Fluid overload and vascular stiffness in hemodialysis patients

by

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Abstract

People requiring hemodialysis (HD) have a high cardiovascular mortality rate. Some of the strongest predictors for the increased cardiovascular disease is related to vascular stiffness and fluid overload. Nevertheless, there is an ongoing debate regarding the importance of fluid overload as an independent factor of vascular stiffness in HD patients. Currently, there are very limited reports that have investigated a correlation between fluid overload and vascular stiffness in HD patients. Therefore, we first explored the current literature by conducting a scoping review to identify all the clinical and epidemiological studies that researched in the similar area of research interest. Then, we performed a pilot observational study to have an impression on the vascular function during the inter- and intradialytic fluid overload changes in fluid overloaded and non-fluid overload HD patients. Fluid status and vascular stiffness were tested in 39 HD patients (20 with fluid overload and 19 without) and compared to 26 healthy controls. Pre-dialysis vascular stiffness measurements were performed for 24 hours and then for 5-hours: starting 30 minutes before and ending 30 minutes after the HD run. Afterward, we designed a randomized controlled trial, using bioimpedance spectroscopy and the time-averaged fluid overload measure, to correct the target weight in the fluid overloaded HD patients, foreseeing an improvement in fluid status and vascular stiffness. However, the study is still ongoing. Altogether, the accumulated results in the scoping review were conflicting as the size and power of the included studies were low, and the approaches varied widely. In the observational study, we found that the inter- and intradialytic changes in fluid overload do not seem a strong determinant of vascular stiffness. It is important to have larger studies to address the effect of fluid overload changes on vascular stiffness in HD patients.

Preface

This thesis is an original work by Aya Lafta. No part of the thesis has been previously published. Two research projects, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board. First project, "Fluid volume overload and vascular stiffness in hemodialysis patients", ethics No. Pro0008086, April 12, 2018. Second project, "Target weight correction and vascular stiffness in hemodialysis patients", ethics No. Pro00086730, March 12, 2019, NCT03929471.

The scoping review in chapter 2 and the observational study in chapter 3 of this thesis will be submitted for publication. The pilot randomized controlled trial is still ongoing (in the appendix). I was responsible for formulating the research proposal plans, Health Canada and study registration at clinicaltrials.gov applications and submitting for ethical approval. Also, I was responsible for data collection, performing the measurements, statistical analysis, and for writing manuscripts. Judy Ukraintz -registered nurse- assisted in data collection and measuring the hemodialysis patients in the clinical studies. Dr. Aminu Bello, assisted with the statistical analysis and with the application for the Strategy for Patients Oriented Research- Alberta Innovates (SPOR) to conduct the scoping review. Dr. Branko Braam – the principal investigator - designed the studies and involved in the research protocols, ethics approval, Health Canada, and clinicaltrials.gov applications as well as data analysis and writing the manuscript.

Dedication

I dedicate this work to the hemodialysis patients who passed away and to those who are still fighting in the University of Alberta Hospital

Acknowledgment

I would like to express my immeasurable appreciation and deepest gratitude to my mentor and supervisor, Dr. Branko Braam, for his unforgettable support, words of encouragement, robust mentorship, and endless help to finish my graduate studies successfully.

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Abbreviations

AIx	Augmentation index
AV	Arteriovenous
BCM	Body composition monitor
BP	Blood pressure
CKD	Chronic kidney disease
DBP	Diastolic blood pressure
DW	Dry weight
ECFV	Extracellular fluid volume
ESKD	End stage kidney disease
FO	Fluid overload
HD	hemodialysis
ICFV	Intracellular fluid volume
IDH	Intradialytic hypotension
IDWG	Inter-dialytic weight gain
MAP	Mean arterial pressure
SBP	Systolic blood pressure
TAFO	Time-averaged fluid overload
TBF	Total body fluid
TW	Target weight
PP	Pulse pressure
PWV	Pulse wave velocity
PWA	Pulse wave analysis
UF	Ultrafiltration
VEGF-C	Vascular endothelial growth factor C

Chapter 1 - Background

This thesis focuses on the effect of the inter- and intradialytic fluid volume changes on vascular stiffness in patients with end-stage kidney disease on hemodialysis (HD). More than 40 % of HD patients have higher cardiovascular mortality rate than general population due to the incidence of fluid overload and impaired vascular function (1-3).

The remodeling in the large arteries structure is one of the strongest predictors of the increased cardiovascular events in HD patients. Essentially, there are two main pathological changes occurred in the vascular structure of HD patients. First, structural changes caused by arteriosclerosis (Monckeberg's sclerosis). Second, functional changes characterized by the increased sympathetic nervous system activity, renin-angiotensin system, and circulatory hypertensive substances like asymmetric dimethyl arginine which inhibits the synthesis of nitric oxide- most potent local vasodilator. These two pathological changes have been recognized for a long time to cause increased vascular stiffness (4, 5).

Fluid overload is another structural factor that has been proposed to be associated with increased vascular stiffness. Reports suggested that fluid overload worsens the vascular stiffness by increasing the vascular wall distension "Laplace's law" (6, 7). However, there are conflicting results in the literature. Some reports demonstrated an improvement in pulse wave velocity (PWV) and augmentation index (AIx) - the two gold standard measures of vascular stiffness - after HD session or after correcting fluid overload in HD patients (1, 8, 9). In contrast, some studies reported no improvement in PWV or AIx. In the current thesis, we aimed to identify the effect of fluid

overload changes on vascular stiffness in HD patients through different research designs: scoping review, observational study, and interventional trial, respectively.

In the scoping review, we explored the available evidence that questioned the effect of acute and chronic fluid overload changes on vascular stiffness in end-stage kidney disease patients on HD. It was difficult to conduct a systematic review due to the limited number of clinical trials in the available literature, whereas the scoping review would essentially identify where the knowledge gaps are. The scoping review was guided by the framework developed by Arksey and O'Malley with revision based on Levac and colleagues (10, 11). We used five electronic bibliographic databases for relevant studies. Two reviewers independently screened the titles, abstracts, and full texts to identify studies that matched with the inclusion criteria of the review. Detailed methodology and results of the scoping review are presented in (**Chapter 2**). From the scoping review, we identified two gaps: First, there was not enough human studies about the behavior of vascular stiffness during the acute HD runs and in between the runs, thus we have done a pilot observational study to get an initial impression of vascular stiffness during inter- and intradialytic fluid overload changes. Also, to test the feasibility of doing such a study design. Second, there were three published randomized controlled trials that assessed the vascular stiffness after a strict fluid overload control (8, 12, 13). The studies had completely different definitions and methodologies to assess fluid status and vascular stiffness and definitions of fluid overload and vascular stiffness. Currently, we are conducting a pilot randomized controlled (RCT) trial to improve fluid status and to assess vascular stiffness. The RCT trial is still ongoing and some details about the study will be discussed in the **appendix**.

In the observational study, we have tested 20 fluid overloaded and 19 non-fluid overloaded HD patients. The fluid overload was defined as an excess of fluid volume of ≥ 1.1 liter (L) above normal extracellular fluid volume, as assessed by the multi-frequency bio-impedance spectroscopy. An ambulatory PWV and AIx measurements were performed for 24 hours before the mid- or end-week of the HD runs, then it was followed by a 5-hour measurement starting 30 minutes before and ending 30 minutes after the HD run. A 5-hour measurement of PWV and AIx in healthy individuals was performed as time control. The primary outcome of this study was to see the behavior of vascular stiffness in HD patients with different fluid status during fluid accumulation and acute fluid removal. The full study is presented in (**Chapter 3**).

In regard to the pilot randomized controlled trial, we formulated a protocol using bio-impedance spectroscopy to correct the target weight in fluid overloaded HD patients and to see the implication of the protocol on the vascular stiffness. The fluid overload measurement was identified by using the time-averaged fluid overload method (TAFO). The latter includes the difference between pre-HD fluid overload, as assessed by bioimpedance, and the average of 3 interdialytic weight gains. So that, it covers the different levels of fluid overload that the HD experience in between HD runs. This study incudes two groups from two different centers; control group who received conventional therapy (without intervention), and intervention group who engaged with the target weight correction protocol for three months. The ultimate goal of this study is to see an improvement in fluid status and vascular stiffness in the intervention group compared to the control ones. The detailed overall design and approach is available in the (**Appendix**).

Chapter 2 - Fluid volume overload and vascular stiffness in hemodialysis patients: are they related?

Introduction

The arterial wall of HD patients has decreased viscoelastic properties leading to increased vascular stiffness. The latter is strongly associated with cardiovascular mortality in patients with chronic kidney disease and particularly, end-stage kidney disease undergoing HD (14-16).

Patients on HD have higher vascular stiffness, as assessed by PWV and AIx, compared to chronic kidney disease patients (17). Some of the independent factors are associated with the mechanical dysfunction and structural alteration in vascular wall of HD patients like: calcification of the large arties (arteriosclerosis), high lipid profile (atherosclerosis), diabetes mellitus, and hypertension (18). Fluid overload is another factor that could increase vascular stiffness in HD patients.

Correction of fluid overload has been associated with improved cardiovascular mortality. It was documented that left ventricular hypertrophy, arterial hypertension, and congestive heart failure were improved after a significant reduction in the fluid overload in HD patients (8). The majority of the studies assumed a possible relationship between fluid overload and vascular stiffness using Laplace's law to support their hypothesis. However, whether fluid overload increases vascular stiffness in HD patients is still under debate. Therefore, we performed a scoping review to identify studies that assessed the effects of interdialytic fluid accumulation and the acute fluid removal on vascular stiffness in adults receiving HD. Also, we attempted to formulate recommendations for future research studies to address the knowledge gaps.

Methods

Approach

We identified the specific criteria for our search strategy: study design, population, and outcomes. We considered all observational (prospective and retrospective cohort studies) and randomized controlled trials that investigated the effect of fluid overload changes and strict fluid volume control on vascular stiffness in HD patients. Our scoping review guided by the framework developed by Arksey and O'Malley (10) with revisions based on Levac and colleagues (11). This framework comprised of five essential elements to guide conduct of scoping reviews: identifying the research question, identifying the relevant studies, study selection, charting the data, and reporting the results.

We identified the studies by conducting comprehensive searches of the following bibliographic databases:

- Ovid Medline 1946 to October 28, 2019
- Ovid Embase 1974 to October 28, 2019
- CINAHL via EBSCOhost 1937 to October 29, 2019
- Wiley Cochrane Library inception to October 29, 2019
- ProQuest Dissertations and thesis global October 29, 2019
- Cochrane library October 29, 2019

Our search used both index (subject headings) and text words, then combined concepts of interest (vascular stiffness, pulse-wave velocity, augmentation index, and fluid overload). All the specified

terms used for the search strategy are presented in (**Table 1**). The specific search strategies (for the selected databases and other data sources) developed and executed by an experienced librarian and peer-reviewed by a second medical librarian. We did not apply language, date restrictions, or source of data to the search strategy. For the strength of evidence, case reports, non-peer-reviewed publications, and editorial were excluded. Finally, through citation chaining (backward by one step) we reviewed the reference lists of systematic and narrative reviews and the included data papers for relevant studies not identified from our initial search until saturation is achieved (i.e. when there is no new study being identified).

Selection criteria of studies for review

We included human studies that reported the outcomes of interest in associations between the inter- and intradialytic fluid overload changes and vascular stiffness in adults with end-stage kidney disease on sustained HD (≥ 18 years old). We did not include animal studies, outcomes related to other dialysis modalities such as peritoneal dialysis, and reports in non-HD chronic kidney disease patients or kidney transplants recipients.

Screening and data selection

All search results were filed and exported in EndNote X8 (Clarivate Analytics) and duplicates references were removed before the file is provided to reviewers for primary and secondary screening as well as data extraction.

Data extraction

We conducted the data extraction in two stages: 1) population, methods, aims, design and conclusions; and 2) the key findings tested against the predefined inclusion and exclusion criteria. Two reviewers: AL and SR are independently screened all identified individual citations for potential inclusion. In the initial screening of title and abstracts, potentially relevant papers identified separately based on the inclusion and exclusion criteria developed a priori. The two lists compiled into single one and full-text papers obtained. Microsoft Excel was used for title, abstract, and full-text screening. The project supervisor – Dr. Branko Braam was consulted for reconciliation when agreement on a citation could not be reached between the two reviewers.

Table 1: Terms used in search strategy

Fluid overload related terms	Vascular stiffness related terms
○ Fluid overload	○ Arterial stiffness
○ Fluid volume overload	○ Vascular stiffness
○ Hydration status	○ Vascular function
○ Overhydration	○ Pulse wave velocity
○ Hypervolemia	○ Pulse wave analysis
○ Extracellular fluid volume expansion	○ Augmentation index
○ Extracellular water expansion	○ Endothelial dysfunction
○ Fluid removal	
○ Ultrafiltration volume and/or rate	
○ Interdialytic weight gain	
○ Intradialytic weight loss	
○ Bio-impedance/electric impedance	
○ Body composition monitor	
○ Fluid volume control	
○ Hemodialysis	

Results

General description of the studies

The search of the current review yielded 666 references, of which 95 studies were included for full-text screening after applying the inclusion/exclusion criteria to titles/abstracts. A total of 24 published papers were included encompassing 21 observational studies and 3 randomized controlled trials (**Figure 1**). To build a better understanding of the included studies' methodological aspects, we have categorized the studies based on their aims and hypothesis into 5 sections: intradialytic, interdialytic, randomized clinical trials, cyclic changes of vascular stiffness, and other studies (**Figure 2**).

Characteristics of included studies

Of the 24 included studies, 11 studies investigated the intradialytic changes of vascular stiffness, as assessed by PWV and AIx. Of these, four studies had multiple measures of vascular stiffness and seven studies looked at the responses of vascular stiffness following the HD run. Four studies assessed vascular function during interdialytic interval. Two randomized controlled trials researched the control of fluid overload guided by bioimpedance on vascular stiffness. Of which, one study was extended and followed with longer follow up in a separate report. Two studies investigated the vascular stiffness during the cyclic changes of fluid overload within one week. Other studies compared pre-HD vascular stiffness in HD groups with different fluid status or PWV level. One study investigated aortic compliance using pulse wave analysis during HD run. The detailed studies characteristics presented in (**Table 2**).

Intradialytic period

Fluid volume and vascular stiffness during single hemodialysis session

This section considers the findings related to the intradialytic changes of vascular function during a single HD run. Four studies with different methodology, fluid overload, and vascular stiffness aspects are available. They either measured both AIx and PWV (19, 20) or AIx only (21, 22). Two studies observed a transient decrease in PWV (19), but not AIx (19, 22). The other two studies reported a transient decrease in AIx (20, 21) but not PWV (20). A common finding was that improved PWV or AIx directly correlated with a decrease in systolic blood pressure or pulse pressure but not with ultrafiltration volume (19-21) or fluid overload measures (19). From this, it becomes clear that a hemodialysis procedure could possibly result in a transient improvement in vascular stiffness. That said, more information is needed to elucidate the determinants of this transient improvement.

Assessment of vascular stiffness before and after HD run

There are seven studies that tested whether a reduction in fluid overload by ultrafiltration improves vascular stiffness in HD cohort. These studies used different vascular stiffness measures and reported different results. Five studies measured both PWV and AIx (1, 6, 9, 23, 24) and two studies used only PWV as a measure of vascular stiffness (25, 26). After the HD run, PWV values improved in four studies (1, 9, 23, 26), worsened in one study (25), and remained unchanged in two studies (6, 24). AIx values improved in four studies (1, 6, 9, 24) and remained unchanged in one study (23). The PWV or AIx changes were not associated with ultrafiltration volume, but mostly with blood pressure variables. Indeed, in the multiple regression analysis of three studies showed that mean arterial pressure, systolic blood pressure, pulse pressure, age, or extracellular

fluid/total body fluid ratio were positively associated with PWV (9, 25, 26) . One of the 3 studies found that the absolute fluid overload is positively associated with pre-HD PWV, and systolic blood pressure was positively correlated with post-HD PWV (25).

Two studies (one Romanian and one Japanese) divided the HD patients into two subgroups based on the fluid status (1) or the fluid removal rate (Δ body weight/dry weight, 5% cutoff) (26). The Romanian study demonstrated that pre-/post-HD PWV and post-HD AIx were significantly higher in overhydrated than the normohydrated HD patients except for the pre-HD AIx which was not different (1). After HD run, the study demonstrated a significant reduction in PWV in the overhydrated group and AIx in the normohydrated group. The Japanese study showed that PWV was reduced significantly in HD patients with a fluid removal rate > 5% and with a water removal rate < 5% (26). The authors claimed that that PWV was not influenced by changes in fluid volume. Whether fluid overload correlated with vascular stiffness is still questionable.

Interdialytic period

Fluid overload and vascular stiffness during interdialytic interval

Four studies investigated the behavior of vascular stiffness during interdialytic days accompanied by gradual fluid accumulation (27-30). They used three different PWV measures; aortic PWV(28, 30), carotid femoral PWV(29), and PWV ratio (carotid-femoral /brachial-radial PWV) (27). Fluid status was defined as either extracellular/intracellular and extracellular/total body fluid ratio (27, 28), amount of ultrafiltration volume (29), or interdialytic weight gain (30). Three studies performed a non-ambulatory vascular stiffness measurement on mid-week non-dialysis day (27-29). They found that total body fluid, intracellular fluid, extracellular/total body fluid, and

extracellular /intracellular ratio (27, 28), but not ultrafiltration volume (29), were positively associated with PWV. The results of the correlation analysis between blood pressure variables and vascular stiffness were different and the interpretation were challenging. Two studies demonstrated a positive association between systolic blood pressure and pulse pressure with PWV (28, 29), whereas the other study did not (27). A study in Greece conducted an ambulatory vascular stiffness measurement for a 72-hour period including the HD run and the subsequent interdialytic interval until the next HD run . The study demonstrated that AIx and PWV increased significantly during a 3-day versus 2-day interdialytic interval. Also, it showed that a gradual increase in systolic and diastolic blood pressure, PWV, and AIx from the end-week HD run onwards, which were positively determined by interdialytic weight gain. However, the study did not report fluid overload measures of the HD patients.

Interventional studies

The effect of fluid overload correction on vascular stiffness

Two prospective randomized controlled trials (one in Turkey and one in Romania) assessed the effect of fluid overload control-guided by bio-impedance spectroscopy on vascular stiffness for 12 months (8, 12, 13). The Turkish study recruited the intervention and control groups from two different centers, as the treating physicians in the control group were blinded to the bioimpedance spectroscopy results (8). In contrast, the Romanian study included the intervention and control groups from a single center where only the co-investigator, who performed the measurements, was blinded to the randomization. The latter study extended the trial to investigate the mortality rate and the changes in vascular stiffness, fluid overload and blood pressure for two more years (13). In regard to the intervention, both studies used bio-impedance spectroscopy to adjust fluid removal

during HD run in the intervention group and conventional therapy in the control group. However, the Turkish study used a method called the time-averaged fluid overload (TAFO) as a representative measure of fluid status. The TAFO method used to minimize the variation of fluid overload during the week, particularly during the 3-day interdialytic day (explained further). The Romanian study used the absolute fluid overload, assessed by bioimpedance, as a fluid overload measure. The results in the Turkish study showed that the absolute fluid overload, TAFO, PWV, and AIx improved in the intervention group and remained unchanged in the control group. The Romanian study found that the fluid overload did not change, whereas, the PWV decreased in the intervention group and increased in the control group after 12 months of the intervention (12). After 2.5 years-end of the intervention- the study found a significant decline in the absolute fluid overload/extracellular fluid ratio and a greater decline in PWV in the intervention group compared to the control group. One year after the end of the intervention, without the fluid overload adjustment, the study demonstrated an increase in PWV in both groups (13).

Vascular function during the cyclic variations of fluid status

Two studies (one German and one Italian) investigated vascular function during the intra-and interdialytic intervals for an entire week (17, 31). Both studies used different approaches to measure PWV. The Italian study measured the PWV immediately before and 1 hour after the end of HD run and in the morning of each interdialytic day, while the German study measured the PWV during each of the three consecutive HD runs. The PWV values were higher before and during the first HD run after the 72-hour interval compared to the second and third HD runs during the week. Although the Italian study showed that the post-HD PWV decreased after the HD run, it found that the intradialytic changes of PWV were not different during the three HD runs. Similarly, the

German study showed that the baseline PWV and systolic blood pressure adjusted PWV did not differ significantly between the three HD runs. During the interdialytic period, the Italian study showed that the PWV increased during the interdialytic days compared to the previous post-HD run. In regard to the determinants of PWV, the two studies reported that the changes in the PWV were not predicted by the change in systolic blood pressure. However, the Italian study showed a weak indirect correlation between blood pressure decline and post-HD PWV. The German study found that the systolic and diastolic blood pressure were positively associated with the mean average of PWV and baseline PWV, but not with the systolic blood pressure adjusted PWV. In regard to the ultrafiltration volume and rate, the Italian study found that the ultrafiltration rate correlated positively with PWV reduction and indirectly with post-HD PWV. In contrast, the German study showed that the adjusted PWV was not predicted by ultrafiltration volume. Although the German study did not evaluate vascular stiffness in the interdialytic days like the Italian study, both did not report measure of fluid overload. Ambulatory PWV and AIx measurements during the acute HD run and in between the runs were not performed.

Other studies

Vascular stiffness measurements in HD patients with different fluid status

A study in Argentina studied the vascular stiffness among three HD groups with different fluid status: over-, normal-, and under-hydrated HD patients (32). The central (carotid-femoral) and peripheral (carotid-brachial) PWV were measured just before the mid-week HD run. The study found that the over-hydrated HD patients had higher central, but not peripheral, PWV than the normal-and under-hydrated groups. The study demonstrated a significant blood pressure dependent correlation between absolute fluid overload and absolute fluid overload/extracellular ratio with

central PWV. However, the correlation between extracellular/intracellular and extracellular/total body fluid ratio with central PWV was positive and independent of blood pressure.

In another study, HD patients were divided into two groups based on the extracellular/intracellular fluid ratio. The cut off ratio was 0.57 based on the maximum discrimination of all-cause mortality survival analysis (3). The study found that the PWV, but not AIx, systolic blood pressure, and pulse pressure were higher in HD patients with high extracellular/intracellular ratio ≥0.57 compared to the lower <0.57. Also, the authors found that PWV values were correlated positively with extracellular/intracellular ratio.

Fluid overload measurements between HD patients with different PWV level

A study in China divided the HD patients into high and low PWV based on the median of the pre-HD PWV value (33). The authors found that the systolic blood pressure, pulse pressure, extracellular/total body fluid ratio were significantly higher in the high PWV compared to the low PWV group. Also, it showed that the pulse pressure and the extracellular/total body fluid ratio were independent determinants of PWV.

Assessment of vascular function during HD run using parameters other than PWV or AIx

A study in USA assessed hemodynamic responses during HD session using pulse wave analysis (34). The study performed an ambulatory measurements of blood pressure and aortic compliance every 30 minutes during the dialysis run. The study observed that the large artery compliance remained unchanged and the small artery compliance consistently reduced during the HD run.

Although the blood pressure variables did not change during the HD, a correlation analysis between aortic compliance and blood pressure variables or ultrafiltration volume was not reported.

Table 2: Summary of the included studies in the scoping review

Authors	Year	Country	Sample size	Age (years)	Diabetes (n, %)	Dialysis vintage (months)	BP drugs (n, %)	Vascular stiffness	Fluid overload	Inclusion/exclusion
1. Intradialytic										
a. Single HD run										
Ogunc, HA (19)	2015	Turkey	30	52 ± 14	(7, 23.3%)	45 ± 53	(12, 40 %)	PWV/&AIx	FO; TBF; ECF; ICF	Inc: on HD > 3 months; no active infection; no valvular heart disease, metallic valve, stent, metallic suture or prosthesis; no peripheral vascular disease; no cancer
Power, AC (20)	2016	Belgium	197	63.3 ± 16.6	(97, 49%)	32 (13-60)	(104, 53%)	PWV &AIx	UF volume	Exc: atrial fibrillation and other cardiac arrhythmias
Mardare, NGG (21)	2005	London	20	55.1± 16.4	(4, 20%)	33.9 ± 26.9	(20, 100%)	AIx	IDWG; Pre- and post-HD weight	Inc: HD > 3 months; no major CVD; no MI, angina, and stroke; no peripheral vascular disease
Thalhammer, CA (22)	2015	Finland	10	72.5 (23–82)		28.4 (2-66)		AIx	FO; TBF; ECF; ICF	Exc: severe arterial hypertension or hypotension; unsuitable superficial veins of the forearm; known subclavian vein obstruction; cardiac arrhythmias
b. Before and after HD run										
Erdan, A (9)	2018	Turkey	75	54± 17	(14, 19%)	26 (16–73)		PWV &AIx	UF volume	Exc: instable hemodynamic parameters; chronic atrial fibrillation, severe aortic stenosis, peripheral artery disease; recent MI; HF; acute infection; morbid obesity; AV fistula
Cakiroglu, UA (23)	2018	Turkey	52	58 ±12	(15, 29%)			PWV &AIx	FO; TBF; ECF; ICF	Inc: just started HD; no cancer; no CAD, cardia arrhythmia; no peripheral arterial disease
Ie, EHDB (24)	2005	Netherland	18	54 (30-85)		31 (4–106)	(8, 44%)	PWV & AIx		Inc: 18 years old and above on maintenance HD
Czyzewski, LW (25)	2017	Poland	71	64 ± 16	(9, 13%)	84 ±71		PWV	FO; TBF; ECF; ICF	Inc: HD > 6 months; no amputations; no metallic implants Exc: atrial fibrillation; active inflammatory process; stage III–IV congestive heart failure
Ueyama, KM (26)	2009	Japan	160	59 ±13	(32, 20%)	8.7 ± 6.9	(60, 10%)	PWV	Pre- and post HD weight	Exc: arterial fibrillation; high frequency of ventricular and arterial premature beats; peripheral arterial disease; HF; pulmonary edema; cancer
Hogas, S (1)	2012	Romania	63	54 ±13		50.8 ± 5.6		PWV &AIx	FO; TBF; ECF; ICF	Inc: adult on HD > 3 months Exc: recent MI; unstable angina; HF; congestive cardiac; peripheral vascular disease; stroke; transient ischemic attacks
Vuurmans, T (6)	2001	Netherland	19	60		13 ± 10	(4, 21%)	PWV &AIx	Pre- post- HD weight	Inc: 8 years old and above on HD > 3 months

Table 2 (continued)

Authors	Year	Country	Sample size	Age (years)	Diabetes (n, %)	Dialysis vintage (months)	BP drugs (n, %)	Vascular stiffness	Fluid overload	Inclusion/exclusion
2. Interdialytic interval										
a. One measurement										
Bia, DG (27)	2015	Argentina	151	58 ±13	(35, 23%)	56 ±51		PWV	TBF; ECF; ICF	Inc: HD > 3 months; no amputation; no metallic implants; no cardiac arrhythmia
Li, XJ (29)	2018	China	82	53 ± 13	(10, 12.5%)	62.6 ± 45.7	(60, 73.2%)	PWV	IDWG; UF volume	Exc: HD < 3 months; uncontrolled malignant hypertension; malignant disease; infection; HF and stroke; history of dilated cardiomyopathy or amyloid degeneration
Lin, YPY (28)	2003	Taiwan	157	55 ±15	(20, 12%)		(85, 54%)	PWV& AIx	FO; TBF; ECF; ICF	Inc: Adult on HD > 3 months; no clinical carotid artery stenosis
***b.* Repeated measurements**										
Koutroumbas, G (30)	2015	Greece	55	63 ± 13	(18, 32%)	29.0 (10.0-75.2)		PWV & AIx	IDWG	Exc: HD< 3months; arrhythmia; old non-functioning fistula; myocardial infarction; unstable angina; ischaemic stroke; HF; malignancy
3. Randomized Controlled trials										
Hur, EU (8)	2013	Turkey	156	51 ± 12	(27, 17%)	61.8 ± 44.8	(32, 20%)	PWV & AIx	FO; TBF; ECF; ICF; IDWG	Exc: Chronic atrial fibrillation; history of missing one or more HD; severe chronic obstructive pulmonary disease; stroke; myocardial infarction
Onofriescu, MM (12)	2012	Romania	135	52 ± 13	(14, 10.3%)	51 (22-102)	(85,62%)	PWV & AIx	FO; TBF; ECF; ICF	Exc: Metallic joint prostheses; cardiac pacemakers; limb amputation;
Onofriescu, MM (13)	2014	Romania	101	53 ± 13	(12, 9.5 %)	105.5 ± 58.5		PWV	RFO	cirrhosis; pregnancy; younger than 18 years old; on HD < 3 months.
4. Cyclic changes										
Di Iorio, BN (17)	2010	Italy	20	56 ± 6		33 ± 29	(5, 25%)	PWV	UF rate	Exc: Acute cardio- vascular accidents; infections in the previous 3 months; HF; arrhythmias; liver disease;cancer; overt oedema
Reshetnik, A (31)	2019	Germany	54	75 (64-85)	(33, 61.1%)	33.1 (4.7-61.5)	(54, 100%)	PWV	UF volume	Exc: Arterial stenosis proximal or at the measurement site; former dialysis fistula at the measurement; active infection; arrhythmias
5. Other										
Bia, D (32)	2015	Argentina	65	58 ± 15		65.3 ± 60.5		PWV	FO; TBF; ECF; ICF	Exc: HD< 3months; extremity amputation; carotid artery stenosis; uncontrolled diabetes mellitus; cardiac arrhythmias; metallic implants
Kim, EJ (3)	2017	Korea	77	52 ±12	(29, 37%)	51.1± 36.9		PWV	FO; TBF; ECF; ICF	Inc: adult on HD > 6 months; no clinical CVD for 3 months preceding enrollment; no renal replacement
Zheng, D (33)	2009	China	73	61 ± 13	(19, 26%)	46.0 ± 8.0	(68, 93%)	PWV	FO; TBF; ECF; ICF	Exc: on HD < 3 months; cardiac event occurred less than 1 month before the study
Gaegbeku, CA (34)	2003	USA	27	55 ± 3	(8, 37%)	47.0 ± 7.0		PWA	UF volume	Exc: on HD < 6months; unresolved infection or illness; BP >200/110 mmHg; SBP <90 mmHg; no palpable pulse for PWA.

HD, hemodialysis; TBF, total body fluid; ECF, extracellular fluid; ICF, intracellular fluid; IDWG, interdialytic weight gain; UF, ultrafiltration; RFO, relative fluid overload; BP, blood pressure; PWA, pulse wave analysis; PWV, pulse wave velocity; AIx , augmentation index; CVD, cardiovascular disease; HF, heart failure; MI, myocardial infarction; CAD, coronary artery disease; AV arteriovenous.

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

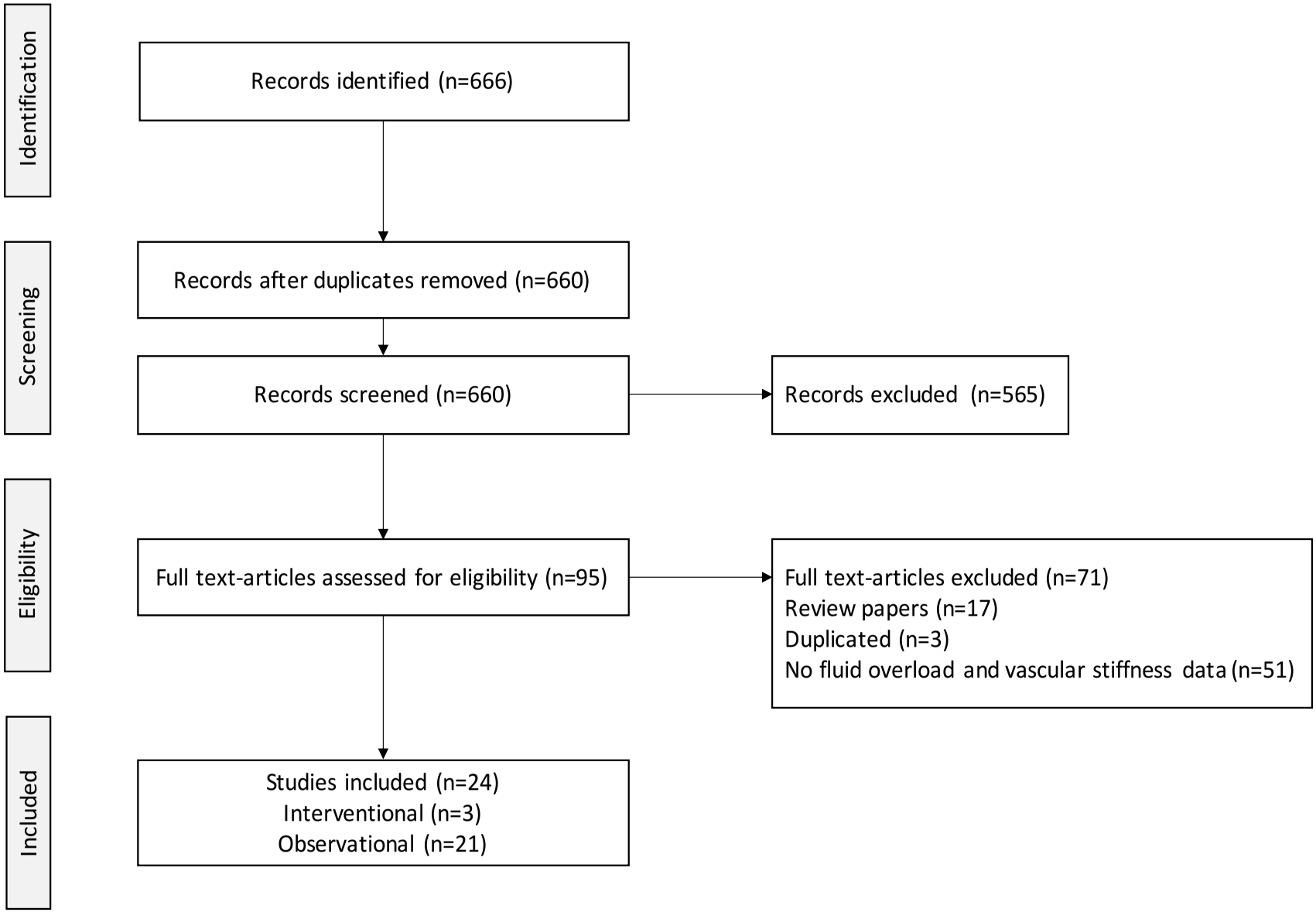
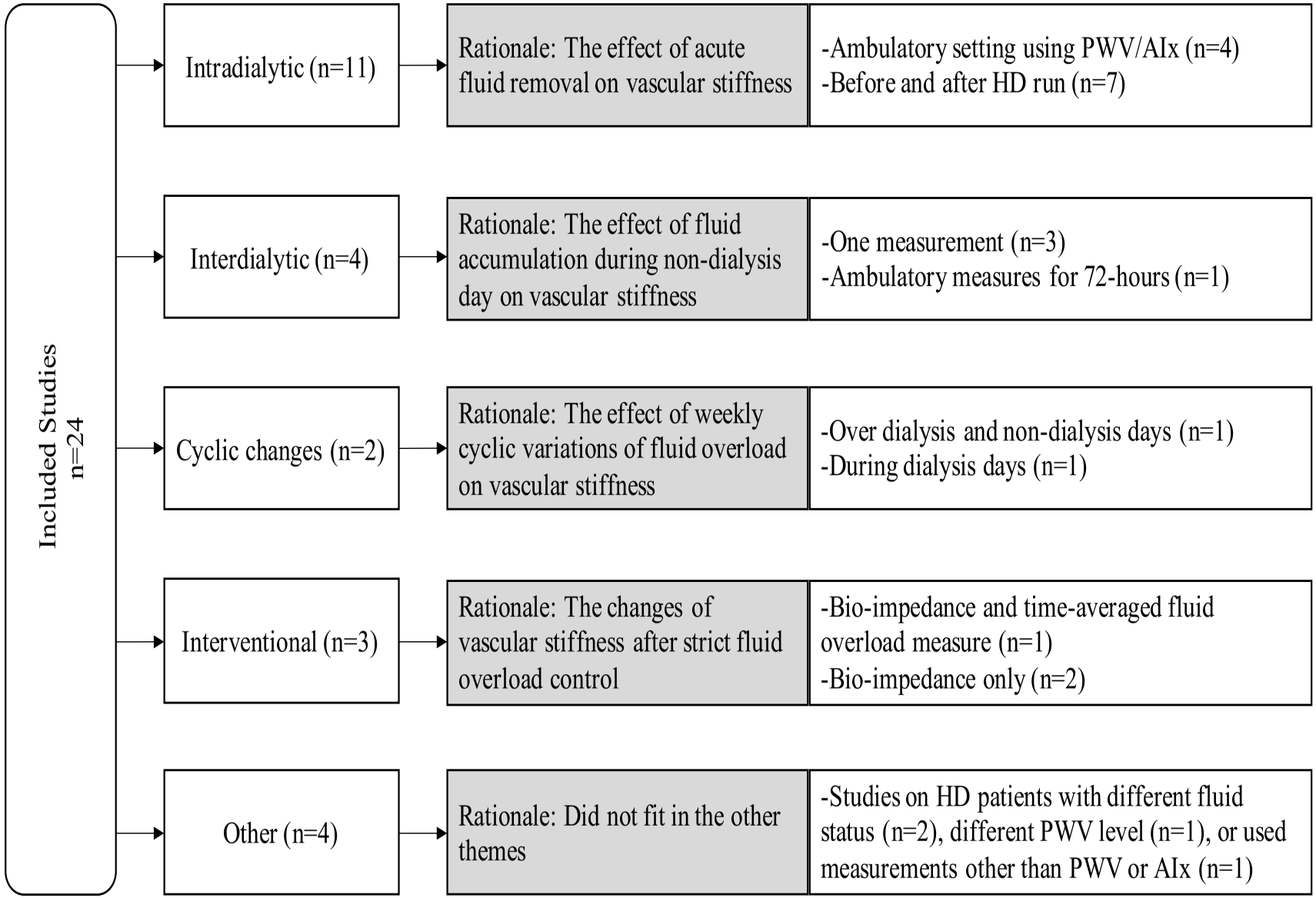


Figure 2: Thematic chart of the included studies



Discussion

In this scoping review, we considered publications regarding effects of intra- and interdialytic fluid volume changes and strict fluid volume control on vascular stiffness in HD patients. Altogether, approaches vary widely, and results are conflicting.

The majority of the observational studies reported that changes in vascular stiffness during the inter- or intradialytic periods were involving several variables such as: blood pressure. However, there was no consensus on how fluid overload and vascular stiffness measured which limits the comparisons. Although the results of bio-impedance-guided interventions for fluid volume control have demonstrated a reduction in vascular stiffness and blood pressure at the end of the treatment, these trials did not specify whether the improvement in vascular stiffness resulted directly from the correction of fluid volume or indirectly from blood pressure improvement (8, 13). This variety in results makes the interpretation and comparison challenging.

As mentioned, studies on this subject have applied different methods. They did not standardize the methodology and analysis of the assessment of vascular stiffness. Similarly, there was no agreement methods and analysis of fluid overload. Using either PWV or AIx as the optimal measure of vascular stiffness measure is debated. PWV and AIx are assessing different aspect of vascular health and they are not interchangeable terms; PWV reflects the vascular elasticity and measures the degree of stiffness, whereas, AIx looks at the impact of vascular stiffness on cardiac load. It should also be noted that other techniques of vascular function measurements have rarely or never been used like direct assessment of microvascular and endothelial function (e.g. forearm

blood flow). The second aspect involves fluid overload. An extracellular/intracellular, or extracellular/total body fluid ratios do not provide enough information about fluid status (32, 35). The fluid of lean tissue mass and adipose tissue mass are different in each individual and it includes different proportion of extracellular and intracellular fluid. In contrast, absolute fluid overload represents the fluid stored exclusively in the extracellular fluid. However, the absolute fluid overload measure varies from one interdialytic day to another, especially after the 72-hours of interdialytic period. Thus, it has been suggested in a few trials that TAFO is a better representative measure of HD patients' fluid overload. It includes the difference between the absolute fluid overload before HD and half of the interdialytic weight gain measure. The studies that used TAFO in their trials, demonstrated significant improvement in fluid status of HD patients (8, 36).

A third aspect is the applied study design. Essentially, comparing vascular stiffness parameters just before or after HD run, without measuring the entire inter- or intradialytic period, does not necessarily reflect the hemodynamic changes that occur during fluid accumulation or removal days. Also, very few studies compared vascular stiffness among HD patients with different fluid status and their measurements were limited to the pre-HD only. Furthermore, trials designed to attempt to correct fluid overload had no or different interventional strategies and had dissimilar definitions of fluid overload. For instance, only one study used TAFO (8). The study found an improvement in fluid status and vascular stiffness. However, the average absolute fluid overload and TAFO were not extremely high, as the fluid status was different in the included HD patients. Therefore, it is difficult to know whether the over-, normo-, or underhydrated HD patients showed the most improvement in vascular stiffness. Finally, the majority of the included studies had a small sample size with short follow up. Taken together, we identified in this scoping review that the fluid

overload and vascular stiffness relationship has not been addressed clearly. To address these gaps, we propose to construct two appropriately powered studies to further unravel the nature of vascular stiffness-fluid overload relationship: first an observational trial and second a randomized controlled trial targeting correction of fluid overload.

In the observational study, two main study components need to be clearly identified: well defined approaches for fluid overload and vascular stiffness and study design. A widely used definition of fluid overload is when the absolute fluid overload $\geq$ 1.1 L above the estimated normal extracellular fluid volume (36, 37). With a 75 mmol/d sodium intake fluid volume in HD patients ideally swings from -1.1 L below the normal extracellular fluid right after the HD session to + 1.1 L above normal extracellular fluid. An absolute fluid overload and TAFO might be better measures to represent fluid overload. As previously discussed, an absolute fluid overload represents the excess fluid stored in the extracellular fluid volume. Furthermore, TAFO includes pre-HD fluid overload and the average of interdialytic weight gain during the week, thus it eliminates the influence of differences in the amount of fluid volume during the interdialytic interval. For vascular stiffness, both PWV and AIx need to be considered since they assess different aspects of vascular function. Aspects of the study design include that the study population should differentiate fluid overloaded versus non fluid overloaded HD patients. Studies should have an appropriately powered sample size to address the aim of the study. For example, around 16 HD patients (with power 0.95, α-error 0.05 and effect size 0.97) are needed to show a significant change in PWV after HD run. This is based on ±4.9 m/s difference in PWV before and after dialysis treatment, as shown before (16, 17, 38). A robust multifactorial analysis of a study would have to exclude several hundreds of patients; such sample size has not been reported. Regarding the timing of the FO measurements using a

validated tool, measurement before the mid-or end-week HD run to avoid the long 72-hour interdialytic interval is preferable, unless the shorter and longer interval are being compared. Regarding vascular function, ambulatory measurements of PWV, AIx, and blood pressure variables during the 24 or even 48 hours in between HD run followed by 4-hours measurements during the run is preferred. Since there are virtually no studies available that have a complete, well defined and designed approach, such a more thoroughly designed study could possibly provide more insight in the relationship between vascular function and fluid overload.

For an intervention trial, similar considerations about methodology and analysis of fluid status and vascular function are applicable. Further, the study design preferably would be a randomized controlled trial and would use a standardized fluid overload reduction protocol. To avoid contamination, it is desirable to run the trial in two different centers. Treating physicians in the control group need to be blinded to the fluid overload results to avoid bias. Characterization of the study groups regarding fluid status should be complete, as mentioned for the observational design. Regarding the primary outcome, a robust measurement of fluid overload and vascular stiffness is preferred which includes the target weight as well as interdialytic weight gain. To this purpose, TAFO seems to be the most suitable parameter. Inclusion of HD patients could then be based on TAFO (for example with a threshold of 1 L or more). To be sufficiently powered, at least 60 HD patients in each arm are needed to be included to show a significant reduction in TAFO by 0.5 L. If a combined parameter for target weight and IDWG is not chosen, it would be desirable to form a 2 by 2 design study, with one target being correction of target weight and one being the correction of excessive IDWG. This design would be quite complicated. Finally, studies should include longer term of follow up with a periodic assessment of fluid overload, vascular stiffness and protocol

adherence to be able to follow the vascular remodeling, if any, after reaching euvolemia. Unfortunately, the current trials do not meet such an approach, and a feasibility study followed by a randomized trial would be needed.

In conclusion, there is no answer whether fluid overload increases vascular stiffness in HD patients due to the conflicting results and a wide variety of methodological aspects. Therefore, larger studies with an appropriate powered sample size, standardized fluid overload and vascular stiffness measurements, and longer term of follow up could identify the ambiguity of fluid overload - vascular stiffness correlation issue.

Chapter 3 - Increased vascular stiffness is not affected by inter- and intradialytic changes in fluid volume in hemodialysis patients

Introduction

Patients on HD have high prevalence of fluid overload and impaired vascular health which strongly predict the cardiovascular events (39, 40). It has been estimated that only 34% of HD patients had fluid overload < 1.1 L of extracellular fluid volume before HD treatment (41).

Vascular stiffness in HD patients is an end result of the damage in the large arterial structure due to multifactorial effects. For instance, atherosclerosis and vascular calcification, increased renin-angiotensin system activity, decreased nitric oxide levels, increased oxidative stress, and systemic inflammatory markers (4-6, 42). Both PWV and AIx are independent factors of vascular outcomes and have been recognized as a gold standard for vascular stiffness measurements (6, 17). The PWV is more related to the vascular wall structure and AIx, derived from pulse wave analysis, is a measure of the pulse pressure waveforms.

The available evidences suggested that fluid overload might be another functional factor that probably increases vascular stiffness through increasing arterial wall tension “Laplace’s law” (6, 43, 44). The cyclic changes of fluid overload due to the intermittent nature of dialysis treatment might also decrease the arterial compliance and increase vascular stiffness in HD patients (40, 43). Reports supported that fluid overload and increased PWV/AIx are highly associated with poor overall survival rate in HD patients (16, 40, 45, 46).

Altogether, the notion of direct effect of fluid overload on vascular stiffness in HD patients remains incomplete. Therefore, the hypothesis of the current study is that interdialytic fluid accumulation increases vascular stiffness which will be corrected by ultrafiltration during HD session. We first confirmed that vascular stiffness is increased in HD patients compared to healthy controls and then studied the inter- and intradialytic dependencies between FO and vascular stiffness in FO and non-FO HD patients.

Methods

Study participants

We recruited 39 out-patients on maintenance HD from dialysis units of University of Alberta Hospital. All patients were 18 years of age and above and had been on dialysis for more than 6 weeks, thrice weekly, 3.5 to 4-hours duration. There are certain conditions can affect the accuracy of the measurements, therefore, exclusion criteria were: acute illnesses that included cardiovascular events and infection, pregnancy, surgery within 6 weeks of the study, nocturnal dialysis, kidney transplantation during the time of the study, implanted pacemaker, defibrillators, pins, metallic stent, artificial joints, and skin lesions at the site where bioimpedance electrodes should be positioned. The same criteria were used to enroll 26 healthy subjects. A health questionnaire was obtained from the healthy subjects. Written informed consent was obtained from all participants. The study was approved by the Human Research Ethics Board at University of Alberta Hospital.

Fluid overload assessment

The body fluid compartments; extracellular fluid volume (ECFV), intracellular fluid volume (ICFV), and total body fluid volume (TBFV) of all study participants were performed using portable bioimpedance spectroscopy monitor (Body composition monitor, BCM® Fresenius Medical Care, Bad Hamburg, Germany) that had been validated previously (47). The average of three consecutive measurements were performed in a quiet room in the dialysis unit and participants were in a supine position with the use of disposable electrodes applied on the wrist and ipsilateral foot. The non-arteriovenous fistula side was used for HD patients. The measurements were performed 15 minutes before the start of mid-week HD session.

FO was defined as an excess of fluid volume of ≥ 1.1 liter (L) above normal ECFV. The HD patients were divided into FO-HD (FO ≥ 1.1 L of normal ECFV) and non-FO HD groups (FO < 1.1 L of normal ECFV). The accuracy of BCM was shown to be within -0.4 ± 1.4 L when compared to dilution methods "considered as gold standard" (37, 47). The 1.1 L cut-off point was based on the calculated 10^{th} and 90^{th} percentiles of the healthy distribution to define the normovolemia range which yielded to -1.1L and +1.1L, respectively. Thereby, subjects were considered fluid overloaded if their FO is greater than 1.1 L and non-fluid overloaded if their FO is lower than 1.1L (48). Also, there is a thought that fluid overload ideally moves back and forth between -1.1 L below normal ECFV after HD run to + 1.1L above normal ECFV before the next HD run with 75 mmol/d sodium intake (37).

Technical aspects and timing of vascular stiffness and blood pressure measurements

We used an oscillometric device called Arteriograph24^{TM} (TensioMed, Budapest, Hungary) to measure aortic PWV, brachial AIx, peripheral systolic and diastolic blood pressure, mean arterial pressure, and pulse pressure using an upper arm cuff (non-arteriovenous fistula arm for HD patients) (49). Arteriograph measures three pulses: systolic wave (P1), reflected wave (P2), and diastolic wave (P3). Then, PWV and AIx are calculated by a software designed for this kind of analysis. Vascular stiffness and blood pressure were performed before the mid-week HD session for 24-hours which was followed by a 5-hours measurement starting 30 minutes before and ending 30 minutes after the dialysis session. In healthy controls, a 5-hour measurement of PWV, AIx and blood pressure was performed as time control.

Clinical data

Dialysis vintage, vascular access, ultrafiltration, and target weight were obtained from the patients record. The interdialytic weight gain (IDWG) estimated by calculating the difference of the patients' weight at the beginning of the HD run and the weight after the previous run. Patients were considered to have diabetes if it was mentioned in their charts or if the patients were on anti-diabetic medications. The antihypertensive medications used were obtained from Nephrology Information System (NIS).

Statistical analysis

Continuous data are expressed as mean ± standard deviation if normally distributed or as median 25-75 percentile if not normally distributed. Categorical variables are expressed as percentage of total. Log transform and Shapiro-Wilk test was used to test the normal distribution of the variables. Chi-square test was used to compare the frequencies in the study groups. Mann-Whitney U test was performed to compare nonparametric parameters between HD group versus healthy controls and between FO-HD versus non-FO HD groups. Two-way repeated measurement ANOVA was used to analyze the variance of vascular stiffness parameters during HD session, the changes of variables in pre-and post-HD session, and during the 24-hours measurements. Tukey multiple comparison test was used to detect the difference between the study groups. The missing data and outliers were imputed by performing a regression analysis using the total data of each parameter per subject. Univariate and multivariate linear regression analysis were performed to determine the factors predicting pre-HD PWV. Correlation between non-parametric data were analyzed with Spearman's test and Pearson's test was used for parametric data. Graph prism (GraphPad 8, San

Diego, CA, USA) and SPSS version 25 (SPSS Inc., Chicago, IL, USA) were used for data analysis. P value <0.05 was considered statistically significant.

Results

Baseline characteristics of the study group

HD patients were significantly older than the healthy controls. The fluid status in HD patients indicated fluid overload. Fifty-nine percent of HD patients were on anti-hypertensive medications; beta-blockers were most frequently prescribed. Both FO-HD and non-FO HD patients had similar prevalence of cardiovascular disease including (angina, coronary artery disease, myocardial infarction, heart failure, and hypertension). However, anti-hypertensive medications were prescribed mostly in FO HD patients compared to non-FO HD patients. FO HD patients had higher dialysis vintage than non-FO HD patients. Demographic and clinical characteristics of the study participants are shown in (**Table 3**).

Blood pressure and vascular stiffness were higher in HD patients compared to healthy controls

At baseline of the 5-hours measurements before initiation of HD session, HD patients had significantly higher systolic blood pressure, mean arterial pressure, pulse pressure, and PWV values compared to the corresponding measurements of healthy controls. The pulse pressure was higher in FO-HD than non-FO HD patients, however, systolic blood pressure, diastolic blood pressure, and mean arterial pressure were not different. The PWV and AIx were not different between FO HD and non-FO HD patients as well. (**Table 3, Figure 3A-C**). After the HD session, fluid overload remained significantly higher in FO HD compared to non-FO HD patients. Yet, no significant changes were observed in PWV and AIx over the HD session in FO HD and non-FO HD patients which was similar to the PWV and AIx measurements of the healthy controls over the 5-hours (**Figure 3D-E**). The mean average 24-hour measurements of systolic blood pressure mean

arterial pressure, pulse pressure, and AIx were significantly higher in FO HD than non-FO HD patients. The two-way repeated measurements ANOVA analysis of AIx was different between the two HD groups, but the PWV was not different (**Table 3, Figure 4A-C**). After the univariate linear regression analysis of baseline PWV predictors, ECFV and TBFV were included in the multivariate linear regression analysis. Neither ECFV nor TBFV were significant predictors for baseline PWV in HD patients (**Table 4**).

Was ultrafiltration (UF) volume related to any improvement in vascular stiffness acutely?

The FO HD patients had higher UF volume than non-FO HD patient, however, this was not significant. There was a weekly reduction in AIx in non-FO HD patients compared to the AIx values before the beginning of the HD session. Contrary to the current hypothesis, PWV values did not decrease after the HD session in either FO HD or non-FO HD patients (**Table 5**).

To test whether fluid removal by UF volume improves PWV and AIx, we performed a regression analysis. There was a positive relationship between UF volume versus post-HD pulse pressure in FO HD patients and delta PWV in non-FO patients. There was a trend toward a positive correlation between UF volume and post-HD AIx in FO HD patients. However, UF volume did not have a direct effect on post-HD systolic blood pressure, diastolic blood pressure, mean arterial pressure, and delta AIx in FO HD and non-FO HD patients (**Table 6**).

Does inter-dialytic fluid accumulation contribute to blood pressure and vascular stiffness in between HD runs?

A significant relationship was observed between FO and inter-dialytic systolic blood pressure, mean arterial pressure, and AIx among HD patients, yet, the analysis failed to show a significant correlation with inter-dialytic PWV (**Figure 5A-D**).

Table 3: Baseline characteristics (of 5-hours) in HD patients and healthy individuals

Parameters	Healthy controls (n=26)	HD group (n=39)	P value	FO HD (n=20)	non-FO HD (n=19)	P value
Gender Male n, %	10 (38.4%)	24 (61.5%)	0.06	14 (70%)	10 (52.6%)	0.26
Age, year	49 (29-56)	60 (49-66)	0.006	62 (51-68)	57 (21-67)	0.05
Body mass index, kg/m2	23.9 ± 3.5	27.0 ± 5.6	0.008	26.2 ± 4.9	27.9 ± 6.2	0.33
Pre-HD weight, kg	68.2 ± 10.9	78.9 ± 17.4	0.003	77.9 ± 16.9	79.9 ± 18.4	0.72
Post-HD weight, kg	-	77.0 ± 17.7	-	75.8 ± 16.9	78.2 ± 18.0	0.66
FO pre-HD, L	-0.16 ± 0.6	1.96 ± 2.4	<0.0001	3.69 ± 2.3	0.13 ± 0.5	< 0.0001
FO post-HD, L	-	0.0 ± 2.2		1.6 ± 1.9	-1.6 ± 1.0	<0.0001
ECFV, L	15.1 ± 2.1	17.8 ± 4.2	0.001	19.1± 4.2	16.5 ± 3.9	0.05
FO/ECFV, %	-0.66 (-4.47-3.06)	7.14 (1.55-19.48)	<0.0001	18.80 (10.0-24.9)	1.55 (-2.0-3.7)	<0.0001
PWV, m/s	8.8 ± 1.4	10.3 ± 1.7	<0.001	10.3 ± 1.4	10.2 ± 1.9	0.83
AIx, %	-22.3 ± 27.1	-10.3 ± 36.6	0.13	-14.8 ± 30.7	-5.5 ± 42.2	0.44
Systolic blood pressure, mmHg	117.0 ± 11.9	138.2 ± 22.2	< 0.0001	144.4 ± 21.5	131.7 ± 21.5	0.07
Diastolic blood pressure, mmHg	69.3 ± 11.3	76.1 ± 15.5	0.04	76.8 ± 11.0	75.4 ± 19.5	0.78
Mean arterial pressure, mmHg	85.2 ± 10.8	95.7 ± 15.4	0.002	99.4 ± 13.0	91.7 ± 17.2	0.12
Pulse pressure, mmHg	49.9 ± 9.9	61 ± 15.8	<0.001	60.0 ± 11.5	50.9 ± 15.3	0.04
Net ultrafiltration, L	-	1.92 ± 1.05	-	2.15 ± 1.12	1.69 ± 0.96	0.19
Dialysis vintage, years	-	2 (0.1-12)	-	3 (2-4)	1 (0.3-3)	0.02
IDWG, kg	-	1.3 (0.0-3.4)	-	1.4 (0.87-2.0)	1.2 (0.6-2.1)	0.66
Anti-hypertensive medications n, %	-	23 (59%)	-	15 (75%)	8 (42%)	0.03
Beta blockers n, %	-	18 (46%)	-	14 (70%)	4 (21%)	0.25
Calcium channel blockers n, %	-	11 (28%)	-	9 (45%)	3 (15.7%	0.16
Diuretics n, %	-	7 (18%)	-	5 (25%)	2 (10.5%)	0.21
Angiotensin receptor blockers n, %	-	5 (12.8%)	-	4 (20%)	1 (5.2%)	0.40
Cardiovascular diseases n, %	-	21 (53%)	-	10 (50%)	11(57%)	0.61
Anuric n, %	-	13 (33%)	-	4 (20%)	9 (47%)	0.07
Diabetic n, %	-	10 (2.5%)	-	6 (30%)	4 (21%)	0.52
24hr-PWV, m/s	-	9.9 ± 1.1	-	9.9 ± 1.1	9.8 ± 1.3	0.80
24hr-AIx, %	-	-12.4 ± 22.3	-	-1.4 ± 21.9	-25.6 ± 18.1	<0.001
24hr-Systolic blood pressure, mmHg	-	131.6 ± 19.5	-	138.2 ± 18.0	123.5 ± 17.2	0.01
24hr-Diastolic blood pressure, mmHg	-	70.8 ± 11.5	-	72.1 ± 8.8	68.8 ± 13.4	0.37
24hr-Mean arterial pressure, mmHg	-	90.4 ± 12.9	-	99.4 ± 13.0	91.7 ± 17.2	0.01
24hr-Pulse pressure, mmHg	-	60.4 ± 13.4	-	65.8 ± 14.4	54.7 ± 9.8	0.008

P value < 0.05 is considered significant; FO, fluid overload; ECFV, extracellular fluid volume; ICFV, intracellular fluid volume; TBFV, total body fluid volume; PWV, pulse wave velocity; AIx, augmentation index; IDWG, intradialytic weight gain. FO post-HD = FO pre-HD- net ultrafiltration.

Table 4: Predictors of baseline PWV in HD patients using univariate and multivariate linear regression analysis

Parameters	Univariate analysis			
	B	t	CI (95%)	P value
Age, year	0.037	1.939	-0.002-0.076	0.06
Body mass index, kg/m2	-0.011	-0.218	-0.112-0.090	0.82
Pre-HD weight, kg	0.026	1.691	-0.005-0.058	0.09
IDWG, kg	-0.22	-0.696	-0.886-0.433	0.49
FO, L	0.088	0.780	-0.141-0.317	0.45
ECFV, L	0.132	2.113	0.005-0.259	0.04
FO / ECFV, %	1.387	0.573	-3.515-6.290	0.57
ICFV, L	0.111	1.817	-0.013-0.234	0.07
TBFV, L	0.069	2.098	0.002-0.135	0.04
ECFV/TBFV, %	2.661	0.389	-11.18-16.51	0.69
ICFV/ECFV, %	0.233	0.137	-3.22-3.69	0.89
Systolic blood pressure, mmHg	0.015	1.24	-0.01-0.041	0.22
Mean arterial pressure, mmHg	0.022	1.21	-0.014-0.058	0.23
Pulse pressure, mmHg	0.027	1.146	-0.021-0.075	0.25
Diabetic n, %	1.174	1.930	-0.05-2.40	0.06
Anti-hypertensive medications n, %	0.540	0.965	-0.59-1.67	0.34
Dialysis vintage, years	-0.039	-0.373	-0.25-0.172	0.71
Net ultrafiltration, L	0.167	0.626	-0.373-0.706	0.53
Multivariate Analysis				
ECFV, L	4.006	1.022	-3.954-11.957	0.74
TBFV, L	-3.945	-0.997	0.053-3.04	0.38

P value < 0.05 is considered significant; IDWG, intradialytic weight gain; FO, fluid overload; ECFV, extracellular fluid volume; ICFV, intracellular fluid volume; TBFV, total body fluid volume.

Table 5: Hemodynamic data of pre-and post-HD session in HD patients

Parameters	FO-HD (n=20)			non-FO (n=19)		
	Pre-HD	Post-HD	P value	Pre-HD	Post-HD	P value
PWV, m/s	10.3 ± 1.4	9.6 ± 2.5	0.99	10.2 ± 1.9	10.4 ± 2.0	0.99
AIx, %	-14.8 ± 30.7	-20.1 ±5 3.1	0.89	-5.5 ± -42.2	-16.3 ± 39.5	0.04
Systolic blood pressure, mmHg	144.4 ± 21.5	143.1 ± 33.9	0.99	131.7 ± 21.5	131.4 ± 24.3	0.99
Diastolic blood pressure, mmHg	76.8 ± 11.0	75.8 ± 17.3	0.99	75.4 ± 19.5	76.0 ± 19.2	0.99
Mean arterial pressure, mmHg	99.4 ± 13.0	97.3 ± 21.1	0.99	91.7 ± 17.1	93.7 ± 19.8	0.99
Pulse pressure, mmHg	66.7 ± 17.7	64.4 ± 23.5	0.97	56.3 ± 11.9	53.7 ± 11.4	0.41
Heart rate, b/m	78.7 ±12.2	77.4 ± 11.9	0.86	80.8 ± 12.6	82.5 ± 14.1	0.99

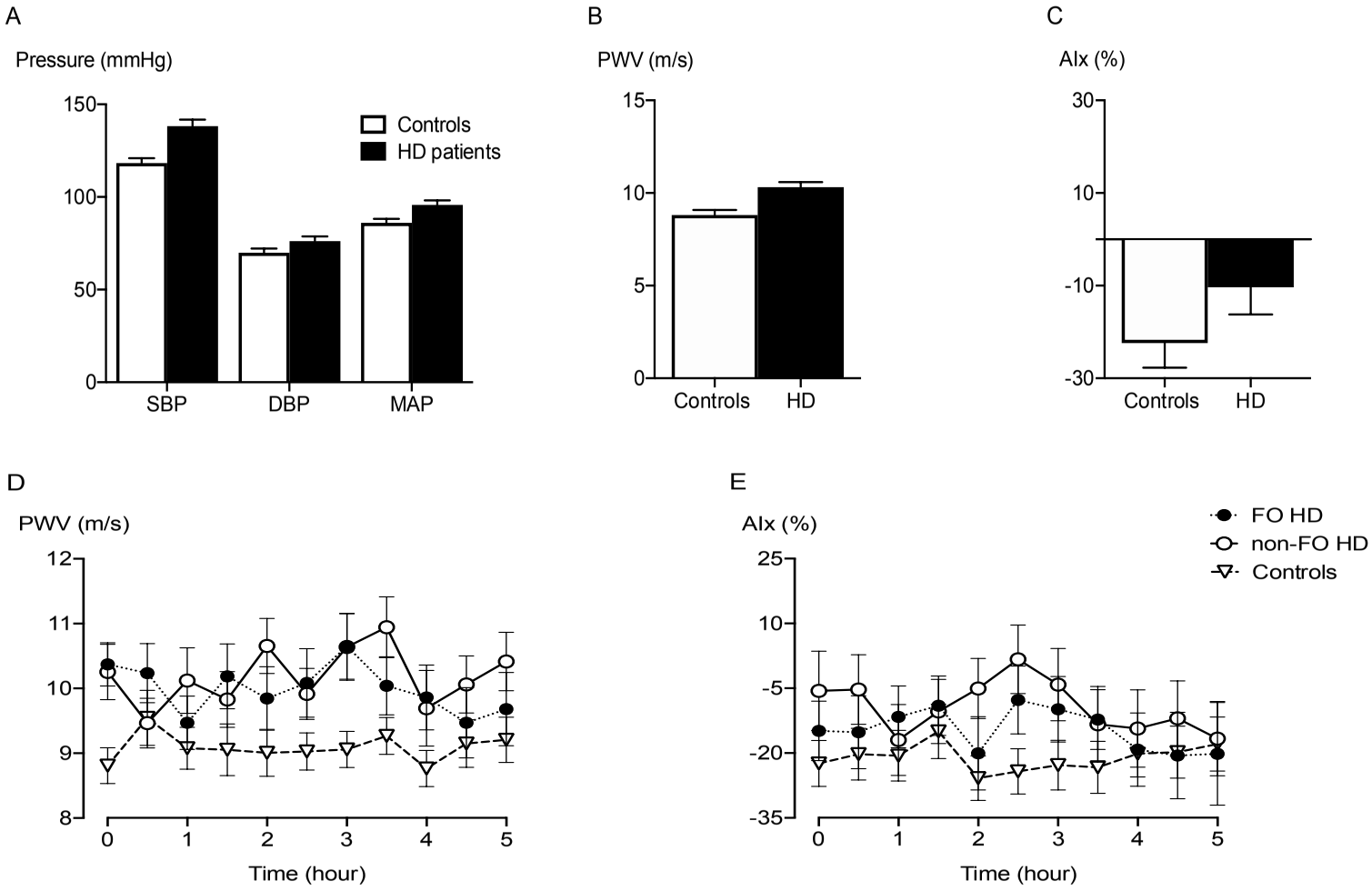
P < 0.05 considered significant; PWV, pulse wave velocity; AIx, augmentation index

Table 6: Linear regression analysis of intradialytic PWV, AIx, and blood pressure vs. UF

Parameters	HD group (n=39)		FO HD group (n=20)		non-FO HD group (n=19)	
	r_2	P value	r_2	P value	r_2	P value
Post-HD PWV, m/s	0.033	0.26	0.033	0.44	0.082	0.23
delta PWV, m/s	0.108	0.04	0.046	0.35	0.300	0.01
Post-HD AIx, %	0.019	0.40	0.193	0.05	0.109	0.16
delta AIx, %	0.002	0.75	0.088	0.20	0.082	0.23
Post-HD Systolic blood pressure, mmHg	0.042	0.20	0.093	0.19	0.003	0.81
Post-HD Diastolic blood pressure, mmHg	0.0008	0.86	4.92e-007	0.99	0.003	0.80
Post-HD Mean arterial pressure, mmHg	0.005	0.66	0.015	0.60	0.001	0.88
Post-HD Pulse pressure, mmHg	0.157	0.01	0.235	0.02	0.006	0.75

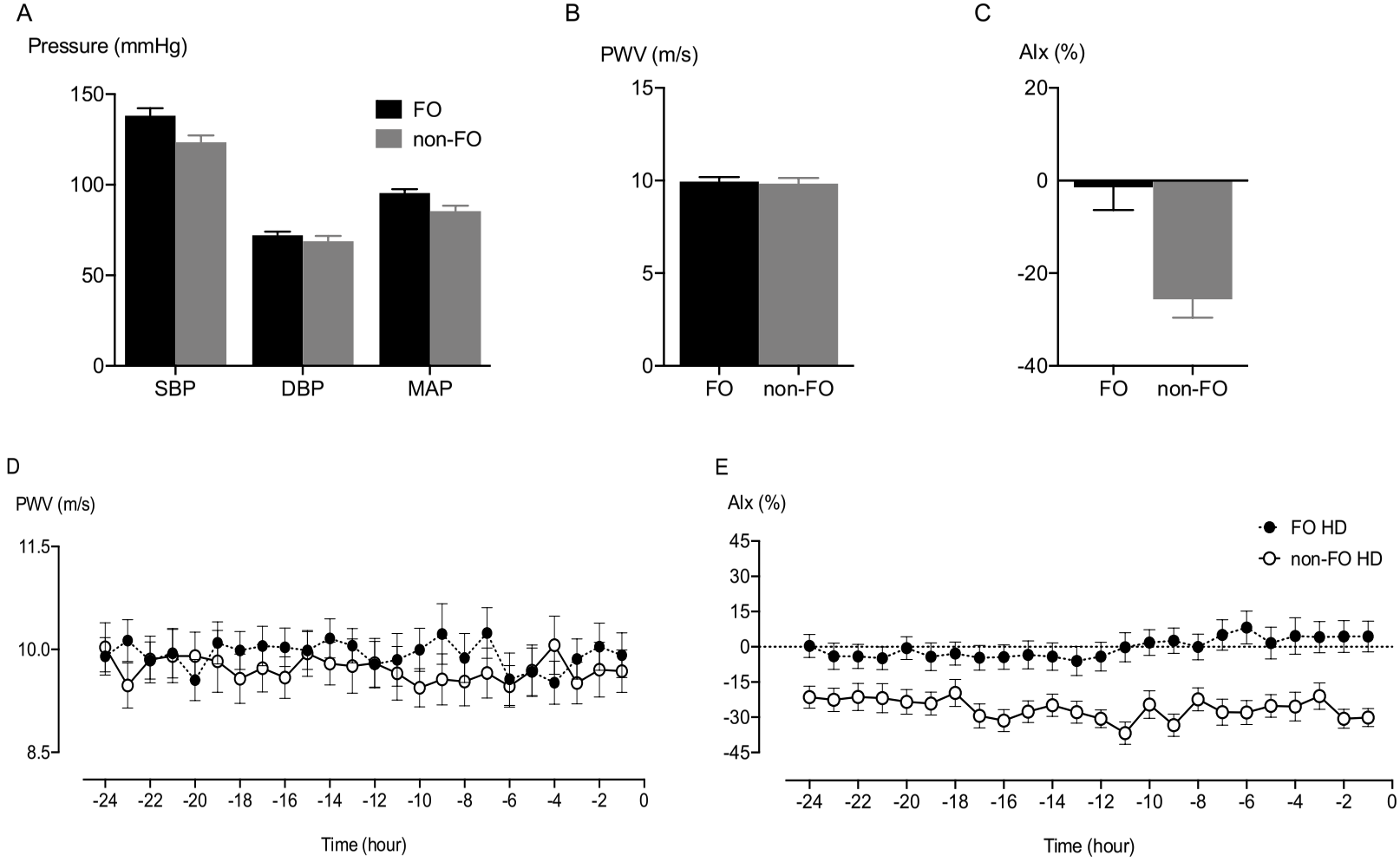
$P < 0.05$ considered significant; delta (post-HD -pre-HD); PWV, pulse wave velocity; AIx, augmentation index

Figure 3: Baseline blood pressure, PWV, and AIx in HD patients and healthy individuals



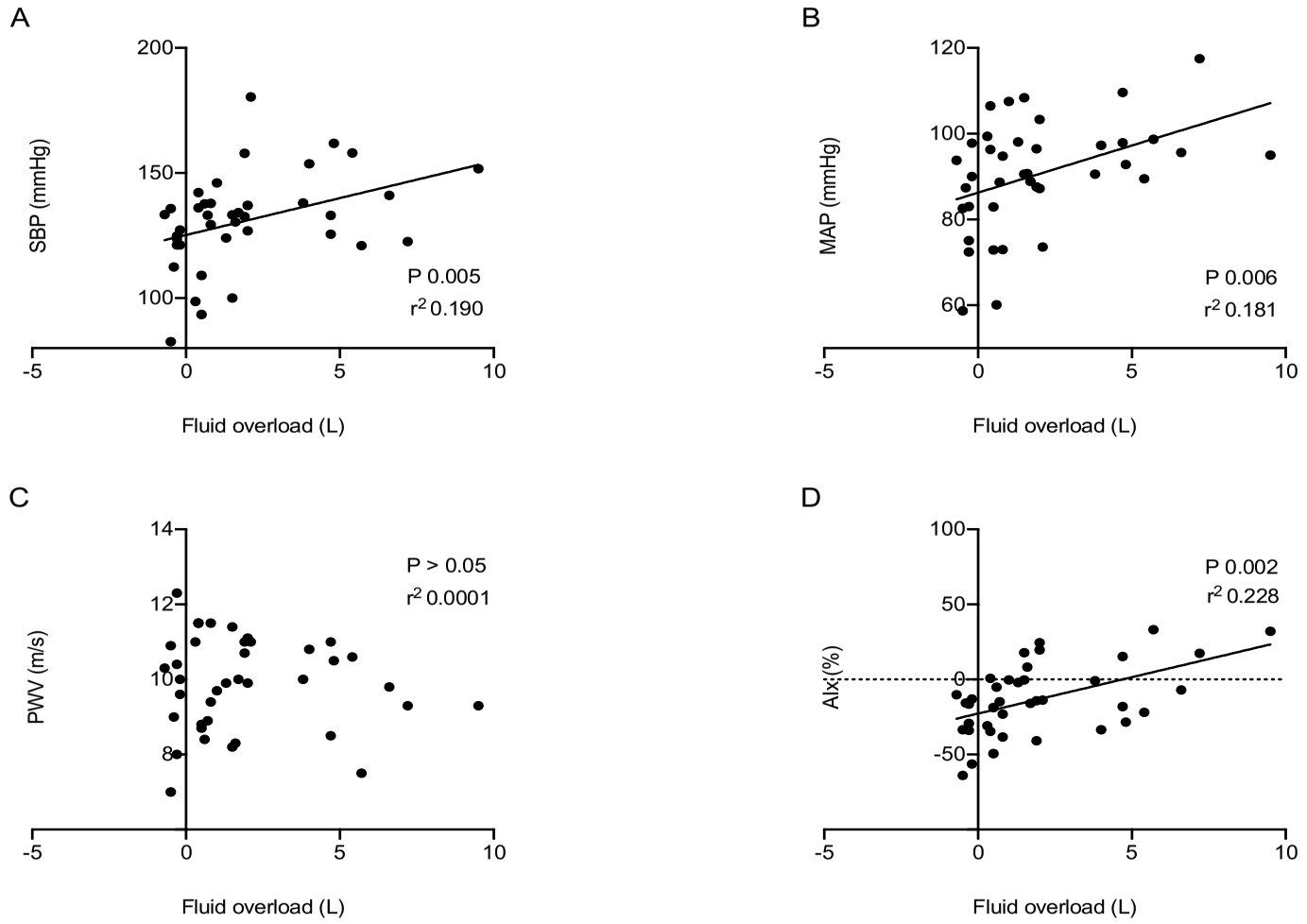
Significant difference in baseline systolic blood pressure (SBP, mmHg), mean arterial pressure (MAP, mmHg), and diastolic blood pressure (DBP, mmHg), was observed in HD patients (black bar) compared to healthy controls (white bar) (**A**). Baseline pulse wave velocity (PWV, m/s) was significantly higher in HD patients than in healthy controls (**B**), but not augmentation index (AIx, %) (**C**). No significant changes in PWV (**D**) and AIx (**E**) during the 5-hours measurements in FO HD (closed symbol; dash dotted-line) and non-FO HD patients (opened symbol; solid line), that was matching with the results of healthy controls (inverted triangle; dash-solid lines).

Figure 4: Average and repeated measures of 24-hr interdialytic in FO and non-FO HD



The mean average of the 24-hour systolic blood pressure (SBP) and mean arterial pressure (MAP), but not mean average of 24-hour diastolic blood pressure (DBP), were higher in FO HD (black bar) versus non-FO HD patients (grey bar) (**A**). No significant difference in mean average of 24-pulse wave velocity (PWV, m/s) was observed between FO HD and non-FO HD patients (**B**). The mean average of 24-hour augmentation index (AIx, %) was higher in FO HD than non-FO HD patients (C). The ambulatory 24-hour measurements of PWV was not different between the two groups (**D**), but the AIx was (**E**).

Figure 5: Correlation analysis between blood pressure, PWV, and AIx vs. fluid overload



Fluid overload (L) was positively correlated with systolic blood pressure (SBP, mmHg; A), mean arterial pressure (MAP, mmHg; B), and augmentation index (AIx, %; D), but it was not correlated with pulse wave velocity (PWV, m/s; C)

Discussion

We investigated the effect of inter- and intradialytic fluid changes on vascular stiffness in HD patients. First, we confirmed that HD patients have higher vascular stiffness than healthy controls which is consistent with other publications (6, 17, 27). Interestingly, the pre-HD PWV and AIx between fluid and non-fluid overloaded HD patients was not different. Two studies evaluated the vascular stiffness in HD patients with different fluid status, as assessed by bio-impedance spectroscopy (1, 32). One study found that the pre-HD PWV was not different among hypervolemic, normovolemic, and hypovolemic HD patients (32). In contrast, another study found that the pre-HD PWV was higher in Fluid overloaded HD patients versus non-fluid overloaded HD patients (1). The differences in vascular stiffness findings could be due to variations in the characteristics of the dialysis patients, such as dialysis vintage, medications and co-morbidities. However, our findings could not show a clear difference in vascular stiffness between HD groups with different fluid volume status.

We hypothesized that interdialytic fluid accumulation would lead to an increase in vascular stiffness in HD patients. There was no gradual increase or decrease in PWV and AIx during the interdialytic interval in HD patients. However, since changes in vascular stiffness could be more pronounced in fluid versus non-fluid overloaded HD patients, we also compared these two groups. we found that the interdialytic changes of AIx, but not PWV, was different between fluid overloaded and non-fluid overloaded HD patients. Two previous reports studied the interdialytic changes of vascular stiffness for 48 hours using two different technologies (50, 51). The two studies found that the interdialytic PWV did not change, whereas AIx increased which is different than our findings. However, these studies did not specify the fluid status of the HD patients which limits the

comparison. There are several explanations for the stability of PWV and AIx during the 24-hour interdialytic period. First, since PWV is strongly related to viscoelastic remodeling of vascular wall, the 24-hour interdialytic time interval might be insufficient to show a clear change in PWV, as suggested before (52, 53). Second, a 48-hour interdialytic interval could produce more pronounced fluctuations in wave reflections, as assessed by AIx, than the 24-hour interdialytic interval we used (51). Third, antihypertensive medications, which were used more frequently by fluid overloaded HD patients, might obscure changes in vascular stiffness (6). All in all, we were unable to show that interdialytic fluid accumulation increases vascular stiffness irrespective of the baseline fluid volume status.

We further investigated whether intradialytic fluid removal through ultrafiltration would improve vascular stiffness in HD patients. We found that both PWV and AIx remained stable during the HD run. A previous study observed a significant decline in intradialytic PWV only at 135 and 210 minutes in to the HD run, whereas intradialytic AIx remained unchanged (19). The latter study has some limitations; the study does not provide fluid volume data of the HD patients, and the two times where PWV reached a significant level followed decreases in blood pressure and did not correlated with fluid volume removal. Along the same lines, ultrafiltration volume and post-HD PWV did not show a correlation which was similar our findings.

While fluid removal could improve vascular stiffness, activation of the sympathetic nervous system and autoregulation responses might offset the overall effect (54, 55). After the fluid removal, stroke volume, cardiac output, and blood pressure decrease. This would trigger the baroreceptor reflex and activate sympathetic nervous system which leads to an increase in peripheral vascular tone.

Another mechanism is autoregulation which in response to a decrease in blood pressure and a reduction in tissue perfusion will maintain blood flow through an autoregulation mediated vasodilation. This could possibly explain why we could not observe changes in vascular stiffness in interdialytic fluid accumulation.

It has been reported that HD treatment may reduce AIx but not PWV following the HD run (1, 6, 56). In the current study, first, post-HD AIx was reduced in non-fluid overloaded HD compared to fluid overload HD patients, while pre-and post-HD PWV remained unchanged in both groups. Second, pulse pressure remained higher in fluid overloaded HD than non-fluid overloaded HD patients after HD treatment. Third, post-HD fluid volume remained over 1.1 L in fluid overloaded HD and further decreased in non-fluid overloaded HD patients. While fluid volume overload increases pulse pressure in HD patients (56), pulse pressure is one of the components of AIx measurement. This could explain the reason why AIx clearly decreased after HD run in non-fluid overloaded HD compared to fluid overloaded HD patients. Our results were supported by another study which demonstrated a decrease in AIx in non-fluid compared to fluid overloaded HD patient after HD run (1). Three more studies showed a significant reduction in AIx after mid-week HD run with unchanged PWV (6, 24, 56), however, fluid volume status in these studies was not reported. Altogether, while both pulse pressure and AIx are considered as an indirect measure of arterial stiffness, yet, we cannot confirm that fluid removal reduces or increases vascular stiffness without having a clear change of PWV coupled with AIx measurements to have complete information on vascular cushioning function.

Although the current study provides a full description of vascular stiffness changes during inter- and intradialytic fluid volume changes, there are limitations in it. Our sample size was relatively small. Yet, our findings are matching the existing literature (6, 19, 50). Another limitation is that we did not study vascular stiffness during the 48-hour interdialytic interval which could provide more obvious change of vascular stiffness than the 24-hour interdialytic interval. However, we believe the assessment of vascular stiffness during the 48-hour interval period might not change our results.

There is another possible explanation why we did not observe changes in vascular stiffness related to the short-term swings in fluid status. It could be high extracellular sodium causes a deterioration in endothelium surface. Endothelial glycocalyx covers the outer surface of endothelium in order to prevent sodium access into the endothelial cells. High sodium reduces the vaso-protective function of the endothelial glycocalyx (57). The latter will be flattened and damaged, herby sodium flux to the endothelial cells increases. Furthermore, studies hypothesized that aldosterone mediates endothelial sodium channel which in turn increases sodium accessibility into the endothelial cells (58). This could lead to endothelial dysfunction, reduce endothelial vasodilator factor (nitric oxide) release, and harden endothelial cells which might explain the poor vascular function. Since sodium intake is widely common among HD patients, estimating sodium storage capacity could give us an image of the endothelial function in fluid and non-fluid overloaded HD patients.

In conclusion, our study clearly reports that fluid accumulation and removal do not have direct effects on vascular stiffness in HD patients. The post-HD AIx reduction in non-fluid overloaded

HD patients might suggest that an adequate ultrafiltration volume could improve the wave reflections but not PWV.

Chapter 4 - General discussion and perspectives

The majority of the current reports assumed the possibility of a relationship between fluid overload and vascular stiffness in HD patients. The assumption was based on Laplace's hypothesis; fluid overload increases the distension of the vascular wall which in turn might determine vascular stiffness. However, this hypothesis is not always applicable, whether during acute or chronic vascular changes. If we look into the Laplace's equation *wall tension = pressure x radius* we could come up with two considerations. First, the radius of the vascular wall needs to be drastically distended to result in a significant change in wall tension and consequently a change in vascular stiffness which, in the physiological setting, does not seem very likely to occur. The second consideration involves the pressure. For instance, If the systolic/diastolic blood pressure is 160/100 mmHg, then the mean arterial pressure would be 120 mmHg, and that equals to 25% of change in the vascular wall, above the normal mean arterial pressure of (120/80 mmHg). This amount of change might not be sufficient to observe a clear vascular stiffness. Chronically, the vascular changes would require prolonged physiological and biological changes to induce vascular damage (remodeling), so in this case Laplace's hypothesis would not be relevant. Up to date, studies that supported the hypothesis whether fluid overload increases vascular stiffness, have demonstrated a positive "blood pressure dependent" effect of fluid overload on vascular stiffness (9, 19, 21, 25, 26, 28) . The fluid overload in these studies was defined as an increase in extracellular/intracellular or extracellular/total body fluid ratio, but not absolute fluid overload. These ratios are not the best representation of fluid overload. As discussed previously, only absolute fluid overload reflects the amount of fluid stored specifically in the extracellular fluid. However, it remains very challenging to investigate changes in fluid overload on vascular stiffness in HD patients.

Understanding the physical aspects of fluid overload in HD patients is very crucial. Before we question the relationship between the fluid overload and increased vascular stiffness in HD patients, it is very important to consider amount and distribution of the fluid overload. The distribution of the fluid overload is relevant, since if it is entirely in the interstitial fluid, then Laplace's law would not apply, and it would be impossible to investigate the correlation between fluid overload and vascular stiffness. Determining the distribution of fluid overload is not trivial.

Another aspect that related to the vascular function is salt in the skin or so called "skin-sodium". The notion of skin sodium has been recently presented in the literature (59, 60) . Reports have found that sodium can be stored as in an osmotically inactive form in the skin and muscles. Skin-sodium increases with age, high sodium intake diet, and in patients with diabetes, chronic kidney disease, and end-stage kidney disease (58, 59, 61, 62) . High level of skin-sodium found to be associated with high blood pressure and poor vascular function in humans. The underlying mechanism is that high sodium increases the local osmolality which triggers the tonicity-enhancer binding protein in macrophages presented in the skin. This tonicity-enhancer binding protein activates vascular endothelial growth factor-C (VEGF-C) that enhances the density of lymph capillary which in turn increases skin-sodium clearance. In contrast, a low VEGF-C level increases fluid retention, decreases the potent vasodilator factor- nitric oxide, and increases salt-sensitivity, herby, high blood pressure. Recent studies demonstrated a lower level of VEGF-C in HD patients, and that was not associated with the sodium and fluid removal by ultrafiltration (59). The latter study showed that sodium-magnetic resonance imaging (Na-MRI) is a practical tool to assess skin-sodium and assist in sodium reduction in end-stage kidney disease patients on dialysis.

As mentioned previously, increased sodium storage in the skin or muscles is associated with impaired vascular function in HD patients. In addition, an increased sodium storage in the layer lining the endothelial luminal surface (glycocalyx) is associated with an improved vascular function (57). The negative charge of the glycocalyx lining the endothelial surface prevents the friction between the red blood cells and the endothelium and the possible shedding of the endothelial glycocalyx. Also, high sodium storage sites determine the quality of the glycocalyx. HD patients have higher plasma sodium than healthy individuals and have a thin and corrosive endothelial glycocalyx (63-65). It has been suggested that the glycocalyx of the red blood cells and endothelium lose the repulsive forces that prevent the friction between the red blood cells and endothelial layer when it is fully occupied by the plasma sodium, therefore, shedding of the endothelium glycocalyx occurs (66).

Overall, it can be concluded that the nature of the fluid overload and vascular stiffness relationship is still unclear. However, three factors should be taken in consideration before investigating the relationship between the two factors (fluid overload and vascular stiffness). First, using Laplace's law hypothesis to define the effect of fluid overload on vascular stiffness is a misguided concept, minor mean arterial pressure changes even with high blood pressure, and the location of fluid overload does not necessarily explain such a hypothesis. Second, plasma sodium and fluid removal through ultrafiltration is not similar to skin sodium removal. Third, identifying the absolute fluid overload is not enough to determine the vascular function. Further, measuring the thickness and function of the endothelial glycocalyx would give us a better impression on the quality of vascular structure in HD patients in response to changes in total body sodium.

Perspectives

In line of the discussion about and given what we have identified from the scoping review and our experience from the observational trial, several steps could be taken to enhance the understanding of the relationship between fluid overload and vascular stiffness: a) determining the distribution of the fluid overload using MRI tool, b) assess the skin sodium level using sodium-MRI, c) measure the quality of the vascular wall through measuring the sodium storage capacity of the endothelial glycocalyx, d) assess the microvascular and endothelial function using one of the 4 arms gold standard methods (Acetyl-choline using nitric oxide, vascular wall tracking, retinoid fluoroscopy, or cheek micro vessel), e) measuring PWV at different sites (Femoral, brachial, radial, and ankle). Also, further studies should subdivide HD patients into fluid overloaded and non-fluid overloaded using TAFO measure instead of the absolute fluid overload. Alternatively, we could subgroup the HD patients into high versus low skin-sodium level using similar fluid overload and vascular stiffness measurements used previously. For the interdialytic vascular stiffness assessment, ambulatory PWV, AIx, and blood pressure measurements for 48-hours instead of 24 hours could improve the assessment of vascular stiffness function.

Without having a full data set, we observed a few difficulties in our pilot randomized controlled trial. After the HD day, some of the HD patients came back with large interdialytic weight gain which impacted the success of the next HD run. Therefore, in further studies a thought could be to correct the high interdialytic weight gain first by implementing fluid and salt education, and only then process with a target weight correction intervention. As followed from the scoping review it seems advisable that future trials should characterize the complete fluid overload measurements, consider assessing microvascular and endothelial function, use larger sample size, and perform a

longer follow up, for studying the possible relationship between fluid overload and vascular stiffness in HD patients.

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Appendix - Target weight correction and vascular stiffness in hemodialysis patients

Preamble

This study proposal is looking at the application of an accurate assessment of fluid status in hemodialysis (HD) patients to correct fluid overload. Patients undergoing hemodialysis suffer from the inability to maintain their normal body fluids and have a high probability to develop hypertension at initiation of dialysis. Long-standing fluid overload is no longer linked solely to high blood pressure, but also to vascular dysfunction and heart failure. Fluid overload is estimated based on the amount of fluids available outside the cells (extracellular fluid volume, ECFV). Thus, determination of the right amount of fluid removal during the dialysis depends on the weight of the patient when the ECFV is normal (referred to as Target Weight, TW). Essentially, TW is assessed by routine clinical judgment, for example, leg and hands swelling (edema), elevation in blood pressure and the expansion of the external jugular vein. Unfortunately, this clinical judgment is not reliable to assess fluid status (8, 37); hence, overestimation of TW occurs and leads to fluid overload. Current technology allows assessing fluid overload accurately by using bio-impedance, a non-invasive method that can be easily used in the HD setting. Despite this, very few studies have been reported where a systematic approach was followed to use bio-impedance to correct a TW that has been set too high, with the ultimate goal to correct fluid overload. ***Therefore, this study aims to provide better fluid control through an intervention to improve fluid status toward normovolemia by using bio-impedance assessment of fluid overload rather than clinical judgments***. First, the fluid status will be measured in all study participants by multifrequency bio-impedance using a 'Body Composition Monitor' (BCM, Fresenius). Second, we will divide the

study subjects into two groups; the control group which will initially receive standard conventional therapy (no intervention) for 3 months, and the intervention group will undergo by BCM measurements and integrated TW correction protocol for 3 months. Third, after three months, the control group will also involve in the same TW correction adjustment. The Primary outcome is improvement in fluid status towards normovolemia (<1.1 L fluid overload). Secondary outcomes are a) improvement in vascular health as assessed by pulse wave velocity and augmentation index and b) a decrease in the use of antihypertensive medications. Altogether, an optimized fluid status via implemented fluid management plan will provide better control of fluid overload, blood pressure, and improvement in vascular function.

Literature review

Fluid overload remains the main clinical problem in the treatment of HD patients. Usually, the fluid volume is maintained within a very narrow range in people with normal kidney function and impaired in patients with failing kidney, therefore, the patients become fluid overloaded (67). Long-term fluid overload is associated with high cardiovascular risk in HD patients; it leads to hypertension, cardiac hypertrophy, and heart failure, respectively (46) (see figure 1). Few studies have investigated whether an improvement in fluid overload will improve arterial wall stiffness which is a measure of vascular health and an independent predictor of cardiovascular mortality (8). Fluid volume contraction, however, is associated with intradialytic hypotension symptoms (explained further) and cardiac stunning. Therefore, controlling fluid overload while prevention fluid underfill is important to improve cardiovascular function, mortality rate, and survival (68).

In the clinical setting, estimation of TW and fluid overload are routinely based on clinical judgment (like edema, high blood pressure, and central venous pressure). This does not necessarily reliably reflect the fluid status of HD patient (45, 65). Edema, for instance, is considered as one of the most reliable clinical indicators of fluid overload, but it takes 3 to 5 L of fluid volume expansion before it becomes obvious. Non-invasive, practical, and accurate bedside devices, like BCM, aid in a proper estimation of TW and management of fluid overload. In fact, Fluid overload is time-dependent, and usually, HD patients are exposed to different amounts of fluids during their dialysis-free period even if the normal fluid volume is achieved after HD run. Moreover, fluid volume is usually higher after the long intradialytic interval. To make different measurements of fluid volume during the week similar, time-averaged fluid overload (TAFO) appears to best representative target of patient's fluid status (36). Although many studies have expressed an improvement in fluid status by using BCM measurement (explained below), few trials have used TAFO concept as a target to achieve TW in HD patients. Given those points, controlling fluid overload in HD patient is crucial to improving cardiovascular parameters. This study is aiming to demonstrate that the fluid management plan based on BCM measurements will improve fluid status, blood pressure and vascular function in HD patients. **This proposal's focus is on improving the set TW to become closer to the actual dry weight (i.e. normovolemia)**.

Hypervolemia in HD patients

Fluid overload, or hypervolemia, is associated with poor cardiovascular survival (69). A study investigated the five years survival of HD patients with concomitant medical conditions (congestive heart failure, fluid overload and pulmonary edema). Congestive heart failure was associated with the lowest survival rates (12.5%), surprisingly survival rates of fluid overload were

lower than pulmonary edema (20.2% and 21.3% respectively). Mortality rate expectations are tended to be high in HD patients with hypervolemia (46).

There are two determinants of fluid overload control in HD patients. The first is the weight set by the medical team as 'normal' which is the above-mentioned TW, which is estimated to be the so-called 'dry weight'. The second is adherence to low sodium intake between dialysis runs. This in turn determines water intake and that so-called interdialytic weight gain, IDWG. The aim of each HD run is to remove fluid so that the patient's weight becomes just below that TW. In between the HD runs, fluids accumulate again in the dialysis-free days because patients ingest sodium, which triggers thirst, water intake and then leads to fluid volume expansion. IDWG is optimally not more than 2 L of ECFV, but many patients return to the units with weight gain that is substantially higher (70-72). Controlling these components represent two of the most challenging aspects of HD treatment (45).

Clinical signs and symptoms do not suffice to estimate the right target weight

As already briefly mentioned, it remains hard to set the TW so that it resembles dry weight in HD patients. Underestimation of TW can lead to undesirable intradialytic hypotension events. Of note, there is a misconception in determining hypovolemia and hypervolemia (73). Being hypotensive does not imply hypovolemia. In fact, a high ultrafiltration rate can cause intradialytic hypotension symptoms without being hypovolemic. Studies have defined intradialytic hypotension as a drop in systolic blood pressure (>20mmHg) or in mean arterial pressure (MAP) (>10mmHg) combined with clinical symptoms and need for medical staff intervention (74). Signs and symptoms other than blood pressure per se, like cramps, dizziness, and blurred vision, may also not reflect

hypovolemia (75). Conversely, hypertension and edema, although more prevalent in fluid overloaded HD patients, does not necessarily imply hypervolemia. For example, sympathetic hyperactivity and increased vascular stiffness can lead to hypertension (73). Finally, normotension does not necessarily mean that a patient is normovolemic (68). Hence, it is imperative to apply more reliable methods to determine the fluid status of HD patients.

Arterial stiffness in HD patients

Renal disease has been recognized for a long time to cause atherosclerosis, presenting as media sclerosis (Monckeberg sclerosis) (42)**.** Also, renal disease causes vascular dysfunction due to a large variety of abnormalities in the internal environment such as increased activity of the Renin-Angiotensin System (RAS), decreased Nitric Oxide (NO) levels, and increased oxidative stress and inflammation (4, 5)**.** This increases arterial stiffness which in turn increases the heart workload. This, in turn, induces ventricular hypertrophy and eventually can lead to heart failure. Furthermore, arterial stiffness increases pulse pressure (the difference between systolic and diastolic blood pressure). A more precise measure of vascular stiffness is the speed at which the pressure wave travels from the heart to the limbs; this is called pulse wave velocity (PWV). The pulse wave bounces back in the limbs to the heart, this creates a pressure load to the heart, which can be assessed using a measurement called pulse wave analysis (PWA). In general, an increase in PWV is associated with increased cardiovascular morbidity and mortality (76-78). In chronic kidney disease and end-stage renal disease patients, increased PWV and PWA has also been associated with a high incidence of cardiovascular events (16, 40, 44, 79)**,** independent of cardiovascular risk factors such as diabetes and hypertension (80-83).

Applanation tonometry and oscillometric methods such as the Arteriograph24™ are non-invasive methods to assess PWV and PWA. For our study, we will use the Arteriograph24™ device to get the full vascular activity of the study subjects. It measures the brachial pressure waveforms (oscillometrically), on the patient's upper arm using a blood pressure cuff. Using Arteriograph24™, multiple measurements can be obtained, in the same fashion as a 24-hour blood pressure measurement (44). Measuring PWV and PWA will help to identify the improvement of vascular health through fluid overload management plan.

fluid overload and arterial stiffness in HD patients

Both fluid overload and vascular stiffness markers (PWV and PWA) are positively correlated with cardiovascular events. Evidence to support a direct connection between fluid overload and arterial stiffness is hardly available. Possible mechanisms include an increase in arterial wall tension (Laplace's law) associated with hypervolemia as well as a change in the reflection point of the pressure waves due to hypervolemia associated changes in peripheral vascular resistance (3). As discussed earlier, chronic kidney disease is associated with increased RAS activity, will contribute to vascular stiffness by functional and structural effects on the vascular wall.

Methods and clinical tools to assist in correcting a too high target weight

Cold dialysate

Intradialytic hypotension is prevalent, occurs in about 20-30% of dialysis sessions and increases mortality (84, 85). Intradialytic hypotension has a multifactorial etiology, including autonomic dysfunction, decreased effective circulating volume and plasma osmolality, impaired venous compliance, decreased cardiac reserve, and changes in serum potassium and calcium

concentrations (86). Cold dialysate can prevent intradialytic hypotension and muscle cramps during, even after dialysis (87). It induces catecholamine release leading to peripheral vasoconstriction and increased cardiac contractility, thus preventing hypotension. It may have some undesirable effects like shivering and cold sensation, which mainly depends on the difference between the dialysate temperature and the patient's pre-dialysis body temperature. In one study, patients dialyzed against 37 ˚C dialysate had the highest incidence of intradialytic hypotension which decreased markedly with the use of 35 ˚C dialysates (15.9% vs. 3.4%, respectively) (88).

Body Composition Monitor

Fluid overload is not easy to assess clinically. Of the various methods to identify the fluid status, the multi-frequency, bio-impedance spectroscopy (Body Composition Monitor, BCM, Fresenius, Germany) has proven to be a reliable and easy to use tool in clinical practice (73, 89, 90). This approach is non-invasive, reproducible and portable. It provides information about the patient's nutritional and fluid status by measuring total body water (TBW), extracellular fluid volume (ECFV), intracellular fluid volume (ICFV), lean tissue index, fat tissue index, and body cell mass. Patients in this study considered fluid overloaded if an estimated extracellular fluid overload is >1.1 L (which in a regularly sized individual is about >7.5% of ECFV) (91). BCM has been demonstrated as one of the promising and well-validated methods to produce an objective assessment of fluid status (36, 37). In the current study, the BCM will be used to assist in the correction of TW when repetitive measurements are done to assure that no changes in dry weight happened while TW correction is being pursued (which can take up to 3 months).

Summary of the literature review

- Fluid overload in HD patients is associated with high mortality and morbidity rates.
- Fluid overload is not well defined in dialysis clinical routine.
- Objective assessment of fluid status is essential in fluid management.
- Body composition monitor is a promising technology to determine fluid status.
- TAFO appears to best target of patient's fluid status.
- Arterial stiffness is a powerful predictor of cardiovascular damage.
- Controlling fluid overload may improve arterial stiffness.
- The link between arterial stiffness and fluid overload has not been defined yet.

Hypothesis and Aims

The hypothesis is that a protocolized adjustment of target weight guided by bio-impedance spectroscopy will improve fluid status, systolic and diastolic blood pressure, and reduce the arterial wall stiffness without increasing the prevalence of intradialytic hypotension.

Aims:

I. To demonstrate improvement in fluid status by a target weight correction protocol which applies BCM measurements

II. To demonstrate that better fluid volume control is associated with a) improvement in vascular health as assessed by pulse wave velocity and augmentation index and b) reduction in antihypertensive medications use

III. To show that this approach does not lead to more episodes with intradialytic hypotension

Overall study design

We will include fluid overloaded HD patients in this randomized controlled trial, for three months in two different dialysis centers (Edmonton General Hospital and University of Alberta Hospital). Subsequently, we will divide the study subjects into a control group and an intervention group. We will assess TW by routine clinical practice and will measure fluid overload in both groups. The control group will initially receive conventional therapy (no intervention) for three months and the intervention group will undergo TW adjustment based on BCM measurements (figure 2 and 3). After three months, TW in the control group will be adjusted in the same fashion as in intervention group. Physicians and in charged nurses will be notified before implementing the intervention. To address the aims of our study: **a)** we will use BCM device to measure the fluid status. We will measure pre-dialysis fluid overload at baseline—which is defined as the day of the BCM measurements—and end of the study in both groups, also we will re-measure the pre-dialysis fluid overload every month-end in the intervention group, **b)** we will use Arteriograph24^{TM} to measure arterial stiffness and blood pressure in both groups in the same time set of fluid overload measurement, **c)** we will record the number of antihypertensive medications on monthly basis, and we will obtain the results of the standard laboratory parameters (including sodium, potassium, hemoglobin, ferritin, C-reactive protein (if any)) from the monthly blood work, and **d)** we will monitor the intradialytic hypotension episodes every hour during HD run in both groups.

Study population, inclusion and exclusion criteria

HD patients who meet the inclusion criteria will be recruited from two different dialysis centers; Edmonton General Hospital (control group) and the 5B1 and 5C2 dialysis units in University of

Alberta Hospital (intervention group). Patients who are willing to participate in the study will receive a detailed description of the study protocol and will be asked for a signed informed consent.

Inclusion criteria

- Adult (>18 years old) outpatients on 3-4 times per week HD sessions for at least 90 days with a life expectancy >6 months
- Fluid overload ≥1.1L
- TAFO >1 L
- Medically stable patient
- Minimum 3 hours of dialysis per session

Exclusion criteria

- Pregnancy or lactation
- Declined informed consent
- Patients with cognitive dysfunction
- Severe life-limiting Comorbidities
- Surgery within six weeks of the study
- Nocturnal dialysis patients
- Patients expected to receive a transplant or move to another center within the duration of the study
- Patients with arteriovenous fistula issues, atrial fibrillation, and metallic implants like pacemaker, cardiac stent, artificial joints, or pins

- Patients with amputated limbs

Measurements

- Fluid overload will be assessed using multifrequency bio-impedance (BCM device, Fresenius)
- PWV/PWA will be measured for 5 hours (approximately 10 PWV/PWA readings) using Arteriograph24^{TM}. For all HD patients; PWV/PWA measurements will start 30 minutes before and end 30 minutes after the dialysis session

Study analysis

- Analysis: Two-way repeated measurement ANOVA.
- Power: we need to recruit around 55 patients to show a significant reduction in TAFO of <1 L (power 0.95, and α I error of 0.05, effect factor 0.5).
- Considering a dropout rate of 20%, we need to recruit 70 patients.

Study duration

Duration of the study will be from 6-12 months depending on the pace of inclusion of patients into the study.

Approach per each aim

Aim I: To demonstrate improvement in fluid status by a target weight correction protocol which applies BCM measurements

Design and groups:

- Intervention group (n=35): see diagrams in figure 2 and 3.
- Control group (n=35): Will undergo conventional therapy in which TW will be adjusted according to the usual clinical practice

TW correction protocol:

- Fluid overload information based on BCM will be used to adjust TW during dialysis
- TW adjustment in the intervention group will be every week over two weeks interval
- TAFO will be calculated based on measurements of predialysis fluid overload (FO_{pre}) and interdialytic weight gain (IDWG); TAFO = FO_{pre} - IDWG/2
- TAFO >2.2L without intradialytic hypotension: we will decrease TW by 0.5L/week
- TAFO > 2.2 L with intradialytic hypotension, or between 1.1and 2.2 L without intradialytic hypotension: we will decrease the TW by 0.2L/ week
- TAFO between 1.1and 2.2 L with intradialytic hypotension: decrease the TW by 0.1L/ week.
- If a patient reaches TW before the three months, we will keep maintaining TW till the end of the study.

Intradialytic hypotension management:

- Intradialytic hypotension events in both groups will be collected during dialysis run, two weeks before the day of BCM measurements. We will continue to assess symptoms during dialysis run over the study period
- If a patient in the intervention group presents intradialytic hypotension events before reaching TW, Alberta Kidney Care (AKC) guideline will be implemented to manage the symptoms. If symptoms continue in two or three consecutive treatment, we will not further adjust TW. For more details, see information in Tables A1-A4.

Measurements: The short intradialytic days (mid- or end- week) will be selected to do BCM measurements which will be performed 15 minutes before dialysis run. Effective ultrafiltration will be recorded at 30-minute intervals also (this can be read from the HD machine).

Anticipated Outcome: TAFO will improve towards the normovolemic range (i.e. <1.1L). To demonstrate that better fluid volume control is associated with a) improvement in vascular health as assessed by pulse wave velocity and augmentation index and b) reduction in antihypertensive medications use.

Aim II: To demonstrate that better fluid volume control is associated with a) improvement in vascular health as assessed by pulse wave velocity and augmentation index and b) reduction in antihypertensive medications use

Design and groups: See above

Measurements: We will measure PWV/PWA and BP at a 30-minutes interval during the full (mid- or end- week HD session) for all patients. We will reassess the measurements at the month-end of the study duration. The number of anti-hypertensive medications will be assessed every month using the patient's chart.

Anticipated outcome: It is anticipated that the intervention group will show an improvement in BP (systolic and diastolic), PWV/PWA readings, and anti-hypertensive medications use compared to the control group.

Aim II: To show that this approach does not lead to more episodes with intradialytic hypotension

Design & groups: See above

Measurements: In both groups, intradialytic hypotension events of six consecutive HD runs will be collected at baseline (prior the day of the BCM measurements) and over three months. The rate of intradialytic hypotension events will be estimated based on the number of intradialytic hypotension events of each dialysis session per hour.

Anticipated outcome: It is anticipated that the intervention group will not have more intradialytic hypotension during their runs.

Knowledge translation

The identified problem is that fluid overload induces cardiovascular damage in HD patients. The current trial will create knowledge effects on the importance of an appropriate assessment of target weight by using reliable diagnostic bedside tools like BCM device. In fact, BCM device is now implemented in the dialysis floor and the medical staff is getting to know about the importance of BCM use to assess the fluid status in HD patients. Basically, we are designing fluid correction protocol to be a reliable and successful method to assess and control fluid overload in HD patients. This will lead to our additional knowledge translation activity, which will be directed toward the knowledge of fluid status management plan (our research and findings will be used as a reliable source). Finally, we will be able to provide well-defined advice to HD patients about the effect of fluid control on cardiovascular health.

Future direction

Since it is anticipated that correcting the target weight, based on BCM measurements, would ameliorate patient's fluid status, blood pressure readings and improve vascular stiffness, our next step is to reduce IDWG by improving the HD patient's health awareness (i.e salt education).

Table A1: Management of Symptomatic intradialytic hypotensive episodes

Symptomatic intradialytic hypotension: is defined as a drop in Systolic BP ≥ 20 mmHg or in MAP≥10 mm Hg of the pre-HD run reading associated with any of the classical symptoms of hypotension (headache, dizziness, nausea, vomiting, cramps, dyspnea, thirst, or angina) and need for medical staff intervention. As soon as you notice an intradialytic hypotension symptom in the patient, consider the following:

1. Trendelenburg
2. Minimum UF rate (0.1 L/hr)
3. Normal Saline 100-300ml IV bolus
4. Reduce the dialysate temperature to 0.5 C◦ lower than the pre-HD temperature. The degree temperature should not be lower than 35C◦ and not higher that 37C◦
5. If symptoms do not resolve, consider an additional 100-300 mL IV bolus of normal saline and turn the UF off for at least 5 minutes
6. Keep monitor BP/HR every 5 minutes from the beginning of the incidence till it resolved
7. If the symptoms couldn't be resolved, then remain the UF off and consult MRHP**

*According to the Alberta Kidney Care (AKC) North intradialytic hypotension guidelines
**MRHP, most responsible healthcare practitioner

Table A2: Re-assessment of fluid removal goal

Level of fluid removal	Action
0.5 L/ week	Consider a reduction in UF goal of 0.2 L/week in the following run
0.2 L/ week	Consider a reduction in UF goal of 0.1L/week in the following run
0.1 L/ week	Isolated ultrafiltration volume*

*After consulting with MRHP, an Isolated UF might be considered by performing an UF for 1 hour then HD for 3 hours or an extra run for UF only might be suggested.

Table A3: Recommendations to manage a symptomatic intradialytic hypotension

Asymptomatic intradialytic hypotension is defined as a fall in Systolic BP < 100 mmHg without symptoms, or a drop in Systolic BP >40 mmHg over 30 minutes.

Consider the following checklists:

1. Advice patients to avoid eating during the HD run
2. Check patient's position (feet up and head down)
3. Consider unwarmed blankets if the patient is using warmed blanket
4. Monitor the BP every 5 minutes as needed
5. Check the lab work including (Hemoglobin, albumin, calcium, and glucose)
6. Reduce the dialysate temperature to 0.5 C◦ lower than the pre-HD temperature. The degree temperature should not be lower than 35C◦ and not higher that 37C◦
7. Review sodium profile and UF profile

* According Alberta Kidney Care (AKC) North intradialytic hypotension guidelines

Figure A1: The consequences of Extracellular expansion in HD patients

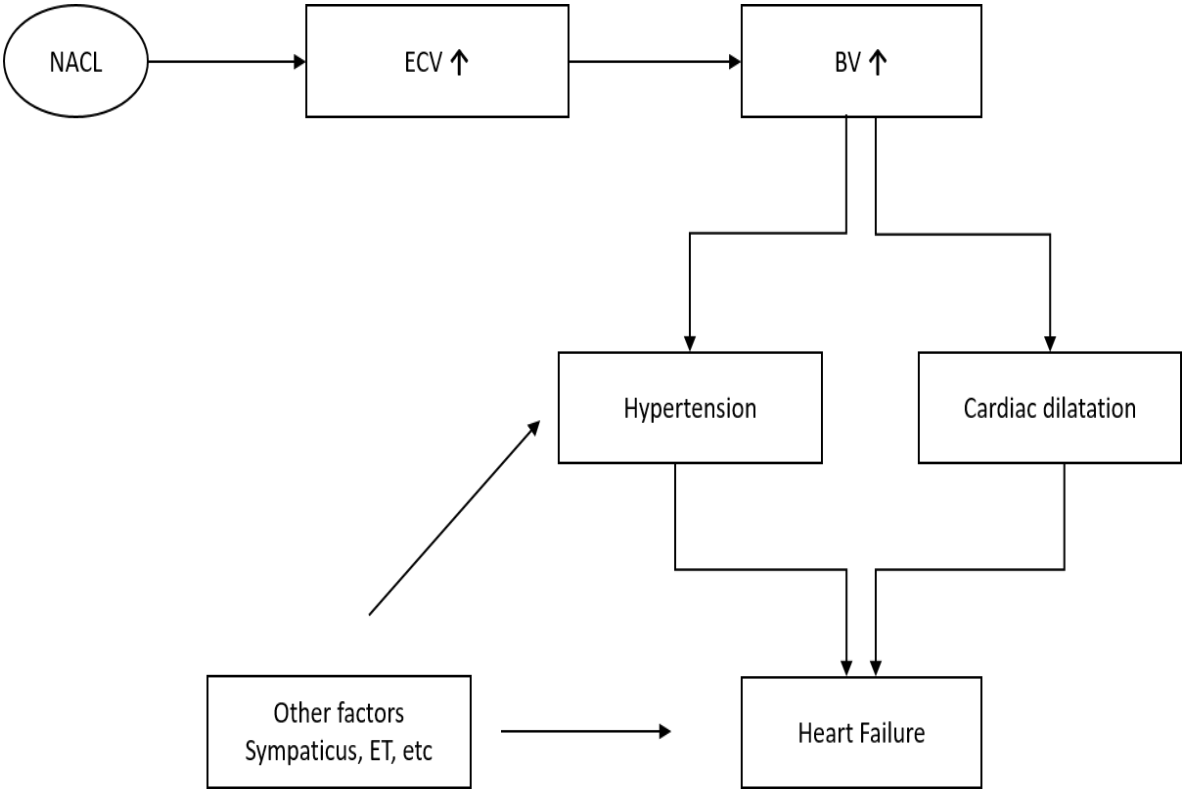

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Figure A2: Overall study design

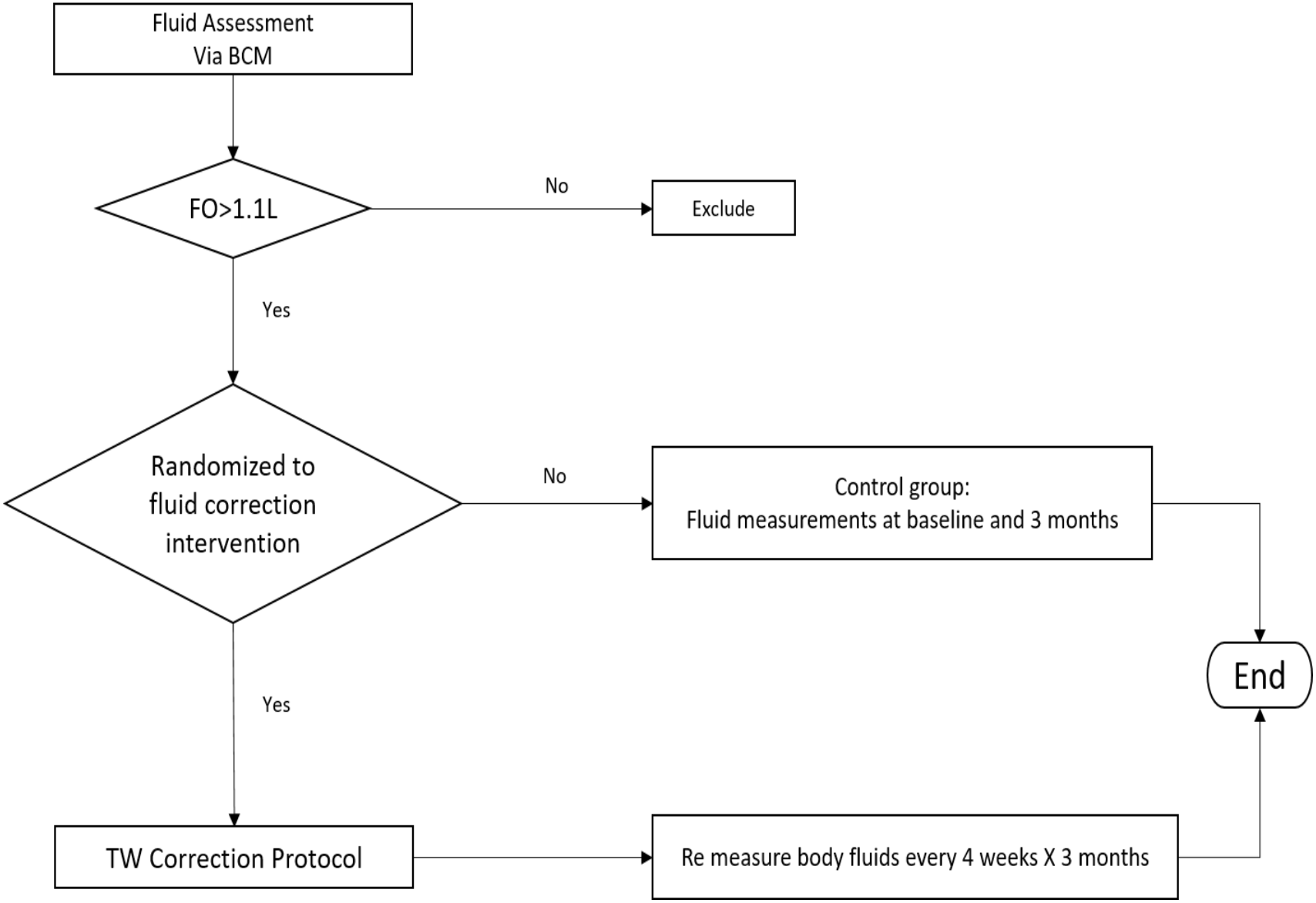


Figure A3: Target Weight Correction Protocol

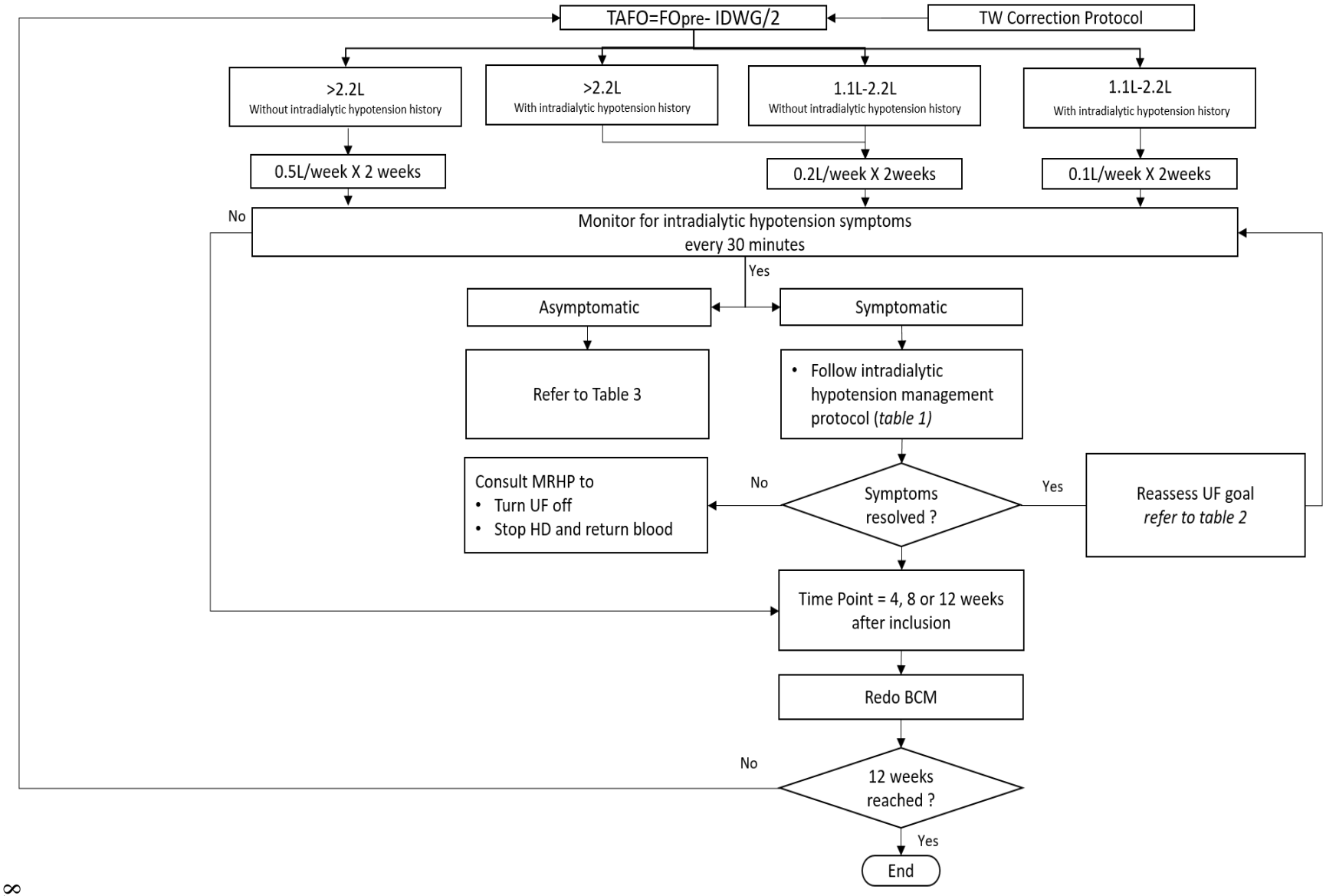
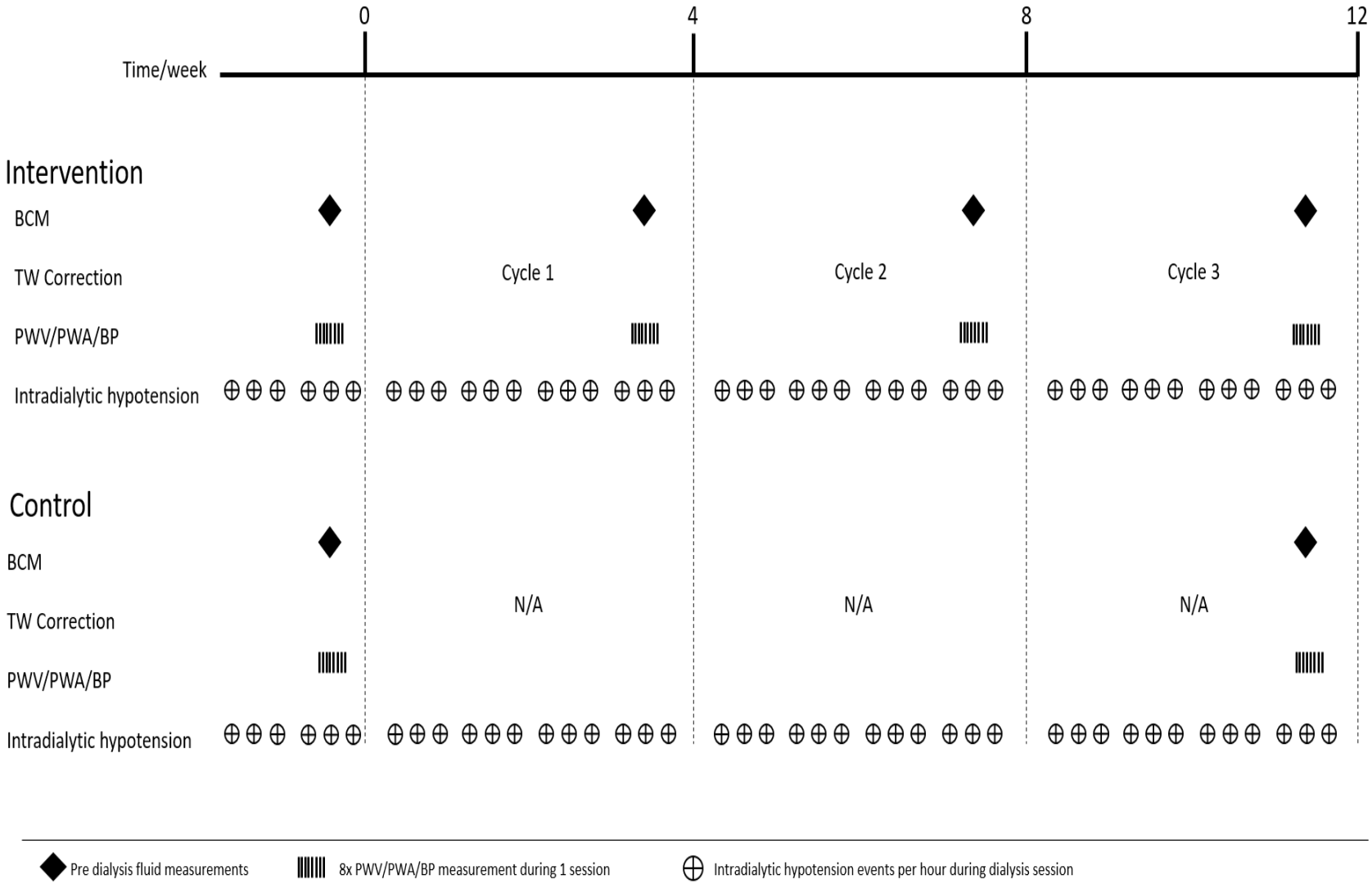


Figure A4: Timeline sketch of the hemodynamic assessment



BCM, Body Composition Monitor; TW, Target Weight; PWV, Pulse Wave Velocity; PWA, Pulse Wave Analysis; BP, Blood Pressure.