The association between heart rhythm complexity and the severity of abdominal aorta calcification in peritoneal dialysis patients

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Abdominal aorta calcification (AAC) has been associated with clinical outcomes in peritoneal dialysis (PD) patients. Heart rhythm complexity analysis has been shown to be a promising tool to predict outcomes in patients with cardiovascular disease. In this study, we aimed to analyze the association between heart rhythm complexity and AAC in PD patients. We prospectively analyzed 133 PD patients. Heart rhythm complexity including detrended fluctuation analysis and multiscale entropy was performed. In linear analysis, the patients in the higher AAC group (AAC ≥ 15%) had a significantly lower standard deviation of normal RR intervals, very low frequency, low frequency, high frequency and low/high frequency ratio. In non-linear analysis, DFAα1, slope 1–5, scale 5 and area 6–20 were significantly lower in the patients with higher AAC. Receiver operating characteristic curve analysis showed that DFAα1 had the greatest discriminatory power to differentiate these two groups. Multivariate logistic regression analysis showed that DFAα1 and HbA1c were significantly associated with higher AAC ratio. Adding DFAα1 significantly improved the discriminatory power of the linear parameters in both net reclassification improvement and integrated discrimination improvement models. In conclusion, DFAα1 is highly associated with AAC and a potential cardiovascular marker in PD patients.

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in end-stage renal disease (ESRD) patients1, accounting for about 40% of deaths among these patients during the first 3 years of dialysis2. Because of the high cardiovascular mortality rate in dialysis patients (almost 5–30 times greater than in the general population)3, the burden of CVD on patients starting dialysis and its impact on the survival of dialysis patients has recently received increasing attention3,4.

Possible explanations for the high rates of CVD mortality and morbidity in ESRD patients include atherosclerosis-related vascular complications and autonomic nervous system dysfunction4. Abdominal aorta calcification (AAC) has been reported to predict CVD events and mortality in ESRD patients, including those undergoing peritoneal dialysis (PD)5–7. The abdominal aorta calcification can be measured via X ray with grading systems8 or computed tomography (CT) with direct measurement of the percentage of AAC (%AAC)9. The %AAC has been shown to be independently associated with mortality and hospitalization in PD patients7. The cutoff value of 15% AAC in CT has been reported that predicting clinical outcomes in PD patients7. However, the clinical use of %AAC measurement is limited by radiation exposure and medical cost.

Analysis of beat-to-beat variation of heart rate, also known as heart rate variability (HRV), is commonly used in cardiovascular researches as a simple and noninvasive approach10. HRV has also been commonly used to...
predict CVD outcomes\(^1\). Newer biological signal analysis methods based on nonlinear signal modeling and complexity evaluation including detrended fractal analysis (DFA) and multiscale entropy (MSE) have been developed in recent years\(^4\). Compared to traditional HRV parameters, nonlinear heart rhythm complexity analysis has a better prognostic power in patients with CVD. In addition, both DFA and MSE have been shown to be useful in predicting survival of heart failure patients\(^13,14\).

To the best of our knowledge, no previous study has investigated the association between heart rhythm complexity and AAC. Therefore, the aim of this study was to analyze the association between heart rhythm complexity and the severity of AAC in PD patients.

**Results**

**Patients.** A total of 133 PD patients (61 men) were enrolled in this study, including 59 (26 men) with AAC \(\geq 15\%\) (higher AAC group) and 74 (35 men) with AAC < 15\% (lower AAC group). The clinical data are shown in Table 1. The AAC ratio of the whole population, AAC \(\geq 15\%\) group and AAC < 15\% were 10.38 (0.53–30.70), 33.87 (27.25–46.39) and 1.32 (0.00–8.74), respectively. Patients in higher AAC group were significantly older and had higher incidences of diabetes mellitus (DM), HbA1c, fasting serum glucose, C-reactive protein (CRP), and lower serum creatinine and left ventricular ejection fraction (LVEF). Other clinical parameters including peritoneal dialysis efficiency (PD KT/V), percentage of beta-blocker and calcium channel blocker usage were comparable in both groups (Table 1).

**Holter data.** In linear analysis, the patients in the higher AAC group had a significantly lower standard deviation of normal RR intervals (SDRR), very low frequency (VLF), low frequency (LF), high frequency (HF) and low frequency to high frequency ratio (LF/HF ratio) than those in the lower AAC group. In non-linear analysis, DFA\(\alpha_1\) was significantly lower in the higher AAC group. The value of DFA\(\alpha_2\) was comparable between the two groups. In MSE analysis, the patients in the higher AAC group had significantly lower slope 1–5, scale 5, and area 6–20 than the patients in the lower AAC group (Table 2).

**Differentiation between the higher and lower AAC groups.** Receiver operating characteristic (ROC) curve analysis showed that DFA\(\alpha_1\) had the greatest discriminatory power to differentiate the two groups compared to other linear, non-linear and clinical parameters (Fig. 1).

The areas under the curves (AUC) of HRV parameters including SDRR, the percentage of absolute differences in normal RR intervals greater than 50 ms (pNN50), pNN20, VLF, LF, HF, LF/HF ratio DFA\(\alpha_1\), DFA\(\alpha_2\), slope 1–5, scale 5, area 1–5, and area 6–20 were 0.663, 0.457, 0.548, 0.752, 0.721, 0.605, 0.723, 0.781, 0.509, 0.707, 0.657, 0.578 and 0.667, respectively.

The AUC of clinical parameters including age, DM, fasting serum glucose, HbA1c, creatinine, CRP and LVEF were 0.641, 0.651, 0.683, 0.729, 0.580, 0.622 and 0.619, respectively.

**Logistic regression analysis to predict the higher AAC group.** In univariate logistic regression analysis, age, DM, fasting serum glucose, HbA1c, creatinine, LVEF, SDRR, VLF, LF, HF, LF/HF ratio DFA\(\alpha_1\), slope 1–5, scale 5, and area 6–20 were significantly associated with the presence of higher AAC. In multivariate logistic regression analysis, only DFA\(\alpha_1\) (OR = 0.032, 95% CI 0.005 to 0.212, \(p < 0.001\)) and HbA1c (OR = 3.497, 95% CI 1.727 to 7.084, \(p = 0.001\)) remained in the model, and both were associated with higher AAC (Table 3).

**Correlations of HRV parameters and percentage of AAC.** In univariate linear regression analysis, age, DM, fasting serum glucose, HbA1c, creatinine, LVEF, SDRR, VLF, LF, HF, LF/HF ratio DFA\(\alpha_1\), slope 1–5, scale 5, and area 6–20 were significantly associated with the percentage of ACC. In the multivariate linear regression model, DFA\(\alpha_1\) (\(\beta = -31.189, 95\% \text{ CI} -44.829 \text{ to } -17.550, p < 0.001\)), LF/HF ratio (\(\beta = 1.111, 95\% \text{ CI} 0.161 \text{ to } 2.060, p = 0.022\)), age (\(\beta = 0.293, 95\% \text{ CI} 0.057 \text{ to } 0.529, p = 0.015\)) and HbA1c (\(\beta = 1.744, 95\% \text{ CI} 1.640 \text{ to } 7.847, p = 0.003\)) were significantly associated with the percentage of AAC (Table 4).

**The advantage of adding DFA or MSE parameters to the linear parameters to discriminate the higher and lower AAC groups.** DFA\(\alpha_1\) and slope 1–5 significantly improved the discriminatory power of SDRR, VLF, LF, HF and LF/HF ratio in both net reclassification improvement (NRI) and integrated discrimination improvement (IDI) models. In addition, area 6–20 significantly improved the discriminatory power of SDRR, LF, HF and LF/HF ratio in the IDI model, and SDRR and HF in the NRI model. Scale 5 significantly improved the discriminatory power of SDRR, HF and LF/HF ratio in the IDI model and HF in NRI model (Table 5).

**Discussion**

There were three major findings in this study. First, the PD patients with higher AAC had worse heart rhythm complexity. Second, in all linear and non-linear parameters, DFA\(\alpha_1\) had the greatest single discriminatory power to detect PD patients with higher AAC. Third, non-linear parameters, especially DFA\(\alpha_1\), significantly improved the discriminatory power of the linear parameters to differentiate PD patients with higher or lower AAC.

In daily practice, predicting the clinical outcomes of PD patients is a challenge. Atherosclerosis-related vascular calcification has been highly associated with morbidity and mortality in ESRD patients\(^4,6,35\). In the advanced stage of atherosclerosis such as atheroma formation, a partial or extensive calcium deposit is frequently observed\(^4,18\). Therefore, blood vessel calcification implies the presence atherosclerosis or subclinical CVD\(^7,18\). Several traditional risk factors for atherosclerosis such as dyslipidemia, hypertension, smoking, and age have also been associated with vascular calcification in ESRD patients\(^9,20\). In addition, uremia, mineral metabolism, chronic inflammation, fetuin-A and osteoprotegerin (OPG) have also been reported to contribute to vascular
calcification\textsuperscript{21,22}. Several studies have reported significant associations between qualitative or semiquantitative evaluations of arterial calcification and all-cause and cardiovascular mortality in hemodialysis patients\textsuperscript{23–25}. In our study, HbA1c and age were significantly associated with %AAC in multivariate linear regression model. Age and HbA1c are known risk factors of vascular calcification\textsuperscript{26,27}. In addition, HbA1c levels are associated with mortality in ESRD patients\textsuperscript{28,29}. Even in PD patients without diabetes, higher HbA1c is still associated with higher cardiovascular events\textsuperscript{30}.

The AAC severity measurements include X ray with Kauppila score\textsuperscript{8} and CT with direct measurement of %AAC\textsuperscript{9}. Previous study supported that CT appeared to be more sensitive than plain X-rays at detecting peripheral and aortic vascular calcifications in hemodialysis patients\textsuperscript{31}. Tsushima et al. developed a method to measure the percentage of calcified volume against whole vascular volume using CT\textsuperscript{9,32} and CT remains the reference standard in AAC evaluation\textsuperscript{33}. AAC was reported to be an important predictor of vascular morbidity and mortality in the Framingham Heart Study\textsuperscript{34}, and it has also been reported to be associated with clinical outcomes in ESRD patients\textsuperscript{5–7}. The percentage of AAC has been shown to be independently associated with mortality and hospitalization in PD patients\textsuperscript{7}. In addition, patients with AAC ≥ 15% had more cardiovascular events than those with AAC < 15\%\textsuperscript{7}. However, despite the usefulness of %AAC by CT, the radiation exposure and cost were limited the use of this tool.

In contrast, electrocardiography (ECG) is an easy, low cost and radiation-free examination. In the present study, we found high correlations among the HRV parameters (especially DFA $\alpha_1$) and AAC. This indicates that ECG recording followed by HRV analysis using linear and non-linear parameters has the potential to be an alternative to AAC in clinical practice.

Previous studies have reported an association between the progression of coronary and carotid artery atherosclerosis and autonomic dysfunction\textsuperscript{35,36}. Despite the reported association between autonomic dysfunction and atherosclerosis\textsuperscript{37}, the mechanisms linking autonomic imbalance to atherosclerosis are still unclear. In addition to atherosclerosis-related autonomic dysfunction, uremic autonomic neuropathy in ESRD patients has frequently been associated with parasympathetic damage and sympathetic nerve overactivity\textsuperscript{38}, both of which have been associated with worse clinical outcomes in ESRD patients\textsuperscript{39}. In our previous study, PD patients had significantly lower values of several linear and nonlinear parameters than those with normal renal function, and this also supports the hypothesis of prominent autonomic dysfunction in ESRD patients\textsuperscript{40}.

|                | AAC <15%  | AAC ≥15% | p value  |
|----------------|---------|---------|---------|
| Age (Years)    |         |         |         |
| (N = 74)       | 52.59 (43.44–59.47) | 58.63 (51.27–64.84) | 0.001   |
| Male, n(%)     | 35 (47%) | 26 (44%) | 0.710   |
| DM, n(%)       | 6 (8%)   | 22 (37%) | <0.001  |
| HTN, n(%)      | 61 (82%) | 52 (88%) | 0.361   |
| Medication     |         |         |         |
| ACEI or ARB    | 33 (45%) | 32 (54%) | 0.269   |
| Beta-blocker   | 41 (55%) | 36 (61%) | 0.515   |
| CCB           | 45 (61%) | 44 (75%) | 0.094   |
| Statin         | 23 (31%) | 23 (39%) | 0.341   |
| Glucose AC, mg/dL | 91.00 (85.75–104.25) | 106.00 (92.00–140.00) | <0.001  |
| HbA1c, %       | 5.30 (5.00–5.65) | 6.00 (5.30–7.00) | <0.001  |
| Creatinine, mg/dL | 11.75 (9.58–13.58) | 10.40 (8.90–12.70) | 0.037   |
| PD KT/V        | 1.87 (1.67–2.05) | 1.92 (1.67–2.17) | 0.203   |
| TG, mg/dL      | 151.50 (86.75–227.00) | 167.00 (101.00–240.00) | 0.377   |
| T-Chol, mg/dL  | 193.00 (166.00–233.00) | 181.00 (149.00–219.00) | 0.118   |
| LDL, mg/dL     | 90.50 (62.75–111.75) | 77.00 (61.00–107.00) | 0.400   |
| HDL, mg/dL     | 39.00 (33.00–50.25) | 36.00 (31.00–43.00) | 0.106   |
| Na, mmol/L     | 136.00 (133.00–138.00) | 136.00 (132.00–138.00) | 0.677   |
| K, mmol/L      | 3.90 (3.40–4.30) | 3.80 (3.10–4.20) | 0.187   |
| Ca, mg/dL      | 9.78 (9.00–10.29) | 9.52 (8.88–9.92) | 0.155   |
| Mg, mg/dL      | 3.20 (2.80–3.60) | 3.20 (2.40–3.60) | 0.612   |
| CRP, mg/dL     | 0.24 (0.10–0.79) | 0.52 (0.17–1.54) | 0.023   |
| LVEF, %        | 70.53 (63.80–75.86) | 65.98 (57.71–73.54) | 0.037   |
| AAC, %         | 1.32 (0.00–8.74) | 33.87 (27.25–46.39) | <0.001  |

Table 1. Clinical data of the patients. Data were presented as median (25th–75th percentile) or number (percentage). AAC = abdominal aorta calcification; DM = diabetes mellitus; HTN = hypertension; ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CCB = calcium channel blocker; PD = peritoneal dialysis; TG = triglycerides; T-Chol = total cholesterol; LDL = Low-density lipoprotein; HDL = high-density lipoprotein; CRP = C-reactive protein; LVEF = left ventricular ejection fraction.
In the present study, DFA $\alpha_1$ had a better correlation with AAC than linear parameters, which implies that non-linear parameters provide more useful information. The non-linear analysis of HRV including MSE and DFA has been reported to be a better predictor of clinical outcomes than traditional linear analysis. MSE has been associated with the prognosis of heart failure\cite{13}, outcome of acute stroke\cite{41}, primary aldosteronism\cite{42}, critical illnesses requiring extracorporeal life support\cite{43}, and post-myocardial infarction heart function\cite{44}. Furthermore, long-time scale parameters (area 6–20) in heart failure patients have been shown to have the best prognostic predictive power\cite{13}, which is similar to our MSE results. DFA as a scaling analysis method to determine the statistical self-affinity of a signal can be used for the evaluation of the fractal behavior in the heart beat dynamics. The short-term ($\alpha_1$; 4–11 beats,) and long-term ($\alpha_2$; 11–64 beats) fractal correlation exponents have been shown to

|                | AAC < 15% (N = 74) | AAC $\geq$ 15% (N = 59) | $p$ value |
|----------------|-------------------|--------------------------|-----------|
| **Time Domain Analysis** |                   |                          |           |
| Mean RR, ms    | 761.29 (660.07–843.09) | 780.67 (713.31–889.30) | 0.084     |
| SDNN, ms       | 46.11 (34.28–58.12) | 33.67 (23.31–46.99) | 0.001     |
| pNN50, %       | 0.33 (0.08–1.77) | 0.48 (0.05–2.20) | 0.515     |
| pNN20, %       | 5.85 (2.15–17.06) | 5.23 (1.34–16.44) | 0.339     |
| **Frequency Domain Analysis** |                   |                          |           |
| VLF            | 789.84 (477.23–1459.70) | 315.90 (154.40–678.98) | $<0.001$  |
| LF             | 152.21 (71.19–295.19) | 49.18 (17.30–147.01) | $<0.001$  |
| HF             | 49.59 (19.05–118.55) | 29.56 (11.24–68.32) | 0.038     |
| LF/HF ratio    | 2.60 (1.57–4.38) | 1.33 (0.91–2.62) | $<0.001$  |
| **Detrended fluctuation analysis** |                   |                          |           |
| DFA$\alpha_1$  | 1.26 (1.13–1.43) | 1.00 (0.85–1.15) | $<0.001$  |
| DFA$\alpha_2$  | 1.24 (1.17–1.31) | 1.22 (1.13–1.30) | 0.853     |
| **Multiscale entropy** |                   |                          |           |
| Slope 1–5      | 0.063 (0.0177–0.1011) | 0.023 (−0.017–0.058) | $<0.001$  |
| Scale 5        | 1.044 (0.898–1.197) | 0.879 (0.722–1.069) | 0.002     |
| Area 1–5       | 4.494 (3.892–5.359) | 4.22 (3.26–5.17) | 0.122     |
| Area 6–20      | 18.50 (16.21–21.59) | 16.14 (13.79–19.26) | 0.001     |

**Table 2.** Holter parameter of the patients with different AAC ratio. Values are median (25th–75th percentile). SDNN = standard deviation of normal RR intervals; pNN20 = percentage of the absolute change in consecutive normal RR interval exceeds 20 ms; pNN50 = percentage of the absolute change in consecutive normal RR interval exceeds 50 ms; VLF = very low frequency; LF: low frequency; HF = high frequency; DFA = detrended fluctuation analyses.

Figure 1. (A, B) Analysis of the discrimination power of linear and non-linear parameters to discriminate patients with higher AAC (AAC $\geq$ 15) by receiver operating characteristic curve analysis (A) The areas under the curve of SDRR, VLF, LF, HF, LF/HF ratio were 0.663, 0.752, 0.721, 0.605 and 0.723 respectively. (B) The areas under the curve of DFA$\alpha_1$, slope 5, scale 5 and area 6–20 were 0.781, 0.707, 0.657 and 0.667, respectively.

In the present study, DFA$\alpha_1$ had a better correlation with AAC than linear parameters, which implies that non-linear parameters provide more useful information. The non-linear analysis of HRV including MSE and DFA has been reported to be a better predictor of clinical outcomes than traditional linear analysis. MSE has been associated with the prognosis of heart failure\cite{13}, outcome of acute stroke\cite{41}, primary aldosteronism\cite{42}, critical illnesses requiring extracorporeal life support\cite{43}, and post-myocardial infarction heart function\cite{44}. Furthermore, long-time scale parameters (area 6–20) in heart failure patients have been shown to have the best prognostic predictive power\cite{13}, which is similar to our MSE results. DFA as a scaling analysis method to determine the statistical self-affinity of a signal can be used for the evaluation of the fractal behavior in the heart beat dynamics. The short-term ($\alpha_1$; 4–11 beats,) and long-term ($\alpha_2$; 11–64 beats) fractal correlation exponents have been shown to
provide a clearer understanding of the fractal correlation property in a physiological system45. DFA has also been associated with the interaction between sympathetic and vagal systems46. In the DIAMOND-CHF trial, after adjusting for clinical parameters, DFA $\alpha_1$ but not linear parameters remained to be an independent predictor of mortality14. Taken together with our findings, non-linear HRV analysis may be a useful tool to evaluate the risk of cardiovascular events.

The traditional linear HRV parameters have also been positively associated with CVD risk factors and multiple cardiovascular outcomes including coronary artery disease and cardiovascular mortality47,48. We also found similar results in that linear HRV parameters including SDRR, VLF, LF, HF and LF/HF ratio were also significantly associated with AAC. In addition, combining linear and non-linear analysis further significantly improved the discriminatory power of the severity of AAC. Combining these linear and non-linear HRV parameters can provide more accurate information to build a ROC curve model to predict the severity of AAC.

There are several limitations to this study. First, this is a small pilot study and the findings should be confirmed by a larger clinical study with long-term follow-up data. Second, our study group is limited to PD patients, and further studies are needed to elucidate whether the same association between AAC and heart rhythm complexity exists in hemodialysis patients.

In conclusion, heart rhythm complexity analysis can predict the severity of AAC in PD patients. DFA $\alpha_1$ had the greatest discriminatory power to differentiate PD patients with higher or lower AAC. In addition, DFA $\alpha_1$ and MSE slope 1–5 significantly improved the discriminatory power of the linear parameters, which suggests the advantage of combining linear and non-linear parameters.

### Table 3. Univariate and multivariate logistic regression model to predict higher abdominal aorta calcification group. PD = peritoneal dialysis; TG = triglycerides; T-Chol = total cholesterol; LDL = Low-density lipoprotein; HDL = high-density lipoprotein; CRP = C-reactive protein; LVEF = left ventricular ejection fraction; SDRR = standard deviation of normal RR intervals; pNN20 = percentage of the absolute change in consecutive normal RR interval exceeds 20 ms; pNN50 = percentage of the absolute change in consecutive normal RR interval exceeds 50 ms; VLF = very low frequency; LF = low frequency; HF = high frequency; DFA = detrended fluctuation analyses.

| Abnormal aorta calcification | Univariate logistic regression | Multivariate logistic regression |
|-----------------------------|-------------------------------|-------------------------------|
|                             | $\beta$ (95% C.I) | p value | OR (95% C.I) | p value |
| Age                         | 1.058 (1.024–1.094) | 0.001 |                |        |
| Sex                         | 1.139 (0.573–2.265) | 0.710 |                |        |
| DM                          | 6.74 (2.51–18.09) | <0.001 |                |        |
| HTN                         | 1.583 (0.588–4.263) | 0.363 |                |        |
| Glucose, mg/dL              | 1.023 (1.009–1.038) | 0.002 |                |        |
| HbA1c, %                    | 4.374 (2.339–8.181) | <0.001 | 3.497 (1.727–7.084) | 0.001 |
| PD/TV                       | 1.479 (0.567–3.854) | 0.424 |                |        |
| Creatinine, mg/dL           | 0.842 (0.734–0.966) | 0.014 |                |        |
| Ca, mg/dL                   | 0.782 (0.539–1.137) | 0.782 |                |        |
| P, mg/dL                    | 0.927 (0.698–1.233) | 0.604 |                |        |
| TG, mg/dL                   | 1.000 (0.998–1.003) | 0.633 |                |        |
| T-Chol, mg/dl               | 0.995 (0.988–1.003) | 0.224 |                |        |
| LDL, mg/dL                  | 0.998 (0.989–1.007) | 0.696 |                |        |
| HDL, mg/dL                  | 0.974 (0.944–1.005) | 0.099 |                |        |
| CRP, mg/dL                  | 0.993 (0.842–1.171) | 0.934 |                |        |
| LVEF, %                     | 0.964 (0.933–0.996) | 0.027 |                |        |
| Mean RR                     | 1.002 (1.000–1.005) | 0.092 |                |        |
| SDRR                        | 0.976 (0.959–0.994) | 0.009 |                |        |
| pNN50                       | 1.003 (0.966–1.042) | 0.860 |                |        |
| pNN20                       | 0.991 (0.969–1.014) | 0.445 |                |        |
| VLF                         | 0.998 (0.998–0.999) | <0.001 |                |        |
| LF                          | 0.998 (0.997–1.000) | 0.046 |                |        |
| HF                          | 1.000 (0.999–1.002) | 0.739 |                |        |
| LF/HF ratio                 | 0.828 (0.709–0.967) | 0.017 |                |        |
| DFA $\alpha_1$              | 0.021 (0.004–0.109) | <0.001 | 0.032 (0.005–0.213) | <0.001 |
| DFA $\alpha_2$              | 0.600 (0.059–6.119) | 0.667 |                |        |
| Slope 1–5                   | <0.001 (<0.001–0.001) | <0.001 |                |        |
| Scale 5                     | 0.203 (0.059–0.705) | 0.012 |                |        |
| Area 1–5                    | 0.844 (0.661–1.078) | 0.175 |                |        |
| Area 6–20                   | 0.875 (0.801–0.956) | 0.003 |                |        |
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|                | Univariate linear regression | Multivariate linear regression |
|----------------|-----------------------------|-------------------------------|
|                | β (95% C.I) | p value   | β (95% C.I) | p value   |
| Age            | 0.557 (0.335–0.778) | <0.001 | 0.293 (0.057–0.529) | 0.015   |
| Sex            | 0.424 (–0.560–6.498) | 0.890 |
| DM             | 9.537 (2.298–16.776) | 0.010 |
| HTN            | 4.854 (–3.572–13.280) | 0.256 |
| Glucose mg/dL  | 0.193 (0.111–0.276)   | <0.001 |
| HbA1c %        | 8.110 (5.047–11.173)  | <0.001 | 4.744 (1.640–7.847) | 0.003   |
| PD KT/V        | 4.508 (–3.821–12.838) | 0.286 |
| Creatinine mg/dL | −1.484 (–2.585–0.384) | 0.009 |
| Ca, mg/dL      | −2.264 (–5.460–0.933) | 0.164 |
| P, mg/dL       | −0.755 (–3.256–1.745) | 0.551 |
| TG, mg/dL      | −0.032 (–0.099–0.034) | 0.336 |
| T-Chol, mg/dL  | 0.011 (–0.007–0.028)  | 0.240 |
| LDL, mg/dL     | −0.001 (–0.08–0.077)   | 0.970 |
| HDL, mg/dL     | −0.241 (–0.502–0.019) | 0.069 |
| CRP, mg/dL     | 0.249 (–1.201–1.700)  | 0.734 |
| LVEF, %        | −0.339 (–0.602–0.077) | 0.012 |
| Mean RR        | 0.011 (–0.011–0.033)  | 0.320 |
| SDRR           | −0.153 (–0.291–0.015) | 0.030 |
| pNN50          | 0.119 (–0.219–0.458)  | 0.486 |
| pNN20          | −0.039 (–0.231–0.154) | 0.693 |
| VLF            | −0.008 (–0.012–0.004) | <0.001 |
| LF             | −0.01 (–0.021–0.001)  | 0.081 |
| HF             | 0.006 (–0.010–0.022)  | 0.481 |
| LF/HF ratio    | −0.092 (–1.758–0.206) | 0.013 | 1.111 (0.161–2.060) | 0.022   |
| DFAa1          | −28.305 (–37.519–19.091) | <0.001 | −31.189 (–44.829–17.550) | <0.001 |
| DFAa2          | −6.613 (–27.159–13.933) | 0.525 |
| Slope 1–5      | −84.854 (–128.136–41.572) | <0.001 |
| Scale 5        | −13.554 (–22.788–4.32) | 0.004 |
| Area 1–5       | −1.816 (–3.852–0.220)  | 0.08 |
| Area 6–20      | −1.116 (–1.777–0.455)  | 0.001 |

Table 4. Univariate and multivariate linear regression to predict percentage (%) of abdominal aorta calcification. Adjusted R2=0.346. PD = peritoneal dialysis; TG = triglycerides; T-Chol = total cholesterol; LDL = low-density lipoprotein; HDL = high-density lipoprotein; CRP = C-reactive protein; LVEF = left ventricular ejection fraction; SDNN = standard deviation of normal RR intervals; pNN20 = percentage of the absolute change in consecutive normal RR interval exceeds 20 ms; pNN50 = percentage of the absolute change in consecutive normal RR interval exceeds 50 ms; VLF = very low frequency; LF = low frequency; HF = high frequency; DFA = detrended fluctuation analyses.

Methods

Patients. We prospectively enrolled 133 patients who received PD with conventional glucose-based lactate-buffered solution (UltraBag; Baxter Healthcare SA, Singapore) for more than 6 months. Patients with chronic atrial fibrillation, clinical signs of acute infection, and those with a prior renal transplant were excluded. The baseline characteristics, medical history and medication usage were carefully recorded, and biochemical parameters were measured during initial evaluation. All patients received 24-h ambulatory ECG Holter recording (ZymedDigiTrak Plus 24-Hour Holter Monitor Recorder and Digitrak XT Holter Recorder 24 Hour, Philips, Amsterdam, Netherlands). This study was approved by the Institutional Review Board of National Taiwan University Hospital, and all research was performed in accordance with relevant guidelines and regulations. All subjects provided written informed consent including for storage of their information in the hospital database and usage for research.

Data pre-processing. A stable 4 hours segment of daytime RR intervals (between 9AM and 5PM) was selected for analysis. The selected electrocardiograms were automatically annotated via an algorithm and carefully examined by two experienced technicians.

Time and frequency domain analysis. All parameters were calculated according to the recommendations of the North American Society of Pacing Electrophysiology and the European Society of Cardiology10. SDRR and the percentage of absolute differences in normal RR intervals greater than 50 ms (pNN50) were calculated to represent the total variance and vagal modulation of heart rate. The frequency domain parameters
Computing the power spectral density (PSD) of the heartbeat series yields information about the fractal characters of the biological system. The PSD is a measure of the power of a signal at different frequencies, and it can be used to determine the fractal dimension of the heartbeat series. The fractal dimension is a measure of the complexity of the signal, and it can be used to quantify the fractal behavior of the heartbeat series over different time scales.

In brief, the time series of different time scales were derived by using a coarse-graining process (i.e. averaging over different time intervals). The sample entropy was adopted to estimate the predictability and extends the entropy of a single timescale to the information richness structure embedded over different time scales. The profile of the sequential changes of the entropies over different time scales can be further quantified. The estimated entropy over different time scales can then be used to represent the complexity (meaningful information richness) of the physiological signals. The linear-fitted slope of scale 1 to scale 5 (slope 1–5), the sum of entropy values of scales 1 to scale 5 (area 1–5) or scale 6 to scale 20 (area 6–20) were calculated to quantify the complexity of the beat-to-beat dynamics exhibited in short and long time scales. The estimated entropy over different time scales was assumed to be the linear or polynomial fitted trends over different scales, and by removing these trends from the integrated time series, the intrinsic fractal behavior could be better quantified. Detrended fluctuation analyses (DFA) is used to evaluate the fractal behavior beneath seemingly nonstationary RR dynamics by eliminating extrinsic trends to remove spurious long-term correlations. The external trends were assumed to be the linear or polynomial fitted trends over different scales, and by removing these trends from the integrated time series, the intrinsic fractal behavior could be better quantified. Detrended fluctuations were calculated by adding up the detrended integrated time series in individual scales. Then, the logarithmic plot of fluctuations against time scales were further constructed. The slope (α exponent) of the log-log plot was used to indicate the fractal correlation characters of time series.

While the respiratory sinus arrhythmia is responsible for most of the short-term RR dynamics in normal subjects, the crossover phenomenon of α exponents of RR dynamics over short (α1; 4–11 beats) and long (α2; 11–64 beats) time scales are of importance. We calculated both short- and long-term α exponents for better probing the fractal characters of the biological system.

### Multiscale entropy (MSE) analysis

MSE takes the predictability of multiple time scales into account and extends the entropy of a single timescale to the information richness structure embedded over different time scales. The profile of the sequential changes of the entropies over different time scales can be further quantifies. In brief, the time series of different time scales were derived by using a coarse-graining process (i.e. averaging consecutive beats to form a new time series), and the sample entropy was adopted to estimate the predictability over different time scales. The estimated entropy over different time scales can then be used to represent the complexity (meaningful information richness) of the physiological signals. The linear-fitted slope of scale 1 to scale 5 (slope 1–5), the sum of entropy values of scales 1 to scale 5 (area 1–5) or scale 6 to scale 20 (area 6–20) were calculated to quantify the complexity of the beat-to-beat dynamics exhibited in short and long time scales.

### Detrended fluctuation analysis (DFA)

DFA is used to evaluate the fractal behavior beneath seemingly nonstationary RR dynamics by eliminating extrinsic trends to remove spurious long-term correlations. The external trends were assumed to be the linear or polynomial fitted trends over different scales, and by removing these trends from the integrated time series, the intrinsic fractal behavior could be better quantified. Detrended fluctuations were calculated by adding up the detrended integrated time series in individual scales. Then, the logarithmic plot of fluctuations against time scales were further constructed. The slope (α exponent) of the log-log plot was used to indicate the fractal correlation characters of time series.

While the respiratory sinus arrhythmia is responsible for most of the short-term RR dynamics in normal subjects, the crossover phenomenon of α exponents of RR dynamics over short (α1; 4–11 beats) and long (α2; 11–64 beats) time scales are of importance. We calculated both short- and long-term α exponents for better probing the fractal characters of the biological system.

### Echocardiography

Transthoracic echocardiography (iE33 xMATRIX Echocardiography System, Philips, Amsterdam, Netherlands) was performed in all patients. The LVEF was quantitated by M-mode measurements or area-length methods.

### Computed tomography

A standard 64-MDCT scan (LightSpeed VCT, GE Healthcare, Milwaukee, WI) was performed in all patients. The calcified area was quantified based on an attenuation range of >150 Hounsfield units.
Statistical analysis. Data were expressed as median (25th and 75th percentiles). Comparisons of data between the higher and lower AAC groups were performed by the Mann-Whitney U test. Differences between proportions were calculated by the chi-square test or Fisher’s exact test. Logistic regression analysis was used to validate associations between parameters and the presence of high AAC. Significant determinants in univariate logistic regression analysis (P < 0.05) were then tested in multivariate logistic regression analysis with stepwise subset selection to identify independent factors to predict the presence of high AAC. Linear regression analysis was used to validate associations between parameters and percentage of AAC. Significant determinants in univariate linear regression analysis (P < 0.05) were then tested in multivariate linear regression analysis with stepwise subset selection to identify independent factors to predict the percentage of AAC. The goodness-of-fit of a logistic model was indicated by R², while the discriminatory power of the model was assessed by the area under the ROC curve (AUC).

Two statistics, net reclassification improvement (NRI) and integrated discrimination improvement (IDI), were used to evaluate improvements in the accuracy of the prediction after adding a single nonlinear parameter into a logistic regression model using only linear parameters81. The significance of NRI and IDI statistics was based on approximate normal distributions. All statistical analyses were performed by R software (http://www.r-project.org/) and SPSS version 25 for Windows (SPSS Inc., IL, USA). The significance level of the statistical analysis was set at 0.05.

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### Author Contributions

Y.H.L. and J.W.H. conceived and designed the experiments. C.L., Y.H.H., L.Y.D.L., and M.T.L. analyzed the data. C.H.T and Y.H.L. wrote the paper. C.T.L. and C.K.P. made scientific comments on the manuscript.

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