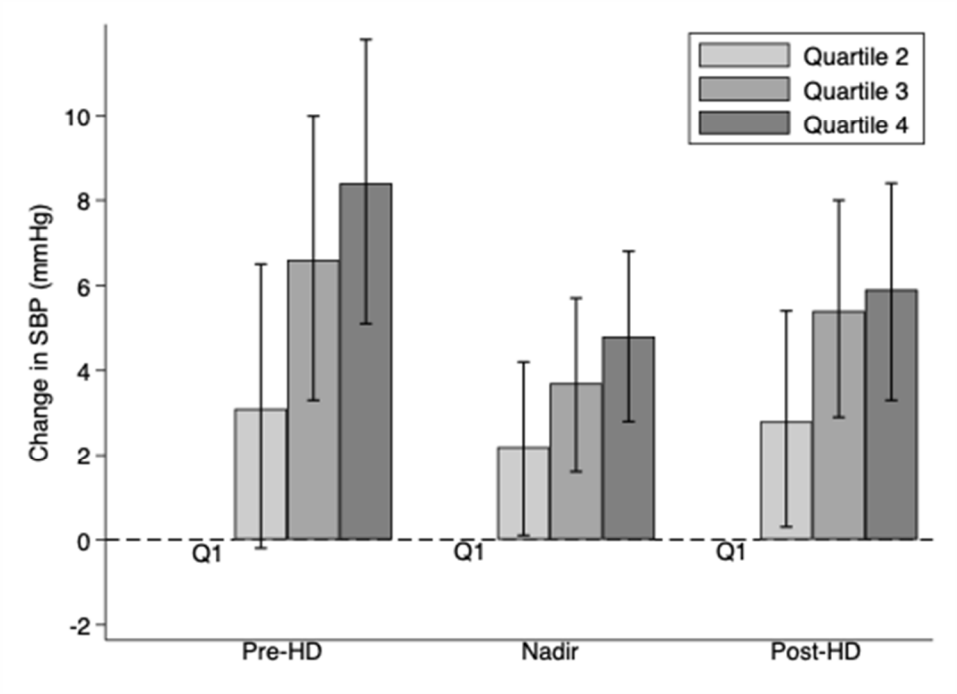


Figure 1. Consort Diagram
976 Total Participants
10 Peritoneal Dialysis
966 on maintenance HD
16 not on thrice-weekly HD
950 on thrice-weekly HD
181 without ET-1
769 included in final analysis

Figure 2:

Figure 2. Categorial Analysis of SBP Change Outcome by Quartiles of ET-1 for SBP Parameters.



References:

1. Cozzolino M, Mangano M, Stucchi A, Ciceri P, Conte F, Galassi A. Cardiovascular disease in dialysis patients. Nephrol Dial Transplant. 2018;33(suppl_3):iii28–iii34. doi:10.1093/ndt/gfy174
2. Agarwal R, Flynn J, Pogue V, Rahman M, Reisin E, Weir MR. Assessment and management of hypertension in patients on dialysis. J Am Soc Nephrol. 2014;25(8):1630-1646. doi:10.1681/ASN.2013060601
3. Assimon MM, Flythe JE. Intradialytic Blood Pressure Abnormalities: The Highs, The Lows and All That Lies Between. Am J Nephrol. 2015;42(5):337-350. doi:10.1159/000441982
4. Van Buren PN, Kim C, Toto R, Inrig JK. Intradialytic hypertension and the association with interdialytic ambulatory blood pressure. Clin J Am Soc Nephrol. 2011 Jul;6(7):1684-91. doi: 10.2215/CJN.11041210.
5. Raj D, Vincent B, Simpson K, Sato E Jones KL, Welbourne TC, Levi M, Shah V, Blandon P, Zager P, Robbins RA. Hemodynamic changes during hemodialysis: Role of nitric oxide and endothelin. Kidney International. 2002; 61:697–704.
6. Inrig JK, Oddone EZ, Hasselblad V, Gillespie B, Patel UD, Reddan D, Toto R, Himmelfarb J, Winchester JF, Stivelman J, Lindsay RM, Szczech LA. Association of intradialytic blood pressure changes with hospitalization and mortality rates in prevalent ESRD patients. Kidney Int. 2007; 71:454–461.
7. Santos SF, Peixoto AJ. Revisiting the dialysate sodium prescription as a tool for better blood pressure and interdialytic weight gain management in hemodialysis patients. Clin J Am Soc Nephrol. 2008 Mar;3(2):522-30. doi: 10.2215/CJN.03360807. Epub 2008 Jan 16.
8. Schlaich MP, Socratous F, Hennebry S, Eikelis N, Lambert EA, Straznicky N, Esler MD, Lambert GW. Sympathetic activation in chronic renal failure. J Am Soc Nephrol. 2009 May;20(5):933-9. doi: 10.1681/ASN.2008040402. Epub 2008 Sep 17.
9. Georgianos PI, Agarwal R. Pharmacotherapy of Hypertension in Chronic Dialysis Patients. Clin J Am Soc Nephrol. 2016;11(11):2062-2075. doi:10.2215/CJN.00870116
10. Krapf R, Hulter HN. Arterial hypertension induced by erythropoietin and erythropoiesis-stimulating agents (ESA). Clin J Am Soc Nephrol. 2009 Feb;4(2):470-80. doi: 10.2215/CJN.05040908.
11. Simeoni M, Perna AF, Fuiano G. Secondary Hyperparathyroidism and Hypertension: An Intriguing Couple. J Clin Med. 2020;9(3):629. Published 2020 Feb 27. doi:10.3390/jcm9030629
12. Böhm F, Pernow J. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. Cardiovasc Res. 2007 Oct 1;76(1):8-18. doi: 10.1016/j.cardiores.2007.06.004. Epub 2007 Jun 16.
13. Kohan DE, Barton M. Endothelin and endothelin antagonists in chronic kidney disease. Kidney Int. 2014;86(5):896-904. doi:10.1038/ki.2014.143
14. Tomić M, Galesić K, Markota I. Endothelin-1 and nitric oxide in patients on chronic hemodialysis. Ren Fail. 2008;30(9):836-42. doi: 10.1080/08860220802356218.
15. Inrig JK, Patel UD, Toto RD, Szczech LA .Association of blood pressure increases during hemodialysis with 2-year mortality in incident hemodialysis patients: a secondary

analysis of the Dialysis Morbidity and Mortality Wave 2 Study.Am J Kidney Dis. 2009; 54:881–890. doi: 10.1053/j.ajkd.2009.05.012.

16. Flythe JE, Xue H, Lynch KE, Curhan GC, Brunelli SM. Association of mortality risk with various definitions of intradialytic hypotension. J Am Soc Nephrol. 2015;26(3):724-734. doi:10.1681/ASN.2014020222
17. Evans RR, Phillips BG, Singh G, Bauman J L, Gulati A. Racial and gender differences in endothelin-1. Am J Cardiol. 1996;78(4):486-488. doi:10.1016/s0002-9149(96)00344-x
18. Ergul S, Parish DC, Puett D, Ergul A. Racial differences in plasma endothelin-1 concentrations in individuals with essential hypertension [published correction appears in Hypertension 1997 Mar;29(3):912]. Hypertension. 1996;28(4):652-655. doi:10.1161/01.hyp.28.4.652
19. Nohria A, Garrett L, Johnson W, Kinlay S, Ganz P, Creager MA. Endothelin-1 and vascular tone in subjects with atherogenic risk factors. Hypertension. 2003;42(1):43-48.
20. Barton M, Tharaux PL. Endothelin and the podocyte. Clin Kidney J. 2012;5(1):17-27. doi:10.1093/ckj/sfs001
21. Barton M. Reversal of proteinuric renal disease and the emerging role of endothelin. Nat Clin Pract Nephrol. 2008 Sep;4(9):490-501. doi: 10.1038/ncpneph0891. Epub 2008 Jul 22.
22. De Miguel C, Speed JS, Kasztan M, Gohar EY, Pollock DM. Endothelin-1 and the kidney: new
23. Peng, T., Li, X., Hu, Z., Yang, X., Ma, C. (2018). Predictive role of endothelin in left ventricular remodeling of chronic kidney disease Renal Failure 40(1), 183-186.
24. Rebholz CM, Harman JL, Grams ME, Correa A, Shimbo D, Coresh J, Young BA. Association between Endothelin-1 Levels and Kidney Disease among Blacks. J Am Soc Nephrol. 2017;28(11):3337-3344. doi:10.1681/ASN.2016111236
25. Fischer A, Bossard M, Aeschbacher S, Egli P, Cordewener C, Estis J, Todd J, Risch M, Risch L, Conen D. Plasma levels of endothelin-1 and renal function among young and healthy adults. Clin Chem Lab Med. 2017 Jul 26;55(8):1202-1208
26. Dhaun N, Moorhouse R, MacIntyre IM, Melville V, Oosthuyzen W, Kimmitt RA, Brown KE, Kennedy ED, Goddard J, Webb DJ. Diurnal variation in blood pressure and arterial stiffness in chronic kidney disease: the role of endothelin-1. Hypertension. 2014 Aug;64(2):296-304.
27. Teng J, Tian J, Lv WL, Zhang XY, Zou JZ, Fang Y, Yu J, Shen B, Liu ZH, Ding XQ. Inappropriately elevated endothelin-1 plays a role in the pathogenesis of intradialytic hypertension [published correction appears in Hemodial Int. 2017 Jan;21(1):148]. Hemodial Int. 2015;19(2):279-286. doi:10.1111/hdi.12238)
28. Chou, K., Lee, P., Chen, C., Chiou, C., Hsu, C., Chung, H., Liu, C., Fang, H. (2006). Physiological changes during hemodialysis in patients with intradialysis hypertension Kidney International 69(10), 1833-1838.
29. Ottosson-Seeberger, A., Ahlborg, G., Hemsén, A., Lundberg, J., Alvestrand, A. (1999). Hemodynamic effects of endothelin-1 and big endothelin-1 in chronic hemodialysis patients. Journal of the American Society of Nephrology : JASN 10(5), 1037-44.

30. Li, P., Schmidt, I., Sabbisetti, V., Tio, M., Opotowsky, A., Waikar, S. (2020). Plasma Endothelin-1 and Risk of Death and Hospitalization in Patients undergoing Maintenance Hemodialysis. Clinical journal of the American Society of Nephrology : CJASN 15(6), 784-793.
31. Seligman, B., Biolo, A., Polanczyk, C., Gross, J., Clausell, N. (2000). Increased plasma levels of endothelin 1 and von Willebrand factor in patients with type 2 diabetes and dyslipidemia. Diabetes Care 23(9), 1395-1400.
32. Schneider, J., Tilly, N., Hierl, T., Sommer, U., Hamann, A., Dugi, K., Leidig-Bruckner, G., Kasperk, C. (2002). Elevated plasma endothelin-1 levels in diabetes mellitus American Journal of Hypertension 15(11), 967-972.
33. De Mattia G, Cassone-Faldetta M, Bellini C, Bravi MC, Laurenti O, Baldoncini R, Santucci A, Ferri C. Role of plasma and urinary endothelin-1 in early diabetic and hypertensive nephropathy. Am J Hypertens. 1998 Aug;11(8 Pt 1):983-8.
34. Kohan DE, Lambers Heerspink HJ, Coll B, Andress D, Brennan JJ, Kitzman DW, Correa-Rotter R, Makino H, Perkovic V, Hou FF, Remuzzi G, Tobe SW, Toto R, Parving HH, de Zeeuw D. Predictors of Atrasentan-Associated Fluid Retention and Change in Albuminuria in Patients with Diabetic Nephropathy. Clin J Am Soc Nephrol. 2015;10(9):1568-1574. doi:10.2215/CJN.00570115
35. Inrig JK, Patel UD, Toto RD, Szczech LA. Association of blood pressure increases during hemodialysis with 2-year mortality in incident hemodialysis patients: a secondary analysis of the Dialysis Morbidity and Mortality Wave 2 Study. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2009;**54**:881–890.
36. Assimon MM, Wang L, Flythe JE. Intradialytic Hypertension Frequency and Short-Term Clinical Outcomes Among Individuals Receiving Maintenance Hemodialysis. *Am J Hypertens* 2017;**31**:329–339.
37. Kohan DE, Pritchett Y, Molitch M, Wen S, Garimella T, Audhya P, Andress DL. Addition of atrasentan to renin-angiotensin system blockade reduces albuminuria in diabetic nephropathy. J Am Soc Nephrol. 2011;22(4):763-772. doi:10.1681/ASN.2010080869
38. Heerspink HJL, Parving HH, Andress DL, Bakris G, Correa-Rotter R, Hou FF, Kitzman DW, Kohan D, Makino H, McMurray JJV, Melnick JZ, Miller MG, Pergola PE, Perkovic V, Tobe S, Yi T, Wigderson M, de Zeeuw D; SONAR Committees and Investigators. Atrasentan and renal events in patients with type 2 diabetes and chronic kidney disease (SONAR): a double-blind, randomised, placebo-controlled trial [published correction appears in Lancet. 2019 May 11;393(10184):1936]. Lancet. 2019;393(10184):1937-1947. doi:10.1016/S0140-6736(19)30772-X

Supplementary Material:

Supplementary Table 1: Comparison between Included and Excluded Participants.

	Included (n=769)	**Excluded (n=207)**	**P**
Age, yrs	52 ± 22	54 ± 23	0.41
Female, n (%)	319 (41.5%)	108 (52.2%)	0.01
Pre-dialysis weight, kg	90.9 ± 24.1	88.3 ± 24.7	0.19
Ultrafiltration volume, L	2.1 ± 1.5	2.0 ± 1.5	0.40
Race, n (%)			<0.001
White	322 (41.9%)	66 (31.9%)	
Black	314 (40.8%)	55 (26.6%)	
Other	133 (17.3%)	86 (41.5%)	
Pre-dialysis systolic blood pressure, mmHg	152 ± 28	154 ± 28	0.47
Access, n (%)			0.32
AVF	521 (67.8%)	137 (66.2%)	
AVG	115 (15.0%)	23 (11.1%)	
Tunneled Catheter	133 (17.3%)	39 (18.8%)	
Peritoneal Dialysis Catheter	0 (0.0 %)	8 (3.9 %)	
Diabetes, n (%)	342 (44.5%)	87 (42.0%)	0.53
Hypertension, n (%)	233 (30.3%)	66 (31.9%)	0.66
Ischemic Heart Disease, n (%)	62 (8.1%)	21 (10.1%)	0.34
Serum Albumin, g/dL	3.5 ± 0.5	3.5 ± 0.6	0.92

Supplementary Table 2: Exploratory linear mixed effects regression models.

SBP Parameters	Change in SBP per SD increase in logET-1 (mmHg)	95% CI	*p*
Pre-SBP	2.8	[1.6, 4.0]	<0.001
Min SBP	1.8	[1.0, 2.5]	<0.001
Post-SBP	2.2	[1.3, 3.2]	<0.001

Models: adjusted for: age, gender, race, HD access, pre-HD systolic BP, categories of session length, ultrafiltration volume, diabetes, congestive heart failure, ischemic heart disease, peripheral vascular disease, lung disease, and hemoglobin.

Supplementary Table 3. Adjusted association of ET-1 with change in SBP over 30-days of follow-up

SBP Parameter	Model	Change in SBP per SD increase in logET-1 (mmHg)	95% CI	P
Pre-HD SBP	Model 2	3.1	[1.8, 4.5]	<0.001
Nadir SBP	Model 2	1.3	[0.3, 2.2]	0.01
Post-HD SBP	Model 2	1.8	[0.6, 3.0]	0.002

Summary of Results and Conclusions

This body of work focuses on two overarching goals: first, exploring different definitions for intradialytic hypertension and identifying which may have the most potent association with mortality. The second, investigating the role of a modifiable factor, ET-1, in contributing to changes in SBP and the development of higher BP during dialysis.

In our first project, we described the associations of three independent definitions of intradialytic hypertension with mortality in a large, contemporary, HD cohort using survival analyses. Our primary observation was that **the most potent association with mortality was observed in participants who experienced *any* increase in systolic BP from pre- to post-HD.** This observation contributes to the body of literature on this topic as it is one of the first to put forth a single definition for intradialytic hypertension relevant to clinical outcomes, which may help guide clinical decision-making in the future.

We also observed effect modification by age and PVD. Specifically, the association between any increase in SBP from pre to post-HD with mortality being most pronounced in those aged 45-70 years, while the association between any increase in SBP from pre to post-HD with mortality was most pronounced in those without PVD at baseline. While these findings should be interpreted with some caution because of lack of statistical power, they may support further investigation into the potential impact of both age and PVD on intradialytic BPs.

Following the results observed in **project 2**, we further aimed to explore potential predictors of SBP change and intradialytic hypertension. As ET-1 is a potent vasoconstrictor and is a known predictor for hypertension in the non-ESKD population, we hypothesized that ET-1 might play a potential role in SBP change in the ESKD population. Herein, we examined the

association of ET-1 with HD-related BP parameters using a large, contemporary, prospective patient cohort. We observed the following**: 1) Higher ET-1 concentrations were independently associated with higher pre-HD SBP, nadir intradialytic SBP, and higher post-HD SBP; and 2) higher endothelin was associated with greater odds of developing intradialytic hypertension.** These observations support a potential role for ET-1 in intradialytic blood pressure variation and intradialytic hypertension. These observations build on earlier data from Li et al. who observed an association between ET-1 and mortality in the maintenance HD population (23) and further suggest a potential therapeutic pathway for pharmacologic management of intradialytic hypertension with already developed ET-receptor antagonists, which, while yet to be evaluated in the ESKD population, have demonstrated safety and efficacy in the CKD population (24). Further, while yet to be developed, these results suggest potential for the development for ET-receptor agonists for those who are more hypotensive-prone on HD, as there are currently limited pharmacologic management strategies for this clinical condition.

Discussion and perspectives:

The present studies highlight the impact intradialytic hypertension has on morbidity and mortality in the HD population and the importance of further investigation into the predictors of intradialytic hypertension and potential modifiable risk factors. Despite a paucity of evidence on this topic, through analyses of large HD cohorts, our results reflect not only a potentially unifying definition for intradialytic hypertension that is relevant to clinical outcomes, but also a potential modifiable risk factor for this disease state.

Hemodialysis (HD) is the primary life-sustaining treatment for those with end-stage kidney disease. (27) However, many patients on HD have comorbid or worsening cardiovascular disease, which may impact their survival on HD therapy. (1) Extensive evidence exists regarding the importance of SBP regulation and hypertension management in this population in order to minimize these risks; however, there have been few biomarkers reported that have demonstrated potential for pharmacologic intervention. Endothelin-1 has been studied in both CKD and non-CKD populations. Pharmacological antagonism has demonstrated efficacy in reducing renal events in patients with type 2 diabetes and appears promising as a potential renal protective early intervention. (23) While this has yet to be studied in the ESKD population, it is biologically plausible that this may have beneficial effects in reducing CV disease and aid in the management of intradialytic hypertension in this population.

In conclusion, this body of work reflects an in-depth analysis of the potential outcomes associated with intradialytic SBP increases and, more specifically which definitions of intradialytic hypertension are associated with greater risks of mortality. Further, we describe

the relationship between a potential predictor for SBP change in this population, ET-1, and outline a potential avenue for intervention.

References:

1. Agarwal R, Flynn J, Pogue V, Rahman M, Reisin E, Weir MR. Assessment and management of hypertension in patients on dialysis. J Am Soc Nephrol. 2014;25(8):1630-1646. doi:10.1681/ASN.2013060601
2. Cozzolino M, Mangano M, Stucchi A, Ciceri P, Conte F, Galassi A. Cardiovascular disease in dialysis patients. Nephrol Dial Transplant. 2018;33(suppl_3):iii28–iii34. doi:10.1093/ndt/gfy174
3. Assimon MM, Flythe JE. Intradialytic Blood Pressure Abnormalities: The Highs, The Lows and All That Lies Between. Am J Nephrol. 2015;42(5):337-350. doi:10.1159/000441982
4. Buren PNV, Kim C, Toto RD, Inrig JK. The Prevalence of Persistent Intradialytic Hypertension in a Hemodialysis Population with Extended Follow-Up. Int J Artif Organs 2012;35:1031–1038.
5. Losito A, Vecchio LD, Rosso GD, Locatelli F. Postdialysis Hypertension: Associated Factors, Patient Profiles, and Cardiovascular Mortality. Am J Hypertens 2016;29:684–689.
6. Inrig JK, Oddone EZ, Hasselblad V, Gillespie B, Patel UD, Reddan D, Toto R, Himmelfarb J, Winchester JF, Stivelman J, Lindsay RM, Szczech LA. Association of intradialytic blood pressure changes with hospitalization and mortality rates in prevalent ESRD patients. Kidney Int 2007;71:454–461.
7. Sherman RA, Daniel A, Cody RP. The effect of interdialytic weight gain on predialysis blood pressure. *Artif Organs.* 1993;17:770–774.
8. Boon D, van Montfrans GA, Koopman MG, et al. Blood pressure response to uncomplicated hemodialysis: the importance of changes in stroke volume. *Nephron Clin Pract.* 2004;96:c82–c87.
9. Dubin R, Owens C, Gasper W, Ganz P, Johansen K. Associations of endothelial dysfunction and arterial stiffness with intradialytic hypotension and hypertension. Hemodial Int 2011;15:350–358.
10. Teng J, Tian J, Lv W, Zhang X, Zou J, Fang Y, Yu J, Shen B, Liu Z, Ding X. Endothelin-1 and dialytic hypertension. Hemodial Int 2015;19:279–286.
11. Raj DSC, Vincent B, Simpson K, Sato E, Jones KL, Welbourne TC, Levi M, Shah V, Blandon P, Zager P, Robbins RA. Hemodynamic changes during hemodialysis: Role of nitric oxide and endothelin. Kidney Int 2002;61:697–704.
12. Chou K-J, Lee P-T, Chen C-L, Chiou C-W, Hsu C-Y, Chung H-M, Liu C-P, Fang H-C. Physiological changes during hemodialysis in patients with intradialysis hypertension. Kidney Int 2006;69:1833–1838.
13. Krapf R, Hulter HN. Arterial Hypertension Induced by Erythropoietin and Erythropoiesis-Stimulating Agents (ESA). Clin J Am Soc Nephro 2009;4:470–480.
14. Chang TI, Abdalla S, London GM, Block GA, Correa-Rotter R, Drüeke TB, Floege J, Herzog CA, Mahaffey KW, Moe SM, Parfrey PS, Wheeler DC, Dehmel B, Goodman

WG, Chertow GM. The effects of cinacalcet on blood pressure, mortality and cardiovascular endpoints in the EVOLVE trial. J Hum Hypertens 2016;30:204–209.
15. Inrig JK, Oddone EZ, Hasselblad V, Gillespie B, Patel UD, Reddan D, Toto R, Himmelfarb J, Winchester JF, Stivelman J, Lindsay RM, Szczech LA. Association of intradialytic blood pressure changes with hospitalization and mortality rates in prevalent ESRD patients. Kidney Int 2007;71:454–461.
16. Inrig JK, Patel UD, Toto RD, Szczech LA. Association of blood pressure increases during hemodialysis with 2-year mortality in incident hemodialysis patients: a secondary analysis of the Dialysis Morbidity and Mortality Wave 2 Study. American journal of kidney diseases : the official journal of the National Kidney Foundation 2009;54:881–890.
17. Park J, Rhee CM, Sim JJ, Kim Y-L, Ricks J, Streja E, Vashistha T, Tolouian R, Kovesdy CP, Kalantar-Zadeh K. A comparative effectiveness research study of the change in blood pressure during hemodialysis treatment and survival. Kidney international 2013;84:795–802.
18. Assimon MM, Wang L, Flythe JE. Intradialytic Hypertension Frequency and Short-Term Clinical Outcomes Among Individuals Receiving Maintenance Hemodialysis. Am J Hypertens 2017;31:329–339.
19. Yang C-Y, Yang W-C, Lin Y-P. Postdialysis blood pressure rise predicts long-term outcomes in chronic hemodialysis patients: a four-year prospective observational cohort study. Bmc Nephrol 2012;13:12.
20. Inrig JK, Oddone EZ, Hasselblad V. et al. Association of intradialytic blood pressure changes with hospitalization and mortality rates in prevalent ESRD patients. Kidney Int 2007; 71: 454–461
21. Inrig JK, Patel UD, Toto RD, Szczech LA . Association of blood pressure increases during hemodialysis with 2-year mortality in incident hemodialysis patients: a secondary analysis of the Dialysis Morbidity and Mortality Wave 2 Study.Am J Kidney Dis. 2009; 54:881–890. doi: 10.1053/j.ajkd.2009.05.012.
22. Van Buren PN. Pathophysiology and implications of intradialytic hypertension. Curr Opin Nephrol Hypertens. 2017;26(4):303-310. doi:10.1097/MNH.0000000000000334
23. Li P, Schmidt IM, Sabbisetti V, Tio MC, Opotowsky AR, Waikar SS. Plasma Endothelin-1 and Risk of Death and Hospitalization in Patients undergoing Maintenance Hemodialysis. Clin J Am Soc Nephrol. 2020 May 7.
24. Kohan DE, Barton M. Endothelin and endothelin antagonists in chronic kidney disease. Kidney Int. 2014;86(5):896-904. doi:10.1038/ki.2014.143
25. Teng J, Tian J, Lv WL, et al. Inappropriately elevated endothelin-1 plays a role in the pathogenesis of intradialytic hypertension [published correction appears in Hemodial Int. 2017 Jan;21(1):148]. *Hemodial Int*. 2015;19(2):279-286. doi:10.1111/hdi.12238
26. Chou KJ, Lee PT, Chen CL, et al. Physiological changes during hemodialysis in patients with intradialysis hypertension. *Kidney Int*. 2006;69(10):1833-1838. doi:10.1038/sj.ki.5000266

27. Heerspink HJL, Parving HH, Andress DL, Bakris G, Correa-Rotter R, Hou FF, Kitzman DW, Kohan D, Makino H, McMurray JJV, Melnick JZ, Miller MG, Pergola PE, Perkovic V, Tobe S, Yi T, Wigderson M, de Zeeuw D; SONAR Committees and Investigators. Atrasentan and renal events in patients with type 2 diabetes and chronic kidney disease (SONAR): a double-blind, randomised, placebo-controlled trial [published correction appears in Lancet. 2019 May 11;393(10184):1936]. Lancet. 2019;393(10184):1937-1947. doi:10.1016/S0140-6736(19)30772-X
28. Robinson BM, Akizawa T, Jager KJ, Kerr PG, Saran R, Pisoni RL. Factors affecting outcomes in patients reaching end-stage kidney disease worldwide: differences in access to renal replacement therapy, modality use, and haemodialysis practices. The Lancet. 2016;388(10041):294-306.