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Periostin is Upregulated in Coronary Arteriopathy in Kawasaki Disease and is a Potential Diagnostic Biomarker

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Abstract

Periostin was upregulated 11-fold in acute and chronic KD coronary arteries compared with controls ($p=0.003$). KD patients had significantly elevated serum periostin values compared with febrile controls ($p=0.0086$). There was no relationship between serum periostin values and age, gender, or acute phase reactants; there was a relationship between serum periostin and maximal coronary artery Z scores that did not reach significance ($p=0.08$). Periostin may prove to be useful as a component of a future diagnostic biomarker panel for KD.

Keywords

Kawasaki Disease; biomarker; periostin

Kawasaki Disease (KD) is a medium-sized muscular arterial disease of early childhood with a predilection for the coronary arteries, resulting in potentially life-threatening dilatation, aneurysms, thrombosis, and stenosis. The etiologic agent remains unknown, and the diagnosis can be difficult because clinical features overlap with other childhood illnesses.¹ Light and transmission electron microscopic studies of KD coronary arteries reveal three

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distinct linked vasculopathic processes, 1) acute self-limited neutrophilic necrotizing arteritis (NA), 2) subacute/chronic (SA/C) vasculitis and 3) luminal myofibroblastic proliferation (LMP), a progressive stenosing intraluminal proliferative lesion composed of smooth muscle cell-derived myofibroblasts and their extracellular matrix (ECM) products.² Serum markers indicating coronary artery damage from the three processes, if identified, could prove useful in KD diagnosis.

We previously reported dysregulation of several ECM molecules in KD coronary arteries by real-time RT-PCR ECM gene array analysis.³ Periostin, a matricellular protein that plays a role in vascular and cardiac responses to injury and repair⁴, was not included on the commercially available ECM array used in our previous study but was of interest to us as a potential biomarker of KD because it is a secreted protein. In this study, we determined whether periostin was upregulated in KD coronary arteries, and whether serum levels of periostin were elevated in children with acute KD.

METHODS

Patients/Tissues/Sera

This study was approved by the Ann and Robert H. Lurie Children's Hospital of Chicago Institutional Review Board. Clinical and demographic information on KD patients and febrile controls are shown in Tables, Supplemental Digital Content 1–4. The mean age of the 15 afebrile controls was 3 years. Coronary arteries from KD patients were individually embedded, while control epicardial coronary arteries with normal pathologic findings were microdissected from myocardial tissue blocks. All KD patients had significant coronary arteriopathy; See Table, Supplemental Digital Content 1, for case numbers from our previously published study reporting the pathologic features of these cases². KD sera were obtained prior to treatment. Coronary artery dilation was defined as a Z score of the right or left anterior descending coronary arteries of >2.

RNA Extraction from formalin-fixed, paraffin embedded (FFPE) tissues

RNA was extracted from FFPE coronary artery tissue sections (7-10 microns) using the Qiagen RNeasy FFPE kit (Qiagen/SA Biosciences, Valencia, CA) as per manufacturer instructions, except that proteinase K lysis was performed for 1 hour at 56° C.

cDNA Synthesis and cDNA quality assessment

Single strand cDNA was synthesized from 300 ng of extracted RNA using the Qiagen/SA Biosciences RT² preAMP cDNA Synthesis Kit. The quality of each cDNA sample was assessed in triplicate by real-time PCR using SYBR Green and primers for the RNA housekeeping gene RPL13A and for human genomic DNA contamination (HGDC), (Qiagen/SA Biosciences). Samples were considered to be of good quality if the C(t) values for HGDC were at least 3 higher (8 fold-change) than for RPL13A, and the RPL13A C(t) was less than 35.

RT-PCR

Real-time PCR was performed using periostin (*POSTN*) primers (SA Biosciences) on an Applied Biosystems Step One Plus Real-time PCR detection system. For differential expression analysis, we used the comparative C_T method.⁵ A two-sample *t*-test was used to compare delta C_T , where delta $C_T = (C_T \text{ POSTN} - C_T \text{ ribosomal protein L13A (RPL13A)})$. The difference in expression levels was determined by comparing delta C_T values between the KD and control groups.

ELISA

ELISA was performed in duplicate on patient sera (at 1:10, with further dilution as necessary) using the Aviscera Biosystems (Santa Clara, CA) Human Periostin and the eBioscience (San Diego, CA) Human sIL-2R Platinum ELISA kits.

Statistical Analysis

Two-sample *t*-tests were used to compare the expression levels of periostin by real time RT-PCR using the comparative ddCt method⁵. The Wilcoxon Rank Sum test was used to compare periostin serum levels, and possible confounding variables were evaluated for association with the levels using simple linear regression models.

RESULTS

Real time PCR

Real-time RT-PCR analysis was performed on 13 KD (7 acute, cases 7-13; 6 chronic, cases 1-6, Table, Supplemental Digital Content 1), and 9 pediatric control (Table, Supplemental Digital Content 2) coronary artery RNAs, and demonstrated an 11-fold increase in expression of periostin in KD compared to control coronary arteries (*p* value <0.001) (Figure 1A). Periostin expression was significantly upregulated in both acute (<7 weeks after onset, *p*=0.005) and chronic (#x0003E;3 months after onset, *p*#x0003C;0.001) KD coronary arteries compared to controls, with no significant difference between acute and chronic KD (*p*=0.56).

ELISA assays

Afebrile childhood controls had mean (+/- standard deviation) periostin levels of 2,244 +/- 2,714 ng/ml. Mean levels of periostin (ng/ml) in febrile controls= 5,647 +/-10,827, KD without coronary artery disease = 35,650 +/-74,485, and KD with coronary artery disease = 51,748 +/-84,763 (Figure 1B). The mean periostin level of all KD patient sera samples (42,549 ng/ml) was significantly higher than that of febrile controls (5,647 ng/ml; *p* = 0.0086). There was also a significant difference in serum periostin levels between KD patients with coronary artery disease and febrile controls (*p*=0.005). The interassay reliability of the ELISA assay was very high with a correlation coefficient of 0.9986.

Lack of relationship of periostin levels with inflammatory markers and other parameters

White blood cell count (*p*=0.87), % neutrophils (*p*=0.26), absolute neutrophil count (*p*=0.7), erythrocyte sedimentation rate (*p*=0.31), C-reactive protein (*p*=0.43), age (*p*=0.89), sex

($p=0.15$), and day of fever ($p=0.47$) were not associated with periostin level. Maximum Z-score showed some relationship to periostin level but was not statistically significant ($p=0.08$). We also performed ELISA assays for soluble IL-2 receptor (sIL-2R) in KD patients and controls as a marker of febrile response.⁶⁻⁸ Levels of sIL-2R were comparable between KD patients (mean = 18.2 ng/ml) and febrile controls (mean = 15.33 ng/ml).

DISCUSSION

We demonstrate that periostin is significantly upregulated in KD arteriopathy in both acute (within 2 months after onset) and chronic (5 months to years after onset) cases. We hypothesize that periostin expression in KD arteriopathy is a consequence of arterial damage from the etiologic agent and the host response, and that after vascular damage, it may play multiple, ongoing functional roles in the subsequent pathologic events, including myofibroblastic differentiation and LMP, collagen production and deposition, and coordination of the ECM response to injury via integrin signaling⁴.

Many biomarkers for KD have been proposed, particularly inflammatory response markers, but no individual biomarker is likely to be diagnostic.⁹⁻¹⁴ We tested periostin as a possible serum biomarker, because markers of cardiac/vascular injury could be very helpful in differentiating children with KD from children with benign febrile illnesses that do not manifest cardiac pathology. The N-terminal moiety of brain natriuretic peptide (NT-proBNP), a cardiac hormone secreted by ventricular cardiomyocytes in response to stress, may also be useful in this regard.^{9-11,14} It is interesting that in our study, serum periostin levels were increased in some KD patients with echocardiographically normal coronary arteries. It is possible that elevated serum periostin levels in acute KD could result from myocarditis, which is common in the acute phase.¹⁵

Our study has several limitations. The target tissue of KD, the coronary artery, is inaccessible to the researcher in living patients, and fortunately, fatalities are unusual. These tissues are available only as autopsy and explanted heart specimens and are thus rare, which accounts for the relatively small sample size of coronary artery tissues in this study. Our lab houses one of the largest collections of such tissues in North America. We performed immunohistochemistry on KD coronary artery tissues using two different commercially available anti-human periostin antibodies, but were unable to determine whether periostin protein was present diffusely in the sections or whether these antibodies resulted in non-specific staining. We plan to perform ELISA studies on serial acute and convalescent KD sera to evaluate periostin expression levels over time in a future study.

In summary, periostin is upregulated in KD coronary tissues, and markedly increased periostin levels are detected in the sera of a subset of KD patients. We propose that a KD biomarker panel be developed and evaluated in a multicenter trial, and that periostin be included in this panel. The development of diagnostic tools to facilitate rapid therapeutic intervention is essential for this potentially devastating childhood disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Delta- C_T values for the POSTN gene in KD and control coronary artery tissues; each point represents a sample and the plus symbol indicates the mean in each group. Lower Delta- C_T values indicate higher expression values. B) Serum periostin levels in KD patients and childhood controls.