
Endothelial microparticles as a diagnostic aid in Churg-Strauss vasculitis-induced cardiomyopathy

P. Kümpers¹, U. Erdbrügger¹, M. Grossheim¹, G.P. Meyer², M. Hiss¹, W. Gwinner¹, H. Haller¹, M. Haubitz¹

¹Department of Nephrology and

²Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany.

Philipp Kümpers, MD; Uta Erdbrügger, MD; Marieke Grossheim; Gerd Peter Meyer, MD; Marcus Hiss, MD; Wilfried Gwinner, MD; Hermann Haller, MD; Marion Haubitz, MD.

Please address correspondence to:

Philipp Kümpers MD, Department of Nephrology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany.

E-mail: keumpers.philipp@mh-hannover.de

Received on October 18, 2007; accepted in revised form on April 24, 2008.

Clin Exp Rheumatol 2008; 26 (Suppl. 49): S87-S90.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2008.

Key words: ANCA-associated vasculitis, Churg-Strauss syndrome, endothelial microparticles, cardiomyopathy, coronary vasculitis.

ABSTRACT

Churg-Strauss Syndrome (CSS) is characterized by allergic rhinitis, asthma and prominent blood and tissue eosinophilia. Although CSS can affect any organ system, isolated cardiac manifestation is a rare feature that is often characterized by rapidly progressive congestive heart failure. We present the case of a 48-year-old woman with acute dyspnoea and chest pain. Her past medical history was significant for asthma and frequently relapsing minimal-change glomerulonephritis. Echocardiogram and coronary angiography revealed cardiomyopathy and coronary small-vessel vasculitis in the presence of blood eosinophilia and elevated IgE. In the absence of infective agents, neoplastic diseases and further vasculitic manifestations, a flow cytometry-based analysis of markedly elevated endothelial microparticles supported the diagnosis of CSS. Cardiomyopathy resolved completely after initiation of immunosuppressive treatment with corticosteroids and cyclophosphamide pulses. Elevated endothelial, leukocytic and platelet-derived microparticles decreased during follow-up and closely paralleled vasculitic activity. Endothelial microparticles might be an additional tool to diagnose and monitor cases of suspected vasculitic cardiac involvement in CSS.

Introduction

Churg-Strauss syndrome (CSS) is characterized by blood and tissue eosinophilia, extravascular granuloma formation, and necrotizing vasculitis of small to medium sized blood vessels. CSS develops typically in patients with asthma and allergic rhinitis and can affect various organ systems, including the cardiovascular, gastrointestinal, renal, and central nervous system (1). Diagnosis of CSS might be difficult since

various allergic, infectious, neoplastic and idiopathic diseases may present with blood as well as tissue eosinophilia, mimicking CSS. Unfortunately, clinical manifestations of CSS can even occur in a single organ, thus obscuring the diagnosis (2). Early recognition and treatment of atypical organ involvement are essential to prevent the potentially devastating complications of this disorder: congestive heart failure, gastrointestinal ischemia, cerebral hemorrhage, renal failure and others. Although CSS responds favorably to high-dose corticosteroid therapy, the presence of severe gastrointestinal, renal or myocardial involvement appears to be associated with a poor clinical outcome and requires the addition of cyclophosphamide (3).

We have earlier established the enumeration of circulating endothelial cells (CECs) as a new marker of vascular damage in new-onset and relapsing ANCA associated vasculitis (4-6). CEC correlate with disease activity and help to distinguish limited granulomatous disease from systemic vasculitis (5). More recently, endothelial microparticles have been described as another new marker of acute vasculitis in a pediatric population (7). We here report the case of CSS with vasculitis-induced cardiomyopathy. Enumeration of CECs and endothelial microparticles was performed to support the diagnosis of coronary vasculitis in CSS.

Case report

A 48-year-old woman presented to our emergency room with acute dyspnoea, mild chest pain, dry cough, and increasing fatigue over the past 6 weeks. Her past medical history was significant for a 5-year history of asthma and frequently relapsing minimal-change glomerulonephritis (MCD). MCD was in remission at presentation, receiving a maintenance immunosuppressive

Competing interests: none declared.

therapy with prednisolone (5mg/day) and cyclosporine (trough level 100 ng/ml). On admission, temperature was 37.5°C, heart rate was 104 bpm, blood pressure was 110/80 mm Hg and respiratory rate was 26/min. Physical examination revealed bilateral fine crackles of the lower lobes. A chest x-ray showed neither cardiomegaly nor pulmonary congestion.

Markedly elevated cardiac enzymes (creatin kinase 550 U/l, MB fraction of 69 U/l, troponin T 2.46 µg/l (<0.01 µg/l)) in the presence of a normal 12 lead ECG lead to the preliminary diagnosis of non-ST segment elevation myocardial infarction (NSTEMI).

Coronary angiography revealed marked caliber changes in the distal position of the epicardial arteries consistent with vasculitis (Fig. 1), whereas atherosclerotic coronary heart disease was not present. Left ventricular function was moderately impaired.

A transthoracic echocardiogram demonstrated cardiomyopathy characterized by moderately reduced left-ventricular function and hypertrophy, pericardial effusion and diastolic dysfunction II° (Fig. 2).

A blood cell count showed leukocytosis of 28.3 Tsd/µl with 17.8 Tsd/µl eosinophils (63%). Pronounced eosinophilia (>60%) was also present in the bone marrow and in bronchoalveolar lavage fluid respectively. Serum IgE was 20-fold elevated, while ANCA-titres were negative. C-reactive protein was moderately increased to 60 mg/l, whereas procalcitonin was negative (0.1 µg/l) suggesting the absence of bacterial infection. Fungal, viral or hematologic neoplastic diseases, including FIP1L1/PDGFRα-mutations (chronic eosinophilic leukemia) were excluded.

Computed tomography of the chest revealed mild bilateral ground-glass opacities of the lower pulmonary lobes. Pulmonary nodules, pleural effusions or pulmonary embolism were absent. No evidence for skin, renal, gastrointestinal or neurologic manifestations of CSS were found.

Based on these facts, we established the diagnosis of ANCA-negative Churg-Strauss Syndrome with clinically prevailing cardiac involvement.

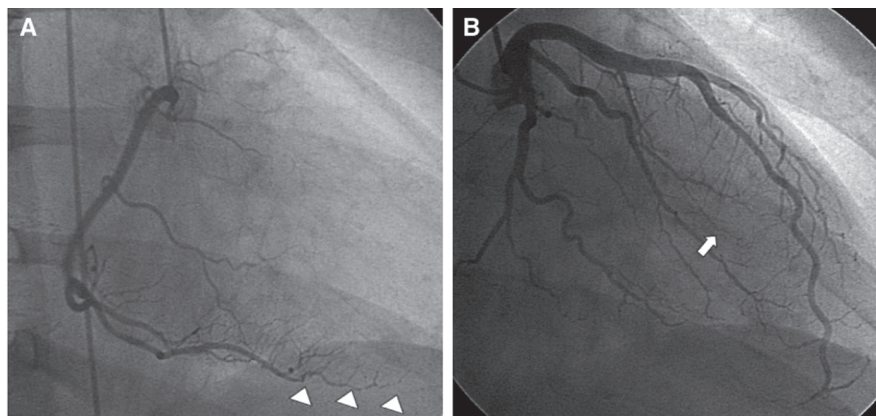


Fig. 1. Coronary angiography immediately after admission showing alterations in the caliber of several small epicardial vessels of **A)** the distal posterior interventricular branch of the RCA and **B)** a distal marginal branch of the LCA, that were suggestive of vasculitis. Left ventricular function was moderately impaired. Coronary heart disease with atherosclerotic disease was not present.

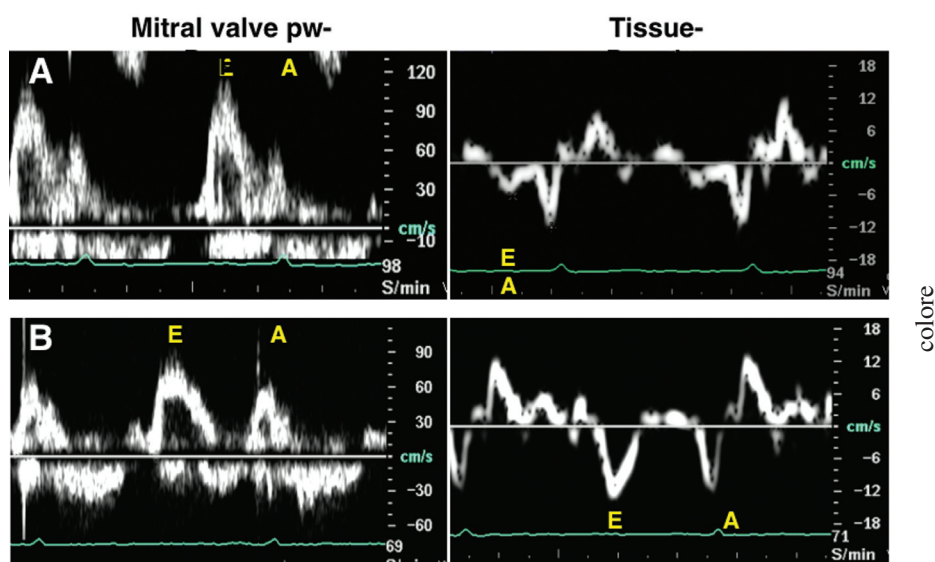


Fig. 2. Echocardiogram showing mitral valve pw-Doppler (left panel) and tissue Doppler mitral annulus velocities (right panel), recorded **(A)** before and **(B)** 4 weeks after the start of immunosuppressive treatment. **A)** Grade II diastolic dysfunction is indicated by a pseudo(normal) filling pattern. **B)** Diastolic dysfunction was no longer detectable at follow-up, as indicated by E/A-ratio >1 (TDI).

High-dose corticosteroids (3 x 500mg prednisolone i.v.) resulted in a rapid normalization of blood eosinophilia within 36h. Left ventricular systolic and diastolic dysfunction resolved during three monthly pulses of intravenous cyclophosphamide (750mg/m² i.v.) (Figs. 2 and 3). During a 10-month follow-up, no recurrence occurred on maintenance therapy with prednisolone and azathioprine.

Methods

To investigate coronary vasculitis in spite of immunosuppression for relapsing MCD (8), which was apparently

inefficacious to prevent the new onset of CSS, we measured disease activity by enumeration of circulating endothelial cells (CECs) (4, 5) as well as flow cytometry-based analysis of endothelial, leukocytic and platelet derived microparticles (MPs) (7). Results from the patient were compared to those of healthy voluntary employees (n=7) from the Department of Nephrology at Hannover Medical School. The study was done in accordance with the declaration of Helsinki. Scientific use of the samples was approved by the institutional review board and informed consent was obtained. Disease activity

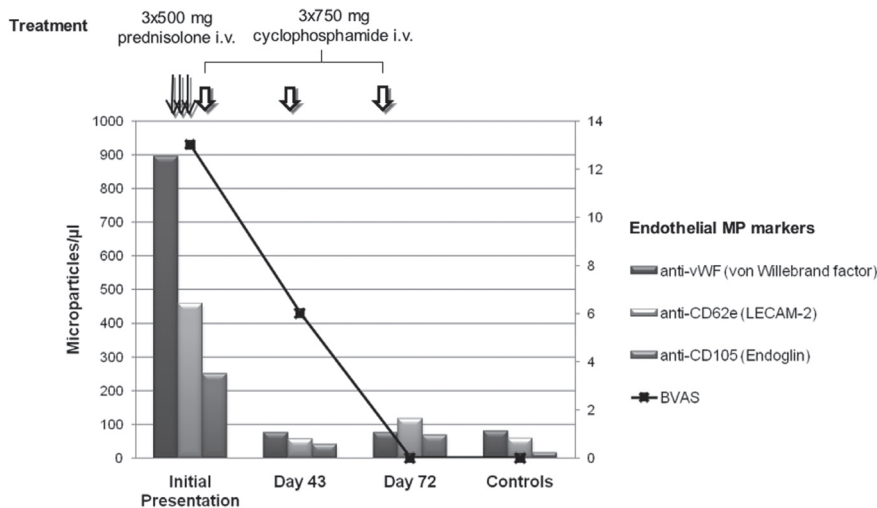


Fig. 3. Course of endothelial microparticles analysed by flow cytometry (bars). Initially elevated microparticles decreased during follow-up and closely paralleled the course of the Birmingham vasculitis activity score (line). Healthy voluntary employees (n=7) from the Department of Nephrology at Hannover Medical School served as controls.

Table I. Enumeration of microparticles and circulating endothelial cells.

	Initial Presentation	Day 43	Day 72	Controls (Mean ± SD)
BVAS	13	6	0	-
CECs /ml	24	20	8	9.33 ± 4.13
Platelet MP markers /µl				
anti-CD42a (platelet glycoprotein)	2420.36	105.99	162.49	108.59 ± 64.3
anti-CD62p (Selectin-P)	535.50	36.50	15.37	27.02 ± 16.30
Leukocytic MP markers /µl				
anti-CD11b (Integrin αM)	429.46	55.26	79.78	27.62 ± 16.70
anti-CD45 (Leukocyte common AG)	564.66	58.99	63.28	49.78 ± 3.91
Endothelial MP markers /µl				
anti-CD62e (LECAM-2)	458.62	55.60	116.62	58.45 ± 40.78
anti-CD105 (Endoglin)	249.19	39.66	66.90	15.48 ± 13.43
anti-vWF (von Willebrand factor)	896.04	75.37	75.00	79.79 ± 57.77

was assessed in accordance with the Birmingham Vasculitis Activity Score (BVAS).

Counting of circulating endothelial cells

CECs were isolated and enumerated as described in detail elsewhere (4, 5). Briefly, anti-CD146 coated M-450 Dynabeads were obtained and stored at 4°C for a maximum of 4 weeks. Venous blood (1 ml) was mixed with 1 ml buffer (phosphate buffered saline, 0.1% bovine serum albumin, 0.1% sodium azide, and 0.6% sodium citrate) at 4°C. FcR blocking agent (20 µl) and 50 µl antibody coated Dynabeads (10 µg/ml) were added and mixed thoroughly. Next, the sample was mixed

for 30 minutes at 4°C and washed with buffer four times inside a magnet at 4°C. Between each washing procedure, the sample was flushed 10 times with buffer in a 100 µl pipette. Ulex Europaeus lectin 1 (UEA-1) solution (100 µl; 2 mg/ml) was added and incubated for 1 hour in darkness. The sample was washed twice and the cell-bead suspension finally dissolved in 200 µl buffer. Cells were counted with fluorescence microscopy using a Nageotte chamber. Conglomerates were counted as one cell.

Flow cytometry analysis of microparticles

Platelet-poor plasma was ultracentrifuged to obtain microparticle pellets.

Pellets were stained with Annexin V, labeled with surface markers of endothelial cells (vWF, CD105, CD62e), leukocytes (CD45, CD11b) and platelets (CD42a, CD62p) and detected using flow cytometry. True count beads were used to optimize quantification of MPs (7). A more detailed description of the methods is given elsewhere (5-7).

Results

CECs in our patient were only slightly elevated at initial presentation (24 CECs/ml (normal range <20 CECs/ml,)) but steadily decreased during treatment at day 43 (20 CECs/ml) and day 72 (8 CECs/ml) (Table I). Healthy controls (n=7) had 9.3±4.1 CECs/ml. Microparticles in our patient were markedly elevated at initial presentation compared to healthy controls (Platelet MPs ~21 fold, leukocytic MPs >13 fold and endothelial MPs ~12 fold) (Table I). Microparticles rapidly decreased during follow-up and closely paralleled the course of the Birmingham vasculitis activity score (Table I and Fig. 3).

Discussion

Cardiac involvement in CSS has been reported, but spectrum of cardiac manifestations, prevalence and diagnostic approaches remain controversial. In 1951 Jacob Churg and Lotte Strauss already noted cardiac failure as the leading cause of death in 3 of 11 (27%) patients. They found eosinophilic inflammation of the myocardium and coronary small-vessel vasculitis in ~60% of the patients at autopsy. In two other series, ~50% of deaths were attributable to cardiovascular disease in CSS (9, 10). Kane *et al.* identified 22 patients (17%) with cardiac involvement (not further specified) among 132 CSS patients that were treated at the Mayo Clinic between 1990 and 2006. Most of them (72%) developed myocardial dysfunction. Female sex, younger age, severe peripheral eosinophilia and lower prevalence of pANCA were associated with cardiac involvement (11). All of these risk factors were present in our patient.

However, CSS with predominant cardiac involvement and subsequent cardiomyopathy or myocardial infarction due

to coronary vasculitis has only been reported in two patients. A myocardial biopsy revealed necrotizing myocardial vasculitis in the first, whereas coronary angiography demonstrated coronary vasculitis in the second case (12, 13). The endothelial layer plays a central pathogenetic role in systemic vasculitides such as the Churg Strauss Syndrome. Circulating endothelial cells (CEC) as a marker of endothelial damage have been shown to be elevated in active ANCA-associated vasculitis (4, 5). More recently, microparticles (MP) were found to be increased in systemic vasculitis in children and adults (7, 14). Release of MPs is triggered by various stimuli as cell activation, apoptosis, cell lysis or oxidative stress. They may originate from vascular endothelium and other circulating blood cells by blebbing and shedding from cell membrane surfaces (15). MPs carry surface markers of the parent cell which is used to detect them by flow cytometry. In our patient, total amount of MPs were dramatically increased on initial presentation, decreased during follow-up and closely paralleled the course of disease activity, gauged by the Birmingham vasculitis activity score. Subgroup analysis showed that these microparticles derived from endothelial cells, leukocytes and platelets. This finding is consistent with data from children with active vasculitis and that of adults (Daniel) which also supported our diagnosis (7, 14). Interestingly, CECs were only slightly elevated in our patient at initial presentation, comparable to the values

seen in patients with Wegener's granulomatosis of the respiratory tract (5). In contrast, CECs are markedly elevated in active systemic AAV (4). As leukocytic MP can be increased in infection, endothelial MP may be the more reliable marker of vascular damage in vasculitis. Nevertheless, larger cohort studies are needed to analyze the diagnostic value of endothelial MPs and other subclasses as platelet and leukocytic derived MP in new onset and relapsing AAV.

As a novel diagnostic tool, endothelial MPs can repeatedly be measured during follow-up compared to invasive procedures like coronary angiography and myocardial biopsy. As far as we know this is the first case of CSS where these novel markers of endothelial damage were used to support diagnosis and monitoring of the disease.

Acknowledgments

We are indebted to Kristin Wyss and Barbara Hertel for their excellent technical assistance.

References

1. CHURG J, STRAUSS L: Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. *Am J Pathol* 1951; 27: 277-301.
2. SPECKS U, DEREMEE RA: Granulomatous vasculitis. Wegener's granulomatosis and Churg-Strauss syndrome. *Rheum Dis Clin North Am* 1990; 16: 377-97.
3. GUILLEVIN L, COHEN P, GAYRAUD M, LHOE F, JARROUSSE B, CASASSUS P: Churg-Strauss syndrome. Clinical study and long-term follow-up of 96 patients. *Medicine (Baltimore)* 1999; 78: 26-37.
4. WOYWODT A, STREIBER F, DE GROOT K, REGELSBERGER H, HALLER H, HAUBITZ M: Circulating endothelial cells as markers for ANCA-associated small-vessel vasculitis. *Lancet* 2003; 361: 206-10.
5. WOYWODT A, GOLDBERG C, KIRSCH T *et al.*: Circulating endothelial cells in relapse and limited granulomatous disease due to ANCA associated vasculitis. *Ann Rheum Dis* 2006; 65: 164-8.
6. ERDBRUEGGER U, HAUBITZ M, WOYWODT A: Circulating endothelial cells: a novel marker of endothelial damage. *Clin Chim Acta* 2006; 373: 17-26.
7. BROGAN PA, SHAH V, BRACHET C *et al.*: Endothelial and platelet microparticles in vasculitis of the young. *Arthritis Rheum* 2004; 50: 927-36.
8. HAUBITZ M, KOCH KM, BRUNKHORST R: Cyclosporin for the prevention of disease reactivation in relapsing ANCA-associated vasculitis. *Nephrol Dial Transplant* 1998; 13: 2074-6.
9. CHUMBLEY LC, HARRISON EG JR, DEREMEE RA: Allergic granulomatosis and angiitis (Churg-Strauss syndrome). Report and analysis of 30 cases. *Mayo Clin Proc* 1977; 52: 477-84.
10. HASLEY PB, FOLLANSBEE WP, COULEHAN JL: Cardiac manifestations of Churg-Strauss syndrome: report of a case and review of the literature. *Am Heart J* 1990; 120: 996-9.
11. KANE GC, CONNOLLY HM, SPECKS U, KEOGH KA: Churg-Strauss syndrome involving the heart: Incidence and clinical characteristics. *Clin Exp Rheumatol* 2007; 25: 100.
12. HELLEMANS S, DENS J, KNOCKAERT D: Coronary involvement in the Churg-Strauss syndrome. *Heart* 1997; 77: 576-8.
13. KOZAK M, GILL EA, GREEN LS: The Churg-Strauss syndrome. A case report with angiographically documented coronary involvement and a review of the literature. *Chest* 1995; 107: 578-80.
14. DANIEL L, FAKHOURI F, JOLY D *et al.*: Increase of circulating neutrophil and platelet microparticles during acute vasculitis and hemodialysis. *Kidney Int* 2006; 69: 1416-23.
15. BOULANGER CM, AMABILE N, TEDGUI A: Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. *Hypertension* 2006; 48: 180-6.