Microparticles and Kawasaki disease: a marker of vascular damage?

S. Guiducci[1], L. Ricci[2], E. Romano[1], C. Ceccarelli[1], J.H.W. Distler[3, 5], I. Miniati[1], G.B. Calabri[4], O. Distler[3], M. Matucci-Cerinic[1], F. Falcini[1]

[1]Department of BioMedicine, Division of Rheumatology, DENOThe Centre, University of Florence, Florence, Italy; [2]Department of Paediatrics, University of Florence, Florence, Italy; [3]Centre of Experimental Rheumatology and Zurich Center of Integrative Human Physiology, University Hospital Zurich, Zurich, Switzerland; [4]Anna Meyer Hospital Cardiology Unit, Florence, Italy; [5]University of Erlangen-Nuremberg, Erlangen, Germany.

Serena Guiducci, MD, PhD
Laura Ricci, MD
Eloisa Romano, MD
Claudia Ceccarelli,
Jörg H.W. Distler, MD, PhD
Irene Miniati, MD, PhD
Giovanni Battista Calabri, MD
Oliver Distler, MD, PhD
Marco Matucci-Cerinic, MD, PhD
Fernanda Falcini, MD

Please address correspondence to:
Dr Serena Guiducci,
Department of BioMedicine,
Division of Rheumatology,
University of Florence,
Viale Pieraccini 18,
50139 Firenze, Italy.
E-mail: s.guiducci@hotmail.com

Received on January 28, 2010; accepted in revised form on September 21, 2010.

Clin Exp Rheumatol 2011; 29 (Suppl. 64): S121-S125.

Key words: microparticles, Kawasaki disease

Competing interests: none declared.

ABSTRACT

Background. *Microparticles (MPs) are increased in diseases characterised by endothelial injury. Kawasaki disease (KD) damages the endothelium provoking life-threatening involvement of coronary arteries.*

Objective. *To compare KD MPs vs. controls.*

Methods. *Thirty KD and 20 controls were enrolled. MPs were stained with monoclonal antibodies against platelets, endothelial cells (EC), monocytes, T and B cells, neutrophils, and quantified by FACS.*

Results. *The total number of MPs was significantly increased in KD versus controls ($193x10^5 \pm 0.6x10^5$ vs. $94x10^5 \pm 0.9x10^5$ million/ml plasma p=0.01) and vs. KD after IVIG therapy ($132x10^5 \pm 0.4x10^5$million/ml plasma p=0.01). EC and T cells were the major source of MPs in KD ($72x10^5 \pm 1x10^5$vs.$3x10^5 \pm 0.9x10^5$million/ml plasma for T cells p=0.005; $76x10^5 \pm 0.7x10^5$ vs. $45x10^5 \pm 0.4x10^5$ million/ml plasma for EC p<0.02) followed by MPs derived from platelets ($13x10^5 \pm 0.3x10^5$ vs. $3x10^5 \pm 0.9x10^5$ million/ml plasma p=0.028). Cell-derived MPs B were $17x10^5 \pm 0.4x10^5$ vs. $20x10^5 \pm 0.8x10^5$million/ml plasma in controls (p=0.7). No significant differences were observed in KD MPs derived from monocytes and neutrophils. After IVIG administration, a significant decrease of MPs derived from platelets ($3x10^5 \pm 0.2x10^5$ million/ml plasma p=0.03), EC ($9x10^5 \pm 0.4x10^5$ million/ml plasma p=0.01), T cells ($72x10^5 \pm 1x10^5$ million/ml plasma p=0.02) and B cells ($7x10^5 \pm 0.3x10^5$ million/ml plasma p=0.02) was observed.*

Conclusion. *The number of KD MPs is significantly increased and EC and T cells are the major source. MPs may develop from endothelial damage and cell activation. Their role as markers of disease activity or as contributors to endothelial derangement in KD has to be further investigated.*

Introduction

Kawasaki disease (KD), an acute febrile systemic vasculitis of small-medium-sized vessels that mainly occurs in young children, damages the endothelium provoking a life threatening involvement of coronary arteries (1-6). Following a complex immune response, a significant overproduction of different cytokines and activation of endothelial cells has been recognised (7-11). During the acute phase increased microvascular permeability, up-regulated expression of adhesion molecules on endothelial cells, infiltrating inflammatory cells, and endothelial dysfunction are likely to contribute to the pathogenesis of vasculitis and aneurysm development (12, 13).

Recently, it has been shown that activated cells (as in vasculitis) and cells undergoing apoptosis release microparticles (MPs) (subcellular elements) responsible for intercellular communication and inflammation. MPs derive from loss of asymmetry of normal cell membrane phospholipids, resulting in an increase in phosphatidylserine on the outer leaflet of the bilipid membrane layer, membrane blebbing with MPs formation, and shedding by a process of exocytic budding. MPs include cytoplasmatic components and membrane-derived elements such as negatively charged phospholipids or cell-surface receptors (14). Cellular MPs are a heterogeneous population, differing in cellular origin, number, size (0.05–1μm), antigenic composition, and functional properties. MPs support coagulation by exposure of negatively charged phos-

pholipids and sometimes tissue factor. MPs may transfer bioactive molecules to other cells or MPs, stimulate cells to produce cytokines, cell adhesion molecules, and modulate endothelium function (15, 16). The complement's protein, C1q, bind to MPs activating the proinflammatory effect on the classical complement pathway (17). Eventually, MPs can both induce and amplify inflammation, thus enhancing tissue damage in vasculitis.

The purpose of our study was to examine the number and profiles of circulating MPs in patients with Kawasaki disease, and compare the number to healthy controls.

Materials and methods

Patients

Thirty consecutive children (19 males, 11 females; median age 17.2±9 months) with the diagnosis of KD according to CDC (Centre for Diseases Controls) criteria were enrolled in the study. Twenty-three of them had typical KD while 7 had incomplete disease. Patients with incomplete KD had high fever lasting more than 5 days and laboratory findings consistent with severe systemic inflammation, but were lacking sufficient clinical manifestations to fulfil the diagnostic criteria (18, 19). All patients were given intravenous immunoglobulin (IVIG) 2g/kg within ten days from the onset of fever, and aspirin (80mg/kg daily divided into three doses) in the acute phase of the disease. Once the fever dropped, the aspirin was reduced to one single daily dose of 3–5mg/kg for 8 weeks in absence of coronary artery damage detected by echocardiography. In patients with coronary artery abnormalities (either aneurysms or dilatations) aspirin was increased to normalisation. Three patients, all with complete KD, received a second cycle of IVIG (2g/kg) since they did not respond to the first administration.

Blood samples were taken in the morning by antecubital vein during routine tests and then stored at -80°C before MPs were studied. Blood samples were collected in each patient at baseline, before IVIG administration, and at 1-month follow-up.

Demographic data (date of birth, age at onset, gender, and ancestry), clinical signs and symptoms at onset, and laboratory variables (erythrocyte sedimentation rate, ESR), C reactive protein (CRP), haemoglobin (Hb), total platelet count (PTL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), sodium (Na), and albumin were recorded at admission. Each patient was evaluated by the cardiologist (EKG, 2D-echocardiography) at admission, at 1, 3, and 6 weeks, and at 6 and 12 months from the onset of fever. Three children developed coronary artery aneurysms (CAA) at 10, 15 and 20 days respectively from the disease onset while 1 out of 30 patients had coronary dilatations. In two patients coronary brightness was observed and in two patients pericardial effusion occured.

Aneurysms were defined in the presence of coronary artery diameter >3mm in children younger than 5 years and >4mm in children older than 5 years or if the internal diameter of a segment measured ≥1.5 times that of an adjacent segment, according to the Japanese Ministry of Health. Because the use of these criteria may result in both underdiagnosis and underestimation of the true prevalence of the true presence of coronary dilation, coronary measurements were also adjusted for body surface area according to De Zorzi *et al*. (19).

Twenty sex- and age-matched healthy subjects recruited among children attending the outpatient clinic for musculoskeletal pain without inflammation were studied as controls. Both KD patients and controls were Caucasians, and all of Italian ancestry. All patients and controls were white. The institutional Ethics Committee of A. Meyer Hospital approved the study, and parents of all the children involved in this study gave informed consent.

Methods

Isolation of microparticles by differential centrifugation

Blood was obtained using a 19-gauge needle and collected into citrated tubes. Immediately after blood was obtained, plasma was centrifuged at 1,500g for 10 minutes, and the supernatant was collected and divided into plasma aliquots that were stored in liquid nitrogen until they were used for analysis. The method for the isolation of MPs by differential centrifugation has been established previously for various cell types (20). Four millilitres of plasma was centrifuged at 1,500g for 5 minutes to remove suspended cells. Afterwards, the cell-free supernatants were centrifuged at 100,000g for 20 minutes using a Centrikon T-1065 centrifuge with a TST28.38 head (Kotron Instruments, Munich, Germany) and 16x76mm centrifugation tubes (Beckman instruments, Fullerton, CA, USA). The supernatant was removed and the pellet was washed twice with 10ml apop buffer (5mM KCl, 1mM $MgCl_2$ and 136mM NaCl, pH 7.4). The MPs were then quantified and characterised by flow cytometry.

Labelling of microparticles and flow cytometry analysis (FACS)

For the differentiation and quantification of MPs derived from platelets, erythrocytes, granulocytes, monocytes, T cells, B cells and endothelial cells by fluorescence-activated cell sorting, MPs were resuspended in apop buffer containing 2.5mM $CaCl_2$ and 1% microparticle-free Foetal Calf Serum (FCS) to a final volume of 500µl as previously described (20). MPs were incubated for 20 minutes at room temperature in the dark with CD42 antibodies for the detection of platelet derived MPs, anti-human CD235 antibodies for erythrocytes, anti human CD66b antibodies for neutrophils, anti-human CD14 antibodies for monocytes, anti-human CD3 antibodies for T cells, anti-human CD19 antibodies for B cells (all antibodies from Becton Dickinson, Basel, Switzerland) or anti-human CD144 antibodies for endothelial cells (Serotec, Dusseldorf, Germany), all of which were labelled with fluoresceine isothiocyanate in the concentrations recommended by the manufacturer. Unbound antibodies were removed with two washes at 100,000g for 20 minutes. Staining with isotype-matched irrelevant antibodies at the same concentration and under the same conditions were used as controls. After the final washing step, MPs were resuspended in 500µl apop buffer,

Table I. The median number ±DS of MPs derived from different blood cell lines in controls, KD patients before IVIG therapy and KD patients after IVIG therapy.

	Controls $x10^5$/ml plasma	KD before IVIG $x10^5$/ml plasma	*p*-value	KD after IVIG $x10^5$/ml plasma	*p*-value
CD 42	3±0.9	13±0.3	0.028	3±0.2	0.03
CD 144	45±0.4	76±0.7	0.02	9±0.4	0.01
CD 235	7±0.3	26±5	0.007	21±2	0.2
CD 3	3±0.9	72±1	0.005	43±1	0.02
CD 66	10±4	11±4	0.3	14±7	0.3
CD 19	20±0.8	17±0.4	0.7	7±0.3	0.02
CD 14	6±4	7±0.4	0.9	2±0.6	0.45

2.5mM $CaCl_2$ and 1% microparticle-free FCS. MPs were counted by measuring for 1 minute at the "high-flow" modus at the FACS Calibur flow cytometre (Becton Dickinson, Mansfield, MA), and the data were evaluated with CellQuest software (Becton Dickinson Immunocytometry System, San Jose, CA). The total number of MPs was calculated by multiplying the ratio of total volume by the measured volume. To calculate the number of MPs per millilitre of plasma, the total number of MPs was divided by 4.

Statistical analysis

Results are expressed as the mean±SEM, unless indicated otherwise. Differences in MPs count were tested between patient groups that were classified by the presence or absence of various categorical parameters, to assess the association of the disease parameters with MPs count. The Mann Witney test was used to compare KD patients *versus* controls, and the Wilcoxon Signed Rank test to compare KD patients before and after IVIG therapy. To assess the correlation of continuous variables with MPs counts, Spearman´s rank correlation coefficients were calculated. Multivariate linear regression models were applied in a stepwise forward and stepwise backward manner to determine an optimal combination of variables to predict the MPs count. Since case numbers were low relative to the examined parameters, an inclusion criterion of $p \leq 0.1$ was used in the stepwise forward procedure. In the stepwise backward procedure, any inclusion criterion of $p \leq 0.05$ was applied. The model was calculated including a constant term. *P*-values <0.05 were considered statistically significant.

Results

At baseline, the median number of MPs was significantly higher in KD than in controls ($193x10^5$±$0.6x10^5$ *vs*. $94x10^5$±0.9x 10^5; p=0.01). At 1 month from IVIG administration, MPs were significantly reduced in comparison to baseline ($132x10^5$±$0.4x10^5$; p=0.01).

The median number of MPs derived from the different blood cell lines is summarised in Table I.

During the acute phase of the disease, plasma *CD42-positive MPs* (platelet-derived) were markedly elevated in KD ($13x10^5$±$0.3x10^5$million/ml of plasma) when compared to controls ($3x10^5$±$0.9x10^5$ million/ml of plasma) and after 1 month from IVIG therapy, were significantly reduced ($3x10^5$±$0.2x10^5$million/ml of plasma). Therefore, there was a significant difference between the amount of CD42-positive MPs in the acute phase of KD and in controls (p=0.028); the same result was detected considering KD patients before and after IVIG therapy (p=0.03).

CD144-positive microparticles (endothelial cells-derived) were significantly higher in KD comparison to controls ($76x10^5$±$0.7x10^5$ million/ml plasma *vs*. $45x10^5$±$0.4x10^5$ million/ml plasma; p<0.02) and were significantly reduced after 1 month from IVIG treatment ($9x10^5$±$0.4x10^5$ million/ml plasma *vs*. $76x10^5$±$0.7x10^5$ million/ml plasma; p=0.01).

The number of MPs derived from red cells *CD235* (erythrocytes-derived) was substantially higher in KD before IVIG administration in comparison to controls ($26x10^5$±$5x10^5$ million/ml of plasma *vs*. $7x10^5$±$0.3x10^5$ million/ml plasma; p<0.007) and were not changed by treatment ($26x10^5$±$5x10^5$ million/ml of plasma *vs*. $21x10^5$±$2x10^5$ million/ml of plasma; p=0.2).

CD3-positive MPs (T cells-derived) were significantly higher in KD than in controls ($72x10^5$±$1x10^5$ million/ml plasma *vs*. $3x10^5$±$0.9x10^5$ million/ml plasma; p=0.005) and were significantly reduced after 1 month from IVIG therapy ($72x10^5$±$1x10^5$ million/ml plasma *vs*. $43x10^5$±$1x10^5$ million/ml plasma; p=0.02).

In KD, at baseline, plasma median value of *CD19-positive* MPs (B cells-derived) was $17x10^5$±$0.4x10^5$ million/ml plasma and $7x10^5$±$0.3x10^5$ million/ml plasma after IVIG therapy, while it resulted $20x10^5$±$0.8x10^5$ million/ml plasma in controls. No significant difference was found in KD before IVIG therapy and controls (p=0.7). A significant decrease in CD19-positive MPs after IVIG therapy (p=0.02) was observed.

The median number of *CD66-positive* MPs (neutrophils-derived) was $11x10^5$±$4x10^5$ million/ml plasma in KD before IVIG, and $14x10^5$±$7x10^5$ million/ml plasma after IVIG therapy ($10x10^5$±$4x10^5$ million/ml in controls). No significant difference was found between children with acute KD and controls (p=0.3), and KD children before and after IVIG therapy (p=0.3).

At baseline, the median number of *CD14-positive* MPs (monocytes-derived) was $7x10^5$±$0.4x10^5$ million/ml plasma (p=0.9), while after IVIG therapy resulted $2x10^5$±$0.6x10^5$ million/ml ($6x10^5$±$4x10^5$ million/ml in controls). No significant difference was found between children with acute KD and controls (p=0.9), and KD children before and after IVIG therapy (p=0.45).

The correlation of concomitant diseases with the increased numbers of MPs in KD patients was searched. All other established factors confounding clinical parameters that could have influenced the results, such as diabetes, hypercholesterolemia and hyperlipidemia were excluded. In Table II, the median values of acute-phase markers are reported. The analysis of conventional acute-phase markers did not show any

correlation with the number of total MPs (R-squared=0.07). No significant association between the total number of MPs and coronary alterations, congiuntivitis, rash, mucositis, lymphoadenopathy, extremities changes, acute phase reactants elevation, white blood cells was observed through multivariate analysis (Table III).

Univariate analysis performed between the MPs levels of various origin and coronary alterations prior IVIG showed a positive correlation for CD144, CD42, CD235, CD19, CD14 this results were unmodified after IVIG (Table IV).

Table II. Laboratory values.

Variable	Values	Day
Age of onset (months)	17.24±14.8	
Day of fever (days)	8.6±4.54	
Erythrocyte sedimentation rate (mm/hr)	76±27.9	5.4±2.7
C-reactive protein (mg/dl)	9.3±5.7	5.9±3.1
Platelets (per mm^3)	492.4±176.3	5.8±2.9
Haemoglobin (g/dl)	10.2±1.6	6.1±2.8
Sodium (mEq/L)	133±4.6	5.5±3.8
Fibrinogen (mg/dl)	837±233	5.7±3.2

Discussion

This is the first study showing that MPs derived from endothelial cells, platelets, erythrocytes, and T cells are significantly elevated in KD, and that MPs plasma levels are reduced by IVIG treatment. Endothelium and T lymphocytes resulted the major source of MPs. Endothelial MPs are novel subcellular elements that may contribute to heighten endothelial dysfunction by enhancing intercellular communication, transferring cytokine receptors among cells, activating complement, promoting leukocyte rolling, and stimulating the release of proinflammatory mediators (21). In addition, endothelial cells derived MPs participate in spreading and amplifying procoagulant cellular response, like tissue factor antigen exposure by monocytic cells (22). According to recent data endothelium is the primary target of KD (23), and therefore MPs could be a marker of monitoring KD vascular injury. Our data also suggest that MPs might be a sign of response to therapy as shown by their reduction after IVIG administration.

In adults, the increase of endothelial cells derived MPs in atherosclerosis, multiple sclerosis, and systemic lupus erythematosus has been reported as an apoptosis or endothelium activation outcome (24-26). In another study, markedly elevated endothelial cells derived MPs supported the diagnosis of Churg-Strauss vasculitis-induced cardiomyopathy (27)

In our study, the paediatric population not charged with other factors involved in endothelial damage (as smoking) is ideal to investigate the endothelium.

Recently, there ha been increasing evidence that KD may predispose to subclinical premature atherosclerosis in adulthood (28). Moreover, an adverse cardiovascular risk profile (low HDL and apo-A1 but high apo-B1 levels), and increased peripheral arterial stiffness after resolution of the acute inflammation has been recognised in KD children, especially in those with coronary damage (29). Given that MPs have been recently isolated in atherosclerotic plaques and in view of their functional properties, they could have a role in the development of early atherosclerosis (30).

Recent studies have outlined the key role of MPs, in particular platelet derived MPs, in promoting coagulation since they provide a negatively charged surface to bind clotting factors. Platelet derived MPs bind to subendothelial matrix and act as a substrate for further platelet binding, promoting platelet adhesion to the site of endothelial injury. Platelet derived MPs also influence endothelial cell function and promote monocyte-endothelial interactions during inflammation (31).

In KD, prothrombotic profile induced by endothelial sufferance along with increased circulating platelet derived MPs may promote coagulation.

After IVIG therapy, levels of platelets derived MPs significantly decrease. No correlation between the median MPs number and markers of acute-phase, like ESR and CRP values was detected. Similarly, no correlation was found between circulating MPs and coronary damage. These negative data could be due to the small number of children enrolled in the study and to the small cohort of children studied, in particular to the small number of children who developed coronary artery involvement. Conversely, in another study (32) a significant positive correlation between endothelial cells derived MPs and the Birmingham Vasculitis Activity Score (BVAS), and the acute phase parameters was found in patients with systemic vasculitis (32, 33). It is difficult to explain the difference of the results, though the enrolled population included adults with different endothelial risk factors, like smoking and drinking.

In conclusion, our study shows that circulating levels of MPs, in particular endothelial cells, platelet and T cells derived MPs, are significantly increased in the acute phase of KD, before IVIG therapy and reduced at one month from IVIG administration. Thus, MPs could contribute to the pathogenesis of vessel damage amplifying inflammation. In the future, larger cohorts of KD patients

Table III. Results of multivariate analysis with the total number of MPs.

Variables	R-squared
Coronary alterations	0.41
Acute phase reactants elevation	0.05
Congiuntivitis	0.35
Rash	0.39
Mucositis	0.39
Lymphoadenopathy	0.40
Extremities changes	0.42
White blood cells	0.43

Table IV. Results of univariate analysis between the MPs levels of various origin and coronary alterations before and after IVIG.

MPs	R-squared Prior IVIG	R-squared Post IVIG
CD42	0.05	0.07
CD144	0.01	0.004
CD235	0.002	0.002
CD3	0.05	0.05
CD66	0.15	0.15
CD19	0.03	0.03
CD14	0.01	0.01

need to be studied in order to provide MPs as useful biomarkers of inflammation and vascular injury in the KD.

References

1. NEWBURGER JW, MASATO T, GERBER MA GEWITZ MH, TONI LY, BURNS JC: COMMITTEE ON RHEUMATIC FEVER, ENDOCARDITIS, AND KAWASAKI DISEASE; COUNCIL ON CARDIOVASCULAR DISEASE IN THE YOUNG; AMERICAN HEART ASSOCIATION: Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation* 2004; 110: 2747-71.
2. CURTIS N: Kawasaki disease. *BMJ* 1997; 315: 322-3.
3. BURNS JC, GLODE MP: Kawasaki syndrome. Lancet 2004; 364: 533-44.
4. TAUBERT KA: Epidemiology of Kawasaki disease in the United States and worldwide. *Prog Pediatr Cardiol* 1997; 6: 181-5.
5. TERAI M, SHULMAN ST: Prevalence of coronary artery abnormalities in Kawasaki disease is highly dependent on gammaglobulin dose but independent of salycilate dose. *J Pediatr* 1997; 131: 888-93.
6. DAJANI AS, TAUBERT KA, GERBER MA *et al*.: Diagnosis and therapy of Kawasaki disease in children. *Circulation* 1993; 87: 1776-80.
7. EBERHARD BA, ANDERSON U, LAXER RM, ROSE V, SILVERMAN ED: Evaluation of cytokine response in Kawasaki *Disease*. *Pediatr Infect Dis J* 1995; 14: 199-203.
8. NAOE S. TAKAHASHI K, MASUDA H, TANAKA N: Kawasaki disease. With particular emphasis on arterial lesions. *Acta Pathol Jpn* 1991; 41: 785-97.
9. JEANETTE JC, SCIARROTA J, TAKAHASHI K: Predominance of monocytes and macrophages in the inflammatory infiltrates of acute Kawasaki disease arteritis. *Pediatr Res* 2002; 53: 94A.
10. SHULMAN ST, ROWLEY AH: Advances in Kawasaki disease. *Eur J Pediatr* 2004; 163: 285-91.
11. ROWLEY AH, SHULMAN ST, GARCIA FL *et al*.: Cloning the arterial IgA antibody response during acute Kawasaki disease. *J Immunol* 2005; 175: 8386-91.
12. BOWN TJ, CRAWFORD SE, CORNWALL ML, GARCIA F, SHULMAN ST, ROWLEY AH: CD8 T limphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. *J Infect Dis* 2000; 182: 1183-91
13. GAVIN PJ, CRAWFORD SE, SHULMAN ST, GARCIA FL, ROWLEY AH: Systemic arterial expression of matrix metalloproteinases 2 and 9 in acute Kawasaki disease. *Arterioscler Thromb Vasc Biol* 2003; 23: 576-81.
14. ZWAAL RFA, SCHROIT AJ: Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood* 1997; 89: 1121-32.
15. DISTLER JHW: Microparticles as regulators of inflammation. *Arthritis Rheum* 2005; 52: 3337-48.
16. DISTLER JHW, HUBER LC, GAY S, DISTLER O, PISETSKY DS: Microparticles as mediators of cellular cross-talk in inflammatory disease. *Autoimmunity* 2006; 39: 683-90.
17. NAUTA AJ, TROUW LA, DAHA MR *et al*.: Direct binding of C1q to apoptotic cells and cells blebs induces complements attivation. *Eur J Immunol* 2002; 32: 1726-36.
18. ROWLEY AH, GONZALES CRUSSI F, GIDDING SS, DUFFY CE, SHULMAN ST: Incomplete Kawasaki Disease with coronary artery involvement. *J Pediatr* 1987; 110: 409-13.
19. DE ZORZI A. COLAN SD, GAUVREU K, BAKER AL, SUNDEL RP, NEWBURGER JW: Coronary artery dimensions may be misclassified as normal in Kawasaki Disease. *J Pediatr* 1998; 133: 254-8.
20. DISTLER JH, JUNGEL A, HUBER LC *et al*.: The induction of matrix metalloproteinase and cytokine expression in synovial fibroblasts stimulated with immune cell microparticles. *Proc Natl Acad Sci USA* 2005; 102: 2892-7.
21. JIMENEZ J, JY W, MAURO LM, SODERLAND C, HORSTMAN C, AHN YS: Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. *Thromb Res* 2003; 109: 175-80.
22. SATTA N, TOTI F, FENGEAS O *et al*.: Monocyte vesiculation is a possible mechanism for dissemination of membrane-associated procoagulant activities and adhesion molecules after stimulation by lipopolysaccharide. *Immunol* 1994; 153: 3245-55.
23. BOOTH AD, WALLACE S, MCENIERY CM, YASMIN BJ, JAYNE DR, WILKINSON IB: Inflammation and arterial stifness in systemic vasculitis: a model of vascular inflammation. *Arthritis Rheum* 2004; 50: 581-8.
24. MINAGAR A, JY W, JIMINEZ JJ: Elevated plasma endothelial microparticles in multiple sclerosis. *Neurology* 2001; 56: 1319-24.
25. BACON PA: Endothelial cell dysfunction in systemic vasculitis: new developments and therapeutic prospects. *Curr Opin Rheumatol* 2005; 17: 49-55.
26. COMBES V, SIMON AC, GRAU GE *et al*.: In vitro generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. *J Clin Invest* 1999; 104: 93-102.
27. KÜMPERS P, ERDBRÜGGER U, GROSSHEIM M *et al*.: Endothelial microparticles as a diagnostic aid in Churg-Strauss vasculitis-induced cardiomyopathy. *Clin Exp Rheumatol* 2008; 26: S86-9.
28. CHEUNG YF, YUNG TC, TAM SC, HO MH, CHAU AK: Novel and traditional cardiovascular risk factors in children after Kawasaki disease: implications for premature atherosclerosis. *J Am Coll Cardiol* 2004; 43: 120-4.
29. SENZAKI H, CHEN CH, ISHIDO H *et al*.: Arterial hemodynamics in patients after Kawasaki disease. *Circulation* 2005; 111: 2119-25.
30. MALLAT Z, HUGEL B, OHAN J, LESECHE G, FREYSSINET A, TEDGUI A: Shed membrane microparticles with procoagulant potential in human atherosclerotic plaque: a role for apoptosis in plaque thrombogenicity. *Circulation* 1999; 99: 348-53.
31. BARRY OP, PRATICÒ D, SAVANI RC, FITZGERALD GA: Modulation of Monocyte-Endothelial Cell Interactions by platelet microparticles. *J Clin Invest* 1998; 102: 136-44.
32. LUQMANI RA, BACON PA, MOOTS RJ *et al*.: Birmingham Vasculitis Activity Score in systemic necrotizing vasculitis. *QJM* 1994; 87: 671-8.
33. BROGAN PA, SHAH V, BRACHET C *et al*.: Endothelial and platelet microparticles in vasculitis of the young. *Arthritis Rheum* 2004; 50: 927-36.