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Association between angiopoietin-2 and functional cardiac remodeling in hemodialysis patients with normal left ventricular ejection

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Abstract

Cardiac remodeling is the initial process in heart failure development. The aim of this study is to evaluate the association between endothelium-related biomarkers and cardiac remodeling in hemodialysis (HD) patients and how the presence of high blood pressure and diabetes mellitus modulates these associations. This was a cross-sectional study with adult HD and normal left ventricular (LV) ejection fraction—LVEF—patients. The authors correlated several endothelium-related biomarkers with echocardiographic indices—LV mass index (LVMi), LVEF, global longitudinal strain, mitral E/e', and aortic root diameter. Seventy-one patients were included, with 37 women (52.1%) and mean age of 54.3 ± 16.8 years. Angiopoietin-2 (AGPT2) was inversely correlated with global longitudinal strain (r = -.374, p = .001) and directly with E/e' (r = .265, p = .025). After adjustment, only AGPT2 was significantly associated with global longitudinal strain. blood pressure and diabetes mellitus were independent moderators for the AGPT2 and global longitudinal strain association. The conditional association was significant only when the mean pre-HD blood pressure was above 97.5 mmHg or in diabetes mellitus patients. Finally, there was an interaction between diabetes mellitus and blood pressure when moderating the conditional effect of AGPT2 on global longitudinal strain. While in non-diabetic patients, the association between AGPT2 with global longitudinal strain was significant only with pre-HD blood pressure levels as high as 110 mmHg, in diabetic patients, this association was significant with pre-HD blood pressure as low as 90 mmHg. Higher levels of AGPT2 were associated with worse cardiac function as determined by lower global longitudinal strain values. This association was moderated by blood pressure and diabetes

mellitus, suggesting that the effects of AGPT2 on cardiac remodeling is dependent of such circumstances.

KEYWORDS
angiopoietin 2, cardiac strain, hemodialysis

1 | INTRODUCTION

Cardiovascular disease is the most common cause of death in hemodialysis (HD) patients and heart failure is a major comorbidity in this population. Up to 40% of HD patients have a diagnosis of heart failure, with an estimated annual incidence of 71 per 1000 person-years, with a mortality ratio of 83% in 3 years.[1,2] In addition to the mortality, the overlap of heart failure and severe renal dysfunction adds complexity to the diagnosis, volume status assessment, and optimal fluid management.[3]

Although the pathophysiology of heart failure in HD patients is a complex one, cardiac remodeling constitutes the initial process, triggered by hemodynamic and neurohormonal stressors, which can result in the subsequent decline of the left ventricular ejection fraction (LVEF).[4,5] Recently, the role of endothelium dysfunction has become a central focus of research, which results in excess interstitial fibrosis and impaired angiogenesis, leading to cardiomyocyte loss and myocardial contractile dysfunction.[6]

Although it is already known that endothelial function is severely compromised in HD patients,[7] there is a scarcity of studies evaluating its association with cardiac remodeling in this population.[8,9] Among the endothelium-related biomarkers, we selected four that comprise different endothelial functions/structures: intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion protein-1 (VCAM-1), which are related to endothelial cell activation; syndecan-1, a rather newly explored marker of endothelial glycocalyx derangement, which is increased in HD patients and angiopoietin-2 (AGPT2), an endothelial growth factor that promotes polymorphonuclear cell infiltration, induces endothelial cell apoptosis, increases vascular permeability and vascular destabilization.[10,11] AGPT2 binds specifically to its receptor (tie-2); however, it has no activating effect and consequently blocks the Angiopoietin-1 activity, being an important angiogenesis regulator. An altered AGPT2/AGPT1 balance has been identified as a cause of cardiac fibrosis.[12] Among the aforementioned biomarkers, AGPT2 has been the most frequently studied endothelial biomarker regarding mortality in HD patients[13]; however, its relationship with cardiac remodeling is not clear. A recent study in healthy adults disclosed that higher levels of AGPT2 are associated with better cardiac remodeling indices detected by two-dimensional ultrasound speckle tracking imaging—a technique that allows the accurate evaluation of cardiac deformation, which can be impaired before clinical manifestations of heart failure and before LVEF is reduced.[14] However, other studies have demonstrated exactly the opposite in older populations with more comorbidities.[15] A better understanding about the association between endothelium-related biomarkers and cardiac remodeling can contribute to advances in developing prognostic models and new therapeutic targets.

Because of different associations in human studies between endothelium-related biomarkers and cardiac function, the aim of the present study is to evaluate the association between endothelium-related biomarkers and cardiac remodeling evaluated by several echocardiographic indices in HD patients with normal LVEF and how the presence of clinical conditions, such as high blood pressure and diabetes mellitus, modulates these associations.

2 | MATERIAL AND METHODS

2.1 | Patients' characteristics

Outpatients were recruited from three centers from a dialysis network located in the city of Fortaleza, state of Ceará, northeast Brazil between August 2020 and January 2021. Patients undergoing maintenance HD for at least 3 months who were community dwellers and ≥18 years of age were eligible. Exclusion criteria included (i) diagnosed coronary disease; (ii) LVEF < 50%; (iii) medium or severe cardiac valvular disease; (iv) cardiac rhythm alterations or regional myocardial contractile dysfunction in the conventional echocardiogram; (v) clinical diagnosis of chronic pulmonary disease; (vi) poor thoracic acoustic window; and (vii) currently unstable patients, defined as patients who were acutely ill or hospitalized at the time of the assessment. The study was approved by the institutional review board (Ethical Committee of Universidade de Fortaleza) and all participants signed the free and informed consent form before the tests were applied.

2.2 | Baseline demographic and clinical characteristics

Demographic, clinical, and laboratory variables were determined at the time of the cardiac image capture. Demographic data were obtained from the participants' self-reports, medical charts and dialysis facility databases. Medical history, including the history of CVD (a composite of either coronary artery disease and/or peripheral vascular disease and/or stroke) and/or heart failure, was defined by patient history or documentation in the patient's electronic chart. Patients were queried about a personal history of myocardial infarction and coronary revascularization (which were used to define coronary disease)

and intermittent claudication and peripheral vascular disease (which were used to define peripheral vascular disease). The data on the presence of diabetes mellitus, use of antihypertensive drugs and dialysis vintage were obtained from the dialysis facilities' electronic records. Pre-dialysis blood tests included blood hemoglobin, calcium and phosphorus levels. Pre-HD blood pressure levels were retrieved from electronic charts in the week blood samples were collected for the measurement of endothelium-related biomarkers and mean pre-HD blood pressure levels was calculated. All dialysis facility laboratory tests were measured in a central laboratory in the city of Fortaleza, Ceará.

2.3 | Echocardiographic measurements

In all patients, comprehensive two-dimensional echocardiographic imaging with tissue and color Doppler with optimization using the Nyquist limit was performed by an expert echocardiographer, who was blinded to biomarker measurements, using an iE33 ultrasound machine with an S5 transducer (Philips Health care, Andover, MA). Data from three cardiac cycles were averaged and used in the off-line QLAB quantification software (version 6.3.3.145HW G1 Philips). Echocardiography was performed during resting conditions approximately 24 h after the last HD session and regional function was evaluated by the standard 17-segment model according to the recommendations of the American Society of Echocardiography. Linear dimensions, displacement, and tissue velocities were measured. LV speckle-tracking echocardiographic strain analysis was also performed using an off-line image analysis program to measure myocardial deformation, based on 2-dimensional images—2D cardiac echo. These images were obtained from the three apical projections: 2-, 3-, and 4-chamber views, with an average of 57 frames per second (SD, four frames per second). Six myocardial walls of interest were examined: the septal, lateral, anterior, inferolateral, inferior, and anteroseptal myocardial walls. Each one was divided into subsegments according to the protocol of the American Society of Echocardiography.[16] In the end-systole, an automated function defined a region of interest, thus allowing tracing of the endocardial border. An average value of peak strain from all projections was used to determine global longitudinal strain. To be more comprehensive, all global longitudinal strain values, which are normally negative, were multiplied by -1, so lower-sign values of global longitudinal strain indicate worse cardiac function. ECG-gated subvolumes from six consecutive cardiac cycles were recorded from the apical approach at end-expiratory breath-hold for multi-beat reconstruction of the entire LV.

Aiming to determine the intra-observer variability, 12 randomly selected cases were analyzed a second time by the same observer, after a minimum period of 6 months to ensure there was no recall bias.

2.4 | Echocardiographic indices

For the current study, we used the following primary echocardiographic traits, capturing selected aspects of cardiac structure and function: LVEF, global longitudinal strain, E/e' and aortic root diameter. LV mass (LVM) was measured using linear methods according to the American Society of Echocardiography guidelines,[17] taking into consideration the LV end-diastolic diameter, and the LV wall thickness. LVM index (LVMi) was calculated by dividing LVM by the body surface area. LV ejection fraction (LVEF) was calculated from LV volumes using the modified biplane Simpson's rule and expressed as a percentage. Systolic LV dysfunction was considered when LVEF was <50% and these patients were excluded as pre-specified. As for the tracing of the endocardium and epicardium, the following views were displayed: the apical four-chamber view, the apical two-chamber view, the apical three-chamber view and the three short-axis views, the LV apex, the LV midlevel, and the LV basal level. The LV endocardium and epicardium were traced automatically, and the tracings were refined with further adjustment. Each was divided into subsegments, according to the protocol of the American Society of Echocardiography. In the end-systole, an automated function defined a region of interest, thus allowing the endocardial border tracing. An average value of peak strain from all projections was used to determine the global longitudinal strain. Higher-signed (less negative) values of global longitudinal strain indicate worse cardiac function. Early transmittal flow velocity (E wave) was measured by pulse wave Doppler from the apical 4-chamber view.[18] Early diastolic mitral annular velocity (e') was measured by tissue Doppler imaging.[19] The E/e' ratio was used as an indicator of LV diastolic function. Aortic root diameter was measured using the leading-edge technique as recommended by the American Society of Echocardiography.[20] To evaluate the fluid status of HD patients,[21,22] the LV end-diastolic volume index and the inferior vena cava diameter were measured—inferior vena cava measurements were made twice at the end of expiration in the sub-xiphoid location, and the average of the measured end-expiratory inferior vena cava diameter.

2.5 | Biomarker measurements

Syndecan-1 was measured as a biomarker of endothelial glycocalyx injury (Abcam, Cambridge, MA, USA). The intra-assay coefficient of variation was 6.2%. ICAM-1, a marker of endothelial cell activation, was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Life Technologies Brasil, São Paulo, Brazil), with an intra-assay coefficient of 8.4%. Additionally, VCAM-1 was measured using a commercially available ELISA kit (Abcam, Cambridge, UK), with an intra-assay coefficient of 5.9%. Angiopoietin-2 was measured using an ELISA kit (R&D Systems, Minneapolis, MN, USA). The intra-assay coefficient of variation was 5.3%. All measurements were performed in duplicate.

2.6 | Statistical analysis

Descriptive characteristics of the study population are reported as proportions for categorical and binary variables, mean with standard deviation (SD) for continuous, normally-distributed variables and median with interquartile range (IQR) for skewed variables.

Differences between categorical variables were assessed using chi-squared tests and the t-test or Wilcoxon's test between continuous variables, as appropriate. Simple correlations between continuous variables were analyzed using Spearman's rank correlation coefficient. The intra-observer agreement was assessed using the intra-class correlation coefficient for absolute agreement and coefficient of variance, defined as the standard deviation of the differences divided by the mean. The Area under the curve of the receiver operating characteristic (AUC-ROC) was calculated for AGPT2 ability to predict a reduced global longitudinal strain. Cutoff points were chosen according to the highest Youden index, which was calculated as [1- (1 - sensitivity) + (1 - specificity)].

For additional analyses, non-normal distributions were natural log-transformed. The generalized linear model (GLM) was performed to evaluate independent associations of clinical, laboratory and endothelium-related variables and echocardiographic indices. The models, including the moderation analysis, were adjusted for demographics (age, sex) and clinical/dialysis variables—comorbidities, antihypertensive medication class, dialysis vintage, hemoglobin level, serum calcium, and phosphorus levels.

For the moderation analysis, the interaction model of the GLM was used, with additional product interaction terms of AGPT2 and pre-HD blood pressure, and diabetes mellitus one at a time and, subsequently, all together. The Johnson–Neyman technique was used to explore significant transition points in the moderation model. The Johnson–Neyman technique aligns the moderating variable (pre-HD blood pressure) in a continuous manner and computes the regions of significance for interactions by analyzing the significance between the predictor and outcome variables.[23] High blood pressure was then defined as the threshold detected by the Johnson-Neyman technique where the significance between predictor and outcome was significative. All the models were adjusted for the abovementioned covariates. A p-value <.05 was considered statistically significant. Taking each moderator and AGPT2 as predictors of global longitudinal strain, an effect size (f^2) of .15 and the abovementioned p value, our study had a post-hoc power of 82% and 95% for blood pressure and diabetes mellitus moderation, respectively.[24] All analyses were performed using SPSS (version 20.0; IBM, Armonk, NY, USA).

3 | RESULTS

3.1 | Study participants

Of 116 patients who met the inclusion criteria for the present study, 71 completed all the tests and were included in the final analysis. Of the 45 excluded patients, 38 did not attend the appointment to undergo the echocardiography, 02 had an inadequate thoracic window, 02 recovered kidney function, 02 were transplanted and 01 died. There was a small predominance of women in the sample (n = 37, 52.1%) and the mean age was 54.3 ± 16.8 years. All patients were on high-flux maintenance HD with a minimal of three sessions per week. The median HD vintage was 24 [IQR 9–60] months. The main causes of end-stage renal disease (ESRD) were hypertensive nephropathy (n = 35, 49.3%) and diabetes mellitus (n = 16, 22.5%). Forty-nine patients (69.0%) had a previous arterial hypertension diagnosis and were on antihypertensive therapy. Thirty-six (50.7%) had high blood pressure (mean pre-HD above the threshold of 97.5 mmHg as explained before) and 25 (35.2%) had diabetes mellitus. Thirteen patients (18.3%) had both diabetes mellitus and high blood pressure. According to the Kidney Disease Improving Global Outcomes (KDIGO) definition,[25] 46 (64.8%) patients had anemia—serum hemoglobin level <13.0 g/dl in males and <12.0 g/dl in females. The main baseline characteristics of the patients are presented in Table 1 and graph distribution of main continuous data are presented in Figure 1A–D.

3.2 | Echocardiographic parameters

The intra-class correlation coefficient and coefficient of variance values for the intra-observer agreement were .95% and 4.3%, respectively. Because it was an exclusion criterion, no patient had reduced LVEF. Only two patients had echocardiographic signs of venous congestion when the images were captured. LV and atrial dilatation was present in 18 (25.4%) and 30 (42.3%) patients, respectively. Eighteen patients (25.4%) had concentric hypertrophy. The majority of the patients (n = 43, 60.6%) had diastolic dysfunction but only 8 patients (10.3%) had grade II/III. Twelve patients (16.9%) had reduced global longitudinal strain (less than 18%, absolute value) and 43 (60.6%) had regional longitudinal strain abnormalities. Eighteen patients (25.4%) had increased LVMi >115 g/m^2 for males and >95 g/m^2 for females and 14 (19.7%) had an E/e' ratio >14.[20] Table 1 displays the main evaluated echocardiographic indices.

3.3 | Evaluation of volume status

LV end-diastolic volume index and end-expiratory inferior vena cava were used to assess patient volume status. There was no significant association between LV end-diastolic volume and global longitudinal strain, or with AGPT2 (r = .11, p = .359 and r = -.097, p = .428, respectively) and only two patients had end-expiratory inferior vena cava >2.1 mm.

3.4 | Endothelium-related biomarkers are associated with global longitudinal strain and E/e'

Table 2 shows there was no difference in endothelium-related biomarkers according to the presence of diabetes mellitus or arterial hypertension. In the univariate analysis, ICAM-1 was associated with LVEF (r = .247, p = .037) and VCAM-1 was associated with E/e' (r = .245, p = .039). AGPT2 was inversely correlated with global longitudinal strain (r = -.374, p = .001) and directly correlated with E/e' (r = .265, p = .025). All correlations between endothelium-related biomarkers and echocardiographic indices are show in supplementary Table S1.

TABLE 1 Baseline demographics, clinical characteristics, endothelium-related biomarkers and echocardiographic indices

	All patients ($n = 71$)
Age (years), mean ± SD	54.3 ± 16.8
Male, *n* (%)	34 (47.9)
Diabetes Mellitus, *n* (%)	25 (35.2)
Hypertension on treatment, *n* (%)	49 (69.0)
Antihypertensives, *n* (%)	
ACE inhibitor/AT1 receptor blocker	37 (52.1)
Beta-blockers	28 (39.4)
Calcium channel blocker	26 (36.6)
Diuretics	18 (25.3)
Others	11(15.5)
CKD presumptive etiology	
Hypertensive	35 (49.3)
Diabetes mellitus	16 (22.5)
Glomerulonephritis	8 (11.3)
Others	12 (16.9)
Dialysis vintage (months), median (IQR)	24 (9–60)
Mean pre-HD blood pressure (mmHg), mean ± SD	101.8 ± 15.4
Residual urine output (>400 ml/day), *n* (%)	17 (23.9)
Hemoglobin (g/dl), mean ± SD	11.7 ± 1.9
Calcium (mg/dl), mean ± SD	8.8 ± .9
Phosphorus (mg/dl), mean ± SD	5.4 ± 1.4
Endothelium-related Biomarkers	
ICAM-1 (ng/ml), median (IQR)	1,241 (928–1666)
VCAM-1 (ng/ml), median (IQR)	1,681 (1284–2000)
AGPT2 (pg/ml), median (IQR)	3.77 (2.13–5.94)
Syndecan-1 (ng/ml), median (IQR)	88 (52–133)
Echocardiographic Indices	
LVEF (%), median (IQR)	63 (60–68)
LVEDV index (ml/m^2)	62.0 (49.8–82.5)
Left atrial volume index (ml/m^2)	33 (25–40)
GLS (%), median (IQR)	21 (19–22)
E/e', median (IQR)	8 (6–12)
AoR (cm), median (IQR)	3.0 (2.7–3.3)
LVMi (g/m^2), median (IQR)	98.9 (70.8–131.7)
Left ventricular geometry pattern	
Normal, *n* (%)	33 (46.5)
Concentric hypertrophy, *n* (%)	18 (25.4)
Eccentric hypertrophy, *n* (%)	14 (19.7)
Concentric remodeling, *n* (%)	06 (8.4)

Abbreviations: ACE, angiotensin converting enzyme; AGPT2, angiopoietin 2; AoR, aortic root diameter; CKD, chronic kidney disease; GLS, global longitudinal strain; HD, hemodialysis; ICAM-1, intercellular adhesion molecule-1; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index; VCAM-1, vascular cell adhesion protein-1.

After adjustment for several factors (age, sex, HD vintage, comorbidities, hemoglobin, calcium and phosphorus levels, antihypertensive medication class), only AGPT2 and global longitudinal strain remained significantly associated—standardized β coefficient -.436, 95%CI -.679 to -.192. AGPT2 had an AUC-ROC of .71 (95%CI .59–.94) for reduced global longitudinal strain and these values increased to .85 (95%CI .65–1.00) and .83 (95%CI .50–1.00) in patients with high blood pressure and diabetes mellitus, respectively—see supplementary Figure S 1. Sensitivity and specificity of the cut-off values for AGPT2 in all patients and subgroups (high blood pressure and diabetes mellitus) are shown in supplementary Table S2.

3.5 | Arterial blood pressure and diabetes mellitus moderates AGPT2 and global longitudinal strain association

Moderated regression analyses were conducted to assess whether there was an interaction between AGPT2 and pre-HD blood pressure and/or diabetes mellitus in global longitudinal strain prediction. A significant moderation effect was observed for the relationship between AGPT2 levels, mean pre-HD blood pressure, and global longitudinal strain (β coefficient -.22, $p = .001$). Figure 2 demonstrates the moderation effects of mean pre-HD blood pressure on the conditional association between AGPT2 and global longitudinal strain. The conditional association was significant when the mean pre-HD blood pressure was above 96.5 mmHg. The conditional association increased when the mean pre-HD blood pressure was higher, with β coefficient values up to -2.4 when mean pre-HD blood pressure was up to 142 mmHg. This means that each increment of 1-SD in AGPT2 showed no association when blood pressure values were lower than 96.5 mmHg but it was associated with a reduction of up to 2.4% in the global longitudinal strain values (reduction of approximately 1SD) in patients with mean pre-HD blood pressure higher than 140 mmHg. Figure 3 illustrates the association between AGPT2 and global longitudinal strain when mean pre-HD blood pressure was 90, 100, or 120 mmHg.

Also, diabetes mellitus was an important moderator for the association between AGPT2 and global longitudinal strain. There was a significant moderation effect for the relationship between diabetes mellitus, AGPT2 and global longitudinal strain—β coefficient -.71, $p = .006$. As shown in Figure 4, probing the moderation effect indicated that individuals without diabetes mellitus did not show any significant association, while a strong negative association was observed between AGPT2 and global longitudinal strain in diabetes mellitus patients.

Finally, we tested pre-HD blood pressure and diabetes mellitus as moderators in the same model. There was an interaction between diabetes mellitus and pre-HD blood pressure in the moderation of the conditional effect of AGPT2 on global longitudinal strain. For example, in non-diabetic patients, the association between AGPT2 with global longitudinal strain was only significant with mean pre-HD blood pressure levels as high as 110 mmHg, while in diabetic patients, this association was significant with pre-HD blood pressure as low as 90 mmHg and it was stronger with increasing blood pressure levels—see Figure 5A,B.

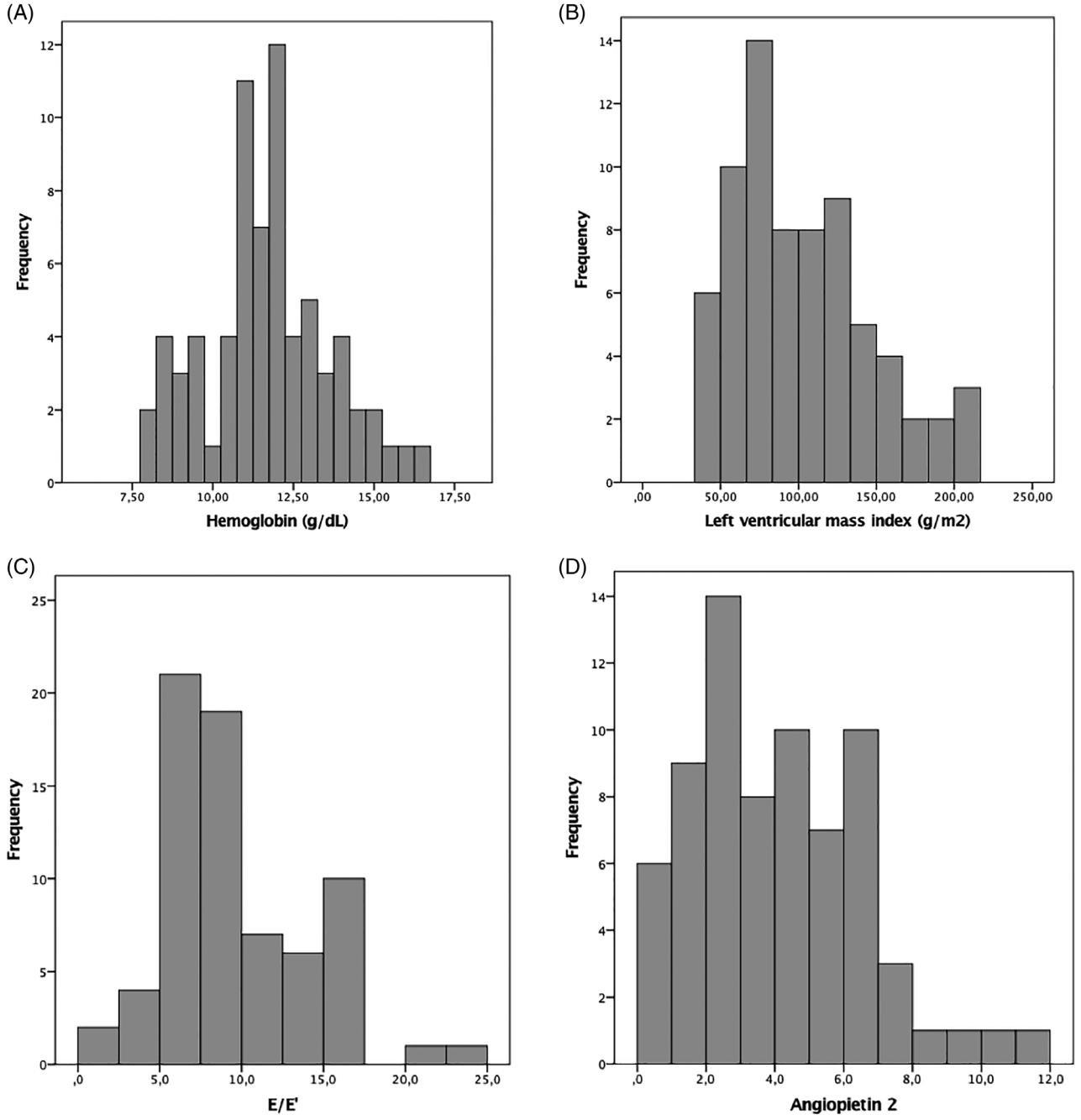

FIGURE 1 Graph distribution of Hemoglobin (A), Left ventricular mass index (B), E/e' (C) and angiopietin 2 (D)

We also tested if the hemoglobin level was a moderator of the association between AGPT2 and global longitudinal strain; however, we observed no interaction between AGPT2 and hemoglobin in diabetes mellitus in the prediction of global longitudinal strain—β coefficient .04, $p = .51$.

4 | DISCUSSION

In the present study, we demonstrated that AGPT2 is associated with global longitudinal strain but not with other echocardiographic remodeling indices in HD patients with normal LVEF. Moreover, we showed that diabetes mellitus and pre-HD blood pressure are important moderators of the association between AGPT2 and global longitudinal strain. AGPT2 was a strong predictor of global longitudinal strain in patients with diabetes mellitus, whereas this association was not observed in patients without diabetes mellitus. Also, there was a growing association between AGPT2 and global longitudinal strain with increasing pre-HD blood pressure and this association is statistically significant only with mean blood pressures >98 mmHg.

Heart failure onset involves the presence of ongoing angiogenesis. In this scenario, vascular endothelial growth factors and

TABLE 2 Baseline demographics, clinical characteristics, endothelium-related biomarkers and echocardiographic indices according presence of high blood pressure or diabetes mellitus

	No-diabetes ($n = 46$)	Diabetes ($n = 25$)	No-high blood pressure ($n = 35$)	High blood pressure ($n = 36$)
Age (years), mean ± SD	52.6 ± 16.8	57.2 ± 16.7	53.4 ± 16.5	55.1 ± 17.3
Male, *n* (%)	21 (45.7)	13 (52.0)	18 (51.4)	16 44.4)
Dialysis vintage (months), median (IQR)	27 (11.5–65.2)	24 (4–42)	36 (12–60)	24 (7.5–54)
Mean pre-HD blood pressure (mmHg), mean ± SD	103.3 ± 15.2	99.3 ± 15.8	90.7 ± 10.1	112.7 ± 11.3*
Hemoglobin (g/dl), mean ± SD	11.5 ± 1.8	12.0 ± 2.2	11.9 ± 1.9	11.5 ± 2.0
Calcium (mg/dl), mean ± SD	8.8 ± 1.0	8.7 ± .5	8.8 ± .8	8.7 ± .9
Phosphorus (mg/dl), mean ± SD	5.4 ± 1.5	5.5 ± 1.4	5.5 ± 1.4	5.4 ± 1.5
Endothelium-related Biomarkers				
ICAM-1 (ng/ml), median (IQR)	1311 (968–1755)	1229 (690–1517)	1225 (980–1745)	1311 (924–1572)
VCAM-1 (ng/ml), median (IQR)	1585 (1197–1965)	1863 (1411–2122)	1,528 (1150–2072)	1710 (1315–1998)
AGPT2 (pg/ml), median (IQR)	3.76 (2.10–6.18)	3.88 (2.25–5.45)	4.04 (2.22–5.45)	3.39 (2.05–6.12)
Syndecan-1 (ng/ml), median (IQR)	88 (52–136)	92 (51–153)	92 (62–184)	85 (47–126)
Echocardiographic Indices				
LVEF (%), median (IQR)	64 (60–68.2)	61 (56.5–64.5)	63 960–68)	63.5 (59.2–66.0)
GLS (%), median (IQR)	21 (19–23)	21 (19–22)	21 (19–23)	20 (19–22)
E/e', median (IQR)	8 (6–12.2)	8.6 (6.2–12.7)	7.5 (5.5–9.0)	10.0 (7.0–15.0)*
AoR (cm), median (IQR)	3.0 (2.6–3.5)	3.0 (2.7–3.3)	3.1 (2.8–3.5)	2.9 (2.6–3.3)
LVMi (g/m^2), median (IQR)	100 (63–134)	81.4 (71.5–128.8)	81.1 (60.2–108.0)	115.2 (78.8–140.4)*

Abbreviations: AGPT2, angiopoietin 2; AoR, aortic root diameter; GLS, global longitudinal strain; ICAM-1, intercellular adhesion molecule-1; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index; VCAM-1, vascular cell adhesion protein-1.
*$p < .05$ HD: hemodialysis.

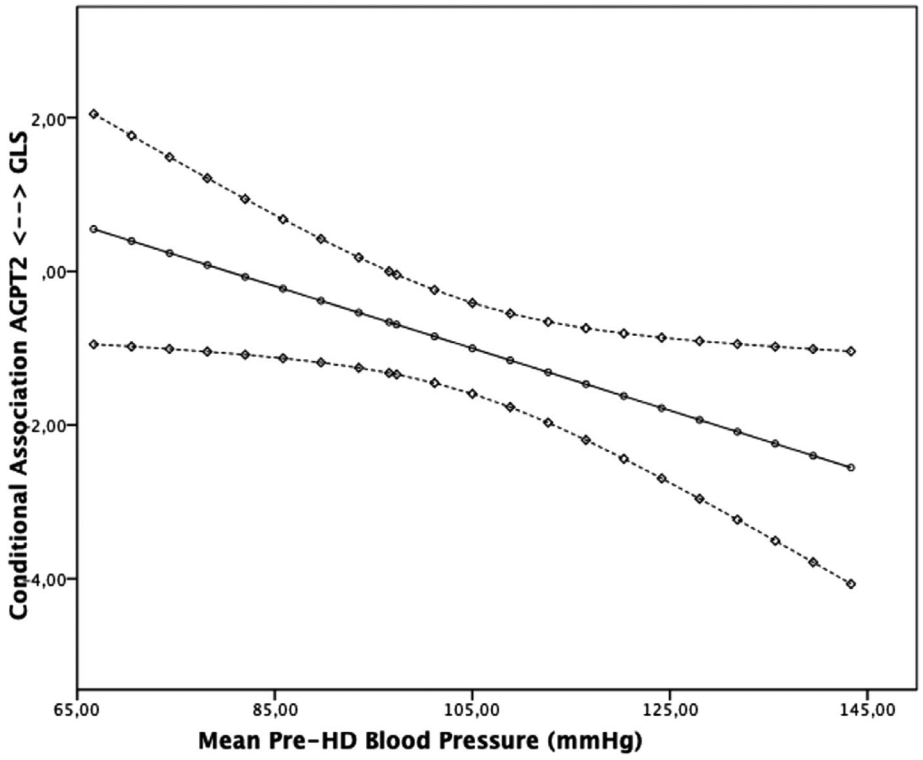

FIGURE 2 Moderation effects of mean pre-HD blood pressure on the conditional association between AGPT2 and GLS. Note that upper 95% confidence interval cross zero value (no association) at values near 97 mmHg. AGPT2, angiopoietin 2; GLS, global longitudinal strain

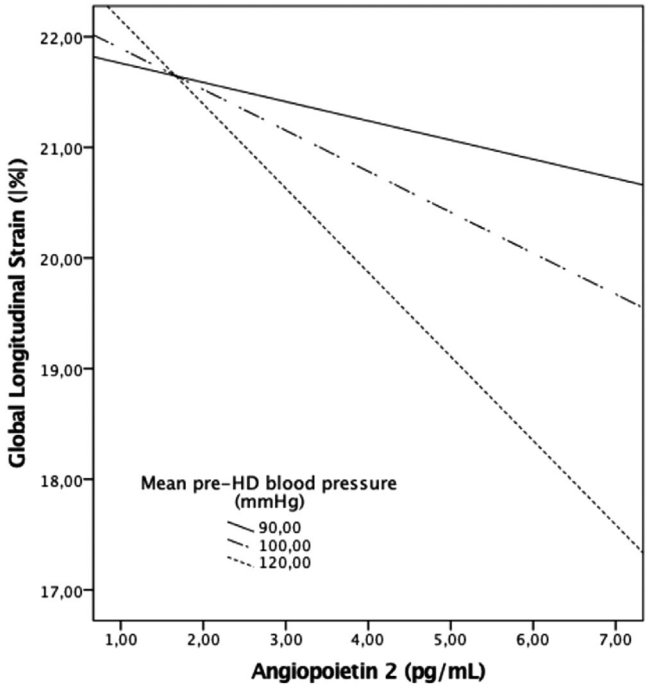

FIGURE 3 Association between AGPT2 and GLS when mean pre-HD blood pressure was 90, 100, or 120 mmHg

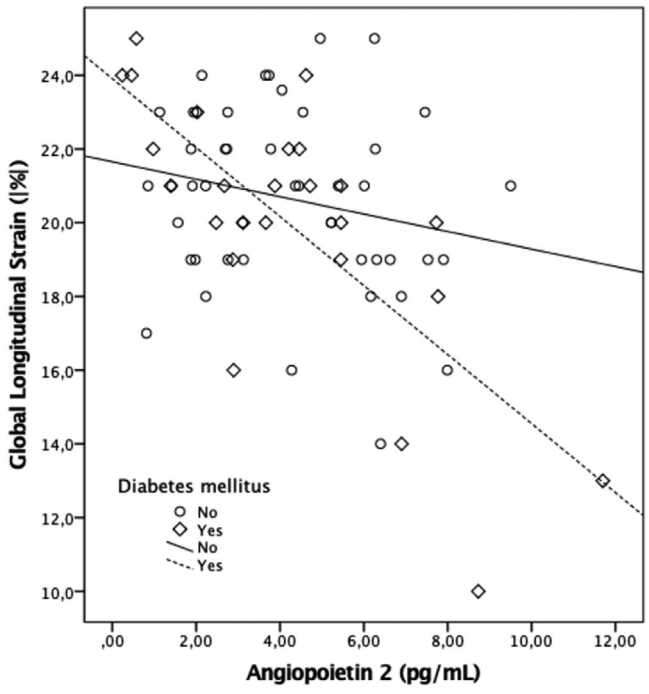

FIGURE 4 Moderation effects of diabetes mellitus on the conditional association between AGPT2 and GLS

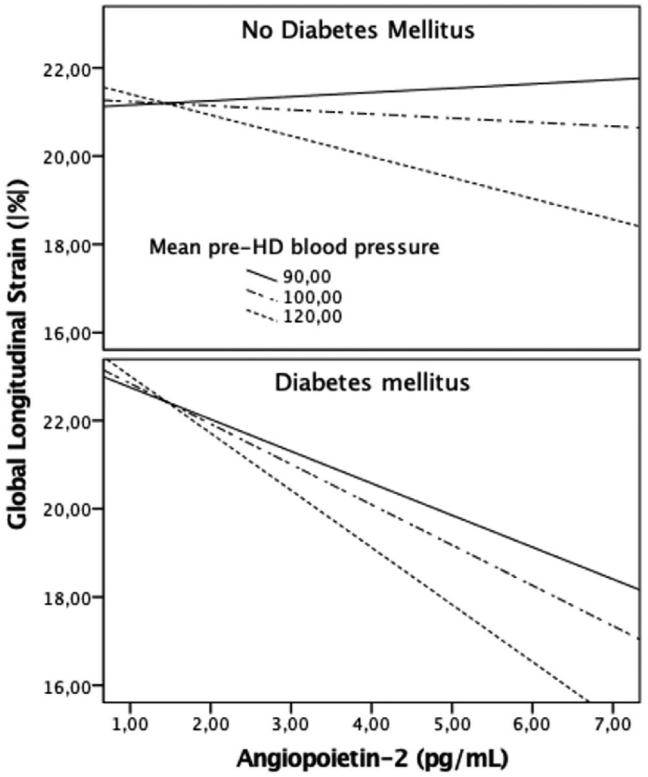

FIGURE 5 Interaction between mean pre-HD blood pressure and diabetes mellitus. In (A) (no diabetes mellitus), only with mean pre-HD blood pressure of 120 mmHg there is association between AGPT2 and GLS. In (B) (diabetes mellitus), such association is present in all three levels of blood pressure

angiopoietin levels respond to tissue hypoxia, initiating the angiogenesis and promoting vascular remodeling, respectively.[26] In addition to traditional factors such as diabetes mellitus, hyperlipidemia, and arterial hypertension, uremia-specific factors such as inflammation and oxidative stress can modulate the process of endothelial dysfunction in HD patients.[27] AGPT2 is increased in patients with chronic kidney disease and HD patients; furthermore, it predicts overall mortality in the latter.[13,28] However, we found that higher AGPT2 levels are associated with worse cardiac remodeling indices, as shown by another recent community study performed in healthy adults, which is in disagreement with our results, showing that higher AGPT2 levels were associated with better global longitudinal strain.[14] Although the results seem to be contradictory, the cohort differences regarding the clinical characteristics supports our findings that other variables can modulate the association between AGPT2 and cardiac remodeling parameters. For example, in a German cohort,[15] higher concentrations of AGPT2 were associated with lower LV systolic function as assessed by echocardiography. This latter sample was more similar to our cohort, with older participants and higher prevalence of hypertension and diabetes. Our findings that high blood pressure and diabetes mellitus can moderate the effects of AGPT2 on cardiac remodeling can help to explain such different results.

In our cohort, the presence of diabetes mellitus and pre-HD blood pressure were moderators of the association between AGPT2 and global longitudinal strain. Interestingly, we did not observe any difference in AGPT2 levels when patients were divided according to presence of diabetes mellitus or the level of pre-HD blood pressure. These findings suggest a different action of AGPT2 in the presence of such

comorbidities. Although highly speculative, studies with diabetic animals demonstrate that an overexpression of AGPT2 impairs myocardial angiogenesis and exacerbates cardiac fibrosis and that hyperglycemia results in dramatic decreases in phosphorylation of the Tie-2 receptor and its downstream signaling partners.[29] Regarding high blood pressure levels, although studies have suggested that endothelial stretch increases AGPT2 release,[30] whether its action is modified by hypertension in the heart failure pathophysiology remains to be clarified and this topic might deserve further investigations.

In the present cohort, we were unable to disclose any significant association between other endothelium-related biomarkers and cardiac remodeling indices. Although some animal studies and specific human cohorts (e.g., post-acute myocardial infarction heart failure) have suggested a role for ICAM-1 and VCAM-1 in cardiac remodeling, animal data are limited[31,32] and, in humans, although the levels of both biomarkers are higher in heart failure patients, their role in early cardiac remodeling is unknown.[33] Finally, regarding syndecan-1, it is associated with the main prognostic outcome in heart failure; however, there are no other studies evaluating its association with ongoing myocardial remodeling.[34]

Because conventional echocardiographic parameters are related to volume change, global longitudinal strain causes concerns about the effect of body fluid load in HD patients. We demonstrated that there was no significant association between LV end-diastolic volume index and end-expiratory inferior vena cava with global longitudinal strain. Furthermore, other studies have proved that global longitudinal strain is not affected by load manipulation.[22,35,36] Also, although our cohort is from maintenance HD patients, all patients had a LVEF >50%, explaining the reduced mean E/e' and LVMi values. Such findings are in accordance with other studies in similar populations.[37,38]

Our study has several limitations. First, the relatively small sample size of the study. Second, due to its cross-sectional design, this study cannot infer causality. Also, we used pre-HD blood pressure as a moderator variable. Although blood pressure measurement before the start and immediately at the end of dialysis is highly variable, as it depends on fluid volume status and the hemodynamic effects of dialysis, it remains the clinical standard according to the guidelines[39] and pre-HD blood pressure has a strong correlation with 24-h ambulatory blood pressure and left ventricular hypertrophy, when evaluated either by echocardiography[40] or by magnetic resonance imaging.[41] Finally, our results cannot be extrapolated to non-HD patients.

In summary, our study suggests that higher AGPT2 is associated with worse cardiac function as determined by lower global longitudinal strain values. Moreover, this association is moderated by pre-HD blood pressure and diabetes mellitus, suggesting different effects of AGPT2 on cardiac remodeling under these circumstances. Although afterload is the main cause of cardiac remodeling in HD patients, as well as in the general population, the clinical implications of our study include different prognostic information on AGPT2 levels according to patients' comorbidities and possible different clinical responses in future clinical trials evaluating AGPT2 as a therapeutic target, including possible effects of non-selective inhibitors of AGPT2, such as Trebananib.[42]

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Ana Aécia Alexandrino de Oliveira e Alexandre Braga Libório contributed to research conception and design. Ana Aécia Alexandrino de Oliveira recruited subjects. Ana Aécia Alexandrino de Oliveira, Thaís Alexandrino de Oliveira, Larissa Alexandrino de Oliveira, Gdayllon Cavalcante Meneses, Gabriela Freire Bezerra and Alice Maria Costa Martins performed experiments. Ana Aécia Alexandrino de Oliveira e Alexandre Braga Libório analyzed data. Ana Aécia Alexandrino de Oliveira e Alexandre Braga Libório Wang drafted manuscript. Thaís Alexandrino de Oliveira, Larissa Alexandrino de Oliveira, Gdayllon Cavalcante Meneses, Gabriela Freire Bezerra and Alice Maria Costa Martins edited and revised manuscript. All authors read, critically revised, and approved the final version of manuscript.

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