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Original Article

A transthoracic Doppler echocardiography study of C-reactive protein and coronary microcirculation in children after open heart surgery

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Abstract *Background:* Systemic inflammation has been suggested to underlie in part the elevated risk of arrhythmias and myocardial dysfunction during the first weeks after cardiac surgery. Recent transthoracic Doppler studies from our centre indicated increased basal coronary arterial flow in children 5 days after cardiopulmonary bypass surgery. In these children, we investigated whether the inflammatory mediator, C-reactive protein, could influence this association. *Methods:* The peak flow velocity, velocity time-integral in diastole and systole, and basal blood flow in the proximal part of the left anterior descending artery, were assessed by transthoracic Doppler echocardiography 1 day before, and 5 days after, cardiac surgery in 17 children with ventricular and atrioventricular septal defects whose mean age at surgery was 6 months. Levels of C-reactive protein in the plasma were measured at both time-points. *Results:* Prior to surgery, all children had levels of C-reactive protein under the limit for detection, that is less than 0.8 milligrams per litre. The levels of the protein had increased significantly by the second day, when the median value was 25, and the range from 20 to 142 milligrams per litre. They remained elevated on the fifth day after surgery, when the median was 11, and the range from 3 to 20 milligrams per litre. On the fifth day, the percentage increase in velocity time integral corrected for left ventricular mass was significantly lower in those patients with C-reactive protein greater than or equal to 10 milligrams per litre than in the remaining patients. Also, both the velocity time integral and the velocity of diastolic peak flow correlated inversely with log C-reactive protein, r being equal to -0.54 and p less than 0.02 and r equal to -0.74 and p less than 0.01, respectively, particularly among those patients in whom clamping of the aorta lasted for more than 1 hour, r for this statistic being equal to -0.8. *Conclusion:* The postsurgical increase in the velocity of coronary arterial flow in children is inversely associated with rising levels of C-reactive protein. The duration of the aortic cross-clamping during surgery strengthens the association between levels of C-reactive protein and the microcirculatory changes.

Keywords: Inflammation; coronary arteries; paediatric cardiology; congenital heart disease

Inflammation has been suggested to underlie in part the risk of arrhythmias and cardiopulmonary dysfunction during the first weeks after cardiac surgery.[1] Cardiopulmonary by-pass elicits an intense inflammatory reaction, with subsequent deleterious effects on the vascular function.[2] C-reactive protein, an important inflammatory mediator mainly synthesized by the liver in response to circulating cytokines, rises rapidly after surgery, reaching a peak after approximately 24 to 48 hours.[3] Several studies have suggested an association of C-reactive protein with the risk of developing atrial fibrillation and myocardial dysfunction after such surgery,[4] while increased levels of the C-reactive protein has also been suggested to have deleterious effects on myocardial

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function even in the absence of cardiac surgery.[5] These observations lend support to the concept that increased levels of the C-reactive protein might be an independent risk factor for myocardial disease.[6]

To date, however, there is no direct evidence to link the possible association between levels of C-reactive protein and the changes in flow through the coronary microcirculation occurring after cardiopulmonary bypass surgery. Data from both experimental and clinical studies using recombinant C-reactive protein, and leading to a wide range of circulating levels, suggested possible dichotomous effects of the C-reactive protein on the vasomotor tone and reactivity of the conduit arteries.[7] Some possible explanations for these contradictory results include contamination of the C-reactive protein extract with other substances, such as endotoxin and sodium azide.[8]

Our recent transthoracic Doppler echocardiographic studies[9] have suggested that basal coronary arterial flow increases in children up to 4 to 6 days after cardiopulmonary bypass surgery. In this same population of children, therefore, we have investigated whether the postsurgical rise in C-reactive protein was correlated with the observed changes in coronary arterial flow.

Subjects and methods

We studied 17 infants referred for cardiopulmonary bypass surgery for closure of ventricular- or atrioventricular septal defects. Their ages ranged from 3 to 18 months. Clinical data was obtained from the hospital records, and written consent had been obtained from the guardians of all the children. The study was approved by the ethics committee for human research at Lund University.

Transthoracic Doppler echocardiography was performed on all children 1 day before, and 5 days plus or minus 1 day postoperatively. The criterions for exclusion were clinical signs of infectious illness, levels of C-reactive protein greater than 1 milligrams per litre prior to surgery, heart failure and preoperative therapy with vasoactive drugs. The Doppler examination was performed using the Sequoia^{TM} C512 machine, manufactured by Acuson in Mountain View, California, United State of America. We used a 7 megaherz transducer, and carried out standard M and B-mode, and Doppler, echocardiographic interrogations to determine the anatomy and function of the heart. Measurements of basal coronary arterial flow, and peak velocity of flow, were performed according to the protocol described in previous studies.[10,11] Pulsed Doppler of 4.5 megaherz, a sweep rate of 100 millimetres per second, and velocity settings of 15 to 60 centimetres per second before, and 30 to 60 centimetres per second after, were used. All measurements were corrected for the angle between the Doppler beam and the direction of coronary arterial flow. True velocity was defined as the measured velocity divided by the cosine of the angle between the Doppler beam and the direction of blood.

The bifurcation of the left coronary artery was imaged from the standard short-axis view of the arterial trunks. The internal dimension of the anterior interventricular artery was measured at end-diastole with callipers applied on the inner borders 2 to 3 millimetres distal to the bifurcation of the left coronary artery. The velocity scale was decreased to the minimum range, and then gradually increased until colour signals were optimized within the lumen of the artery. After obtaining good signals of flow, the sample volume was adjusted to 0.5 to1.0 millimetres. The sample was positioned on the flow signal 2 to 3 millimetres distal to the bifurcation, and the velocity of flow recorded. We chose a sample volume that gave the best possible quality envelope, and pure sound throughout the cardiac cycle.

The images were saved on a magnetic optical disc. They were reviewed offline in slow motion, and analyzed in single frame advance mode. The diameter of the aortic root was measured in M-mode using a long axis view at the level of the proximal hinge points of the valvar leaflets. Left ventricular mass was calculated according to Devereux and colleagues,[12] and as recommended by the American Society of Echocardiography.[13]

Left ventricular mass per gram is equal to 0.8 [1.04 (left ventricular internal diameter in diastole plus ventricular septal thickness in diastole plus left ventricular inferior mural thickness in diastole)^3 (minus left ventricular internal diameter in diastole)^3] plus 0.6.

We corrected the increase in the velocity time integral for the left anterior interventricular artery for left ventricular mass.[14]

The velocity time integral corrected in centimetres per 100 grams is equal to [(velocity time integral on the fifth day minus velocity time integral pre-operatively) per velocity time integral pre-operatively] multiplied by the left ventricular mass pre-operatively^{-1} multiplied by 100.

Left ventricular fractional shortening was computed from the standard formula. Arterial blood pressure was measured by an automatic oscillometer cuff sphygmomanometer (Dynamap, Critikon Inc, Tampa, Florida, United States of America).[15] The product of rate and pressure was calculated by multiplying heart rate with the systolic blood pressure.[16] The analysis package of the ultrasound

unit was used for manual tracing of the spectral envelope. The measurements of the velocity of flow across the aortic valve were averaged over three consecutive cardiac cycles, and included in measurements of cardiac output. We measured the peak velocity of flow in diastole, and the velocity time integral in diastole and systole. The velocity time integral per minute is equal to the velocity time integral in systole and diastole multiplied by the heart rate. The flow of blood per minute was calculated as the velocity time integral per minute multiplied by cross-sectional area, where cross-sectional area is equal to pi multiplied by the square of the coronary arterial diameter divided by 2.

Postoperative therapy

All children received opioid derivatives in the form of intravenous ketobemidone hydrochloride, given at 25 micrograms per kilogram during the first 3 postsurgical days, and oxicodon, given at 0.1 milligram per kilogram orally between the third and fifth postoperative days. None of our patients received inhibitors of angiotensin converting enzyme, inotropes, beta-blockers or other vasoactive medications.

Statistics

We used paired Student's t test for comparison of the data obtained before and after surgery. The effects postsurgically of the levels of C-reactive protein on the variations in coronary arterial flow were assessed by ANOVA. Simple regression was used to assess the correlation, if any, between the variations in coronary arterial flow and the levels of C-reactive protein in the plasma. The levels of C-reactive protein were log-transformed, given their skewed distribution. All statistical analyses were performed using StatView® (SAS Institute, Cary, NC, USA), version 5.0 as a statistical software package. A value of p of less than 0.05 was considered statistically significant. Results are presented as mean values plus or minus the standard deviation.

Results

All children had similar pre- and peri-operative characteristics, and no adverse clinical events occurred during the postoperative period. Prior to surgery, levels of C-reactive protein were below the limits for detection in all the children, in other words less than 0.8 milligrams per litre. Levels of the C-reactive protein increased significantly by the second day, reaching a median of 25, and a range from 4 to 142 milligrams per litre. They remained elevated on the fifth day after surgery, when the median was 11, and the range from 3 to 20 milligrams per litre.

Compared to the preoperative values, blood flow in the left anterior interventricular artery, the velocity time integral in diastole and systole, and peak velocity of flow in diastole, all increased significantly on the fifth day, at 27 plus or minus 10 versus 47 plus or minus 15 millilitres per minute, p equal to 0.0001; 9 plus or minus 3 to 14 plus or minus 5 centimetres, p equal to 0.05; and 36 plus or minus 10 to 49 plus or minus 16 centimetres per second, p equal to 0.05, respectively.

The velocity time integral after surgery, and the peak velocity of flow in diastole, correlated inversely with log-transformed values for C-reactive protein, with r equal to -0.54, p less than 0.02, and r equal to -0.74, p less than 0.01, respectively (Fig. 1, Panels A and B). When patients were further divided in relation to the period of aortic cross-clamping, which had a mean of 59 minutes, and ranged from 33 to 121 minutes, these associations remained significant only in those in whom the period of clamping exceeded 1 hour (Fig. 2). The increase in postsurgical velocity time integral corrected for left ventricular mass was significantly lower among those patients with levels of C-reactive protein greater than 10 milligrams per litre than in the remaining patients, at 5 plus or minus 2 versus 2 plus or minus 1, respectively; p less than 0.05 (Fig. 3).

On the fifth day postoperatively, neither the diameter of the left anterior interventricular artery, nor flow through the artery, correlated with the levels of C-reactive protein, p being greater than 0.5. Similarly, levels of C-reactive protein showed no association with left ventricle fractional shortening, cardiac output, the product of rate and pressure, and mean arterial blood pressure, the value of p being greater than 0.5 for all.

Discussion

We have shown that, in children undergoing cardiopulmonary bypass surgery, the rise in the velocity of flow through the coronary arteries after the procedures was attenuated in those with higher levels of C-reactive protein. This inverse association between levels of C-reactive protein and the velocity of coronary arterial flow was strengthened by prolonged aortic cross-clamping during the period of surgery. Although these findings do not prove a causal relationship, they are in keeping with the hypothesis that systemic inflammation might be an important mechanism in the pathogenesis of postsurgical myocardial dysfunction, acting in part

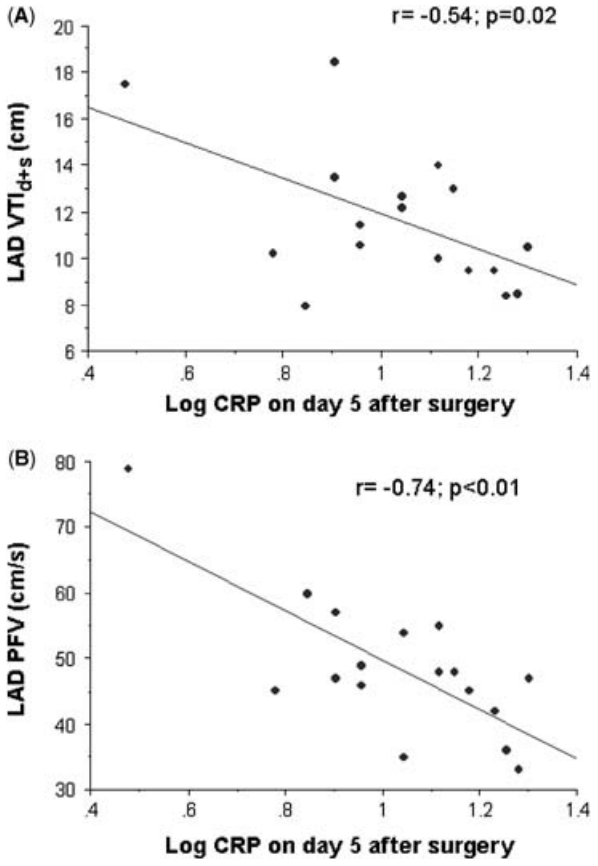

Figure 1.
Panel A shows the velocity time integral for the left anterior interventricular artery in centimetres (LAD VTI, Panel A), while Panel B shows the diastolic peak velocity of flow (LAD PFV, Panel B) both inversely correlated with log C-reactive protein on the fifth day after surgery ($r = -0.54$, $p < 0.02$, *and* $r = -0.74$, $p < 0.01$, *respectively).*

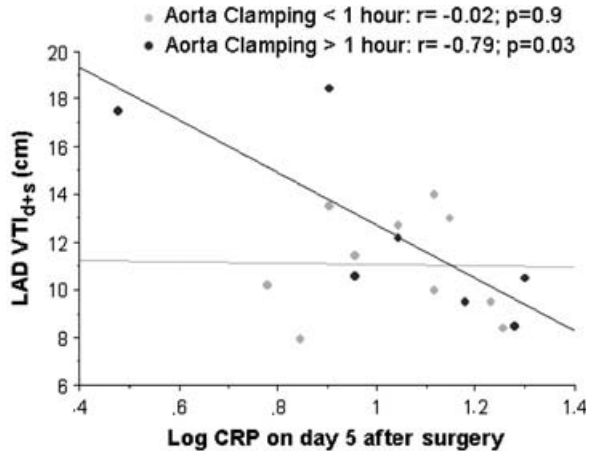

Figure 2.
The effect of the duration of aorta cross-clamping on the association between the velocity time integral in diastole and systole (LAD VTI) and log C-reactive protein on the fifth day after surgery.

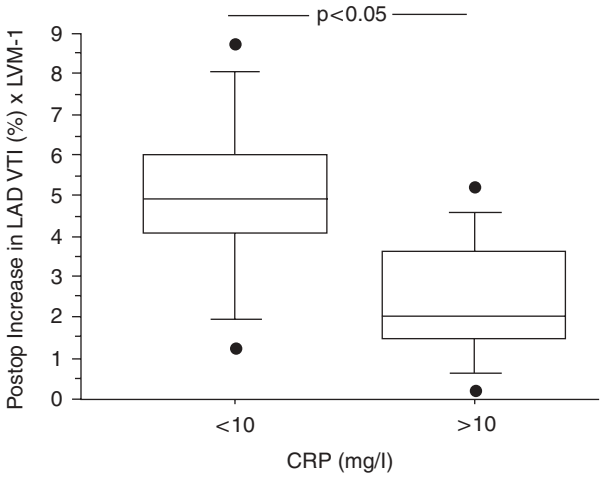

Figure 3.
The effect of C-reactive protein on the velocity time integral for the left anterior interventricular coronary artery (LAD VTI) corrected for left ventricular mass on the fifth day after surgery.

by possible vasoconstrictive effects on the coronary circulation.

Cardiopulmonary bypass and surgery are strong stimuluses for systemic inflammation in both children and adults, being characterized by an intense and rapid rise of inflammatory cytokines, followed from the second day by 100 to 200 fold increase in levels of C-reactive protein.[17] This postoperative rise in levels of the C-reactive protein, particularly in adults, coincides with an increased risk for cardiovascular complications, such as haemodynamic instability, myocardial ischaemia, arrhythmias, and even multi-organ failure in severe cases.[4,18]

After cardiopulmonary bypass surgery, the coronary microcirculatory phenotype is shifted to a highly disturbed state, characterized by decreased myogenic tone, and impaired ability to dilate in response to physiological or pharmacological stimuluses.[19,20] The mechanisms underlying the impaired coronary flow reserve appear to be manifold, and may include an imbalance between dilating and constricting compounds synthesized by the arterioles. Importantly, C-reactive protein stimulates release from endothelial cells of endothelin-1, which is a powerful vasoconstrictor.[21] Cardiopulmonary bypass surgery is an important source of endothelin-1.[22] C-reactive protein also poses a down-regulatory action on the constitutive form of nitric oxide synthase via decreased stability of endothelial nitric oxide synthase messenger ribonucleic acid, thereby resulting in decreased amounts of nitric oxide, which is a potent vasodilator. In one previous experimental study,[23] exposure

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of coronary arterioles to human recombinant C-reactive protein at levels comparable to those attained in our study, namely 7 versus 11 milligrams per litre, resulted in significant inhibition of the endothelium-dependent nitric oxide-mediated dilation of the arterioles by increasing production of superoxide from nicotinamide adenine dinucleotide phosphate oxidase via activation of the p38 kinase.

Alternatively, the coronary microcirculatory phenotype associated with C-reactive protein in our study might be a consequence of the surgically induced disturbances in the adjacent ventricular myocytes, which closely interact with the arteriolar smooth muscle cells. Particularly during cardiopulmonary bypass surgery, the myocardial cells are highly susceptible to damage via the inflammatory response, ischaemia-reperfusion, and surgery as such, with subsequent infiltration of leucocytes and oedema surrounding the arterioles, and hence, arteriolar compression.[24] This hypothesis, however, is less plausible, since neither the haemodynamic parameters relating to the myocardium, such as heart rate, contractile function and blood pressure, nor the surgical parameters, such as clamping, perfusion and overall duration of surgery, correlated with the measured levels of C-reactive protein.

Our study does have its limitations, the main one residing in the lack of cardiovascular complications in our material, which precludes conclusion on the possible impact of the observed association on the clinical outcome. It is also the case that screening for additional markers of inflammation, such as interleukins, might have provided additional mechanistic clues.

We conclude, nonetheless, that the rise we observed in C-reactive protein after cardiopulmonary bypass surgery is inversely associated with the velocity of coronary arterial flow as assessed by transthoracic Doppler echocardiography. Although this finding in itself is not proof of a causative relationship, it is in keeping with the hypothesis that systemic inflammation following cardiac surgery could be an important mechanism in the onset of myocardial complications due to dysfunction of the coronary microvasculature. Additional studies are needed to investigate whether this association could, in part, account for the previously suggested adverse effects of inflammation on myocardial physiology during the immediate postoperative period.

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References

1. Hassanabad ZF, Furman BL, Parratt JR, Aughey E. Coronary endothelial dysfunction increases the severity of ischaemia-induced ventricular arrhythmias in rat isolated perfused hearts. Basic Res Cardiol 1998; 93: 241–249.
2. Verrier ED, Boyle Jr EM. Endothelial cell injury in cardiovascular surgery. Ann Thorac Surg 1996; 62: 915–922.
3. Franke A, Lante W, Fackeldey V, et al. Pro-inflammatory cytokines after different kinds of cardiothoracic surgical procedures: is what we see what we know? Eur J Cardiothorac Surg 2005; 28: 569–575.
4. Bruins P, te Velthuis H, Yazdanbakhsh AP, et al. Activation of the complement system during and after cardiopulmonary bypass surgery: postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. Circulation 1997; 96: 3542–3548.
5. Wiklund L, Ronquist G, Roomans GM, Rubertsson S, Waldenstrom A. Response of myocardial cellular energy metabolism to variation of buffer composition during open-chest experimental cardiopulmonary resuscitation in the pig. Eur J Clin Invest 1997; 27: 417–426.
6. Paul M. Ridker. C-reactive protein, inflammation, and cardiovascular disease clinical update. Tex Heart Inst J 2005; 32: 384–386.
7. Sternik L, Samee S, Schaff HV, et al. C-reactive protein relaxes human vessels in vitro Arterioscler. Thromb Vasc Biol 2002; 22: 1865–1868.
8. Zhong Y, Li SH, Liu SM, et al. C-Reactive protein upregulates receptor for advanced glycation end products expression in human endothelial cells. Hypertension 2006; 48: 504–511.
9. Aburawi EH, Berg A, Liuba P, Pesonen E. Effects of cardiopulmonary bypass surgery on coronary flow in children assessed with transthoracic Doppler echocardiography. Am J Physiol Heart Circ Physiol. 2007 May 4; [Epub ahead of print].
10. Hozumi T, Yoshida K, Ogata Y, et al. Noninvasive assessment of significant left anterior descending coronary artery stenosis by coronary flow velocity reserve with transthoracic color Doppler echocardiography. Circulation 1998; 97: 1557–1562.
11. Saraste M, Koskenvuo J, Knuuti J, et al. Coronary flow reserve: measurement with transthoracic Doppler echocardiography is reproducible and comparable with positron emission tomography. Clin Physiol 2001; 21: 114–122.
12. Devereux RB, Alonso DR, Lutas EM, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986; 57: 450–458.
13. American Society of Echocardiography Committee. Recommendations for quantification of the left ventricle by two-dimensional echocardiography. J Am Soc Echocardiogr 1989; 2: 358–367.
14. Hildick-Smith DJ, Johnson PJ, Wisbey CR, Winter EM, Shapiro LM. Coronary flow reserve is supranormal in endurance athletes: an adenosine transthoracic echocardiography study. Heart 2000; 84: 383–389.
15. Czernin J, Muller P, Chan S, et al. Influence of age and haemodynamics on myocardial blood flow and flow reserve. Circulation 1993; 88: 62–69.
16. Baller D, Bretschneider HJ, Hellige G. A critical look at currently used indirect indices of myocardial oxygen consumption. Basic Res Cardiol 1981; 76: 163–181.
17. Paparella D, Galeone A, Venneri MT, et al. Blood damage related to cardiopulmonary bypass: in vivo and in vitro comparison of two different centrifugal pumps. ASAIO J 2004; 50: 473–478.

18. Gaspardone A, Versaci F, Tomai F, et al. C-Reactive protein, clinical outcome, and restenosis rates after implantation of different drug-eluting stents. Am J Cardiol 2006; 97: 1311–1316.
19. Wang SY, Friedman M, Franklin A, Sellke FW. Myogenic reactivity of coronary resistance arteries after cardiopulmonary bypass and hyperkalemic cardioplegia. Circulation 1995; 92: 1590–1596.
20. Hiratzka LF, Eastham CL, Carter JG, et al. The effects of cardiopulmonary bypass and cold cardioplegia on coronary flow velocity and the reactive hyperemic response in patients and dogs. Ann Thorac Surg 1988; 45: 474–481.
21. Verma S, Li SH, Badiwala MV, et al. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. Circulation 2002; 105: 1890–1896.
22. Pernow J, Wang QD. Endothelin in myocardial ischaemia and reperfusion. Cardiovasc Res 1997; 33: 518–526.
23. Lafuente N, Azcutia V, Matesanz N, et al. Evidence for sodium azide as an artefact mediating the modulation of inducible nitric oxide synthase by C-reactive protein. J Cardiovasc Pharmacol 2005; 45: 193–196.
24. Heltne JK, Koller ME, Lund T, et al. Dynamic evaluation of fluid shifts during normothermic and hypothermic cardiopulmonary bypass in piglets. Acta Anaesthesiol Scand 2000; 44: 1220–1225.