

High sensitivity C-reactive protein: a biomarker for heart failure in children with univentricular heart disease

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Abstract: Children with univentricular congenital heart disease are at increased risk for developing heart failure. However, detecting early heart failure in these patients is especially difficult because few objective measures have been validated in this cohort. The aim of this study is to determine the usefulness of high sensitivity C-reactive protein (hsCRP) as a biomarker for detecting heart failure in univentricular heart disease patients, and to define the impact of univentricular morphology and stage of palliation on its utility. A cross-sectional observational study of children with univentricular congenital heart disease aged 1 month to 7 years was conducted. The presence of heart failure was defined as a Ross score > 2 . The association of hsCRP with heart failure was assessed using logistic regression and receiver operating characteristic curves. Of the 50 included children, 16 (32%) had clinical heart failure. A doubling of hsCRP resulted in a 0.31 unit increase in Ross score (95% confidence interval: $-0.07, 0.56$; $P = 0.01$) with a c-statistic of 68%. When stratified by ventricular morphology, the left univentricular morphology group exceeded the threshold of $\geq 75\%$ (c-statistic = 81%) as did those at stage II of palliation (c-statistic = 85%). A cut point of ≥ 0.8 mg/mL correctly classified 75% of patients with left univentricular morphology, and 73% of patients regardless of morphology at stage II of palliation. From these results, we conclude that hsCRP is a useful biomarker of clinical heart failure in univentricular patients with left ventricular morphology at all stages of surgical palliation, and in all univentricular patients at stage II of palliation.

Keywords: congenital heart disease, single ventricle, heart failure, biomarker, high sensitivity C-reactive protein

Introduction

Children with univentricular congenital heart disease (UVD) have increased ventricular work and are at greater risk for developing heart failure (HF) than children with other congenital heart defects. Furthermore, the diagnosis of early or mild HF is especially difficult because there are few, if any, objective and quantitative measures of cardiac dysfunction that have been validated in this cohort.¹ Many biomarkers have been studied extensively in adults with structurally normal hearts, however, whether these correlate with HF in infants and young children with UVD is not known. In adult HF, the etiology is typically coronary heart disease, whereas HF in children with UVD is presumed to be due to primary myocardial dysfunction. Furthermore, in the UVD patient, ventricular “cross talk” may be compromised or even absent. These important pathophysiological differences render it difficult to extrapolate adult data to pediatric UVD patients. In this study, we analyzed high sensitivity C-reactive protein (hsCRP) levels in 50 pediatric patients with UVD. We analyzed HF versus

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hsCRP based on ventricular morphology as well as stage of surgical palliation.

Patients and methods

Patients and study design

A single site cross-sectional observational study utilizing a secondary study base was conducted. All children 1 month to 7 years old with UVD presenting to the UCSF Pediatric Heart Center between February 2007 and May 2010 were eligible for the study. Patients were excluded if they had renal failure, trisomy 21, an acute intercurrent illness, a congenital defect that interfered with feeding (eg, cleft palate, esophageal atresia), or had participated in an investigational drug or device study in the last 6 months. In addition, patients for whom a valid Ross score could not be determined (eg, those receiving nasogastric tube feedings, those with postoperative diaphragmatic paralysis) were excluded. This study was approved by the UCSF Institutional Review Board, and written consent was obtained from the guardians of all subjects.

Clinical heart failure scoring

Each child was assigned an HF score based on the Ross classification system² immediately prior to phlebotomy. The Ross classification relies on historical data, vital signs, and physical exam findings that were validated in infants,³⁻⁵ and has been adapted for children.^{3,4,6} For this study, HF scoring was recorded independent of presumed mechanism, since the goal was to identify biomarkers in UVD patients that would be clinically useful across etiologies. The primary outcome was HF (Ross class II–IV) versus no HF (Ross class I). Predictor measurement occurred subsequent to outcome determination, allowing assessors to be blinded to plasma protein levels.

Plasma biomarker determination

At the time of cardiac catheterization, pre-operative evaluation for cardiac surgery, or medical admission, 6 mL of whole blood was obtained in conjunction with clinically indicated phlebotomy. For hsCRP, 2 mL of whole blood was sent to the UCSF Clinical Laboratory for assay by rate turbidimetry. As a previously established negative control,⁷ cardiac troponin I (cTnI) was assayed from 2 mL of whole blood by fluorescent enzyme immunoassay. Each sample was assayed in duplicate. Additional data collected included patient name, medical record number, sex, age, weight, UVD morphology, diagnosis, and surgical history.

Statistical analysis

The primary outcome was HF (Ross class II–IV) versus no HF (Ross class I). The relationship between raw Ross score and plasma proteins was summarized using scatter plots, and analyzed using both linear and logistic regression. Univariate logistic regression was used to assess the crude association between hsCRP or cTnI levels and clinical HF. A *P*-value < 0.05 was considered statistically significant. Receiver operator characteristic (ROC) curves were generated to evaluate proteins as biomarkers in the entire cohort and stratified groups. Assays were deemed useful if they carried a c-statistic ≥ 0.75 (graph area encompassed by the curve). For a useful test, a clinically relevant cut point was identified that would distinguish UVD children in HF from those free of HF at the time of evaluation. All statistical analyses were performed using Stata software (v 10; StataCorp LP, College Station, TX).

Results

We approached 64 patients with UVD meeting the inclusion criteria for this study and presenting to the UCSF Pediatric Heart Center between February 2007 and May 2010 for enrollment. Ten (15%) declined, and of the 54 remaining subjects, four (5%) were subsequently excluded because of missing data. Thus, 50 children were studied (Table 1). Of those, 38 (76%) had single morphologically right ventricles (RV), 11 (22%) had single morphologically left ventricles (LV), and one (2%) had a primitive ventricle of indeterminate morphology. Of the single RV patients, 19 had hypoplastic left heart syndrome (ten with mitral and aortic atresia, two with mitral stenosis and aortic atresia, and seven with mitral and aortic stenosis), 12 had a right dominant (unbalanced) atrioventricular canal defect, three had double-outlet RV, two had interrupted aortic arch with a large ventricular septal defect and small LV, and there was one patient each with aortic stenosis and severe LV dysfunction, or L-transposition of the great arteries with severe pulmonary stenosis and a small LV. Of the 11 patients with single LV, five had tricuspid atresia, three had pulmonary atresia with intact ventricular septum, two had double-inlet LV, and one had critical pulmonary stenosis with a small RV. The patient with indeterminate ventricular morphology had a primitive ventricle with heterotaxy syndrome. While patients at stage I of palliation can be subject to volume overload contributing to HF because of the physiology associated with a Norwood or Sano procedure, pulmonary artery band, Blalock-Taussig shunt, or central shunt, later stages of palliation serve to unload the single ventricle and HF is more commonly due to

Table 1 Patient data

Ventricular morphology	Stage of palliation ^a	Total patients	Female/male	Median age, months (range)	Ross class I	Ross class II–IV
Right	Total	38	17/21	20 (2–80)	26	12
	Stage I	15	7/8	4.4 (2–6)	10	5
	Stage II	16	8/8	35 (5–54)	11	5
	Stage III	7	2/5	71 (55–80)	5	2
Left	Total	11	4/7	42 (3–78)	7	4
	Stage I	3	1/2	4 (3–8)	3	0
	Stage II	7	3/4	45 (17–69)	3	4
	Stage III	1	0/1	73	1	0
Indeterminate	Total	1	1	60 (56–66)	2	0
	Stage I	0	0	0	0	0
	Stage II	1	0/1	66	1	0
	Stage III	0	0	0	0	0

Notes: ^aStage I: stabilization of aortic and pulmonary blood flow, eg, Norwood, Sano, pulmonary artery band, Blalock–Taussig shunt, central shunt; Stage II: establishment of partial cavopulmonary circulation between the superior vena cava and pulmonary arteries, eg, Glenn shunt, Kawashima; Stage III: completion of cavopulmonary circulation, eg, Fontan.

primary myocardial dysfunction. At our institution, patients with significant atrioventricular valvar insufficiency are not considered candidates for a cavopulmonary circulation.

Patients were assigned into a Ross class as a measure of clinical HF as previously described^{7,8} and the surgical stage of palliation was noted (Table 1). Of the 34 children free of clinical HF (Ross class I), the median Ross score was 1 ± 0.8 . For the 16 children with clinical HF (Ross class II–IV), the median Ross score was 4.5 ± 1.8 (Ross class II). The overall prevalence of HF in the included sample was 32%, with a morphology-specific prevalence of 32% in those with single RV and 36% in those with single LV.

Figure 1 shows scatter plots of hsCRP or cTnI versus Ross score that provide a visual appraisal of the linearity of the relationship between the candidate biomarkers and the Ross score. Notably, after \log_2 transformation, hsCRP demonstrated a linear relationship with the Ross score. A doubling of hsCRP resulted in a 0.31 unit increase in Ross score (95% confidence interval [CI]: $-0.07, 0.56$; $P = 0.01$). As expected, cTnI (0.1 mg/L increase associated with a 0.027 unit increase in Ross score [95% CI: $-0.21, 0.39$, $P = 0.86$]) was not associated with increased Ross score in our sample. Exclusion of influential data points for these assays did not alter the results. Using single-predictor logistic regression, a doubling of hsCRP was associated with an odds ratio for HF of 1.41 (95% CI: 1.03, 1.92; $P = 0.03$). cTnI (OR: 0.52, 95% CI: 0.26, 10.1; $P = 0.67$) was not associated with clinically relevant alterations in the odds of HF.

ROC curve analysis of hsCRP data for the entire study cohort gave a c-statistic of 68%, which came close to but did not exceed our pre-specified threshold of $\geq 75\%$ area contained by the ROC curve (Figure 2). ROC analysis stratified

by ventricular morphology demonstrated that the left UVD group exceeded the threshold of $\geq 75\%$ (c-statistic = 81%), however, right UVD patients did not (c-statistic = 59%; Figure 3). ROC analysis stratified by stage of surgical palliation demonstrated that patients at stage II exceeded the usefulness threshold (c-statistic = 85%), however, those at stages I and III did not (c-statistic = 38% and 50%, respectively; Figure 3). As expected, cTnI failed to distinguish children in HF from those free of HF, with relatively poor sensitivities and specificities for HF (data not shown).

The test characteristics for various plasma levels of hsCRP as a marker of current clinical HF were examined for the single LV group and for all morphologies at stage II of palliation (those that demonstrated c-statistic values $\geq 75\%$). For all patients with a single LV, a cut point of ≥ 0.8 mg/mL showed both sensitivity (100%) and specificity (63%), thus correctly classifying 75% of the patients. When all UVD patients were stratified by stage of surgical palliation, a cut point of ≥ 0.8 mg/mL was also needed to correctly classify 73% of patients at stage II of palliation, with a sensitivity of 100% and a specificity of 57%. Logistic regression, ROC curve analysis, and evaluation of test characteristics suggest that hsCRP can be a useful biomarker of mild to severe HF in single LV patients and all UVD patients at stage II of surgical palliation, with a cut point of ≥ 0.8 mg/mL.

Discussion

In this study, we have demonstrated that hsCRP can be a useful biomarker of clinical HF in UVD patients with left ventricular morphology at all stages of surgical palliation, and in all UVD patients at stage II of palliation, with a cut point of ≥ 0.8 mg/mL. To our knowledge, this is the first

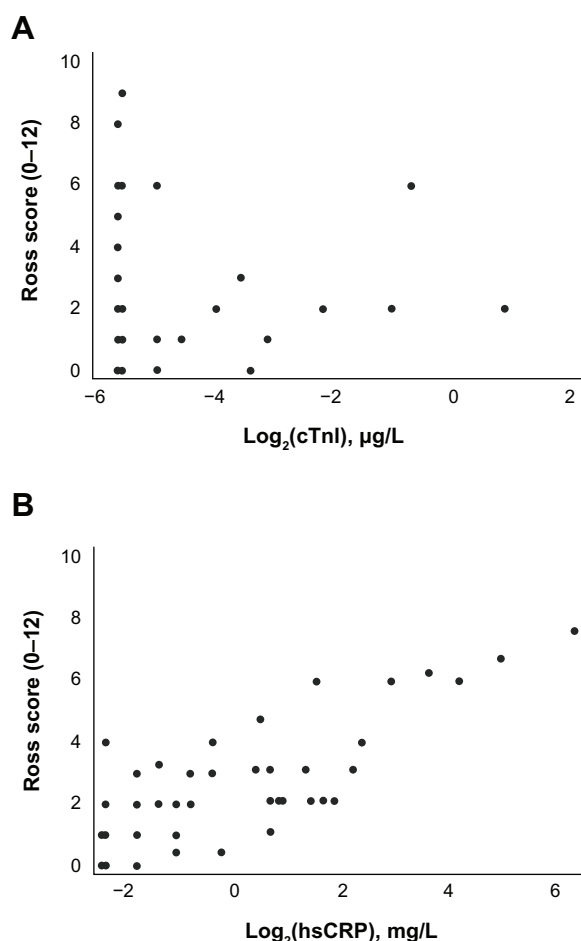


Figure 1 Evaluation of hsCRP and cTnI versus Ross scores. Scatter plot of plasma cTnI (**A**) and hsCRP (**B**) concentration versus raw Ross scores in 50 UVD patients. **Note:** Log₂ transformation of hsCRP revealed a roughly linear relationship with raw Ross score.

Abbreviations: hsCRP, high sensitivity C-reactive protein; cTnI, cardiac troponin I; UVD, univentricular congenital heart disease.

report of a relationship between hsCRP and clinical HF in young children with congenital heart disease, and UVD in particular.

In the pediatric population with normal cardiac anatomy, hsCRP has been helpful in distinguishing constrictive pericarditis from restrictive cardiomyopathy,⁹ infectious from non-infectious causes of fever following cardiopulmonary bypass,¹⁰ and has been shown to correlate with arterial stiffness¹¹ and long-term sequelae¹² in Kawasaki's disease. hsCRP also has been identified as a biomarker of acute and chronic HF in adults with ischemic heart disease.¹³ The mechanism has been thought to be due to the general inflammatory response that accompanies atherosclerotic plaque formation.¹⁴ Atherogenesis, however, is not a typical mechanism of HF in children with UVD. Recent studies suggest that this may not be the only source of C-reactive protein elevation. Anzulovic-Mirosevic et al have shown that C-reactive protein levels are increased in adult patients with non-ischemic

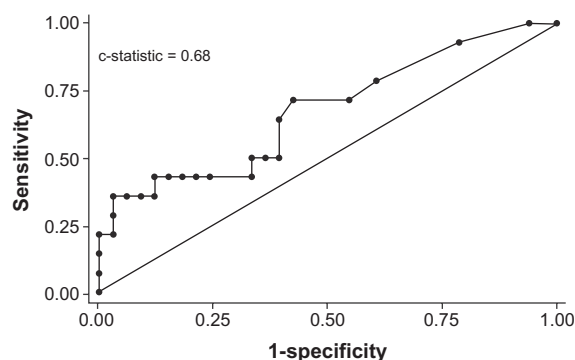


Figure 2 Receiver operator characteristic curve for hsCRP in UVD patients.

Notes: False positives (1-specificity) were plotted against true positives (sensitivity) for all patients. The area under the curve (c-statistic) was 0.68, which approached significance.

Abbreviations: hsCRP, high sensitivity C-reactive protein; UVD, univentricular congenital heart disease.

cardiomyopathy proportionate to the severity of the symptoms and systolic impairment.¹⁵ Furthermore, in situ hybridization experiments have shown significant myocardial production of C-reactive protein mRNA in cardiomyocytes of patients with non-ischemic cardiomyopathy.¹⁶

Interestingly, hsCRP in UVD patients was found to be more indicative of HF in those patients with left ventricular morphology and those at the second stage of palliation. This is a particularly important time during staged palliation of UVD because patients with a partial cavopulmonary connection are relatively stable from a hemodynamic standpoint, and early detection of cardiac dysfunction by elevated hsCRP levels might guide planning for completion of the cavopulmonary connection (ie, Fontan), as well as facilitate risk stratification. For the patients included in this study, the median ages at stages II and III palliation were 5.3 months (range 2.8–13.5 months) and 4.3 years (range 1.5–6.6 years), respectively; however, early detection of heart failure could prompt an accelerated timeline for unloading the ventricle and more aggressive medical optimization prior to surgery for individual UVD patients.

An acknowledged limitation of this study is the use of the Ross classification system for HF, which has not been specifically validated in the UVD population. However, it has been utilized extensively in pediatric HF studies,⁶ including the multicenter trials of carvedilol therapy for pediatric HF that included UVD patients,^{17–19} and enalapril in infants with UVD.^{17–21} As such, we considered it the most relevant outcome measure for this study.

In conclusion, we show that a hsCRP cut point value of ≥ 0.8 mg/mL is associated with HF specifically in those UVD patients at the second stage of surgical palliation, implying that hsCRP provides a useful tool with which to monitor HF in this unique population. We believe that this

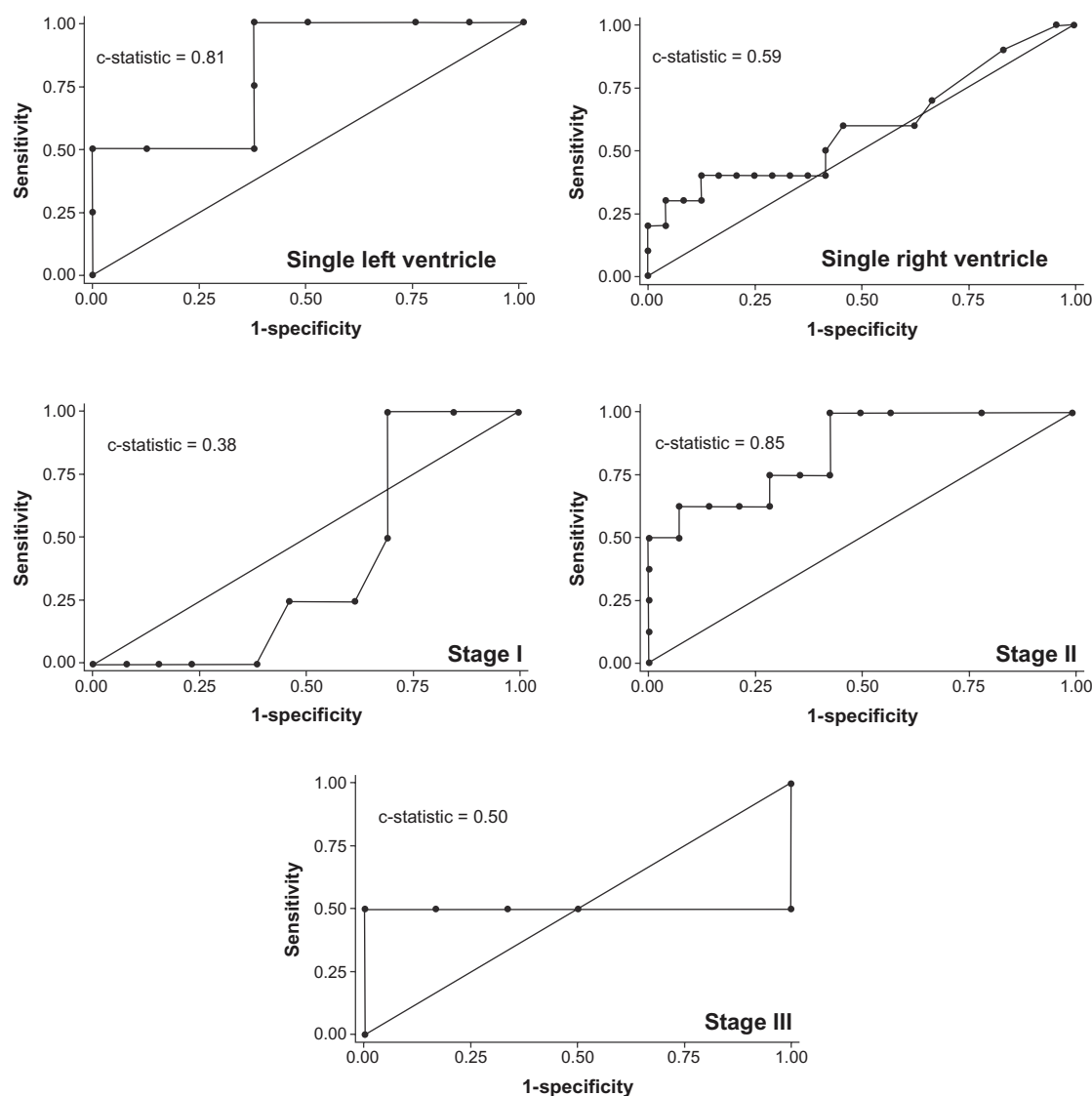


Figure 3 Receiver operator characteristic curves for hsCRP by patient group.

Notes: False positives (1-specificity) were plotted against true positives (sensitivity) for patients with single left or right ventricle, and for patients with single ventricles who had undergone Stage I (Norwood, Sano, pulmonary artery band, Blalock-Taussig shunt, or central shunt), Stage II (partial cavopulmonary shunt, ie, Glenn or Kawashima), or Stage III (complete cavopulmonary shunt, ie, Fontan) surgeries. Patient groups with a single left ventricle at any stage of surgical palliation, and all patients at stage II of palliation, demonstrated an area under the curve (c-statistic) ≥ 0.75 , indicating a useful test.

Abbreviation: hsCRP, high sensitivity C-reactive protein.

value should form the basis of future longitudinal studies to determine the value of hsCRP in monitoring the severity of HF, and the ability of hsCRP to predict cardiac dysfunction and other important clinical outcomes.

Acknowledgments

The authors thank the UCSF pediatric cardiology fellows and the UCSF Congenital Cardiac Catheterization Laboratory staff for their invaluable assistance with the study.

Disclosure

Financial support was received from the Lorraine Newman Fund of the UCSF Division of Pediatric Cardiology and

a Strategic Opportunity Award from the UCSF Clinical and Translational Science Institute (National Institutes of Health RR024131) to HSB.

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