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The role of cystatin C as a biomarker for prognosis in pulmonary arterial hypertension due to congenital heart disease

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ABSTRACT

Background: Adults with pulmonary arterial hypertension due to congenital heart disease (PAH-CHD) have a poor prognosis. Identifying patients with a high risk for clinical events and death is important because their prognosis can be improved by intensifying their treatment. Cystatin C, a novel cardiac biomarker, correlates with right ventricular dimensions in patients with idiopathic PAH, giving it potential to determine prognosis in PAH-CHD patients. We investigated the predictive value of cystatin C for long-term mortality and clinical events.
Methods: Fifty-nine PAH-CHD patients (mean age 42 SD 13 years, 42% male) were included in this prospective observational study, with cystatin C measurements between 2005 and 2015 on the outpatient clinic. Patients were evaluated with a standardized evaluation protocol including laboratory, functional and echocardiographic variables. Clinical events comprised worsening functional classification, worsening heart failure, symptomatic hyperviscosity, haemoptysis and arrhythmia. We used Cox regression to determine predictors for mortality and clinical events.
Results: Mean follow-up was 4.4 years, during which 12 (20%) patients died. Cystatin C (HR 1.3, $p < 0.001$), creatinine (HR 1.2, $p < 0.001$), NT-pro-BNP (HR 2.0, $p = 0.012$), hs-troponin T (HR 1.9, $p = 0.005$), 6-MWD (HR 0.8, $p = 0.044$) and TAPSE (HR 0.8, $p < 0.001$) predicted mortality. Similar results were found for the prediction of clinical events. When adjusted for NT-pro-BNP or glomerular filtration rate in multivariate analysis, cystatin C remained predictive for mortality.
Conclusions: Cystatin C, a novel cardiac biomarker, predicts long-term mortality and clinical events in patients with PAH-CHD. Consequently, cystatin C may attribute to clinical decision making regarding treatment intensity.

1. Introduction

In congenital heart disease (CHD), the presence of pulmonary arterial hypertension (PAH) is associated with poor prognosis [1–4]. PAH-CHD is characterized by increased pulmonary vascular resistance resulting in right ventricular (RV) remodeling, dysfunction and eventually failure. During this process, PAH-CHD patients are at risk for clinical events such as hospitalization for heart failure, arrhythmias and ultimately death.

Identifying patients with a high risk for clinical events and death is important because their prognosis can be improved by intensifying their treatment [5]. Currently timing of initiation of PAH-specific combination therapy and determination of follow-up intensity depend on parameters with an established association with mortality. These parameters, such as six-minute walk distance (6-MWD) and World Health Organization (WHO) functional classification, are formulated as treatment goals in both the American and European PAH guidelines [6, 7]. However, the PAH guidelines are based on studies combining various PAH etiologies, thus hampering its use in PAH-CHD patients specifically. Within the last decade, biomarkers emerged as important prognostic markers for clinical events and death in patients with PAH-CHD [8–11]. Recently cystatin C, a novel cardiac biomarker, has been suggested as potential prognostic biomarker in idiopathic PAH patients [12]. Cystatin C correlates with RV pressures, function and morphology [12] and reflects renal function [13], inflammation and vascular and

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myocardial remodeling [14, 15], all pathways with relevance in PAH-CHD. Moreover, cystatin C is inexpensive, widely available, minimally invasive and simple to determine [13]. However, its prognostic value for patients with PAH-CHD is still not established.

In the current study we investigated the predictive value of the novel cardiac biomarker cystatin C for long-term mortality and clinical events in patients with PAH-CHD.

2. Methods

2.1. Study population

The current study was part of a prospective observational study on PAH-specific therapy in adult patients with PAH-CHD [2], including patients with Down syndrome [16, 17]. All PAH-CHD patients with cystatin C measurements between March 2005 and July 2015 were included, on the outpatient clinic of two tertiary referral centers. Both patients with open or closed systemic-to-pulmonary shunts were included. PAH was defined upon the echocardiographic PAH probability (tricuspid regurgitation velocity ≥2.9 m/s). Right heart catheterization was only performed in case diagnosis of PAH was not clearly evident at echocardiography. In patients who were PAH-therapy naïve, bosentan or macitentan monotherapy was started. Eisenmenger syndrome, the most advanced stage of PAH-CHD, was defined as a net right-to-left shunt over the congenital heart defect due to an increased pulmonary vascular resistance. Approval of the research protocol by the local ethics committee was obtained. Informed consent was not required, as all investigations were performed for routine clinical care.

2.2. Data collection

Patients on the outpatient clinic were evaluated every three to six months with a standardized evaluation protocol including laboratory, six minute walk test and echocardiographic parameters. In order to perform biomarker measurements at a later stage, blood obtained from peripheral venous sampling was collected. This was stored in a −80 °C frozen state until biomarker measurements were performed. This stored blood was used in 30 (51%) patients for cystatin C and high-sensitivity troponin T (hs-troponin T) measurements.

The six minute walk test was performed according to the American Thoracic Society guidelines with continuous pulse oximetry monitoring [18]. Baseline echocardiography was performed with a Vivid 7 ultrasound system (General Electric). Pulmonary stenosis was ruled out in all patients [19]. Tricuspid annular plane systolic excursion (TAPSE) was measured in the lateral tricuspid valve annulus using M-mode in the apical 4-chamber view. Right ventricular systolic pressure (RVSP) was obtained from Doppler recording of tricuspid regurgitation using the modified Bernoulli equation.

N-terminal pro brain natriuretic peptide (NT-pro-BNP) levels were determined by electrochemiluminescence immunoassay on an Elecsys 2010 analyzer (Roche Diagnostics, Almere, The Netherlands). Hs-Troponin T levels were determined with an enzyme-linked immunosorbent assay method on the same analyzer. Cystatin C levels were determined with an immunonefelometry method on an ProSpec analyzer (BN ProSpec, Siemens, Frimley, United Kingdom) using latex enhanced particles coated with anti-cystatin C antibodies. The reference values for young healthy persons range from 0.53 to 0.95 mg/l. The coefficient of variation was 1.8% within one run and 2.0% during reproducibility tests. Freezing and long-term storage up to 25 years has a small impact on stability of cystatin C [20].

2.3. Definition of clinical events

Clinical events comprised worsening WHO functional classification, worsening heart failure, symptomatic hyperviscosity, haemoptysis and arrhythmia. Worsening heart failure was defined as start or increase of diuretics or hospital admission, for worsening symptoms of heart failure. Arrhythmias were defined as any episode of documented supraventricular tachycardia that required electrocardioversion or change of medication. Symptomatic hyperviscosity was defined as two or more symptoms of hyperviscosity (headache, faintness, dizziness, fatigue, tinnitus, blurred vision, paraesthesia of fingers, toes, and lips, muscle pain, and weakness) in combination with an elevated hematocrit (male 0.50 l/l, female 0.45 l/l). Worsening WHO functional classification was defined as the first increase in WHO classification during follow-up compared to the baseline value. Haemoptysis was defined as expectoration of blood ranging from blood-streaking of sputum to the presence of gross blood in the absence of any accompanying sputum.

2.4. Statistical analysis

Descriptive data were presented as mean ± SD if normally distributed or median with IQR, as appropriate. Categorical data were evaluated using the chi-square statistic. The change of continuous variables was evaluated using a 2-tailed paired t-test. Independent samples t-test or Mann–Whitney U test was used for comparison of continuous variables between two groups. Time to event analysis was performed with Kaplan Meier estimates of survival. Log rank test was performed to determine significant differences in mortality rate between two groups. Associations between predictors and outcome were evaluated using univariate Cox-regression analysis. The baseline visit was defined as the evaluation on the outpatient clinic during which cystatin C was measured. This date was used as start date in the Cox analysis. Relevant cut-off for cystatin C and NT-pro-BNP were obtained using a receiver operating characteristics curve. All reported p values were two-sided, and values of $p < 0.05$ were considered significant. Statistical analysis was performed with SPSS 22.0 (IBM Corp, Armonk, NY).

3. Results

3.1. Patient cohort

Fifty-nine adults (42 SD 13 years, 42% male) with PAH-CHD were included in the study. Table 1 summarizes the baseline characteristics. Of all patients 41% had Down syndrome and 81% Eisenmenger syndrome. Most patients had a normal renal function with a median creatinine of 80 μmol/l (upper limit of normal 110 μmol/l). Baseline therapy included use of bosentan (20%), macitentan (17%), a combination of bosentan or macitentan with sildenafil (8%), or no PAH-therapy (55%). Following baseline measurements, all of the therapy naïve patients started with either bosentan or macitentan within two weeks, except for one patient in whose case reimbursement of bosentan was rejected by the health insurance. Mean follow-up was 4.4 years, during which 12 (20%) patients died. Causes of death were right-sided heart failure (n = 6), sudden cardiac death (n = 2), sepsis (n = 2), and unknown (n = 2). Seven out of 24 patients with Down syndrome died during follow-up compared to 5 out of 35 non-Down patients ($p = 0.163$). There were several clinical events: fourteen patients had a worsening WHO functional classification, eight patients experienced arrhythmia, fourteen had worsening heart failure (all patients received treatment with diuretics), six had symptomatic hyperviscosity and six patients had haemoptysis.

3.2. Predictors of outcome in PAH-CHD

Fig. 1 shows the predictors of outcome, including mortality and the clinical events arrhythmia and worsening heart failure. An overview of all predictors and clinical events is listed in supplementary Table 1. Using univariate Cox-regression analysis, significant determinants of mortality were cystatin C (HR 1.3, $p < 0.001$), creatinine (HR 1.2, $p < 0.001$), NT-pro-BNP (HR 2.0, $p = 0.012$), hs-troponin T (HR 1.9, $p = 0.005$), 6-MWD (HR 0.8, $p = 0.044$) and TAPSE (HR 0.8,

Table 1
Baseline characteristics.

	All patients n = 59	Deceased n = 12	Survivors n = 47	*p*
Demographics				
Age, years	42 SD 13	45 SD 11	41 SD 14	0.322
Male, n (%)	25 (42)	8 (67)	17 (36)	0.056
Down syndrome, n (%)	24 (41)	7 (58)	17 (36)	0.163
Clinical subgroup				
Eisenmenger syndrome, n (%)	48 (81)	11 (92)	37 (79)	0.677
Systemic to pulmonary shunt, n (%)	4 (7)	0 (0)	4 (9)	
Small defect, n (%)	1 (2)	0 (0)	1 (2)	
Closed defect, n (%)	6 (10)	1 (8)	5 (11)	
PAH-therapy				
Bosentan monotherapy, n (%)	12 (20)	1 (8)	11 (23)	0.148
Macitentan monotherapy, n (%)	10 (17)	1 (8)	9 (19)	
Combination ERA/PDE-5 inhibitor, n (%)	5 (8)	0 (0)	5 (11)	
PAH-therapy naïve, n (%)	32 (55)	10 (83)	22 (47)	
WHO functional class				
WHO II, n (%)	26 (44)	5 (42)	21 (45)	0.851
WHO III, n (%)	33 (56)	7 (58)	26 (55)	
WHO IV, n (%)	0 (0)	0 (0)	0 (0)	
Laboratory				
Cystatin C, mg/l	0.88 (0.79 − 1.14)	1.19 (0.88 − 2.03)	0.87 (0.78 − 1.08)	**0.004**
High sensitive Troponin T, μg/l	0.008 (0.005 − 0.017)	0.017 (0.007 − 0.030)	0.006 (0.005 − 0.015)	**0.021**
NT-pro-BNP, ng/l	430 (227 − 1100)	827 (385 − 2235)	339 (218 − 959)	0.066
Creatinine, μmol/l	80 (67 − 98)	98 (79 − 154)	78 (67 − 92)	**0.005**
Potassium, mmol/l	4.2 SD 0.4	4.5 SD 0.5	4.2 SD 0.4	**0.010**
ASAT, U/l	31 SD 10	37 SD 14	29 SD 8	0.078
CRP, mg/l	4.1 (2.2 − 9.4)	3.1 (2.4 − 9.2)	4.4 (1.6 − 9.9)	0.800
Hemoglobin, mmol/l	11.2 SD 2.5	11.4 SD 2.7	11.1 SD 2.5	0.675
Echocardiography				
Right ventricular systolic pressure, mmHg	83 SD 22	90 SD 14	81 SD 24	0.103
TAPSE, mm	19 SD 5	15 SD 5	20 SD 5	<**0.001**
Moderate or severely impaired LV function, n (%)	5 (9)	2 (17)	3 (6)	0.254
Exercise testing				
Six-minute walking distance, m	379 SD 118	306 SD 115	398 SD 113	**0.015**
Resting arterial oxygen saturation, %	87 SD 7	84 SD 8	87 SD 7	0.188
Resting heart rate, bpm	80 SD 13	87 SD 14	78 SD 12	**0.034**

Data are mean ± standard deviation or median (IQR).
μg/l = microgram per liter; ng/l = nanogram per liter; mg/l = milligram per liter; ml/min = milliliter per minute; μmol/l = micromole per liter; U/l = units per liter.
PAH, pulmonary arterial hypertension; ERA, endothelin receptor antagonist; PDE-5, phosphodiesterase type 5; WHO, World Health Organization; NT-pro-BNP, N-terminal pro brain natriuretic peptide; CRP, C-reactive protein; ASAT, aspartate aminotransferase; TAPSE, tricuspid annular plane systolic excursion; LV, left ventricular.

$p < 0.001$). Moreover, TAPSE and the biomarkers cystatin C, creatinine, NT-pro-BNP and hs-troponin T were predictive for all clinical events. This accounted both for clinical events individually and for the combined endpoint of any event.

3.3. Cystatin C in PAH-CHD

Cystatin C was measured with a median level of 0.88 mg/l (upper limit of normal 0.95 mg/l). Twenty-five patients had elevated cystatin C levels (range 0.95 to 5.20 mg/l). Patients with Down syndrome were more likely to have elevated levels of cystatin C ($p = 0.010$; Table S2). Six minute walk distance (319 vs 424 m, $p < 0.001$) and estimated glomerular filtration rate (52 vs 60 ml/min/1.73 m^2, $p < 0.001$) were significantly lower in patients with elevated cystatin C compared to patients with normal cystatin C levels. Finally, patients with elevated cystatin C levels showed higher C-reactive protein levels (6.1 vs 2.9 mg/l, $p = 0.002$). The receiver operating characteristic analysis showed that a cystatin C level of 1.10 mg/l was the best cut-off value to predict mortality with a sensitivity of 67% and a specificity of 83% (area under the curve (AUC) 0.77). Patients with cystatin C levels above the 1.10 mg/l cut-off value showed a higher mortality rate (67% versus 36%, $p = 0.001$, Fig. 2). The best cut-off for NT-pro-BNP to predict mortality was 350 ng/l (sensitivity 83%, specificity 51%, AUC 0.67). A predicted survival model on baseline cystatin C and NT-pro-BNP serum levels is shown in Fig. 3. Patients with both a baseline cystatin C level above 1.10 mg/l and NT-pro-BNP above 350 ng/l had a mortality rate of 58% versus 11% in patients without both risk factors (log rank 16; $p < 0.001$). When adjusted for NT-pro-BNP or glomerular filtration rate in multivariate analysis, cystatin C remained predictive for mortality (Table 2).

4. Discussion

4.1. Interpretation

The current study is the first to indicate that the novel cardiac biomarker cystatin C predicts long-term mortality and clinical events in patients with PAH-CHD. After dichotomizing for survival analysis based on receiver operating characteristics, cystatin C above the optimal cut-off (1.10 mg/l) was associated with a higher mortality rate (67% versus 36%, $p = 0.001$, Fig. 2). Cystatin C levels in our study were relatively low (mean 1.08 mg/l) compared to studies on the effect of cystatin C on mortality in acquired heart disease (mean 1.20–1.51 mg/l) [21–24], presumably because our patient cohort was relatively young and had preserved renal function. Similarities between these studies and the current study were that, even with different cut-off points, all studies found that increasing levels of cystatin C were associated with worse survival. Additionally we showed cystatin C also predicts clinical events such as arrhythmia and worsening heart failure.

It is well established that renal insufficiency predicts mortality in PAH-CHD [11, 25, 26]. Because cystatin C is a sensitive indicator of renal filtration [13], it is conceivable cystatin C merely reflects renal dysfunction that by itself predicts mortality. However, cystatin C remained predictive for mortality after adjustment for glomerular filtration rate in multivariate analysis (Table 2). Moreover, three large studies, including 990, 480 and 279 patients with acquired heart disease, showed that

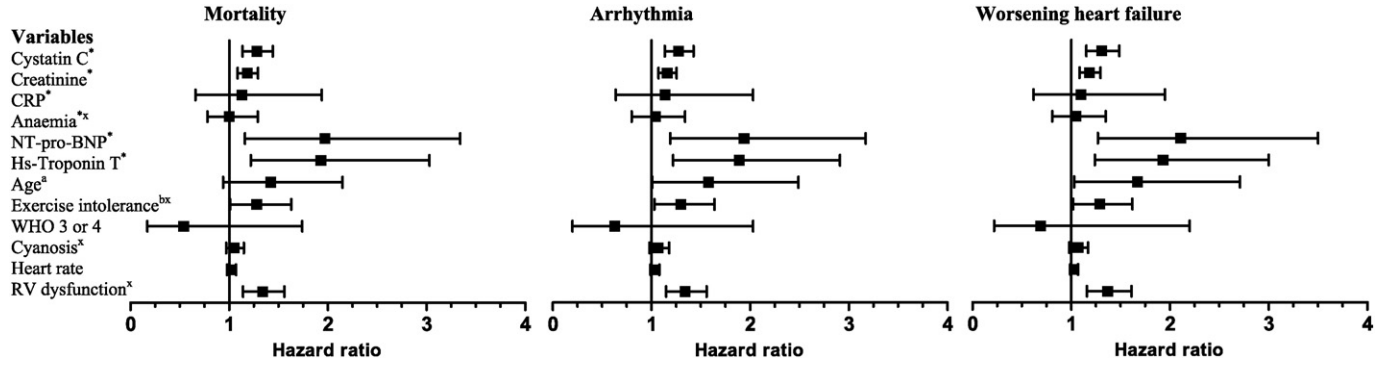

Fig. 1. Univariate predictors of outcome. To improve reader convenience all predictors with a protecting effect on survival were multiplied with minus one. Thus an increase of a predictor's value equals a worse prognosis.

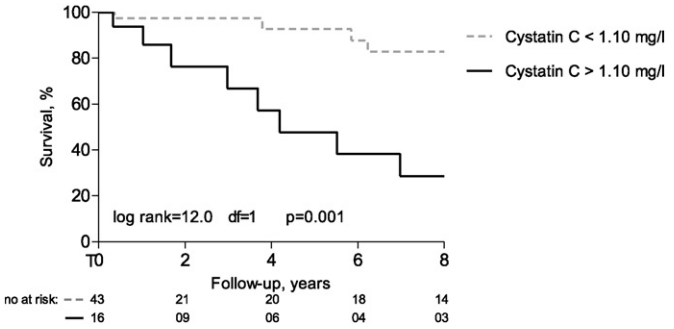

Fig. 2. Kaplan Meijer cystatin C above 1.10 mg/l.

cystatin C predicts mortality independently from renal function [21–23]. Other potential contributing mechanisms that could be responsible for the prognostic role of cystatin C are the association between cystatin C and inflammation, direct role of cystatin C in the vascular wall remodeling in atherosclerosis and role in remodeling of heart extracellular matrix [14, 15].

Cystatin C levels were higher in Down syndrome patients. The result can be explained by multiple factors. Down syndrome patients in our cohort had a more advanced PAH disease severity, indicated by higher numbers of Eisenmenger syndrome, RVSP, and lower 6-MWD (Table S2). PAH influences vascular wall remodeling [27]. More advanced PAH disease severity may have more effect on vascular wall remodeling, which could have increased cystatin C levels. Furthermore, Down syndrome patients had a higher CRP (although non-significant), possibly indicating more inflammation. Lastly, Down syndrome patients received PAH-specific combination therapy less frequently, which in turn could have influenced disease severity. To our best knowledge, a direct effect of PAH-specific therapy on cystatin C levels is unknown.

The development of PAH in patients with CHD is associated with increased mortality and high morbidity [28]. However, it is believed that the prognosis of patients with PAH-CHD can be improved by intensifying their treatment. A recent study has shown that a more aggressive approach using upfront combination therapy is more effective than monotherapy [5]. Consequently, it is particularly important that simple markers are identified that can risk-stratify patients to allow optimal medical management. The PAH guidelines describe several treatment goals which incorporate parameters with an established association with prognosis [6, 7], but the guidelines are mainly based on studies with idiopathic PAH patients. Since there are significant differences in pathophysiology and prognosis between PAH-CHD and idiopathic PAH [29], it could well be that the effect size of the predictors for mortality also differs.

4.2. Clinical impact

A recent study of Diller and coworkers in Eisenmenger patients challenged the traditional view of benign survival prospects of patients with Eisenmenger syndrome and suggested a proactive treatment strategy including a more aggressive approach trying to avoid the development of the condition [30]. Traditionally, determination of follow-up intensity and timing of initiation of PAH-specific combination therapy depended on worsening of functional parameters such as 6-MWD and TAPSE. Our data suggests that several simple biomarkers, next to these functional parameters, can be used to evaluate prognosis of patients with PAH-CHD. This way a timely start of aggressive treatment is possible and disease progression might be delayed.

The use of multiple cardiac biomarkers is currently advocated in acquired heart failure because it achieves greater predictive accuracy [31]. NT-pro-BNP is an already established biomarker of prognosis in patients with PAH-CHD [6, 7]. In addition, we demonstrated the added value of combining NT-pro-BNP with cystatin C. Our prediction model of NT-

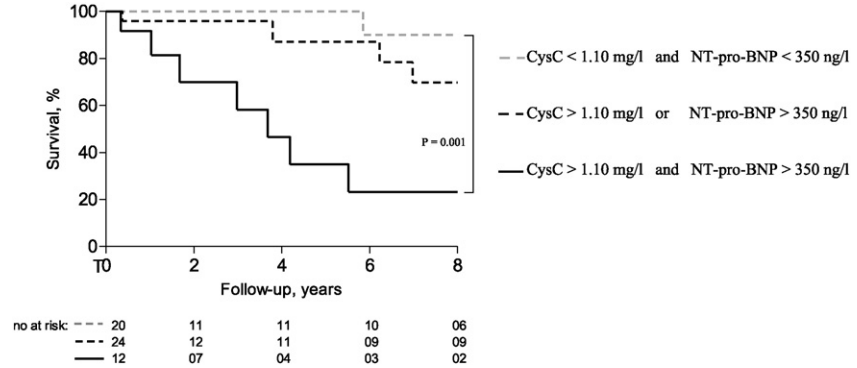

Fig. 3. Predicted survival model based on cystatin C and NT-pro-BNP serum levels. CysC = cystatin C.

Table 2
Multivariate analysis of cystatin C on mortality.

	HR	*p*
A		
Cystatin C*[a]	1.3	**0.009**
NTproBNP*	1.1	**0.771**
B		
Cystatin C*[a]	1.2	**0.028**
MDRD GFR	1.0	**0.749**

A: multivariate analysis of cystatin C adjusted for NT-pro-BNP.
B: multivariate analysis of cystatin C adjusted for MDRD-GFR.
* = log-transformed; a = per 0.1 mg/l; HR, Hazard ratio; NT-pro-BNP, N-terminal pro brain natriuretic peptide; MDRD GFR, modification of diet in renal disease glomerular filtration rate.

pro-BNP and cystatin C demonstrated that patients with both a baseline cystatin C level above 1.10 mg/l and NT-pro-BNP above 350 ng/l had higher mortality rates compared to patients without both risk factors, which favors the use of multiple biomarkers. For the prediction of mortality in PAH-CHD, we suggest the use of biomarkers which reflect different pathological pathways, for instance inflammation, volume overload, RV and vascular wall remodeling. Cystatin C is an inexpensive, widely available, minimally invasive and simple to determine laboratory biomarker which can give additional information about the prognosis of patients with PAH-CHD.

4.3. Limitations

A potential limitation was the relatively small study size, as in most studies in patients with CHD. Secondly, invasive hemodynamics are recommended in the guidelines for the diagnosis of PAH. However, echocardiography is an adequate non-invasive modality in patients with evident diagnosis of PAH [32] in patients with CHD. The vast majority of patients (81%) had Eisenmenger syndrome. Due to the higher complication risk in patients with PAH-CHD, cardiac catheterization was not performed routinely in these patients. Patients with PAH-CHD often have abnormal hemostasis, including thrombocytopenia, making them at risk for both bleeding and thrombosis [33]. In particular, parietal thrombosis of enlarged proximal pulmonary arteries can be found in up to 20% of patients. Catheterisation in these patients may cause peripheral embolization and pulmonary infarctions, and is associated with biventricular dysfunction and reduced pulmonary flow velocity [34]. Right heart catheterization was only performed at baseline in case diagnosis of PAH was not clearly evident at echocardiography.

5. Conclusion

Prognosis of adult PAH-CHD patients remains poor. Our study in PAH-CHD patients showed that cystatin C, a novel cardiac biomarker, predicts long-term mortality and clinical events. Consequently, cystatin C may attribute to clinical decision making regarding treatment intensity.

Author contributions

All authors attributed in both the conception, design, critical revision and final approval of this manuscript. Ilja M. Blok analyzed and interpreted the data and drafted the manuscript under the supervision of senior authors Barbara J.M. Mulder and Berto J. Bouma.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2016.02.003.

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