**Beat to beat alterations of acoustic intensity and frequency at the maximum power of heart sounds are associated with NT-proBNP levels**

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Supplementary tables S1-4

Supplementary figures S1-7

Table S1. Clinical characteristics in HF and non-HF

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **All**  **n = 40** | **HF**  **n = 13** | **Non-HF**  **n = 27** | ***p*-value**  **between HF**  **and Non-HF** | **High risk HF**  **n = 5** | **Non-high risk HF**  **n = 35** | ***p*-value**  **between high risk HF**  **and Non-high risk HF** |
| Male, n (%) | 29 (73) | 10 (77) | 19 (70) | 0..664 | 4 (80) | 25 (71) | 0.688 |
| Type of CVD |  |  |  |  |  |  |  |
| IHD, n (%) | 24 (60) | 6 (46) | 18 (67) | 0.215 | 1 (20) | 23 (65) | 0.051 |
| AF, n (%) | 7 (18) | 6 (46) | 1 (4) | < 0.001 | 4 (80) | 3 (9) | < 0.001 |
| AS, n (%) | 11 (28) | 5 (38) | 6 (22) | 0.281 | 2 (40) | 9 (26) | 0.503 |
| AR, n (%) | 7 (18) | 4 (31) | 3 (11) | 0.125 | 2 (40) | 5 (14) | 0.157 |
| MR, n (%) | 15 (38) | 5 (38) | 10 (37) | 0.930 | 4 (80) | 11 (31) | 0.036 |
| TR, n (%) | 20 (50) | 10 (76) | 10 (37) | 0.018 | 5 (100) | 15 (43) | 0.017 |
| Type of HF |  |  |  |  |  |  |  |
| HFpEF | 22 (55) | 9 (69) | 13 (48) | 0.209 | 3 (60) | 19 (54) | 0.810 |
| HFmrEF | 12 (30) | 2 (15) | 11 (84) | 0.162 | 1 (20) | 11 (31) | 0.602 |
| HFrEF | 6 (15) | 2 (15) | 4 (15) | 0.962 | 1 (20) | 5 (14) | 0.738 |
| Medications |  |  |  |  |  |  |  |
| ACEI/ARB, n (%) | 28 (70) | 9 (69) | 19 (70) | 0.941 | 3 (60) | 25 (71) | 0.602 |
| ARNI, n (%) | 0 (0) | 0 (0) | 0 (0) | - | 0 (0) | 0 (0) | - |
| Beta-blocker, n (%) | 35 (88) | 11 (84) | 24 (89) | 0.702 | 5 (100) | 30 (86) | 0.366 |
| Diuretics, n (%) | 8 (20) | 5 (38) | 3 (11) | 0.043 | 3 (60) | 5 (14) | 0.017 |
| SGLT2i, n (%) | 2 (5) | 1 (8) | 1 (4) | 0.588 | 0 (0) | 2 (6) | 0.583 |
| Statin, n (%) | 27 (68) | 7 (54) | 20 (74) | 0.201 | 1 (20) | 26 (74) | 0.015 |

Data are presented as n (%). HF, heart failure is defined as NT-proBNP > 300 pg/mL; High risk HF is defined as NT-proBNP > 900 pg/mL; ACEI, angiotensin converting enzyme; AF, atrial fibrillation; AR, aortic valve regurgitation; ARB, angiotensin II receptor blocker; ARNI, angiotensin receptor neprilysin inhibitor; AS, aortic valve stenosis; CVD, cardiovascular disease; HF, heart failure; HFpEF, HF preserved ejection fraction; HFmrEF, HF mild reduced ejection fraction; HFrEF, HF reduced ejection fraction; IHD, ischemic heart disease; MR, mitral valve regurgitation; SGLT2i, sodium glucose cotransporter inhibitor; TR, tricuspid valve regurgitation.

Table S2. Clinical characteristics and it’s relation to heart sound

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | ***fS1* (Hz)** | ***fS2* (Hz)** | ***feS3* (Hz)** | ***feS4* (Hz)** |  | **Δ*fS1* (Hz)** | **Δ*fS2* (Hz)** | **Δ*feS3* (Hz)** | **Δ*feS4* (Hz)** |
| ***r***  ***p*-value** | ***r***  ***p*-value** | ***r***  ***p*-value** | ***r***  ***p*-value** | ***r***  ***p*-value** | ***r***  ***p*-value** | ***r***  ***p*-value** | ***r***  ***p*-value** |
| Age, y | - 0.096  0.554 | 0.116  0.478 | - 0.057  0.725 | 0.118  0.469 |  | 0.060  0.713 | - 0.049  0.765 | - 0.085  0.603 | - 0.044  0.786 |
| BMI, kg/m2 | 0.320  0.043 | - 0.348  0.078 | 0.177  0.264 | - 0.085  0.600 |  | 0.116  0.476 | 0.197  0.223 | 0.016  0.923 | 0.132  0.416 |
| NT-proBNP, pg/mL | - 0.070  0.668 | - 0.050  0.759 | - 0.008  0.962 | - 0.070  0.668 |  | 0.458  0.003 | 0.011  0.945 | 0.043  0.792 | - 0.133  0.412 |
| Cr, mg/dL | - 0.019  0.906 | 0.166  0.307 | 0.070  0.666 | 0.006  0.970 |  | 0.166  0.306 | - 0.039  0.813 | - 0.070  0.668 | 0.033  0.841 |
| eGFR, mL/min/1.73m2 | - 0.083  0.612 | - 0.286  0.074 | - 0.059  0.717 | - 0.071  0.665 |  | - 0.167  0.303 | - 0.043  0.792 | - 0.065  0.692 | - 0.167  0.302 |
| LVEF, % | 0.199  0.218 | 0.036  0.825 | - 0.005  0.976 | - 0.047  0.774 |  | 0.033  0.838 | - 0.024  0.885 | 0.032  0.846 | 0.149  0.358 |
| E wave, cm/s | 0.159  0.327 | 0.009  0.958 | - 0.081  0.619 | - 0.070  0.668 |  | 0.019  0.906 | 0.027  0.869 | - 0.198  0.220 | 0.103  0.528 |
| A wave, cm/s | - 0.003  0.988 | 0.193  0.282 | 0.135  0.455 | - 0.177  0.325 |  | - 0.250  0.075 | 0.089  0.622 | - 0.006  0.997 | 0.184  0.305 |
| E/A | - 0.126  0.486 | - 0.255  0.152 | - 0.232  0.194 | - 0.006  0.974 |  | 0.068  0.708 | - 0.285  0.107 | - 0.242  0.174 | - 0.066  0.713 |
| DcT, msec | 0.348  0.088 | 0.228  0.273 | - 0.032  0.878 | 0.068  0.746 |  | 0.189  0.365 | 0.344  0.086 | - 0.007  0.971 | 0.051  0.807 |
| E/e’ | - 0.038  0.818 | 0.191  0.245 | - 0.282  0.082 | - 0.124  0.452 |  | - 0.119  0.471 | 0.055  0.738 | -0.221  0.177 | - 0.191  0.244 |
| TRPG, mmHg | 0.032  0.849 | 0.005  0.977 | - 0.047  0.781 | - 0.213  0.206 |  | 0.276  0.099 | - 0.104  0.540 | - 0.038  0.823 | - 0.130  0.443 |
| QRS, msec | - 0.307  0.054 | - 0.026  0.876 | 0.120  0.459 | 0.068  0.675 |  | - 0.011  0.947 | 0.002  0.986 | - 0.010  0.952 | - 0.001  0.994 |
| SV1+RV5, mV | - 0.002  0.992 | 0.142  0.384 | 0.109  0.501 | 0.099  0.543 |  | - 0.093  0.566 | 0.269  0.093 | - 0.669  0.669 | 0.043  0.789 |
| CTR, % | 0.404  0.010 | 0.082  0.613 | 0.109  0.501 | 0.003  0.985 |  | 0.372  0.018 | 0.103  0.527 | - 0.077  0.638 | 0.094  0.564 |

Data are presented as means ± SD; BMI, body mass index; CTR, cardio-thoracic ratio; Cr, creatinine; DcT, deceleration time; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; TRPG, tricuspid regurgitation peak gradient.

Table S3. Univariate/Multivariate analysis for high risk HF

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Univariate analysis for high risk HF** | | |  | **Multivariate analysis for high risk HF** | | |
| **OR** | **95% CI** | ***p*-value** |  | **OR** | **95% CI** | ***p*-value** |
| Age, y | 1.084 | 0.949 - 1.240 | 0.193 |  | - | - | - |
| Male, n (%) | 1.600 | 0.159 - 16.13 | 0.690 |  | - | - | - |
| BMI, kg/m2 | 0.917 | 0.701 - 1.200 | 0.523 |  | - | - | - |
| AF, n (%) | 42.67 | 3.536 - 514.8 | < 0.001 |  | - | - | - |
| IHD, n (%) | 0.130 | 0.013 - 1.301 | 0.083 |  | - | - | - |
| Cr, mg/dL | 10.04 | 0.210 - 48.05 | 0.256 |  |  |  |  |
| eGFR, mL/min/1.73m2 | 0.946 | 0.859 - 1.042 | 0.217 |  |  |  |  |
| LVEF, % | 1.005 | 0.895 - 1.127 | 0.937 |  | - | - | - |
| E wave, cm/s | 1.014 | 0.984 - 1.045 | 0.370 |  | - | - | - |
| DcT, msec | 0.986 | 0.954 - 1.020 | 0.411 |  | - | - | - |
| E/e’ | 1.219 | 0.962 - 1.546 | 0.105 |  | - | - | - |
| TRPG, mmHg | 1.150 | 0.996 - 1.327 | 0.045 |  | - | - | - |
| S1, dB | 0.958 | 0.831 - 1.105 | 0.551 |  | - | - | - |
| S2, dB | 1.014 | 0.854 - 1.203 | 0.876 |  | - | - | - |
| eS3, dB | 0.904 | 0.754- 1.084 | 0.264 |  | - | - | - |
| eS4, dB | 0.932 | 0.816 - 1.067 | 0.325 |  | - | - | - |
| ΔS1, dB | 1.623 | 1.007 - 2.617 | 0.036 |  | 1.651 | 0.851 - 3.203 | 0.090 |
| ΔS2, dB | 1.948 | 0.542 - 6.999 | 0.316 |  | - | - | - |
| ΔeS3, dB | 0.582 | 0.076 - 4.464 | 0.575 |  | - | - | - |
| ΔeS4, dB | 1.470 | 0.327 - 6.600 | 0.621 |  | - | - | - |
| *fS1*, Hz | 0.978 | 0.905 - 1.156 | 0.710 |  | - | - | - |
| *fS2*, Hz | 0.950 | 0.904 - 1.225 | 0.491 |  | - | - | - |
| *feS3*, Hz | 1.024 | 0.794 - 1.200 | 0.820 |  | - | - | - |
| *feS4*, Hz | 1.016 | 0.801 - 1.210 | 0.884 |  | - | - | - |
| Δ*fS1*, Hz | 1.758 | 1.051 - 2.937 | 0.011 |  | 1.690 | 0.987 - 2.893 | 0.027 |
| Δ*fS2*, Hz | 0.937 | 0.621 - 1.832 | 0.813 |  | - | - | - |
| Δ*feS3*, Hz | 1.214 | 0.512 - 1.322 | 0.442 |  | - | - | - |
| Δ*feS4*, Hz | 0.584 | 0.559 - 5.244 | 0.262 |  | - | - | - |

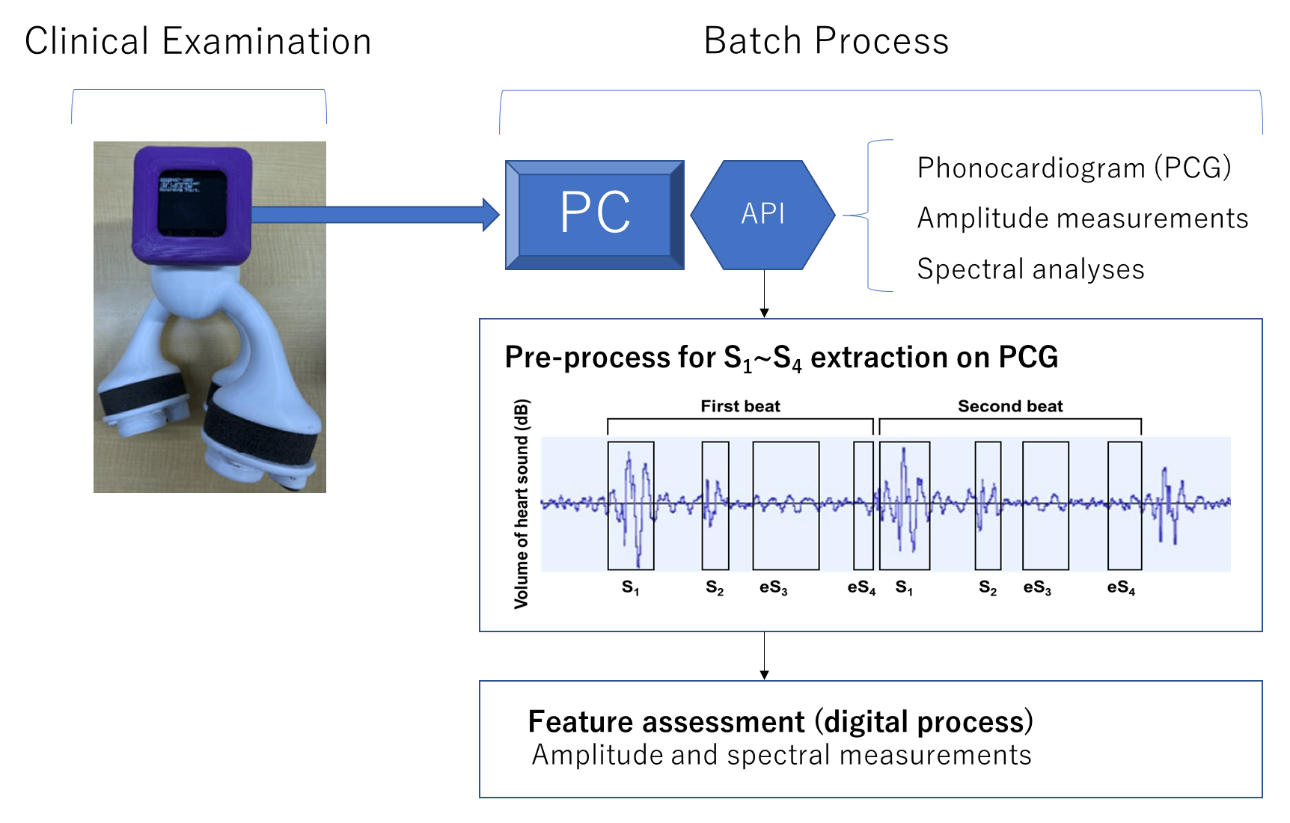
AF, atrial fibrillation; BMI, body mass index; CI, confidence interval; CTR, cardio-thoracic ratio; Cr, creatinine; DcT, deceleration time; eGFR, estimated glomerular filtration rate; HF, heart failure is defined as NT-proBNP > 300 pg/mL; IHD, ischemic heart disease, LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; OR, odd ratio; S1, first heart sound; S2, second heart sound; eS3, equivalent to third heart sound; eS4, equivalent to forth heart sound, TRPG, tricuspid regurgitation peak gradient.

Table S4. Multivariate analysis for HF and high risk HF

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Multivariate analysis**  **for HF** | | |  | **Multivariate analysis**  **for HF** | | |  | **Multivariate analysis**  **for high risk HF** | | |  | **Multivariate analysis**  **for high risk HF** | | |
| **OR** | **95% CI** | ***p*-value** |  | **OR** | **95% CI** | ***p*-value** |  | **OR** | **95% CI** | ***p*-value** |  | **OR** | **95% CI** | ***p*-value** |
| AF, n (%) | 15.31 | 1.219 - 192.3 | 0.035 |  | 17.31 | 1.699 - 176.5 | 0.016 |  | 36.90 | 2.224 - 612.3 | 0.012 |  | 37.74 | 2.215 - 576.7 | 0.012 |
| ΔS1, dB | 4.507 | 0.018 - 113.5 | 0.579 |  | - | - | - |  | 1.061 | 0.618 - 1.819 | 0.829 |  | - | - | - |
| Δ*fS1*, Hz | - | - | - |  | 5.512 | 0.397 - 76.60 | 0.201 |  | - | - | - |  | 1.799 | 0.845 - 3.827 | 0.061 |

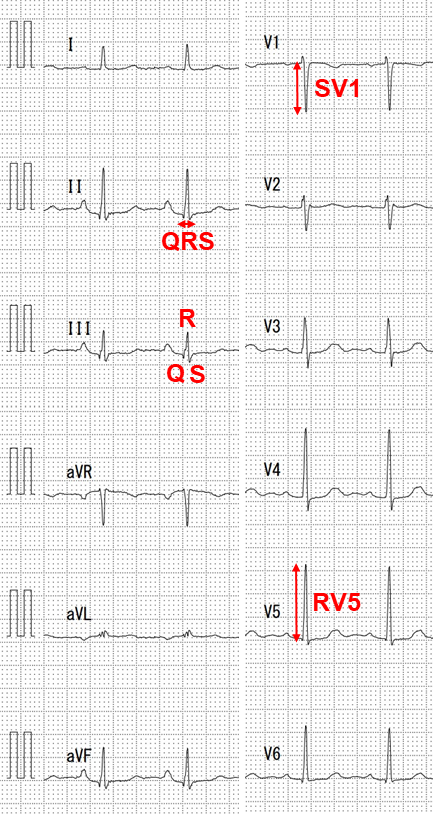
AF, atrial fibrillation; HF, heart failure is defined as NT-proBNP > 300 pg/mL; NT-proBNP, N-terminal pro-brain natriuretic peptide; OR, odd ratio; S1, first heart sound.

**Figure S1. Heart sound collection**

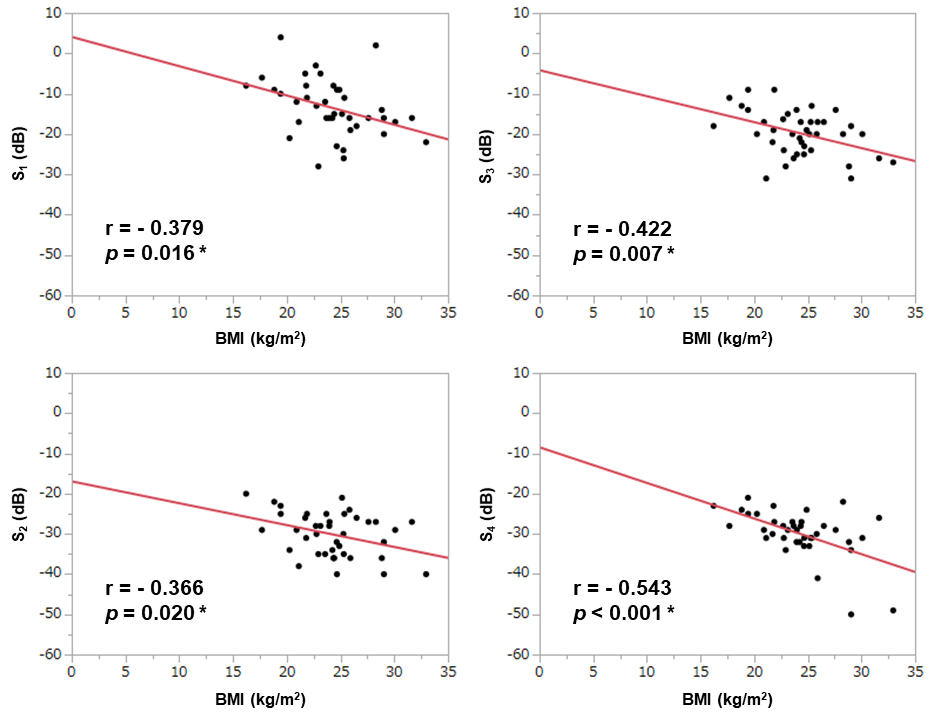


API, application programming interface; S1, first heart sound; S2, second heart sound; eS3, equivalent to third heart sound; eS4, equivalent to forth heart sound; PC, personal computer; PCG, phonocardiogram.

**Figure S2. Definitions of ECG wave**



**Figure S3. Correlations between heart sounds and BMI**



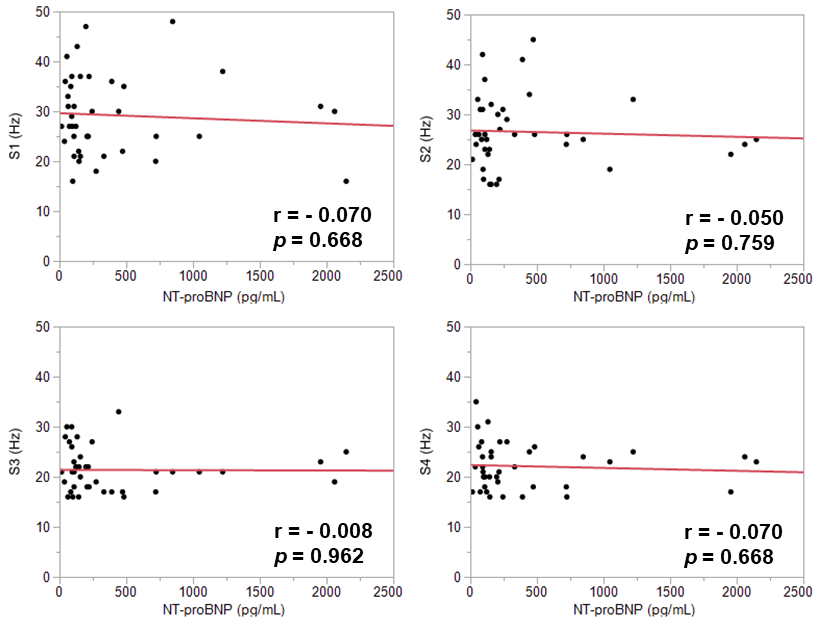
\*, *p* <0.050; BMI, body mass index; S1, first heart sound; S2, second heart sound; eS3, equivalent to third heart sound; eS4, equivalent to forth heart sound.

**Figure S4. Correlations between heart sounds intensities and NT-proBNP**



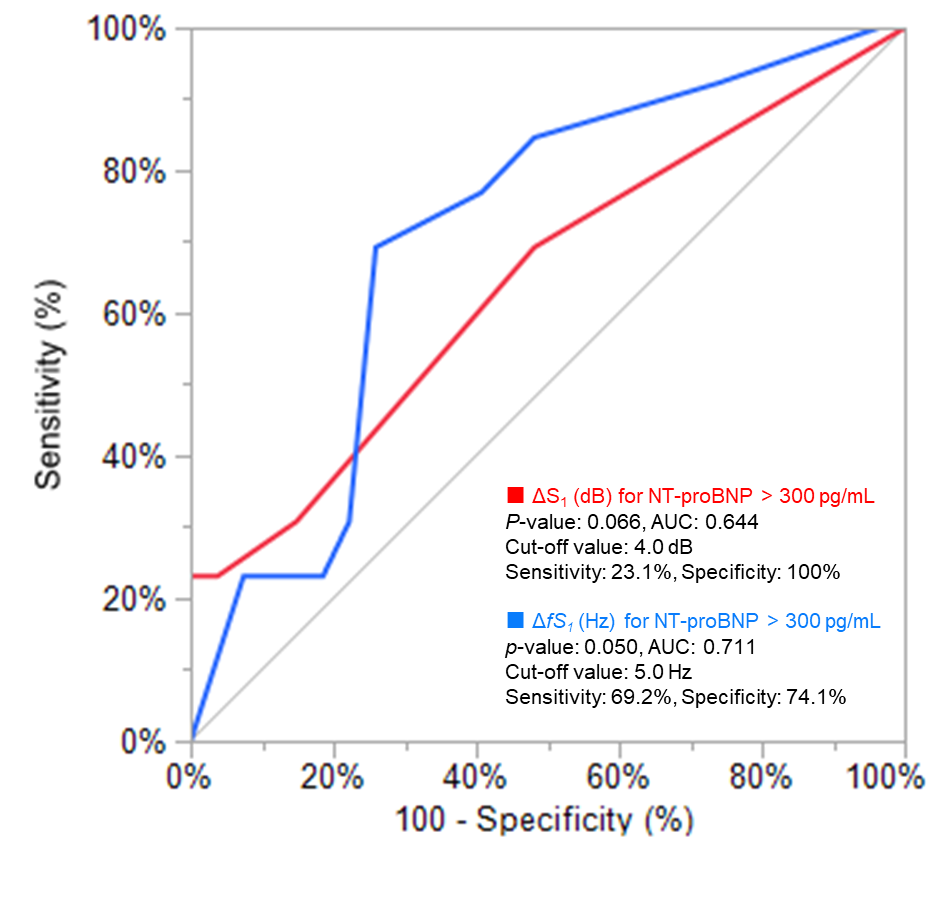
NTproBNP, N-terminal pro-brain natriuretic peptide; S1, first heart sound; S2, second heart sound; eS3, equivalent to third heart sound; eS4, equivalent to forth heart sound.

**Figure S5. Correlations between heart sounds frequencies and NT-proBNP**



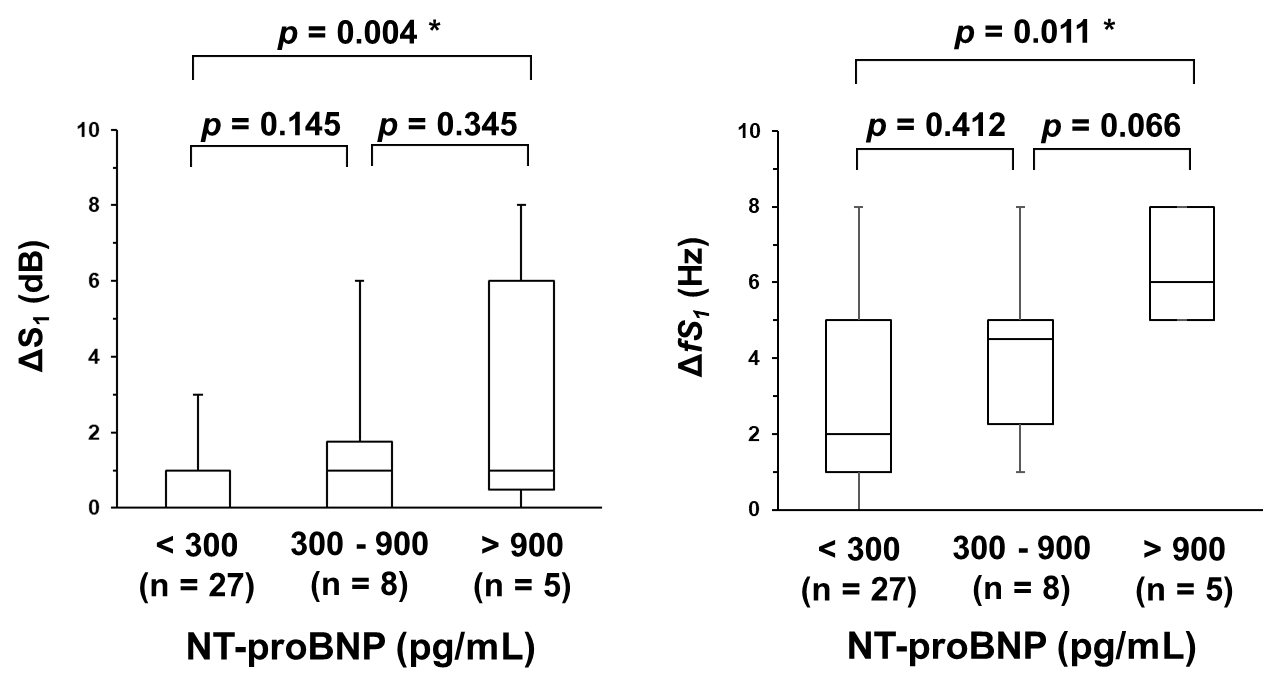
NT-proBNP, N-terminal pro-brain natriuretic peptide; S1, first heart sound; S2, second heart sound; eS3, equivalent to third heart sound; eS4, equivalent to forth heart sound.

**Figure S6. Receiver operating characteristic analysis for heart failure**

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AUC, area under curve; ΔS1, absolute value of difference between first heart sound continuous 2 beat; NT-proBNP, N-terminal pro-brain natriuretic peptide.

**Figure S7. ΔS1 intensities according to NT-proBNP**



\*, *p* < 0.050; NT-proBNP, N-terminal pro-brain natriuretic peptide; ΔS1, absolute value of difference between first heart sound continuous 2 beat