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Chronotropic dysfunction determines multiorgan complications in adults after Fontan surgery

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Short title: Chronotropic dysfunction and organ complications after Fontan surgery

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What’s new?

Despite the normal or close-to-normal function of the systemic ventricle, patients undergoing Fontan operation (FO) are unable to generate cardiac output or exhibit similar exercise capacity as their healthy peers. Impaired exercise capacity can result from widespread chronotropic dysfunction and multiorgan complications.

We demonstrated an existing relationship between heart rate reserve (HRR) and selected multiorgan complications. HRR can be a promising indicator of organ complications in patients after FO and may be helpful in selecting patients for pacemaker implantation.
Abstract

**Background:** Despite the normal or close-to-normal function of systemic ventricle, patients undergoing Fontan operation (FO) are unable to generate cardiac output or exhibit similar exercise capacity as their healthy peers. This can be attributed to chronotropic dysfunction and multiorgan complications.

**Aims:** We evaluated the prevalence of chronotropic incompetence in adults after FO and assessed the relationship between heart rate reserve (HRR) and multiorgan complications.

**Methods:** Data were obtained from 50 FO patients (mean [SD] age = 27 [6.6] years) and 30 healthy controls matched for age and sex. All patients were subjected to clinical examination, laboratory tests, echocardiography, and cardiopulmonary exercise test (CPET) and assessed for chronotropic function.

**Results:** The CPET parameters were impaired in the FO group. Chronotropic incompetence was identified in 46 patients (92%), who also showed a lower median (Q1-Q3) chronotropic index (0.55 [0.47-0.62] vs. 0.93 [0.88-0.99], \( P < 0.001 \)) and higher median HRR (32 [24–60] vs. 8 [1–14] bpm, \( P < 0.001 \)). A negative correlation was observed between HRR and peak oxygen uptake and positive correlation between HRR and peak ventilatory equivalent of CO\(_2\) and mean platelet volume (MPV). The study revealed the significant diagnostic utility of HRR in detecting abnormal peak ventilatory equivalent of O\(_2\), ALP level, ratio of aspartate to alanine transaminase, and MPV.

**Conclusions:** Chronotropic insufficiency correlates with impaired exercise capacity, liver dysfunction, and blood platelet pathology in FO patients. HRR may be a promising indicator of organ complications and a sign of future bradyarrhythmia and the need for cardiac pacing.

**Key words:** chronotropic insufficiency, exercise capacity, Fontan operation, heart rate reserve, pacemaker
Introduction

Fontan operation (FO) remains the preferred treatment for most patients with single-ventricle physiology. This surgical procedure aims to restore the balance between pulmonary and systemic circulation and achieve normal or near-normal blood oxygenation. However, patients undergoing FO develop a number of cardiac and extracardiac complications over time. The commonly reported cardiac complications are systolic and diastolic single-ventricle dysfunction [1], increase in atrioventricular valve regurgitation, and arrhythmias [2], while the extracardiac complications include plastic bronchitis, chronic kidney disease, liver and thyroid dysfunctions, thromboembolic complications, and exudative enteropathy [3–6]. Moreover, after FO, patients have reduced cardiac output and experience a progressive decline in exercise capacity [2,7]. The determinants of cardiac output are stroke volume (SV) and heart rate (HR). Available data confirm the influence of an impaired SV on the reduced exercise capacity of the patients [8,9].

However, when the SV depending on the ejection fraction is constant, the main mechanism that increases the cardiac output during exercise is increased HR response. Exercise capacity is affected by the systemic ventricle preload (which is determined by the volume of blood flowing through the heart) and pulmonary resistance [2].

In general, chronotropic incompetence is defined as the inability to sufficiently increase HR in response to increased activity or demand. Thus far, only a few reports have been published analyzing the influence of impaired chronotropic response on cardiac output, and thus on the exercise capacity of patients who underwent FO. In patients not presenting a clear cardiac disease, the prognostic parameter beyond the physical capacity is the HR response to exercise. Chronotropic incompetence and slow HR reduction after exercise are the risk factors of sudden cardiac death and serious cardiac events in patients having cardiovascular diseases.
However, data on these conditions in patients with adult congenital heart diseases are sparse.

In clinical practice, we observe that despite the normal or close-to-normal function of the systemic ventricle, patients undergoing FO are still unable to generate cardiac output or exhibit similar physical capacity as their healthy peers. It seems likely that this may be due to chronotropic insufficiency and the presence of organ complications, which together lead to reduced exercise capacity in these patients.

In this study, we evaluated the prevalence of an abnormal HR response to exercise (chronotropic incompetence) in adults who underwent FO and assessed the relationship between the HR reserve and other selected multiorgan dysfunctions.

Patients and methods

Study participants

This retrospective study included adults over 18 years of age, who underwent FO for a diagnosis of functionally single ventricular heart and remain under the medical supervision of the John Paul II Hospital. The main exclusion criteria of the study were as follows: asthma, pulmonary artery hypertension requiring vasodilator therapy, atrial fibrillation, atrial flutter, history of pacemaker placement, current infection, inflammation, pregnancy, diabetes, major trauma, therapy with vitamin K antagonists and beta-blockers, neoplastic disease, and history of alcohol abuse. The control group comprised healthy, age- and sex-matched volunteers.

The study used clinical, demographic, and anatomic data derived from the medical records of the patients. Each patient was subjected to a physical examination and assessment of body mass index (BMI), arterial oxygen saturation, and ejection fraction of the systemic ventricle.
BMI was calculated as weight of the patient (kg) divided by height (m$^2$). Oxygen saturation was measured by pulse oximetry while breathing room air.

**Echocardiography**

Ejection fraction of the single ventricle was assessed using Simpson’s method. In addition, valvular competence was evaluated. This examination was performed by two experienced, independent cardiologists using echocardiography (Vivid 7, GE Medical Systems, Milwaukee, WI, USA), as previously described [1].

**Cardiopulmonary exercise test**

To evaluate exercise tolerance, a cardiopulmonary exercise test (CPET) was performed using a modified Bruce protocol (Reynols Medical System, ZAN-600). The following parameters were recorded during the test: time of exercise; 12-lead electrocardiogram, blood pressure; minute ventilation (VE); peak oxygen uptake (VO$_2$ peak); respiratory exchange ratio (RER); peak ventilatory equivalent of oxygen (VE/VO$_2$); peak ventilatory equivalent of carbon dioxide (VE/VCO$_2$); breathing reserve; and oxygen saturation. VO$_2$ peak was estimated as the highest value of oxygen uptake at peak exercise in ml/kg/min, and the percentage of the predicted value was calculated. Ventilatory anaerobic threshold was measured using the V-slope method. Oxygen pulse was defined as the amount of oxygen consumed per heartbeat. The ventilatory equivalent of oxygen (VE/VO$_2$) was defined as the amount of ventilation needed for the uptake of a given amount of oxygen, while the ventilatory equivalent of carbon dioxide (VE/VCO$_2$) was defined as the amount of ventilation needed for the elimination of a given amount of carbon dioxide. RER was calculated by dividing VO$_2$ by VCO$_2$.

**Chronotropic incompetence**

Chronotropic index (CI) was determined by applying the chronotropic metabolic relationship concept introduced by Wilkoff et al [11]. To calculate CI, we used the following rule: (peak
HR - resting HR)/(220 - age - resting HR). Chronotropic incompetence was confirmed if CI was <0.8.

The heart rate reserve (HRR) was defined as the difference between maximal HR (HRmax) and peak HR (HRpeak). HRmax was calculated according to the rule: 220 - age. Accordingly, HRR was calculated using the following equation: HRR = HRmax - HRpeak = 220 - age - HRpeak [12,13].

**Laboratory tests**

Blood samples were collected from the antecubital vein from patients after overnight fasting (for at least 12 hours). These samples were evaluated for the following laboratory parameters: red blood cells, hemoglobin, hematocrit, red blood cell distribution width, white blood cells, platelets, mean platelet volume (MPV), N-terminal pro-B-type natriuretic peptide (NT-proBNP), as well as liver function markers including serum protein electrophoresis, alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyltranspeptidase, alkaline phosphatase (ALP), total bilirubin level, α-fetoprotein, international normalized ratio, creatinine, and cystatin C. Furthermore, we assessed liver dysfunction by calculating the following parameters using specific formulas: AST/ALT ratio, AST/platelet ratio index, Forns index, and MELD-XI score [14].

This study was approved by the University Ethical Committee. All patients provided written informed consent (No. 1072.6120.110.2017).

**Statistical analysis**

The distribution of data was presented as numbers and percentages for categorical variables, as means with SDs for normally distributed continuous variables, and as medians with lower and upper quartiles (Q1–Q3) for continuous variables with a nonnormal distribution. The Kolmogorov–Smirnov test was used to verify the normality of the data distribution. Patients
who underwent Fontan procedure and control participants were compared using two-tailed Student’s t-test or Mann–Whitney U test for quantitative variables, and using the chi-square test for qualitative variables. The diagnostic usefulness of HRR in predicting abnormal values of selected parameters describing multiorgan complications (VE/VO₂, ALP, AST/ALT ratio, and MPV) was evaluated by receiver operating characteristic (ROC) curves with area under the curve (AUC) values. A cutoff value corresponding to the highest accuracy was determined, and the related sensitivities and specificities, as well as the Youden index, were calculated. The relationships among clinical data, CPET results, and chronotropic and biochemical parameters were evaluated by calculating Spearman’s rank correlation coefficients. All the analyses were performed in IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Statistical significance was defined as P < 0.05.

Results

Patients characteristics

We enrolled 50 adult patients, including 34 men (68%) with a mean (SD) age of 27 (6.6) years. Patients who underwent FO did not differ from the controls in age, sex, and BMI. Of the 50 patients in the FO group, 45 (90%) underwent total cavopulmonary connection (TCPC) and 5 (10%) underwent direct right atrium-pulmonary artery connection (APC). There were 32 (64%) patients who had fenestration and 18 (36%) without fenestration. The median (Q1-Q3) age of patients at the time of surgery was 4 (2–5) years, and the median time after surgery was 19 (2–5) years. The mean (SD) SVEF was 52% (9.1). The baseline characteristics of the study group and the control group are presented in Table 1.

CPET results

Patients in the FO group had more frequently decreased VO₂ peak (82% vs. 0%, P < 0.001), increased VE/VCO₂ (98% vs. 77%, P = 0.004), and increased VE/VO₂ (14% vs. 0%, P =
The CPET results obtained for the FO group and the control group are presented in Table 2. Chronotropic incompetence was identified in 46 patients (92%) after the Fontan procedure. When compared to the controls, patients in the FO group had a lower median (Q1-Q3) chronotropic index (0.55 [0.47-0.62] vs. 0.93 [0.88-0.99], P < 0.001) and higher median HRR (32 [24–60] vs. 8 [1–14] bpm, P < 0.001). We observed a negative correlation between HRR and VO2 peak (R = -0.40, P < 0.001) (Figure 1A) and a positive correlation between HRR and VE/VCO2 (R = 0.49, P < 0.001) (Figure 1B).

**Biochemical test results**

We found an elevated level of ALP in six patients (12%) and increased AST/ALT ratio in 21 patients (42%). Furthermore, an increased MPV was observed in 14 (28%) patients and an elevated level of NT-proBNP (>125 pg/ml) in 27 patients (54%).

The laboratory parameters determined for the FO group and the control group are presented in Table 3.

We observed a positive correlation between the HRR and MPV (R = 0.3, P = 0.04) (Figure 2A) and a trend toward positive correlation between the HRR and ALP level (R = 0.24, P = 0.09) (Figure 2B).

A tendency toward positive correlation was observed between the HRR and NT-proBNP (R = 0.26, P = 0.07). A similar association was observed for the SVEF values as we found a trend toward negative correlation between the HRR and these values (R = -0.24, P = 0.09).

We did not observe any correlation between HRR and liver fibrosis parameters such as AST/ALT ratio, AST/platelet ratio index, Forns index, and MELD-XI score.
**Receiver operating characteristic**

Table 4 shows the results of the ROC curve analysis on the ability of HRR to predict abnormal liver function and hematological and CPET parameters.

The ROC curve analysis revealed the significant diagnostic utility of HRR to detect the abnormal value of four parameters. The highest AUC value was observed for abnormal MPV (AUC = 0.74), and the lowest value for abnormal AST/ALT ratio (AUC = 0.67).

Figure 3 presents the ROC curve analysis of the ability of HRR to detect the abnormal values of VE/VO₂, ALP level, ASP/ALT ratio, and MPV.

**Discussion**

Our study showed the significant influence of chronotropic incompetence on exercise capacity and the relationship between HRR and selected parameters of multiorgan malfunction in adults who underwent Fontan surgery.

CPET is a widely acknowledged tool as it provides information about not only exercise capacity but also prognosis, including the need for heart transplantation [15]. The results of the present study corroborate with a well-known observation that patients with Fontan physiology have reduced exercise capacity. Compared to the controls, patients in the FO group demonstrated significantly lower exercise time, lower saturation both at rest and during exercise, lower VO₂ peak/kg as well as lower VO₂ peak (%N). Of the patients in the FO group, 98% had an increased VE/VCO₂. These results concur with the previous studies and show that exercise limitation is common in this special group of patients [2,15,16]. The reasons for the reduced exercise performance in patients after FO seem to be associated with a combination of different mechanisms. One of them might be the lack of ability to increase and maintain the cardiac output in response to an increased workload [2].
Chronotropic incompetence is a widespread problem in patients undergoing FO, as sinus node damage occurs during surgery or structural alterations result from hemodynamic changes after the Fontan procedure. In the present study, these were detected in 46 patients (92%). Diller et al. found that chronotropic incompetence, within a group of patients with adult congenital heart diseases, was the most common observed among the patients who underwent FO [17]. Some authors have also reported that abnormal chronotropic response is observed during exercise in patients who had FO [18,19]. HR during exercise is determined by the sinus node function, local effect of autonomic innervation, circulating catecholamines, and increased ventricular preload [20]. In our study, the ejection fraction of patients was close to normal. In this situation, the main mechanism contributing to increased cardiac output in exercise should be increased HR. We observed that even though the patients in the FO group performed a diagnostic CPET (RER 1.04), they had significantly lower peak oxygen consumption and that HRR was moderately associated with VO₂ and VE/VCO₂. Furthermore, we were able to establish a precise HRR of ≥41, which indicated those individuals expected to have worse VE/VO₂. Therefore, HRR might be used as another parameter suggesting potential benefits from rate-responsive cardiac pacing in FO patients with chronotropic incompetence which influences physical activity during day-to-day living.

In the present study, we noticed elevated levels of single biochemical markers (such as γ-glutamyltranspeptidase, total bilirubin, international normalized ratio, ALP) as well as increased AST/ALT ratio. Moreover, we found the relationships between increased levels of ALP, AST/ALT ratio, and HRR. The increase of HRR value to ≥26 allowed predicting the increase in the AST/ALT ratio in patients in the FO group. Further increase in HRR to ≥31 was found in subjects with elevated ALP levels. One possible explanation for this observation can be the fact that due to chronotropic incompetence and reduced cardiac output, the central venous pressure increases. Chronic increase of central venous pressure and impaired liver
perfusion observed in Fontan circulation leads to hepatic dysfunction and widening of the liver sinuses. This is manifested as an increase in liver dysfunction parameters and the development of Fontan-associated liver disease [6]. In conclusion, chronotropic dysfunction expressed as increasing HRR can be a marker to identify people with impaired liver function. Similar observations were found by Italian researchers assessing a pediatric group receiving FO. They found hepatic abnormalities in up to 53% of patients and reported that hepatic dysfunction was correlated with reduced HR and low cardiac index [5]. It should be noted that the mechanisms associated with hepatological disorders are complicated and unclear and require further research.

In our study, patients in the FO group demonstrated various significant hematological disorders compared to the controls. We explored abnormalities in the platelet system and found irregularities in the number of platelets in 38% of patients. An increased MPV was observed in 28% of patients and was found to be associated with HRR. Moreover, we observed that an HRR of ≥59 indicates diagnostic usefulness in detecting patients with abnormal MPV. A possible explanation for the association between MPV and HRR can be that an increased HRR results in a reduced cardiac output. This in turn leads to impaired organ perfusion and increased venous congestion in the liver, spleen, and marrow [21]. Impaired marrow perfusion can cause disturbances in thrombopoiesis. On the other hand, splenomegaly, liver cirrhosis, and portal hypertension (which are frequent in Fontan circulation) may be responsible for increased platelet destruction [22]. In patients with fenestration (64% in our study), an additional reason for thrombocytopenia and changes in MPV might be a right-to-left shunt, resulting in the delivery of megakaryocytes into the system arterial circulation, thus bypassing the lungs where the megakaryocyte cytoplasm is fragmented into platelets [23].
The association between HRR and the analyzed biochemical and morphotic parameters was not found to be significant; however, we believe that it is worth analyzing.

In the present study, 45 patients (90%) in the FO group underwent TCPC and five (10%) underwent direct APC. The presence of fenestration did not show any influence on HRR. Available data indicate that it is often necessary to implant a pacemaker in patients undergoing TCPC compared to patients undergoing APC [24,25]. However, a team from Boston demonstrated that the incidence of arrhythmia and pacemaker implantation was not significantly different between patients undergoing TCPC and APC patients. [26]. One possible explanation for this finding can be the fact that the development of chronotropic insufficiency and the need for pacemaker implantation are determined not only by structural changes in the heart but also by a combination of other hemodynamic parameters and organ complications associated with Fontan circulation. However, this requires further analysis and research.

Summarizing the results of our study, chronotropic dysfunction may cause complications, and in some cases, may correspond with them. Our observations demonstrated that patients with chronotropic dysfunction present an impaired exercise capacity, liver complications, and changes in the platelet system. The chronotropic incompetence parameters (for HRR calculation) can be measured during any exercise test. In the present study, we used the data from CPET which is a recommended method for the clinical evaluation of exercise capacity in adults with congenital heart disease [27]. We believe that HRR, as a relatively simple and generally available diagnostic parameter during CPET, may be a promising indicator for identifying those patients with expected specific organ complications. Moreover, chronotropic incompetence parameters and HRR may be a sign of future bradyarrhythmia in patients with Fontan physiology and indicate the need for regular follow-up in the case of cardiac pacing. Nevertheless, every single decision about this procedure should be made with great caution,
and taking into consideration the patient’s age, potential need for epicardial pacing, and possible complications in the long-term follow-up.

**Limitations of the study**

The study has several limitations to be acknowledged. Firstly, it was retrospective. Secondly, the number of patients in the study group was small and relatively mixed as it included both patients with intracardiac and extracardiac conduits as well as different ventricular geometry. However, the dominant feature of all patients was Fontan physiology. SVEF in the FO group was lower than in the control group, but it was close to normal. The present study did not include the results of invasive diagnostic tests. The ROC curve analysis showed significant results, but the number of patients in the study group was small. However, the values of sensitivity and specificity of the methods used in the study indicate that the HRR parameter may be a potential factor. Nevertheless, further analysis on a larger number of patients is required.

There is a need for further studies to explore the combination of dynamic physiological variables such as chronotropic performance parameters and biomarkers related to multiorgan complications, in order to improve the risk assessment in patients undergoing FO.

**Acknowledgments**

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References:


Table 1. Baseline characteristics of the study group and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fontan patients (n = 50)</th>
<th>Controls (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>27 (6.6)</td>
<td>29.9 (4.1)</td>
<td>0.82</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>16 (32)</td>
<td>9 (30)</td>
<td>0.89</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171 (8.1)</td>
<td>174 (6.9)</td>
<td>0.06</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66.2 (12.2)</td>
<td>69.0 (9.3)</td>
<td>0.28</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.7 (3.4)</td>
<td>22.7 (2.2)</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean (SD) and categorical data as number (percentage).

Abbreviation: BMI, body mass index.
**Table 2.** Cardiopulmonary exercise test results of patients in the Fontan operation group and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fontan patients (n = 50)</th>
<th>Controls (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiopulmonary exercise test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise time, min</td>
<td>13.5 (3.4)</td>
<td>16.65 (2.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sat. O(_2) rest, %</td>
<td>91.0 (87.0–94.0)</td>
<td>97.0 (96.0–98.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sat. O(_2) ex., %</td>
<td>85.0 (82.0–86.0)</td>
<td>96.5 (96.0–97.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VO(_2) peak/kg, ml/kg/min</td>
<td>23.9 (7.6)</td>
<td>49.2 (7.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VO(_2) peak, %N</td>
<td>60.6 (17.5)</td>
<td>95.9 (4.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VE/VCO(_2), l/l</td>
<td>33.6 (5.2)</td>
<td>26.5 (2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VE/VO(_2), l/l</td>
<td>33.5 (31.5–36.0)</td>
<td>28.85(25.5–31.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RER peak</td>
<td>1.0 (0.1)</td>
<td>1.1 (0.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>CI</td>
<td>0.55 (0.47-0.62)</td>
<td>0.93 (0.88-0.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HRR</td>
<td>32.0 (24.0–60.0)</td>
<td>8.0 (1.0–14.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean (SD) or median (Q1–Q3).

Abbreviations: CI, chronotropic index; HRR, heart rate reserve; RER peak, peak respiratory exchange ratio; Sat. O\(_2\) ex., oxygen saturation during exercise; Sat. O\(_2\) rest, rest oxygen saturation; VE/VCO\(_2\), peak ventilatory equivalent of CO\(_2\); VE/VO\(_2\), peak ventilatory equivalent of O\(_2\); VO\(_2\) peak/kg, peak oxygen uptake per kg; VO\(_2\) peak (%N), percentage of predicted value for peak oxygen uptake.
Table 3. Laboratory parameters in patients after the Fontan procedure and in control group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fontan patients (n = 50)</th>
<th>Controls (n = 30)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP, pg/ml</td>
<td>140.5 (72.0–331.0)</td>
<td>24.5 (6.0–35.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RBC, 10^9/μl</td>
<td>5.6 (0.5)</td>
<td>4.9 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HGB, g/dl</td>
<td>16.3 (1.9)</td>
<td>14.7 (1.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCT, %</td>
<td>48.3 (6.4)</td>
<td>43.0 (3.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RDW, %</td>
<td>13.2 (12.8–13.9)</td>
<td>12.4 (12.0–12.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelet count, 10^9/μl</td>
<td>159.6 (61.7)</td>
<td>228.2 (38.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PDW, fL</td>
<td>15.8 (2.7)</td>
<td>12.2 (2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MPV, fL</td>
<td>11.9 (1.0)</td>
<td>10.4 (1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystatin C, mg/l</td>
<td>0.9 (0.8–1.1)</td>
<td>0.9 (0.8–0.9)</td>
<td>0.007</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.9 (0.8–1.0)</td>
<td>0.9 (0.7–1.0)</td>
<td>0.67</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73 m²</td>
<td>108.5 (97.0–123.0)</td>
<td>111.0 (105.0–124.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>AST, IU/l</td>
<td>24.0 (20.0–29.0)</td>
<td>19.5 (17.0–22.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>25.0 (20.0–34.0)</td>
<td>20.5 (17.0–23.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>GGTP, U/l</td>
<td>73.0 (52.0–120.0)</td>
<td>15.5 (14.0–18.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin, μmol/l</td>
<td>17.9 (13.6–24.5)</td>
<td>12.0 (7.7–17.0)</td>
<td>0.0005</td>
</tr>
<tr>
<td>α-Fetoprotein, ng/ml</td>
<td>2.8 (1.9–4.1)</td>
<td>2.3 (1.9–3.4)</td>
<td>0.14</td>
</tr>
<tr>
<td>ALP, U/l</td>
<td>78.5 (65.0–88.0)</td>
<td>67.0 (55.0–89.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total protein, g/dl</td>
<td>75.7 (71.4–78.6)</td>
<td>75.0 (73.0–78.6)</td>
<td>0.95</td>
</tr>
<tr>
<td>PT, s</td>
<td>13.8 (13.1–17.2)</td>
<td>11.9 (11.4–12.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INR</td>
<td>1.2 (1.2–1.6)</td>
<td>1.0 (1.0–1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST/ALT ratio</td>
<td>1.0 (0.8–1.1)</td>
<td>0.9 (0.8–1.1)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean (SD) or median (Q1–Q3).
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AST/ALT ratio, ratio of aspartate transaminase to alanine transaminase; eGFR, estimated glomerular filtration rate; GGTP, \( \gamma \)-glutamyltranspeptidase; HCT, hematocrit; HGB, hemoglobin; INR, international normalized ratio; MPV, mean platelet volume; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PDW, platelet distribution width; PT, prothrombin time; RBC, red blood cells; RDW, red cell distribution width.
Table 4. Receiver operating characteristic curve analysis testing the ability of heart rate reserve to predict abnormal liver function and hematological and cardiopulmonary exercise test parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>95% CI</th>
<th>P value</th>
<th>Cutoff point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal VE/VO₂ &gt; 40 l/l</td>
<td>0.73</td>
<td>0.51–0.96</td>
<td>0.05</td>
<td>41</td>
<td>0.86</td>
<td>0.65</td>
<td>0.51</td>
</tr>
<tr>
<td>Abnormal ALP &gt; 40 U/l</td>
<td>0.71</td>
<td>0.53–0.88</td>
<td>0.04</td>
<td>31</td>
<td>1.00</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Abnormal AST/ALT ratio &gt; 1.1</td>
<td>0.67</td>
<td>0.52–0.82</td>
<td>0.04</td>
<td>26</td>
<td>0.91</td>
<td>0.48</td>
<td>0.39</td>
</tr>
<tr>
<td>Abnormal MPV &gt; 12.5 fL</td>
<td>0.74</td>
<td>0.56–0.93</td>
<td>0.009</td>
<td>59</td>
<td>0.64</td>
<td>0.89</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Abbreviations: ALP, alkaline phosphatase; AST/ALT ratio, ratio of aspartate transaminase to alanine transaminase; AUC, area under the curve; CI, confidence interval; MPV, mean platelet volume, VE/VO₂, peak ventilatory equivalent of O₂.
Figure 1. A) Relationship between heart rate reserve and peak oxygen uptake; B) relationship between heart rate reserve and peak ventilatory equivalent of CO₂.
Figure 2. A) Relationship between heart rate reserve and mean platelet volume; B) relationship between heart rate reserve and alkaline phosphatase level.
Figure 3. Receiver operating characteristic curve analysis of heart rate reserve for detecting:

A) abnormal peak ventilatory equivalent of O\textsubscript{2}, B) abnormal level of alkaline phosphatase, C) abnormal ratio of aspartate transaminase to alanine transaminase, and D) abnormal mean platelet volume.