Comprehensive Use of Cardiopulmonary Exercise Testing Identifies Adults with Congenital Heart Disease at Increased Mortality Risk in the Medium Term

Running title: Inuzuka et al.; Exercise testing in congenital heart disease

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Abstract:

Background - Parameters of cardiopulmonary exercise testing (CPX) were recently identified as strong predictors of mortality in adults with congenital heart disease (ACHD). We hypothesized that combinations of CPX parameters may provide optimal prognostic information on mid-term survival in this population.

Methods and Results - 1,375 consecutive ACHD patients (age 33±13 years) underwent CPX at a single center over a period of 10 years. Peak oxygen consumption (peak VO₂), ventilation per unit of carbon dioxide production (VE/VCO₂ slope) and heart rate reserve (HRR) were measured. During a median follow-up of 5.8 years, 117 patients died. Peak VO₂, HRR and VE/VCO₂ slope were related to mid-term survival in ACHD patients. Risk of death increased with lower peak VO₂ and HRR. A higher VE/VCO₂ slope was also related to increased risk of death in non-cyanotic patients, while VE/VCO₂ slope was not predictive of mortality in cyanotic patients. The combination of peak VO₂ and HRR provided the greatest predictive information after adjusting for clinical parameters, such as negative chronotropic agents, age and presence of cyanosis. However, the incremental value of these exercise parameters was reduced in patients with peak respiratory exchange ratio below 1.0.

Conclusions - CPX provides strong prognostic information in patients with ACHD. Prognostication should be approached differently depending on the presence of cyanosis, the use of rate lowering medications and achieved level of exercise. We provide 5-year survival prospects based on CPX parameters in this growing population.

Key words: congenital heart disease, exercise testing, peak oxygen consumption, prognosis
Introduction

Adults with congenital heart disease (ACHD) are at increased risk of mortality and morbidity.\textsuperscript{1,2} Cardiopulmonary exercise testing (CPX) has emerged as an important tool for risk stratification and may guide clinicians in assessing prognosis and planning interventions.\textsuperscript{3} Although, various studies have linked individual parameters of CPX, such as peak oxygen consumption (peak VO\textsubscript{2}), heart rate increase during exercise and ventilatory efficiency to prognosis in ACHD patients,\textsuperscript{4-6} it remains uncertain how best to apply these parameters in clinical practice. In particular, it is unclear how these parameters should be best combined to obtain prognostic information. Furthermore, no previous study has attempted to relate these parameters or algorithmic combination of them directly to mid-term survival rates in ACHD. Such information would be a useful adjunct to the clinician when assessing, for example, the risk of an intervention compared with an estimation of risk under conservative management. Available data employing conventional statistical analyses are not particularly useful in this regard, as they provide relative risk estimates rather than actual projected risk. We therefore assessed the relation between parameters of CPX and expected survival in a large number of ACHD patients undergoing CPX at a tertiary center over a 10 year period. Furthermore, we investigated how exercise parameters can be best combined to predict mid-term survival.

Methods

We retrospectively analyzed all CPX performed in ACHD patients (> 14 years of age) between January 1999 and December 2008 at the Royal Brompton Hospital, London. Patients were referred for exercise testing as part of our routine clinical follow-up protocols. The
study population comprised 1,375 ACHD patients with various diagnoses (Table 1). This study was approved by the local ethics committee. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Clinical Data**

Demographic information, medication, medical and surgical history refer to the time of exercise testing. We addressed only the first exercise test for those who underwent multiple tests. A main diagnosis was determined for all patients from hospital records. If more than one cardiac lesion was present, the lesion considered hemodynamically most significant was recorded as the main diagnosis. Patients with “simple” lesions were defined as those with an atrial or ventricular septal defect, patent ductus arteriosus or aortic coarctation. Patients with “complex cyanotic” heart disease were those with a functionally univentricular heart without Fontan type repair, and those with unrepaired pulmonary atresia and aortopulmonary collateral arteries. Cyanotic patients were defined as those with oxygen saturation of <90% at rest or patients who were known to desaturate with exercise. Negative chronotropic agents were defined as beta-blockers, non-dihydropyridine calcium channel blockers, sotalol and amiodarone.

**Cardiopulmonary Exercise Testing**

Cardiopulmonary exercise testing was performed on a treadmill according to a modified Bruce protocol. This includes a stage 0 in which patients walk at a velocity of 1 mph at a 5% gradient. All patients were encouraged to exercise to exhaustion. Ventilation, oxygen consumption, and carbon dioxide production were measured continuously with a respiratory mass spectrometer (Amis 2000, Innovision, Odense, Denmark), as described previously. Heart rate was measured by continuous electrocardiography, and blood pressure was recorded.
manually by sphygmomanometry. Oxygen saturation was continuously monitored by pulse oximetry. Supplemental oxygen was not given during the CPX.

**Cardiopulmonary Parameters**

Peak VO₂ was defined as the mean of the highest 2 consecutive values of 15-second averages of VO₂. Percentage predicted VO₂ was calculated using the regression equations published by Wasserman et al.⁸ The ventilation per unit of carbon dioxide production (VE/VCO₂ slope) was obtained by linear regression analysis of the data acquired throughout the entire period of exercise.⁶, ⁹ The ventilatory threshold (VT) was measured by the V-slope method supplemented by additional parameters, such as change in respiratory exchange ratio, PETO₂ increase and VE/VO₂ increase as suggested by Wasserman in previous publications.⁸, ¹⁰-¹² Heart rate reserve (HRR) was calculated as the difference between peak and resting heart rates.⁵ Patients with a cardiac pacemaker, or those who had sustained tachyarrhythmia, or a higher degree AV block during exercise testing were excluded from the HRR analyses. The maximal voluntary ventilation (MVV) was estimated by multiplying the forced expiratory volume in the first second (FEV₁) by 40. The breathing reserve was calculated from the following equation:

Breathing Reserve (%) = 100 × (MVV – peak minute ventilation)/MVV.¹³ The respiratory exchange ratio (RER) was defined as the ratio between VCO₂ and VO₂.

**Follow-Up**

All patients were followed at the Royal Brompton Hospital. Survival status and time to death was assessed through the health service computer system, which is linked to a national database held by the Office of National Statistics. We opted to use all-cause mortality as our end
point to eliminate any possibility of bias arising from incorrect classification of the cause of death.

**Statistical Analysis**

Categorical variables are presented as %, and comparisons between groups were performed using the chi-square tests. Numerical variables are expressed as mean ± standard deviation or median (interquartile range), and comparisons between groups were performed using the Wilcoxon rank sum tests.

Univariate Cox proportional-hazards analysis was used to assess the association between variables and all-cause mortality. Significant parameters were subsequently included into a multivariate Cox regression model in a stepwise forward procedure. Model selection was performed by minimization of the Akaike information criterion and retention criteria were set at P< 0.05. To account for the potential confounding effect of negative chronotropic agents when analysing HRR, analyses were performed, both, with stratification by the use of negative chronotropic agents and with exclusion of patients who were on such medications. Time-dependent receiver operator characteristics (ROC) curves from censored survival data using the nearest neighbor estimator (the weighted Kaplan-Meier estimator) was used to compare the predictive value of variables at 5 years. Sensitivity and specificity of a variable x (at a value of c) was calculated as follows: sensitivity = (Pc-Sc)/(1-St) and specificity = (St-Sc)/St, where, Sc= estimated 5-year survival rate in patients with the variable x>c, St= estimated 5-year survival rate in the whole population and Pc= proportion of patients with the variable x>c. Cut-off values for variables were determined from the ROC curves so that the sum of sensitivity and specificity was maximized.

A random survival forest method was used to account for potential non-linear effects of
variables and to estimate mid-term survival.\textsuperscript{16, 17} The random survival forest method involves obtaining bootstrap samples from the original cohort and using each sample to compute a prediction model based on a splitting rule. Predicted survival for each patient was calculated from each model and averaged over each model. The prediction model was applied to the out-of-bag (OOB) data (i.e. 36.7\% of the whole data not used for model construction) to calculate OOB c-index, a measure of model discrimination, which is conceptually similar to the area under receiver curve. The predictive value of each variable was assessed with minimal depth.\textsuperscript{18} Variables with a large minimal depth split the decision tree near the terminal and, thus are unlikely to greatly affect prediction, while those with a small minimal depth split the tree close to the root and are considered as highly predictive. The variable importance represents another measure of the predictive value of a variable. It was calculated as the difference between the OOB c-index obtained from the original OOB data and that obtained from the permuted OOB data, in which variables are randomly rearranged.\textsuperscript{16} It thus tests how robust a variable is to random noise. A \textit{P}-value < 0.05 was considered statistically significant. Analyses were performed using R version 2.10.1 and the packages survival, survivalROC, gamlss and randomSurvivalForest.\textsuperscript{16, 17, 19, 20} Examples of R Codes used for statistical analysis are included as part of the online-only data supplement.

\section*{Results}

Baseline characteristics of the 1,375 consecutive ACHD patients are presented in Table 1. Seventy percent of patients had undergone previous sternotomy, while 28\% of patients had a history of thoracotomy (not mutually exclusive). Overall, 51\% of patients were in NYHA class I, 39\% in class II and only 10\% in class III. The vast majority of patients (89\%) were in sinus
rhythm at the time of exercise testing. Nine percent of patients had a cardiac pacemaker.
Cyanosis was present in 16.4% of patients, the majority of whom were those with Eisenmenger syndrome, followed by patients with a functionally univentricular heart who had not undergone a Fontan type of repair (Table 1).

A gradual decline in percentage predicted peak VO₂ was observed across the spectrum of congenital heart disease (Figure 1). The lowest values were found in patients with Eisenmenger syndrome, followed by “complex cyanotic” heart disease, with 81% and 60% having severe exercise limitation (<50% predicted peak VO₂), respectively. This was compared to only 19% of patients with “simple” lesions having severe limitation. VT was achieved by 81% of patients. Factors associated with failure to achieve VT included cyanosis (P<0.001) and NYHA class III (P<0.001). The majority of patients terminated exercise because of fatigue (n=671) or dyspnea (n=484). Other reasons for test termination included dizziness (n=74), chest discomfort (n=48), relevant arrhythmias (n=35), ST-elevation on ECG (n=8), high or low blood pressure (n=7) or a substantial drop in oxygen saturation (n=3).

**Exercise parameters and outcome**

During a median follow-up period of 5.8 (interquartile range 3.3-8.2) years, 117 patients (8.5%) died. The mode of death included heart failure (n=41), sudden cardiac death (n=34), perioperative death (n=12), infection (n=5), pulmonary embolism (n=1), haemoptysis (n=1), brain hemorrhage (n=1) and malignancy (n=1). There were 21 unexpected out-of-hospital deaths in which we could not establish exact modes of death as no autopsy data was available. Table 2 illustrates mortality rates according to underlying diagnostic subgroup. Peak VO₂, VT, HRR and VE/VCO₂ slope were identified as univariate predictors of death on Cox proportional-hazards
analysis, whereas breathing reserve was not related to survival (Table 3). Other univariate predictors included age, resting oxygen saturation and use of negative chronotropic agents.

To assess the impact of underlying diagnosis on the predictive value of parameters of CPX, hazard ratios were compared between diagnostic groups. While confidence intervals varied as expected due to group size and different numbers of events, the hazard ratios were similar between different diagnoses (Figure 2).

Peak VO$_2$ (ml/kg/min) and percentage predicted peak VO$_2$ correlated strongly with each other ($r=0.81$, $P<0.001$). Therefore, we chose percentage predicted VO$_2$ for multivariate analysis, as the area under the time-dependent ROC curve (AUC) was higher for percentage predicted VO$_2$ compared to the absolute value of peak VO$_2$ in predicting prognosis (AUC 0.71 versus 0.68).

On multivariate Cox analysis stratified by the use of negative chronotropic agents, peak VO$_2$ (Hazard ratio 0.78 per 10% increase [95% CI: 0.69-0.90]; $P<0.001$), HRR (Hazard ratio 0.85 per 10 beats/min increase [95% CI: 0.77-0.94]; $P<0.001$) and resting oxygen saturation per 1% (Hazard ratio 0.96 [95% CI: 0.93-0.99]; $P=0.009$) emerged as independent predictors of mortality. The same independent predictors were identified when patients on negative chronotropic agents were excluded from the analysis (Hazard ratio 0.79 per 10% [95% CI: 0.66-0.96]; $P=0.02$, hazard ratio 0.85 per 10 beats/min [95% CI: 0.75-0.97]; $P=0.01$ and hazard ratio 0.94 per 1% [95% CI: 0.90-0.98]; $P=0.002$, for peak VO$_2$, HRR and resting oxygen saturation, respectively).

Sub-analysis using the VE/VCO$_2$ slope up to ventilatory compensation point instead of calculating the slope from entire exercise period was also performed. No major differences in the predictive value of these two methods of calculating VE/VCO$_2$ slope were found. Similarly to
the VE/VCO₂ slope calculated over the entire exercise period, VE/VCO₂ slope calculated up to the ventilatory compensation point was a univariate predictor (Hazard ratio 1.03 [95% CI: 1.02-1.05]; \( P < 0.001 \)), but not a multivariate predictor of outcome. As the AUC on ROC analysis was slightly higher for the VE/VCO₂ slope from the entire exercise period compared to VE/VCO₂ slope up to the ventilatory compensation point (AUC=0.68 and 0.61, respectively), we decided to use this parameter for all subsequent analyses.

**Subgroup analysis for cyanotic patients**

Of the 225 cyanotic patients, 38 patients (16.9%) died during a follow-up period of 6.2 (interquartile range 3.4-7.9) years. HRR was a univariate predictor of death both with stratification by the use of negative chronotropic agents (hazard ratio 0.81 per 10 beats/min [95% CI: 0.70-0.93]; \( P = 0.004 \)) and with exclusion of patients on negative chronotropic agents (hazard ratio 0.78 per 10 beats/min [95% CI: 0.64-0.94]; \( P = 0.01 \)). Other univariate predictors were peak VO₂ (Hazard ratio 0.77 per 10% [95% CI: 0.63-0.95]; \( P = 0.01 \)) and resting oxygen saturation (Hazard ratio 0.93 [95% CI: 0.89-0.97]; \( P < 0.001 \)). VE/VCO₂ slope and VT were not related to outcome (\( P = 0.59 \) and 0.26, respectively). On multivariate analysis, HRR (hazard ratio 0.83 per 10 beats/min [95% CI: 0.72-0.97]; \( P = 0.02 \)) and resting oxygen saturation (Hazard ratio 0.95 [95% CI: 0.90-0.99]; \( P < 0.04 \)) were independent predictors of death with stratification by the use of negative chronotropic agents. When patients on negative chronotropic agents were excluded, HRR was the only independent predictor of outcome.

**Estimation of mid-term survival using a non-parametric model**

To account for non-linear effects of variables on survival and interaction between variables, models based on a random survival forest were created. Estimated 5-year survival as a
function of peak VO$_2$, HRR and VE/VCO$_2$ slope are provided in **Figure 3**. The cut-off values of 64% for percentage predicted peak VO$_2$ and 71 bpm for HRR were suggested by time-dependent ROC as being optimal. While VE/VCO$_2$ slope was not found to be predictive in cyanotic patients, a higher VE/VCO$_2$ slope was related to increased risk of death in the non-cyanotic cohort, with a VE/VCO$_2$ slope of 39 suggested as an optimal cut-off value based on the results of time dependent ROC analysis. Peak VO$_2$ and HRR were found to be predictive both in cyanotic and acyanotic patients. HRR was related to outcome even in patients treated with negative chronotropic agents.

Peak VO$_2$, HRR, the use of negative chronotropic agents, age and oxygen saturation at rest were identified as the strongest predictive markers on random survival forest analysis. OOB c-index for nested random survival forest models with variables ordered according to their predictive value are shown in **Figure 4**. While the top 5 variables improved overall OOB c-index and thus had complementary value in terms of prediction, others, such as anatomical diagnosis, did not further add significantly to the accuracy of prediction. Based on a model using these 5 strongest variables, contour plots were constructed to provide estimated 5-year survival for different combinations of peak VO$_2$ and HRR (**Figure 5**). OOB c-indices for random survival forest models using different combinations of variables were compared (**Table 4**). The model containing only peak VO$_2$, HRR, the use of negative chronotropic agents and cyanosis achieved an OOB c-index of 0.774, which was comparable to the best model using all of the top 5 variables (OOB c-index=0.784).

To further elucidate the effect of the exercise level on predictive value of CPX parameters, variable importance was compared between patients with low and high peak RER. As expected, the variable importance for peak VO$_2$ and HRR was smaller in patients with peak
RER <1.0 compared to in those with RER ≥ 1.0 (0.012 vs. 0.072), suggesting that peak VO₂ and HRR in patients with low RER add little predictive information to pre-test risk factors (i.e. age, the use of negative chronotropic agents and low oxygen saturation). The same variables were identified as prognostic markers when patients with RER<1.0 were excluded from the analysis.

Discussion

This study shows that peak oxygen consumption, heart rate reserve and VE/VCO₂ slope are related to mid-term survival in ACHD patients. While VE/VCO₂ slope is not predictive of outcome in cyanotic patients, a high VE/VCO₂ slope is related to increased risk of death in non-cyanotic patients. The combination of peak VO₂ and HRR provides the greatest predictive value in addition to readily available clinical parameters, such as the use of negative chronotropic agents, age and low oxygen saturation. Furthermore, our study suggests the prognostic value of exercise parameters is compromised for patients unable to achieve a peak RER above 1.0.

Consistent with the results of previous studies, exercise capacity was found to be reduced in ACHD patients (mean peak VO₂=66% of predicted value, Table 1). The degree of exercise limitation varied across the spectrum of ACHD, with cyanotic patients representing the most limited subgroup (Table 1, Figure 1). The current study provides information on the distribution of percentage predicted peak VO₂ in different diagnostic subgroups from a large number of contemporary patients (Figure 1). This data may be useful to clinicians to compare exercise capacity of a particular patient with that of patients of the same diagnostic group.

It has been argued that parameters that are largely effort independent, such as VT or VE/VCO₂ slope, may be better suited for estimating prognosis compared to peak VO₂. The VE/VCO₂ slope reflects ventilation–perfusion mismatch as well as derangement of peripheral
The main advantage of VE/VCO₂ slope lies in its robustness and the fact that it can be obtained reliably with submaximal exercise, as the relationship between ventilation and carbon dioxide production is linear up to the ventilatory compensation point, which occurs after the VT. In contrast, peak VO₂ and heart rate reserve require maximal exercise tests and, thus, depend on patient effort. Perhaps, surprisingly in the present study, however, these effort-dependent parameters were much stronger compared to the submaximal parameters (VT and VE/VCO₂ slope) in predicting outcome.

According to current guidelines for CPX, peak RER is the most accurate and reliable marker of adequate effort and a peak RER<1.0 should generally reflect submaximal cardiovascular effort. It has been suggested that the prognostic value of peak VO₂ may be reduced in the presence of a low peak RER. Consistent with these results, we found that a RER below 1.0 was related to substantial reduction of the predictive value of peak VO₂ and HRR in the current study. Thus, while RER itself was not a strong predictor of survival, it affected the accuracy of the prediction based on parameters of CPX.

In the present study, 18% of patients failed to achieve VT. Factors affecting the ability to achieve VT were the presence of cyanosis and more pronounced heart failure symptoms. The precise mechanism by which cyanosis affect the achievement of VT is not well understood. Excessive ventilatory response and gas exchange abnormalities due to ventilatory/perfusion mismatch have been reported in cyanotic patients, which may result in ventilatory limitation during exercise. Another possible cause of failure to achieve VT in ACHD population may be skeletal muscle deconditioning. There is evidence that adults with congenital heart disease are deconditioned due to inappropriate restriction from participation in physical activities. In fact, Greutmann et al. recently showed that the degree of respiratory and skeletal muscle weakness
commonly present in ACHD patients is comparable to patients with advanced heart failure in acquired heart disease.\textsuperscript{39} It is possible that patients with muscle weakness terminate CPX before the limits of the cardiovascular system are reached, which, in turn, would result in failure to achieve VT.

Chronotropic incompetence, a blunted increase in heart rate during exercise, is an established predictor of all-cause mortality in patients with ischemic heart disease\textsuperscript{40, 41} and ACHD.\textsuperscript{5} It has been suggested that HRR is related to autonomic dysfunction, neurohormonal activation and cardiac arrhythmias.\textsuperscript{5, 42-44} In acquired heart failure, chronotropic response has been shown to relate to outcome even in patients treated with beta-blockers.\textsuperscript{45} Our study demonstrates, for the first time in a congenital heart setting, that the prognostic value of HRR is maintained even in patients treated with medications affecting chronotropic response. The prognostic power of HRR may reflect the high predisposition to arrhythmic events and sudden cardiac death of the ACHD population.\textsuperscript{46, 47} Further studies are required to assess the relationship between HRR and sudden cardiac death as well as other parameters of autonomic dysfunction (e.g. heart rate variability and heart rate turbulence).

The current study reveals that the combination of peak VO\textsubscript{2} and HRR is a strong predictor of mid-term mortality in ACHD patients. We speculate that this can be explained, at least in part, by a synergistic effect of the prognostic value of peak VO\textsubscript{2} in predicting mainly heart failure-related death and that of HRR for arrhythmia-related mortality. As arrhythmia and heart failure are the two most common modes of death in the ACHD population,\textsuperscript{1} combination of the two is not only statistically significant but also theoretically appealing.

Cut-off values for CPX parameters in the present study were obtained from time-dependent ROC curve analysis. These cut-off values were related to a more pronounced decline
in survival as estimated by the random survival forest model. Although using dichotomous cut-off values is clinically convenient, these cut-off values are by necessity artificial and we contend that CPX parameters should be interpreted as continuous values. Furthermore, the prognostic value is greatest when parameters are interpreted in combination. To enable clinicians to estimate survival prospects for different combinations of peak VO\textsubscript{2} and HRR, we provide contour plots for mid-term survival in Figure 5.

**Study limitations**

Cardiopulmonary exercise testing was performed as part of our routine evaluation of patients in the ACHD clinic. All patients were at a tertiary ACHD center and therefore it is possible that they may not represent the pattern of ACHD that may exist in the community. While this may affect the prevalence of exercise intolerance, it is unlikely to affect the relation between exercise parameters and outcome, which was the main aim of the study.

Assessing the prognostic value of CPX parameters in specific diagnostic subgroups of ACHD was beyond the scope of this study. Other, smaller studies have provided such information. We aimed to develop an algorithm applicable to all ACHD patients independently of diagnosis. While prognosis is undoubtedly influenced by the different pathophysiological mechanisms present in different diagnostic groups, the result of the current study supports the notion that hazard ratios of CPX parameters are largely comparable across the spectrum of ACHD (Figure 2) and anatomical diagnosis has limited impact on estimates of survival as long as oxygen saturations are taken into account (Figure 4).
Conclusions

The combination of peak VO\(_2\) and HRR as measured on CPX provides the greatest predictive information in addition to readily available clinical risk factors, such as use of negative chronotropic agents, age and low oxygen saturation in patients with ACHD. As CPX parameters are influenced by patient effort, prediction is more reliable when a peak RER over 1.0 is achieved. Although the results of the current study require further external validation, this study provides estimates of 5-year survival, based on CPX parameter for this growing population.

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Conflict of Interest Disclosures: None

References:


Table 1. Baseline characteristics

<table>
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<tr>
<th></th>
<th>All</th>
<th>Non-cyanotic</th>
<th>Cyanotic</th>
<th>P-value</th>
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<tbody>
<tr>
<td>n</td>
<td>1375</td>
<td>1150</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.4±13.4</td>
<td>33.3±13.6</td>
<td>34.1±12.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Female (%)</td>
<td>642 (46.7)</td>
<td>516 (44.9)</td>
<td>126 (56.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.8±4.7</td>
<td>24.1±4.7</td>
<td>22.3±4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NYHA III (%)</td>
<td>140 (10.4)</td>
<td>74 (6.6)</td>
<td>66 (29.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiac anatomy (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Simple lesion</td>
<td>215 (15.6)</td>
<td>210 (18.3)</td>
<td>5 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Valvular disease</td>
<td>166 (12.1)</td>
<td>165 (14.3)</td>
<td>1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>Repaired TOF</td>
<td>377 (27.4)</td>
<td>376 (32.7)</td>
<td>1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>Ebstein’s anomaly</td>
<td>66 (4.8)</td>
<td>54 (4.7)</td>
<td>12 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Systemic right ventricle</td>
<td>148 (10.8)</td>
<td>130 (11.3)</td>
<td>18 (8.0)</td>
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<tr>
<td>Fontan</td>
<td>92 (6.7)</td>
<td>62 (5.4)</td>
<td>30 (13.3)</td>
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<tr>
<td>Eisenmenger syndrome</td>
<td>81 (5.9)</td>
<td>0</td>
<td>81 (36.0)</td>
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<tr>
<td>Complex cyanotic disease</td>
<td>65 (4.7)</td>
<td>0</td>
<td>65 (28.9)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>165 (12.0)</td>
<td>153 (13.3)</td>
<td>12 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Resting oxygen saturation (%)</td>
<td>97±5</td>
<td>99±1</td>
<td>88±7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sternotomy (%)</td>
<td>953 (69.6)</td>
<td>874 (76.3)</td>
<td>79 (35.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thoracotomy (%)</td>
<td>380 (27.8)</td>
<td>296 (25.9)</td>
<td>84 (37.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sinus rhythm (%)</td>
<td>1226 (89.2)</td>
<td>1022 (88.9)</td>
<td>204 (90.7)</td>
<td>0.50</td>
</tr>
<tr>
<td>Pacemaker (%)</td>
<td>123 (9.0)</td>
<td>103 (9.0)</td>
<td>20 (9.0)</td>
<td>0.93</td>
</tr>
<tr>
<td>Negative chronotropic agents (%)</td>
<td>303 (22.0)</td>
<td>230 (20.0)</td>
<td>73 (32.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak respiratory exchange ratio</td>
<td>1.07±0.13</td>
<td>1.08±0.13</td>
<td>1.02±0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VT reached (%)</td>
<td>1119 (81.4)</td>
<td>963 (83.7)</td>
<td>156 (69.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VT (ml/kg/min)</td>
<td>15.3±5.6</td>
<td>15.8±5.6</td>
<td>11.9±4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak VO2 (ml/kg/min)</td>
<td>22.3±9.3</td>
<td>23.6±9.3</td>
<td>15.5±5.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage predicted peak VO2 (%)</td>
<td>65.9±23.7</td>
<td>69.7±22.7</td>
<td>46.5±18.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VE/VCO2 slope</td>
<td>37.7±16.1</td>
<td>34.1±11.6</td>
<td>53.0±22.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate reserve (beats/min)</td>
<td>76.7±28.2</td>
<td>81.0±27.0</td>
<td>54.9±23.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Breathing reserve (%)</td>
<td>33.9±18.3</td>
<td>35.0±18.0</td>
<td>29.5±19.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Median follow-up period (years)</td>
<td>5.8 (3.3-8.2)</td>
<td>5.7 (3.3-8.3)</td>
<td>6.2 (3.4-7.9)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

VT indicates ventilatory threshold; BMI, body mass index; VO2, oxygen consumption; TOF tetralogy of Fallot; VE/VCO2 slope, ventilation per unit increase in carbon dioxide production. Negative chronotropic agents were defined as beta-blockers, non-dihydropyridine calcium channel blockers, sotalol and amiodarone.
Table 2. Number of deaths in each anatomical subgroup

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Number of deaths</th>
<th>5-year mortality rate*, % (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=1375)</td>
<td>117</td>
<td><strong>6.8 (5.3-8.2)</strong></td>
</tr>
<tr>
<td>Simple lesion (n=215)</td>
<td>13</td>
<td>4.0 (0.96-7.0)</td>
</tr>
<tr>
<td>Valvular disease (n=166)</td>
<td>8</td>
<td>5.5 (1.3-9.6)</td>
</tr>
<tr>
<td>Repaired TOF (n=377)</td>
<td>12</td>
<td>2.9 (1.1-4.7)</td>
</tr>
<tr>
<td>Ebstein’s anomaly (n=66)</td>
<td>5</td>
<td>8.4 (0.96-15.3)</td>
</tr>
<tr>
<td>Systemic right ventricle (n=148)</td>
<td>13</td>
<td>6.8 (2.6-10.8)</td>
</tr>
<tr>
<td>Fontan (n=92)</td>
<td>16</td>
<td>16.3 (8.0-23.8)</td>
</tr>
<tr>
<td>Eisenmenger syndrome (n=81)</td>
<td>17</td>
<td>16.7 (7.9-24.7)</td>
</tr>
<tr>
<td>Complex cyanotic disease (n=65)</td>
<td>19</td>
<td>21.1 (9.4-31.3)</td>
</tr>
<tr>
<td>Others (n=165)</td>
<td>14</td>
<td>5.0 (1.3-8.6)</td>
</tr>
</tbody>
</table>

TOF indicates tetralogy of Fallot.
* Mortality rates are based on the Kaplan-Meier estimator.

Table 3. Univariate predictors of all-cause mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio (95% Confidence Interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak respiratory exchange ratio</td>
<td>0.80 (0.69-0.93)</td>
<td>0.004</td>
</tr>
<tr>
<td>Ventilatory threshold (ml/kg/min)</td>
<td>0.86 (0.83-0.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak VO$_2$(ml/kg/min)</td>
<td>0.89 (0.87-0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage predicted peak VO$_2$(10%)</td>
<td>0.67 (0.61-0.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate reserve (10beats/min) with exclusion of patients on NCAs.</td>
<td>0.74 (0.67-0.83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate reserve (10 beats/min) with stratification by the use of NCAs</td>
<td>0.75 (0.69-0.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VE/VCO$_2$ slope</td>
<td>1.02 (1.02-1.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fall in oxygen saturation &gt; 5% during exercise</td>
<td>2.9 (2.01-4.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Other univariate predictors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.03 (1.02-1.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resting oxygen saturation (%)</td>
<td>0.91 (0.89-0.93)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NCAs</td>
<td>3.35 (2.33-4.81)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NCAs indicates negative chronotropic agents; VE/VCO$_2$ slope, ventilation per unit increase in carbon dioxide production; VO$_2$, oxygen consumption.

Table 4. Out-of-bag (OOB) concordance index for random survival forest models

<table>
<thead>
<tr>
<th>Variables for random survival forest model</th>
<th>OOB c-index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate prediction</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.549</td>
</tr>
<tr>
<td>Oxygen saturation at rest</td>
<td>0.678</td>
</tr>
<tr>
<td>Ventilatory threshold</td>
<td>0.609</td>
</tr>
<tr>
<td>VE/VCO$_2$ slope (for non-cyanotic patients)</td>
<td>0.617</td>
</tr>
<tr>
<td>Percentage predicted peak VO$_2$</td>
<td>0.676</td>
</tr>
<tr>
<td>HRR</td>
<td>0.711</td>
</tr>
<tr>
<td><strong>Multivariate prediction</strong></td>
<td></td>
</tr>
<tr>
<td>Peak VO$_2$ + NCAs</td>
<td>0.723</td>
</tr>
<tr>
<td>VE/VCO$_2$ slope + NCAs (for non-cyanotic patients)</td>
<td>0.724</td>
</tr>
<tr>
<td>HRR + NCAs</td>
<td>0.739</td>
</tr>
<tr>
<td>NCAs + Age + Oxygen saturation at rest</td>
<td>0.739</td>
</tr>
<tr>
<td>Peak VO$_2$ + HRR + NCAs + Cyanosis</td>
<td>0.774</td>
</tr>
<tr>
<td>Peak VO$_2$ + HRR + NCAs + Age + Oxygen saturation at rest</td>
<td>0.784</td>
</tr>
</tbody>
</table>

HRR indicates heart rate reserve; NCAs, negative chronotropic agents; VE/VCO$_2$ slope, ventilation per unit increase in carbon dioxide production; VO$_2$, oxygen consumption.
Figure Legends:

**Figure 1.** Distribution of percentage predicted peak oxygen consumption (peak VO₂) in different diagnostic groups. AVSD = atrioventricular septal defect, med = median, q25 and q75 = 25th and 75th percentile, RV = right ventricle.

**Figure 2.** Hazard ratio for 10% increase in percentage predicted peak oxygen consumption (upper panel) and 10 beats/min increase in heart rate reserve (lower panel) in different diagnostic subgroups. Hazard ratios for heart rate reserve were calculated after stratifying by the use of negative chronotropic agents. The horizontal lines represent the confidential interval for the hazard ratio for each anatomical subgroup. The width of diamonds corresponds to the confidence interval for the entire group’s hazard ratio. This Figure illustrates that hazard ratios are similar between diagnostic groups, suggesting that these parameters provide similar prognostic information across different diagnoses.

**Figure 3.** Univariate estimation of 5-year survival rate using exercise parameters based on a random survival forest analysis. (Upper panels) The dots illustrate estimated 5-year survival as a function of (left panel) peak oxygen uptake (VO₂), (middle panel) heart rate reserve and (right panel) VE/VCO₂ slope for the whole population. These are based on the results of a random survival forest analysis as described in detail by Ishwaran et al. The vertical dotted line shows cut-off values obtained by time-dependent ROC analysis. (Lower panels) Survival functions for non-cyanotic patients (black solid lines), cyanotic patients (black dotted lines), patients off negative chronotropic agents (NCA) (gray solid lines) and patients on NCA (gray dotted lines).
The vertical dotted line for VE/VCO₂ slope reflects the cut-off value for non-cyanotic patients as obtained by time-dependent ROC analysis.

**Figure 4.** Predictive value of variables. Variables are ordered according to predictive value assessed by the minimal depth method according to nested random survival forest (RSF) models. Improvement in out-of-bag concordant index (OOB c-index) was observed when the top 5 variables (closed circle) were added to the models, whereas other variables (open circle) did not further improve the accuracy of prediction. HRR = heart rate reserve, NCAs=negative chronotropic agents, Peak VO₂ = percentage predicted peak oxygen consumption, RER= respiratory exchange ratio and VT = ventilatory threshold.

**Figure 5.** Estimated 5-year survival rate for different combinations of peak oxygen consumption and heart rate reserve. 5-year survival was estimated based on the results of the random survival forest model using peak oxygen consumption (peak VO₂), heart rate reserve, the use of negative chronotropic agents, age and oxygen saturation at rest. Estimated 5-year survival is illustrated as contour plots for different combination of peak VO₂ and heart rate reserve. The contour plots are provided separately depending on the presence of cyanosis and the use of negative chronotropic agents.
Percentage predicted VO2 (10% increase)

<table>
<thead>
<tr>
<th>Anatomical diagnosis</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple lesions</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Valvular lesions</td>
<td>0.01</td>
</tr>
<tr>
<td>Repaired tetralogy of Fallot</td>
<td>0.13</td>
</tr>
<tr>
<td>Systemic right ventricle</td>
<td>0.01</td>
</tr>
<tr>
<td>Ebstein's anomaly</td>
<td>0.05</td>
</tr>
<tr>
<td>Fontan</td>
<td>0.58</td>
</tr>
<tr>
<td>Complex cyanotic</td>
<td>0.15</td>
</tr>
<tr>
<td>Eisenmenger syndrome</td>
<td>0.04</td>
</tr>
<tr>
<td>All</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Hazard ratio

Heart rate reserve (10 beats/min increase)

<table>
<thead>
<tr>
<th>Anatomical diagnosis</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple lesions</td>
<td>0.08</td>
</tr>
<tr>
<td>Valvular lesions</td>
<td>0.08</td>
</tr>
<tr>
<td>Repaired tetralogy of Fallot</td>
<td>0.98</td>
</tr>
<tr>
<td>Systemic right ventricle</td>
<td>0.23</td>
</tr>
<tr>
<td>Ebstein's anomaly</td>
<td>0.44</td>
</tr>
<tr>
<td>Fontan</td>
<td>0.04</td>
</tr>
<tr>
<td>Complex cyanotic</td>
<td>0.14</td>
</tr>
<tr>
<td>Eisenmenger syndrome</td>
<td>0.2</td>
</tr>
<tr>
<td>All</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Hazard ratio
Comprehensive Use of Cardiopulmonary Exercise Testing Identifies Adults with Congenital Heart Disease at Increased Mortality Risk in the Medium Term

Ryo Inuzuka, Gerhard-Paul Diller, Francesco Borgia, Leah Benson, Edgar L.W. Tay, Rafael Alonso-Gonzalez, Margarida Silva, Menelaos Charalambides, Lorna Swan, Konstantinos Dimopoulos and Michael A. Gatzoulis

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Examples of R Code used for statistical analysis
For correspondence relating to details of the statistical analysis and R codes, please contact Dr. Inuzuka (inuzukar-tky@umin.ac.jp) or Dr. Diller (gerhard.diller@googlemail.com) directly.
Analyses were performed using R version 2.10.1 and the packages survival, survivalROC, gamlss and randomSurvivalForest.
This is an example, the dataset is named 'data', and the following variables are used:

PVO2 = percentage predicted peak oxygen consumption
fu.time = follow up time (in months)
death = event of all-cause mortality (1 and 0)

R code starts here:

library(survival)
library(survivalROC)
library(gamlss)
library(randomSurvivalForest)
ntree <- 1000
month<-60 # follow up time set to 5 years

object<- rsf(Survrsf(fu.time, death)~ .,data = data, ntree=ntree, splitrule = "logrank", forest=T)
prob<-length(object$time[object$time<month])/length(object$time)
baseForest <- object$forest
predictors <- object$predictors
npts<-25
class(baseForest) <- c("rsf", "partial")
x <- predictors[, object$predictorNames == "PVO2"]
n.x <- length(unique(x))
x.uniq <- sort(unique(x))[unique(as.integer(seq(1, n.x, length = min(npts, n.x)))))]
n.x <- length(x.uniq)
yhat <- yhat.se <- NULL
newdata.x <- predictors
n<-nrow(newdata.x)
for (l in 1:n.x) {
  newdata.x[, object$predictorNames == "PVO2"] <- rep(x.uniq[l],
    n)
  pred.temp <- 100 * exp(-predict.rsf(baseForest,
    newdata.x)$ensemble[, max(which(object$timeInterest <=
      quantile(object$time, probs = prob,
      na.rm = TRUE)))]
  yhat <- c(yhat, mean(pred.temp, na.rm = TRUE))
  yhat.se <- c(yhat.se, sd(pred.temp/sqrt(n),
    na.rm = TRUE))
}

# cut-off value

nobs <- NROW(data)
ROC<- survivalROC(Stime=data$fu.time, status=data$death, marker = data$PVO2,
predict.time = 60,span = 0.25*nobs^(-0.20))
cutoff<-ROC$cut.values[ROC$TP-ROC$FP==min((ROC$TP-ROC$FP))]

# plot figure
plot(x.uniq, yhat, pch = 19, cex = 1, col = 1,xlim= c(20,100),ylim=c(85,98),
xlab="Percent of predicted VO2,  %",ylab="Estimated 5-year survival,  %",las=1)

ast <- data.frame(cbind(x.uniq, yhat))
xmax=150
mod4 <- gamlss(yhat ~ lo(x.uniq), data=ast, trace=FALSE)
aa <- centiles.pred(mod4, xname="x.uniq", xvalues=4: xmax, cent=c(2.5,50,97.5))
points(4: xmax, aa[4: xmax,2], type="l", lty=2,lwd =1.0)
points(4: xmax, aa[4: xmax,3], type="l",lwd=2.0)
points(4: xmax, aa[4: xmax,4], type="l", lty=2,lwd=1.0)
abline(v=cutoff,lty=2,col="gray50")
text(cutoff,85,round(cutoff))
object<-varSel(Survrsf(fu.time, death)~.,data = data, method="md",do.trace=T)
pnames.order<-rownames(object$varselect)
n.pred <- length(pnames.order)
err <- rep(0, n.pred)
for (k in 1:n.pred){
  rsf.f <- "Survrsf(fu.time, death)~"
  rsf.f <- as.formula(paste(rsf.f,paste(pnames.order[1:k],collapse="+")))
  err[k] <- rsf(rsf.f, data, ntree=ntree,splitrule="logrank")$err.rate[ntree]
}
imp.out <-
  as.data.frame(cbind(object$varselect$depth,round(object$varselect$vimp,5),round(err,5)
  ,round(-diff(c(0.5,err)),5)),row.names=as.character(pnames.order))
  colnames(imp.out) <-c("Depth","vimp","Err","Drop Err")
print(imp.out)

plot(1:10,as.numeric(1-imp.out$Err[1:10]),pch=c(19,19,19,19,19,21,21,21,21,21),type="b",xlab="",ylab="OOB c-index for nested RSF models",xaxt="n",cex.lab=1.1)
text(1:10,as.numeric(1-imp.out$Err[1:10])+0.005,labels=rownames(imp.out)[1:10],srt=90,adj=0)
text(5.5,0.45,"Variables added according to predictive value",xpd=T,adj=0.5,cex=1.1)

pv<0.4

plot(1:10,as.numeric(1-imp.out$Err[1:10]),pch=c(19,19,19,19,19,21,21,21,21,21),type="b",xlab="",ylab="OOB c-index for nested RSF models",xaxt="n",cex.lab=1.1)

plot(1:10,as.numeric(1-imp.out$Err[1:10])+0.005,labels=rownames(imp.out)[1:10],srt=90,adj=0)
text(5.5,0.45,"Variables added according to predictive value",xpd=T,adj=0.5,cex=1.1)

pv<0.4

plot(1:10,as.numeric(1-imp.out$Err[1:10]),pch=c(19,19,19,19,19,21,21,21,21,21),type="b",xlab="",ylab="OOB c-index for nested RSF models",xaxt="n",cex.lab=1.1)

plot(1:10,as.numeric(1-imp.out$Err[1:10])+0.005,labels=rownames(imp.out)[1:10],srt=90,adj=0)
text(5.5,0.45,"Variables added according to predictive value",xpd=T,adj=0.5,cex=1.1)

pv<0.4

plot(1:10,as.numeric(1-imp.out$Err[1:10]),pch=c(19,19,19,19,19,21,21,21,21,21),type="b",xlab="",ylab="OOB c-index for nested RSF models",xaxt="n",cex.lab=1.1)

plot(1:10,as.numeric(1-imp.out$Err[1:10])+0.005,labels=rownames(imp.out)[1:10],srt=90,adj=0)
text(5.5,0.45,"Variables added according to predictive value",xpd=T,adj=0.5,cex=1.1)