Heart rate variability changes in mild-symptomatic, physically fit male in 4-6 weeks from the end of SARS-Cov-2 infection

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Abstract

SARS-Cov-2 infection, due to inflammation processes, can affect autonomic nervous system and heart rate variability (HRV) even after disease. Previous studies showed significant changes in HRV parameters in severe (including fatal) infection of SARS-Cov-2. However, HRV analysis for the asymptomatic or mild-symptomatic Covid-19 patients have not been reported. In this study, we suggested that there is an influence of a SARS-Cov-2 infection on the HRV in such patients after weeks form disease.

Sixty-five ECG Holter recordings from young (mean age 22.6 ± 3.4 years), physically fit male subjects after 4-6 weeks from the second negative test (considered to be the beginning of recovery) and twenty-six control male subjects (mean age 23.2 ± 2.9 years) were considered in the study. Night-time RR time series were extracted from ECG signals. Selected linear, frequency as well as nonlinear HRV parameters were calculated.

We found significant differences in Porta's symbolic analysis parameters V0 and V2 ($p<0.001$), α_2 ($p<0.001$), very low frequency component (VLF; $p=0.022$), and respiratory peak (from PRSA method; $p=0.012$). These differences may be caused by the changes of the parasympathetic autonomic nervous system as well as by the coupling of respiratory rhythm with heart rate due to an increase in pulmonary arterial vascular resistance.

The results suggest that the changes in the HRV, thus autonomic nervous system, are measurable after a few weeks from the beginning of the recovery even in the post-Covid group of young and physically active population. We indicated HRV sensitive markers which could be used in the long-term monitoring of recovered patients.

1. Introduction

The autonomic nervous system has access to information about the state of the human homeostasis at the deepest level. The non-invasive tests of simple clinical parameters, which are subject to autonomous regulation, have the potential to provide non-specific but unique knowledge that cannot be given by any other method of modern medical diagnosis. Probably, this knowledge could be obtained from a combination of microneurography and continuous biochemical measurements, like catecholamine levels, but currently, such measurements are impossible. However, having access to non-invasive data such as RR intervals, respiratory intervals or blood pressure recordings, advanced methods of signal analysis can be used for an objective assessment of the clinical condition of the patient. It has been shown, that autonomic activity can be assessed by the dedicated analysis of heart rate variability (HRV) [1] [2]. However, as the diagnostic method is indirect, it requires special language of description. This language is defined by a set of computational methods, that assess such exotic properties of the signal as complexity, regularity or scaling. Although this approach is considered esoteric and far too complex for regular clinical use, there exist certain cases, in which this unique information was proven useful.

The autonomic system modulates heart rate variability in viral infections due to inflammation, being developed by the activity of the immune system. Autonomic changes are visible in HRV during sepsis as a result of the presence of the pathogen, gram-negative bacteria [3]; they may also precede this infection [4] [5], which is specifically important in case of septic neonates with low birth weight - a clinical group which requires special care [5]. In the study by Mattei et al. [6], a decrease of the spectral indices in low (LF), high (HF), very low (VLF) frequencies as well in total power was observed in infants over one year of age, who have been infected with the H1N1 flu virus. La-Orkhun et al. [7] investigated the occurrence of arrhythmias and changes in HRV in children as a result of Dengue virus infection by comparing the results of the HRV analysis in the convalescent stage and during the follow-up visit (at least 14 days after defervescence).

Since the beginning of the SARS-CoV-2 virus pandemic, only a few reports focusing on the HRV analysis related to COVID-19 infection have been published. One of the classical markers of HRV assessment, the standard deviation of the NN intervals (SDNN), was studied in [6]. Its drops preceded an increase of the inflammatory marker CRP [8]. SDNN as well as other spectral parameters were shown as predictors of illness severity and mortality in critically ill patients with COVID-19 [9]. The studies of Aragón-Benedí et al. [9] and Kaliyaperumal et al. [10] showed an increase in parasympathetic activity and a decrease in sympathetic activity reflecting a compensatory anti-inflammatory response in patients who died due to infection. Single clinical cases of continuous monitoring of the patient during the inflammation process of COVID-19 were also reported, where a parallel decrease in the heart rate (HR) and SDNN at the onset of the disease were found [11]. Differences in the autonomic responses between patients with COVID-19 in relation to patients with sepsis (due to various causes) have also been described, indicating the unique expression of SARS-CoV-2 infection [12]. More detailed discussion about the prognostic value of HRV markers and their correlations with inflammatory markers in COVID-19 disease can be found in [9].

To our knowledge, no previous studies have been done for assessment changes of the activity of the autonomic nervous system in subjects encompassing asymptomatic or mild symptoms of SARS-CoV-2 infection after a few weeks from the beginning the recovery. Thus, the main research hypothesis here is verifying the influence of a SARS-Cov-2 infection on the HRV after 4–6 weeks from the second negative test (considered to be the beginning of recovery) in a group of young men (20–25 years). Convalescents were encompassing asymptomatic or had mild symptoms of SARS-CoV-2 infection who were without accompanying diseases. In the HRV study, we propose selected parameters, especially these that characterize the activity of the autonomic nervous system. In case of significant differences in HRV markers with the matched control group, it should be possible to discuss the source of HRV changes during the recovery process. The proposed HRV study, in a relatively long period after infection, selects sensitive parameters that reflect the compensatory mechanisms in the circulatory system due to COVID-19 and inflammatory process. We conclude that such non-invasive markers should be verified in further studies as screening parameters in groups of patients affected by COVID-19.

2. Methods

Participants

Seventy physically fit male patients without comorbidities who were diagnosed with COVID-19 by RT-PCR on swab test of the upper respiratory tract. After 4–6 weeks from the second negative PCR test for SARS-CoV-2 virus were enrolled in the study group. Participants were asymptomatic or showed only mild symptoms during the infection (reported symptoms: dry cough – 2 subjects, dyspnea – 1 subject, taste disorders – 6 subjects, smell disorders – 9 subjects). The mean age of these convalescents was: 22.6 ± 3.4 years. Twenty-six healthy men, mean age 23.2 ± 2.9 years, without a history of SARS-Cov-2 infection, were recruited to the control group. In the controls, the inclusion criteria were: i) no previous confirmed infection with SARS-CoV-2 virus, ii) age 20–25 years; iii) male gender iv) no diagnosed cardiovascular diseases.

The results of the HRV analysis are based on sixty five recordings from the study group. Five time series were rejected due to a low quality of the signal. All sets from control group were used in the comparison study. Baseline characteristics, including clinical and biological parameters are showed in Table 1. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments and approved by The Local Committee of Ethics (Ethics and Supervision Committee for Research on Humans and Animals at the Central Clinical Hospital of the Ministry of Interior in Warsaw, Poland) no 89/2020. All participants gave informed written consent to participate in the study.

Table 1
Baseline characteristics of the study population.

Parameter	Study group (post-Covid)	Control group	p-value
n	65	26	-
age	22.6 (3.4)	23.2 (2.7)	0.134
BMI [kg/m^2]	24.0 (2.0)	23.8 (2.5)	0.775
BSA	2.00 (0.13)	2.04 (0.17)	0.272
SBP [mmHg]	128.4 (12.1)	124.5 (12.4)	0.175
DBP [mmHg]	71.3 (7.5)	71.6 (10.0)	0.913
Biological characteristics			
Interleukin 6 (ng/L)	2.08 (0.78)	1.83 (0.36)	0.202
C reactive protein	0.74 (1.32)	0.76 (1.08)	0.491
Leucocytes (G/L)	5.68 (1.12)	5.59 (1.46)	0.443
Procalcitonin (µg/L)	0.06 (0.03)	0.05 (0.03)	0.757
Troponin I (ng/ml)	2.72 (4.56)	3.84 (2.72)	< 0.001
D-dimer (µg/L)	218.5 (186.8)	266.8 (131.3)	< 0.001
Potassium (mmol/L)	4.29 (0.24)	4.24 (0.32)	0.564
Sodium (mmol/L)	140.8 (1.38)	140.9 (2.21)	0.902

Heart rate variability

Six hours length, night-time RR time interval series were extracted from twenty-four hours Holter (LifeCard, Reynolds Medical) recordings and used for HRV analysis. Signals were previously verified and corrected by a specialist. HRV parameters considered in the study are described below.

Linear methods

Linear methods (both in the time domain and in the frequency domain) are the most popular and commonly used measures of HRV analysis [13]. The main advantage of these methods is the low computational complexity, which results in fast calculations and well-studied clinical interpretation of the parameters determined. In this paper, the following markers were selected for the analysis of the HRV: mean RR interval (meanRR), SDNN, mean square root of differences between successive RR intervals (RMSSD), percentage of consecutive differences intervals greater than 50 ms (pNN50). The first metric, meanRR is expressed in milliseconds and carries information about the average duration of a single RR interval. It is the resultant balance between sympathetic and parasympathetic nervous system activity.

This measure is supported by another indicator, SDNN, which is often used as the main HRV marker. SDNN shows the scatter of RR intervals around the meanRR. The smaller the value of the SDNN, the lower the signal variability. In the study of the SDNN results, the length of the HRV record should be considered. Note, that the SDNN determined from the short signal in the range of minutes is not comparable with the SDNN from the Holter recordings, which are characterized by circadian variations. The next two markers, RMSSD and pNN50, are indicators of short-term variability and reflect activity of the parasympathetic system [14].

Frequency domain

Signal analysis in the frequency domain is based on calculation of the power spectrum (e.g. determined by the Fourier Transform) in the specific frequency ranges. In the spectrum of the HRV, the following frequency bands are usually examined [13]: very low (VLF; 0.003–0.04 Hz), low (LF; 0.04–0.15 Hz) and high (HF; 0.15–0.40 Hz).

HRV Task Force [13] proposed a physiological interpretation of these frequency bands in the RR interval series. LF range is mainly associated with the activity of the sympathetic component of the autonomic nervous system, while the power in the HF band describes the activity of the parasympathetic system and breathing influence [15]. However, the frequency bands cannot be completely assigned to specific parts of the autonomous system and this assignment has raised many objections [16] [17]. The interpretation of the VLF component modulation is not completely clear. Traditionally it is being associated with thermoregulation and/or humoral regulation [18] [19] and more recently it was shown to correlate with the occurrence of the U-shaped patterns [20] that have a form of infrequent events in the heart rate rather than a constant modulating frequency.

Non-linear methods

Another group of methods proposed in our study is non-linear. They refer to the "complexity" description of the RR series. Complexity is related to irregularity of RR intervals in the records. In the extreme case, when the same value of the RR time interval (the ideal case of the pacemaker activity) is present, there is a lack of complexity. Another subgroup of parameters are symbolic - V0 and V2 proposed by Porta et al. [21]. In this approach, the values of the datasets are divided into N equal ranges and every range is denoted by a different symbol. Then sequences of the successive three RR intervals are collected and the RR intervals are replaced with symbols, according to the previously constructed ranges. Every three symbols represent a symbolic word. The V0 parameter determines the percentage of words consisting of the same symbols (which is related to low variability in the time series), while the V2 parameter is equal to the percentage of words composed by three different symbols (high variability). Both indexes may be related to the activity of the sympathetic and parasympathetic branches of the autonomic nervous system [21]. In the case that the value of V0 decreases and the value of V2 increases, it is suggested that the activity of the parasympathetic branch increases in the relation to the sympathetic one. The opposite case indicates sympathetic activity, which is related to a longer (compared to the parasympathetic) delay in the response of the sinus node cells to sympathetic stimulation [22].

Other non-linear measures are derived from Detrended Fluctuation Analysis (DFA) [23]. There are two scaling exponents α1 and α2, which refer to selected time scales in HRV data. As a result, they can be used to assess the correlations in short (4–16 beats) and long (16–64 RR intervals) time windows. Additionally, the latter is correlated with the power of the spectrum in the LF and VLF bands [24]. A significant increasing in the value of this exponent α2 was observed in patients with heart failure (CHF) [23]. Correlations were also observed between α2 and short-term changes in heart rhythm resulting from physiological arousals in a polysomnographic study [20] [25].

The next group of parameters was determined from the phase rectified signal averaging (PRSA) method, which is briefly described below. The PRSA method was introduced for the analysis of non-stationary and noisy data with periodicities [26], which are often difficult to observe in the frequency domain. Therefore, the study of long signals is reduced to shorter by a certain averaging. The first step of the method is defining the anchor points. An anchor can be every RR interval, which is longer than the previous one, which means that it reflects the ability of the system to lengthen the heart cycle, i.e. decelerate. The second step of the PRSA method is the definition of the radius. Usually, the radius parameter is strictly related to the frequency scale of the periodicities obtained around the anchor points. Slow oscillations require a larger radius than the fast ones. In our study, we used 1024 points. Such length was chosen as satisfactory for Fast Fourier Transformation (FFT) and enough for observation of respiratory oscillations. Each anchor point and the radius parameter define a window. The anchor point is denoted as RR(0), negative integers number previous intervals, and the following ones the anchor, by positive indexes. Windows are aligned such a way that the anchor points coincide. The final PRSA curve is obtained from averaging the RR intervals in aligned windows.

So far, the PRSA technique has been used to analyze the parameters of the averaged curve. For this purpose, diagnostic markers DC, AC (ability to slow down and accelerate) were introduced. The DC parameter was used as a predictor of mortality in patients after myocardial infarction [27] as well as a discriminating factor in patients with heart failure [28]. Previous reports indicated the relationship of the above parameters with the activity of the autonomic nervous system. For example, a decreased DC value was interpreted as the dominance of the sympathetic part in various clinical groups [28] [29].

In our paper, we expanded the analysis of the PRSA curve to explore the respiratory oscillations from RR intervals. Instead of original HRV data, the PRSA curve was used for FFT. The averaged signal shows the properties of the power spectrum (like characteristic frequencies), which were not observable in the classical analysis [26]. In the FFT technique applied to the PRSA curve, the sampling frequency is the reciprocal of the mean RR interval. As a result, the frequency representation is not given in Hz but as a multiplicity of the mean RR interval reciprocal. Looking for the spectral power maximum in the corresponding range of HF, we determined its position (denoted as Resp. Peak). The Resp. Peak is a marker, which specifies the number of averaged RR intervals per breath. It can be easily used for assessing the coupling between heart rhythm and respiration [30] [31]. We also propose a parameter, which estimates the width of the respiratory peak (Resp. range). The wider the range, the larger variability of the breath length. Both proposed markers characterize respiration oscillations by HRV data.

Statistical analysis

Comparison of the mean values of HRV parameters between the study and the control group was undertaken using the parametric t Student test for independent groups or the nonparametric

Mann-Whitney test if the distribution were not normal (tested by Shapiro-Wilk test); $p < 0.05$ was considered as significant. Statistical analysis were performed using the R package (ver. 3.6.0) with R-Studio software (ver. 1.2.1335).

3. Results

The quantitative results were presented as X(Y), where X is the mean and Y standard deviation. Table 2 showed the results for the linear, nonlinear and PRSA parameters, respectively. Significant differences was found for VLF range ($p = 0.02$), symbolic analysis parameters: V0 and V2 (both $p < 0.001$), α_2 ($p < 0.001$) and Resp. Peak. ($p = 0.012$) parameter from PRSA method.

Table 2
Comparison of the mean HRV parameters between the study groups.

Parameter	Study group (post-Covid) (n = 65)	Control group (n = 26)	p-value
Linear parameters in time domain			
Mean RR [ms]	1162 (111)	1115 (122)	0.093
SDNN [ms]	139 (33)	129 (30)	0.166
RMSSD [ms]	90 (39)	91 (35)	0.857
pNN50 [%]	47.8 (18.4)	41.9 (17.4)	0.109
Frequency domain			
VLF [ms^2]	13826 (6600)	10490 (4762)	0.022
LF [ms^2]	2889 (1606)	2879 (1471)	0.742
HF [ms^2]	3063 (2665)	3084 (2704)	0.816
Non-linear analysis			
V0 [%]	49.0 (15.4)	66.5 (18.2)	< 0.001
V2 [%]	14.7 (6.9)	7.5 (6.3)	< 0.001
Sample entropy	1.32 (0.18)	1.27 (0.20)	0.357
α_1	1.05 (0.16)	0.99 (0.15)	0.082
α_2	1.11 (0.08)	1.03 (0.10)	< 0.001
PRSA			
DC [a.u.*]	26.6 (9.9)	26.8 (10.1)	0.878
AC [a.u.]	-23.1 (7.5)	-23.9 (7.0)	0.816
Resp. peak [a.u.]	3.91 (0.56)	4.25 (0.61)	0.012
Resp. range [a.u.]	0.275 (0.030)	0.281 (0.031)	0.425
* a.u.- arbitrary units, see details in the sec. 2.2.2			

4. Discussion

In the study, we obtained significant differences between the convalescents and the control group in the symbolic patterns represented by parameters V0 and V2. The value of V0 was lower, and the value of V2 was higher for convalescents. Following the interpretation proposed by Guzzetti et al. [32], such representation may be explained as an increase in parasympathetic cardiac modulation. The interpretation of V2 related to the parasympathetic activity seems quite probable if one recalls that the parasympathetic branch has a potence to introduce momentary (beat to beat) changes in the heart rate, which may even lead to asystole. Even changes of sympathetic activity imposed by pheochromocytoma don't have such a potence [33]. The effect of autonomic dysfunction in early stage of COVID-19 was observed previously in patients with different intensities of symptoms[34] as well as in critical conditions [9]. However, autonomic activity was characterized in these reports by spectral components and time domain parameters. In our study, these markers are non-significant in comparison between the convalescents and the control group. Our results indicates that symbolic parameters studied here are more sensitive to detecting autonomic modulation changes and more resistant to non-stationarity than other classical HRV indexes.

Another result is the occurrence of a significant difference in the Resp. Peak parameter, related to the coupling of respiratory rhythm with heart rate. The Buchner et al. [30] study proved the significance of this parameter (more specifically of the equivalent parameter: mean rhythm ratio) in the analysis of this coupling showing an increase in this parameter from values 3–4 to 8 after a complete pharmacological blockade of the autonomic system by administration of a beta-blocker. The role of such coupling is to maintain the homeostasis (perhaps a better term here would be: allostasis) between ventilation and perfusion [35]. The differences in respiratory rhythm between the study and the control group might be related to the long-term effects of SARS-CoV-2 virus on the respiratory system, for example, due to a reduction in tidal volume, or to an increase in pulmonary arterial vascular resistance, which is observed in patients after COVID-19 even with mild symptoms of the disease [36]. It is interesting, that possible increase of vascular resistance can be indicated by a simple parameter based only on 1024 consecutive heart beats, which are easy to measure in a noninvasive screening scenario, even using modern heart rhythm monitors, which are very popular nowadays. This primary observation provides an open field for a subsequent pilot study.

The significant statistical differences for the nonlinear parameters of heart rate variability provide some support of the hypothesis for the persistent changes in the autonomic nervous system as a result of infection, manifested by increased parasympathetic activity. However, the significant difference for the Resp. Peak parameter suggests an alternative explanation. Statistical significance for the markers describing the activity of parasympathetic branch of the autonomic system (i.e. decreased values for the parameter V0 and increased values for the parameter V2) could result not from pathological changes in the ANS but from the modulation of the coupling resulting from adaptation processes during the convalescence period.

The differences of VLF and α_2 may be associated with the different number of the U-shaped patterns, characteristic changes in RR time interval values, lasting approximately 20–40 seconds, occurring during sleep [20] [25]. U-shaped patterns are a common phenomenon in humans, occurred mostly during sleep and appear to be a cardiovascular response to the occurrence of cortical arousal. Cortical arousal is a physiological adaptive mechanism for the occurrence of external or internal stimuli that disrupt the state of homeostasis [25]. The study by Soliński et al.[20] demonstrated the relation between the number of U-shaped patterns detected in night-time RR time interval series and the values of HRV parameters related to its long-time correlation, multifractal properties and very low frequency component. Referring to the methodology used in that report, we compared the mean values of U-shaped patterns between the study and the control group obtaining a significant statistical difference (study group: 17.1 (95%CI: 15.6–18.5) patterns vs. control group 12.9 (95%CI 10.9–14.8) patterns, $p < 0.001$). The U-shaped patterns were then artificially removed from the RR time series and the mean values of the HRV parameters used in the data analysis were again compared. Re-analysis comparing HRV parameters between groups showed non-significance for VLF, $\alpha 2$ parameters (p-value: 0.062 and 0.092, respectively), however, significance for V0 and V2 parameters persisted ($p < 0.001$). It is difficult to determine whether differences in the number of U-shaped patterns could be related to SARS-Cov-2 virus infection. Additional polysomnography studies should be performed to investigate possible differences in sleep dynamics between groups. Also the etiology of U-shaped patterns is not fully understood.

A strength of the conducted study, taking into account the limited number of the subjects, is the selection of fairly homogeneous populations for comparative analysis of HRV parameters (population of males in a narrow age range). The results of the study may be an input to create more complex indexes and methods such as multiparameters or artificial intelligent algorithms to diagnose autonomic dysregulation which use either HRV measures and other physiological signals, similar to the index described in [37].

Limitations

This study has potential limitations. RR time interval series were measured in uncontrolled conditions, according to a standard Holter protocol, thus the results may have been affected to some extent by unknown external factors. In addition, we did not measure Holter recordings in the study group before SARS-Cov-2 infection, so that a direct comparison of HRV analysis results before and after the advent of Covid-19 was not possible. However, repetition of the Holter measurements after relatively long time from the infection (e.g. 1–2 year) can provide new information about the study group.

5. Conclusions

The study showed significant differences of HRV parameters related to the parasympathetic nervous system activity and the level of the coupling between cardiac and respiratory rhythm between post-Covid male subjects with mild symptoms and the control group. The results suggest that even in the group of relative young and physically active population the infection of SARS-Cov-2 virus may cause changes in the ANS and cardio-respiratory system measurable after a few weeks from the beginning of the recovery. This result is consistent with the previous study, which indicated the occurrence of pathological changes in mild-symptomatic COVID-19 patients [37].

One of the clinical endpoints of the present study could be the development of a methodology for monitoring the post SARS-Cov-2 patient including analysis of HRV performed by Holter monitoring. In order to validate the application of HRV measures, we suggest to perform regular Holter recordings in the study group several months after Covid-19. In our report, the study group was characterized by a mild (or asymptomatic) course of the disease, which may indicate that significant HRV parameters are sensitive markers in the long-term monitoring of recovered patients.

Declarations

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Author contributions

A.P., R.G. and Z.K. planned and performed the experiments. T.B., M.P., J.Ż. and M.S. designed the data study. M.S., M.P., J.A. did the calculations and statistical analysis. M.S., M.P., T.B., J.A., J.Z. were responsible for results presentation and interpretation. All authors participated in manuscript construction. All authors read and approved the final version of article.

Competing interests

The authors declare no competing interests.

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