Heart Rate Variability as a Biomarker to Characterize Autonomic Responses to Different COVID-19 Vaccine Technologies Under Orthostatic Stress

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Summary

Heart rate variability (HRV) is a non-invasive biomarker of cardiovascular autonomic modulation. This study evaluated HRV during a standardized Tilt Test in 27 participants grouped by first COVID-19 vaccine technology: Vaccine 1, viral vector (n=11); Vaccine 2, inactivated virus (n=4); and Vaccine 3, mRNA (n=12). ECG-derived NN intervals were analyzed globally and during baseline (0-15 min), orthostatic stress (15-30 min), and recovery (30-50 min). Clinical data were integrated using Power BI, statistical analyses were performed in Jamovi, and phase-specific analysis used PyBioS. Global HRV showed significant between-group differences in SDNN (p=0.0189), HF (p=0.0476), and heart rate variation (p=0.0124). Phase-specific HRV parameters showed no significant between-group differences (p>0.05), indicating that the clearest differences were detected in global HRV rather than in an isolated Tilt Test phase. Age differed among groups and should be considered in future studies. These findings support HRV as a signal-derived biomarker for characterizing autonomic response patterns across vaccine technology groups, without establishing causality.

Keywords: heart rate variability; Tilt Test; autonomic nervous system; ECG; COVID-19 vaccine technologies.

1. Introduction

The Tilt Test is used to assess cardiovascular responses to orthostatic stress and to investigate the regulation of the autonomic nervous system. The protocol used in this project lasts 50 minutes, with 15 minutes in the horizontal position, 15 minutes with a 70° tilt and 20 minutes of recovery in the horizontal position. During orthostatic stress, changes in heart rate and beat-to-beat variability provide quantitative information about cardiovascular autonomic regulation.

HRV is a non-invasive biomarker obtained from ECG-derived NN intervals and has been applied in the evaluation of autonomic modulation. Previous work by this research group has used HRV during the Tilt Test to investigate autonomic regulation in post-COVID-19 participants [2,9,18]. Current work extends this signal processing approach to groups defined by COVID-19 vaccine technology.

Previous reports have discussed cardiovascular and autonomic events following SARS-CoV-2 infection and vaccination [1,3–6,17,19,20]. However, the present analysis was not designed to establish vaccine causality. Its originality lies in combining a standardized orthostatic stimulus with global and phase-specific features derived from HRV NN in the same computational flow. Therefore, HRV is investigated as a signal-derived biomarker to characterize measured autonomic responses, rather than as proof of an effect on the vaccine platform.

The hypothesis is that HRV parameters obtained during orthostatic stress can be investigated as signal-derived biomarkers to characterize cardiovascular autonomic regulation between vaccine technology groups, without assuming a priori that the groups differ.

2. Objective

To investigate cardiovascular autonomic regulation associated with different COVID-19 vaccine technologies using global and phase-specific HRV parameters extracted from ECG-derived NN intervals during the Tilt Test, along with clinical profile.

3. Materials and Methods

Data were collected at the Polyclinic Hospital/University of Mogi das Cruzes (UMC). The study is part of the FAPESP project (Scholarship 21/14231-0) and was approved by the Institutional Research Ethics Committee (64561022.7.0000.5497). Data collection was performed using the PC1000 ECG system (TEB, Brazil), electrodes, a reclining table, and an automatic digital sphygmomanometer (HEM-7320-Br, OMRON).

The project database contains 71 participants. In the present analysis, only participants with a clinical profile and corresponding export in the NN interval were retained. Three clinical records with no NN exports were excluded, resulting in 27 participants with no history of COVID-19. Participants had to be between the ages of 18 and 75, have negative pregnancy and PCR tests when applicable, have no heart disease, and not continuously use medications that alter the autonomic nervous system.

Participants were classified according to the technology of the first dose of the vaccine: Vaccine 1 comprised the viral vector group (n=11), Vaccine 2 the inactivated virus

group (n=4) and Vaccine 3 the mRNA group (n=12). Trade names were not used for group identification.

The workflow of signal processing and data integration was: clinical interview and Tilt Test; ECG acquisition; Export of NN ranges; global HRV analysis; phase-based analysis of cardiovascular signals; integration with clinical variables; and statistical comparison. Global statistical analysis of HRV was performed in Jamovi. Phase-based analysis was performed using PyBioS [16]. Power BI was used to integrate anamnesia information and physiological data, and to support data visualization [8].

For phase analysis, the NN intervals were separated according to the standardized periods of the Tilt Test: baseline (0-15 min), tilted/orthostatic stress (15-30 min), and recovery (30-50 min). Each period was analyzed separately. The phase characteristics reported were mean heart rate, mean NN, SDNN, RMSSD, NN50 and pNN50.

Age was maintained in the clinical profile as a factor to be considered in interpretation and in future studies, while the main inferential focus of the present study was the comparison of HRV between vaccine technology groups.

4. Results

The final analysis included 27 participants: Vaccine 1 (n=11), Vaccine 2 (n=4), and Vaccine 3 (n=12). The results are presented as clinical profile, global HRV and specific HRV by phase.

Table 1. Clinical profile by vaccine technology group.

Variable	Vaccine 1 (n=11)	Vaccine 2 (n=4)	Vaccine 3 (n=12)	p
Weight (kg)	72.00 [64.00-76.93]	61.00 [59.68-68.25]	77.50 [68.50-89.50]	0.2722
Height (cm)	166.00 [162.00-172.00]	166.50 [164.25-171.50]	177.00 [170.75-179.25]	0.0871
Age (years)	42.00 [27.00-46.50]	29.50 [22.00-40.75]	21.00 [20.75-23.00]	**0.0035**
BMI (kg/m²)	25.61 [22.50-28.50]	22.19 [21.75-23.28]	23.85 [21.42-28.25]	0.5939
Sex (F/M)	5/6	2/2	4/8	0.7726

Among the clinical profile variables, weight (p=0.2722), height (p=0.0871), BMI (p=0.5939) and sex distribution (p=0.7726) did not differ significantly between the groups. Age varied between the groups (p=0.0035); however, age is not treated as a primary outcome of this study and should be considered as a potential factor in future studies evaluating HRV among vaccine technology groups.

Table 2. Global HRV parameters by vaccine technology group.

Variable	Vaccine 1 (n=11)	Vaccine 2 (n=4)	Vaccine 3 (n=12)	p
Mean HR (bpm)	73.00 [67.50-79.50]	71.50 [66.00-77.25]	79.00 [66.50-79.50]	0.9282
Mean NN (ms)	833.54 [763.09-901.23]	858.77 [795.42-935.26]	814.34 [772.62-920.12]	0.8981
SDNN (ms)	109.00 [62.98-135.98]	161.67 [153.06-177.31]	181.69 [126.60-224.22]	**0.0189**
RMSSD (ms)	34.34 [27.02-103.41]	92.88 [59.50-133.28]	99.39 [61.00-110.79]	0.2370
NN50	320.00 [78.50-609.50]	412.00 [199.25-610.50]	645.00 [357.50-1204.25]	0.1508
pNN50 (%)	9.80 [2.15-16.90]	11.05 [5.03-17.80]	20.05 [10.62-32.62]	0.1786
Triangular index	18.86 [17.15-24.20]	26.32 [24.70-27.84]	25.60 [16.46-32.93]	0.3184
VLF	586.46 [404.62-1382.12]	4624.62 [1619.63-15763.98]	4895.54 [1362.36-13756.79]	0.0603
LF (ms²)	439.56 [313.46-2339.81]	9025.91 [2216.44-40950.94]	8915.58 [964.25-25327.83]	0.0843
HF (ms²)	431.42 [182.48-2150.92]	3746.03 [1797.23-20436.90]	6729.34 [1643.87-17331.64]	**0.0476**
LF/HF	1.90 [0.86-2.58]	1.54 [1.18-2.06]	1.40 [0.96-1.62]	0.6942
HR variation	50.39 [37.55-77.05]	153.93 [142.29-162.85]	203.39 [84.05-240.04]	**0.0124**

Table 4. HRV parameters for 15-30 minutes

Variable	Vaccine 1 (n=11)	Vaccine 2 (n=4)	Vaccine 3 (n=12)	p
Mean HR (bpm)	68.81 [62.57-76.63]	65.41 [59.65-70.27]	63.30 [58.91-76.03]	0.5798
Mean NN (ms)	878.32 [788.14-961.45]	921.51 [858.69-1016.23]	965.57 [791.65-1030.34]	0.4959
SDNN (ms)	43.09 [34.25-75.90]	65.55 [46.15-87.57]	78.36 [61.94-94.33]	0.3603
RMSSD (ms)	40.18 [23.43-77.43]	58.94 [43.22-76.40]	71.53 [36.44-107.43]	0.4812
NN50	34.00 [8.50-198.50]	172.50 [63.25-277.75]	283.00 [112.50-526.75]	0.1156
pNN50 (%)	3.79 [0.78-20.42]	16.54 [5.81-29.48]	34.39 [10.67-56.96]	0.1288

Table 5. HRV parameters for 30-50 minutes

Variable	Vaccine 1 (n=11)	Vaccine 2 (n=4)	Vaccine 3 (n=12)	p
Mean HR (bpm)	85.08 [76.52-90.73]	86.67 [80.86-93.91]	91.26 [78.82-102.27]	0.4642
Mean NN (ms)	709.03 [664.04-794.35]	701.41 [646.67-754.11]	661.21 [594.56-767.01]	0.4655
SDNN (ms)	45.14 [38.12-65.60]	127.11 [87.15-162.09]	82.16 [59.66-109.71]	0.0568
RMSSD (ms)	22.78 [15.56-33.42]	116.10 [30.49-215.47]	106.39 [19.96-125.25]	0.1828
NN50	23.00 [11.50-48.50]	44.50 [40.50-62.00]	27.50 [9.50-94.25]	0.3952
pNN50 (%)	1.63 [0.87-3.73]	3.48 [2.92-5.27]	2.04 [0.72-7.09]	0.4578

Variable	Vaccine 1 (n=11)	Vaccine 2 (n=4)	Vaccine 3 (n=12)	p
Mean HR (bpm)	65.97 [64.19-74.47]	62.45 [57.12-67.54]	62.83 [59.36-72.02]	0.4580
Mean NN (ms)	918.61 [809.16-943.00]	968.98 [896.42-1063.01]	967.67 [842.91-1023.50]	0.4365
SDNN (ms)	71.48 [41.88-89.95]	68.56 [65.75-85.09]	92.78 [74.98-121.69]	0.2019
RMSSD (ms)	42.07 [32.70-102.09]	46.41 [32.62-64.46]	84.33 [42.84-104.99]	0.4485
NN50	245.00 [46.50-380.00]	174.50 [105.75-248.75]	299.00 [178.75-695.00]	0.2861
pNN50 (%)	17.56 [3.14-29.35]	15.51 [7.48-22.99]	29.86 [12.49-57.18]	0.1908

No phase-specific between-group comparisons reached $p<0.05$ in the periods 0-15 min, 15-30 min, or 30-50 min. Thus, statistically significant HRV differences in this dataset were observed in the global analysis, and not in the

individual phases of the Tilt Test.

5. Discussion

The main HRV findings were the overall differences between groups in SDNN, HF and heart rate variation. SDNN reflects the overall variability, these results demonstrate measurable differences in ECG-derived variability characteristics between the vaccine technology groups of the present dataset. Age also varied between groups, and should be treated as a potential factor to be examined more specifically in future studies with larger and more balanced samples.

Phase-specific analysis provides a complementary view of the orthostatic stress response. The periods of 0-15 min, 15-30 min of orthostatic stress and 30-50 min of recovery were analyzed separately. Although HRV parameters changed throughout the Incline Test, no specific between-group comparisons per phase reached $p<0.05$. Therefore, the clearest differences between the groups were identified in the overall HRV, and not in the isolated phases.

The main limitations remain the modest and unequal sizes of the groups, especially Vaccine 2. The statistical results describe associations in physiological signals measured according to vaccine technology.

6. Conclusion

HRV obtained during a standardized Tilt Test can be used as a signal-derived biomarker to characterize cardiovascular autonomic responses between vaccine technology groups. In the updated sample, the clearest between-group differences were observed in global variation in SDNN, HF, and heart rate, while phase-specific HRV showed no significant differences between groups. Age should be considered as a potential factor in future studies, especially in larger, more balanced samples.

Recognition

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