The relationship between plasma microparticles and disease manifestations in patients with systemic sclerosis


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Abstract

OBJECTIVE: Microparticles are small, membrane-coated vesicles that can serve as novel signaling structures between cells. The aim of the present study was to analyze the profile of microparticles in the blood of patients with systemic sclerosis (SSc; scleroderma) and healthy controls. METHODS: The study population consisted of 37 patients with SSc and 15 healthy subjects of comparable sex and age. Microparticles were isolated from plasma by high-speed differential centrifugation. Microparticles were stained with monoclonal antibodies against cell type-specific markers and were quantified by fluorescence-activated cell sorting analyses. RESULTS: The total number of microparticles was strongly increased in patients with SSc compared with healthy controls (mean +/- SEM 88.0 +/- 4.8 x 10^5 microparticles/ml plasma versus 42.3 +/- 9.4 x 10^5 microparticles/ml plasma; P < 0.001). Similarly, significant increases were found for microparticles derived from platelets, endothelial cells, monocytes, and T cells, reflecting the activation of these cells in SSc. Platelets were the most common source of microparticles in the blood of patients with SSc (66.9 +/- 5.2% of all microparticles) and healthy donors, followed by microparticles derived from endothelial cells (8.8 +/- 0.9% in SSc patients). The modified Rodnan skin thickness score (MRSS) was inversely correlated with the total number of microparticles. Furthermore, patients with cutaneous ulcers showed a significantly lower total number of microparticles. In multivariate analysis, an additive model of age, C-reactive protein, MRSS, and subtype of disease accounted for 55% of the variability of the total microparticle count (r = 0.744). CONCLUSION: The number of microparticles from different cellular sources is increased in the blood of SSc patients. Considering their role as important mediators of intercellular communication, microparticles could be a novel link between activated cellular compartments in the pathogenesis of SSc.
THE RELATIONSHIP BETWEEN PLASMA MICROPARTICLES AND DISEASE MANIFESTATIONS IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Abstract

Objective: Microparticles are small membrane-coated vesicles that can serve as novel signaling structures between cells. The aim of the present study was to analyze the profile of microparticles in the blood of patients with systemic sclerosis (SSc) and healthy controls.

Methods: The study population consisted of 37 patients with SSc and 15 healthy subjects of comparable sex and age. Microparticles were isolated from plasma by high speed differential centrifugation. Microparticles were stained with monoclonal antibodies against cell type specific markers and quantified by FACS analyses.

Results: The total number of microparticles per ml plasma was strongly increased in patients with SSc compared to healthy controls (88.0 ± 4.8 x 10^5 microparticles versus 42.3 ± 9.4 x 10^5; p < 0.001). Similarly, significant increases were found for microparticles derived from platelets, endothelial cells, monocytes and T cells, reflecting the activation of these cells in SSc. Platelets were the most common source of microparticles in the blood of patients with systemic sclerosis and healthy donors (SSc: 66.9 ± 5.2% of all microparticles), followed by microparticles derived from endothelial cells (SSc: 8.8 ± 0.9%). The modified Rodnan skin score was inversely correlated with the total number of microparticles. Furthermore, patients with cutaneous ulcers showed significantly lower number of total microparticles. In multivariate analysis, an additive model of age, CRP, modified Rodnan skin score and discrimination of diffuse or limited SSc turned out to account for 55% of the variability of the total microparticle count (r = 0.744).

Conclusion: The number of microparticles from different cellular sources is increased in the blood of SSc patients. Considering their role as important mediators
of intercellular communication, microparticles could be a novel link between activated cellular compartments in the pathogenesis of SSc.
Introduction

Systemic sclerosis (SSc) is a connective tissue disease of unknown etiology, which affects the skin and various internal organs. SSc is characterized by an activation of immune cells, peripheral vasculopathy, the occurrence of autoantibodies, and tissue fibrosis. The clinical manifestations of SSc are the result of a complex interaction of a variety of different cell types. Endothelial cell damage with apoptosis resulting in the loss of capillaries is considered as one of the earliest changes in the pathogenesis of the disease (1). There are also inadequate attempts to compensate for the reduced capillary density leading to the formation of tortuous, bushy and giant capillaries (2). Activation of the immune system with the formation of perivascular infiltrates is another early feature of SSc. These infiltrates are dominated by T cells and are thought to contribute to the activation of fibroblasts by the release of profibrotic cytokines such as IL-4 (3).

Microparticles are a heterogeneous population of small, membrane-coated vesicles. They are released from a variety of cells during activation and apoptosis via an exocytic budding process. Long considered as inert debris, microparticles are now appreciated as important mediators of cellular crosstalk (4). Microparticles expose an array of antigens from their parental cells on their surface that can be used to identify the cell types from which they originate. Microparticles regulate inflammation, stimulate coagulation, affect vascular functions and have also been implicated in apoptosis and cell proliferation (4). Interestingly, increased numbers of microparticles are found in systemic inflammatory conditions such as sepsis (5) and multiple sclerosis (6). Recently, microparticles have also been suggested as markers of endothelial cell damage in vasculitis and acute coronary syndromes (5, 7).

Since both inflammation and endothelial cell damage are hallmarks of SSc, we speculated that the number of microparticles might be elevated in SSc. To test this
hypothesis, microparticles derived from different cell types were isolated from the plasma of patients with diffuse and limited SSc, and the microparticle count was compared to that of healthy individuals. In addition, we searched for associations of microparticles with major clinical manifestations of SSc. Results of these studies indicate that levels of microparticles are elevated in the blood of patients with SSc. The inverse correlation with skin fibrosis suggests that microparticles may attenuate fibrotic disease manifestations.
Material and methods

Patients

Thirty-seven consecutive, unselected patients with SSc were recruited at the Department of Rheumatology of the University of Florence. All patients fulfilled the American College of Rheumatology criteria for SSc (8). There were 33 women and 4 men with a median age of 63 years (range 29-82 years). Patients with overlap syndromes/mixed connective tissue disease and other connective tissue diseases were excluded from the study.

Healthy volunteers (n = 15) were used as controls. The control group consisted of 12 women and 3 men with a median age of 55 years (range 42–70 years). All patients and controls were of Caucasian origin. All patients signed a consent form approved by the ethical committee.

Clinical assessment

An extensive clinical profile was established for each SSc patient. Patients’ characteristics are summarized in Table 1. SSc patients were classified as limited or diffuse SSc according to the criteria proposed by LeRoy et al. (9). The presence of fingertip ulcers at the time of blood drawing, other skin ulcers (“cutaneous ulcers”, e.g. at the lower extremities, elbows, forearms), teleangiectasias and disease duration since first non Raynaud symptoms were recorded. All patients reported the occurrence of Raynaud's phenomenon after exposure to low temperatures. The modified Rodnan skin score was assessed by the same experienced rheumatologist in all patients at 17 body areas by clinical palpation and was rated 0–3, with a maximum total score of 51. Disease activity was measured according to Valentini et al (10).
Nailfold videocapillaroscopy was performed in a blinded manner for the analysis of microvascular abnormalities. Patients were allowed to adapt to room temperature (20–22°C) for at least 15 min before the examination was started. The nailfolds of all 10 fingers were analyzed for the following parameters: presence of enlarged and giant capillaries, pericapillary edema, hemorrhages, loss of capillaries, ramified/bushy capillaries and disorganization of the vascular distribution. According to these features, patients were grouped into capillaroscopy changes with an early, active and late pattern using the criteria proposed by Cutolo et al. (11). The early pattern included the criteria of few giant capillaries and capillary hemorrhages, relatively well preserved capillary distribution and no evident loss of capillaries. The criteria for the active pattern were frequent capillary hemorrhages and giant capillaries, moderate loss of capillaries with some avascular areas, mild disorganization of the capillary architecture and absent or some ramified capillaries. Finally, the late pattern criteria were irregular enlargement of capillaries, few or absent giant capillaries, absence of hemorrhages, severe loss of capillaries with large avascular areas, severe disorganization of the normal capillary distribution and frequent ramified/ bushy capillaries. It should be noted that these criteria are frequently used, but that some aspects of this classification are not yet validated.

Pulmonary involvement was examined by the carbon monoxide diffusion capacity using the single-breath method standardized for hemoglobin. Antinuclear antibodies were determined by ELISA, anti-centromere antibodies determined on Hep-2 cells and anti-topoisomerase I (Scl-70) antibodies were determined by immunoblot analysis. Plasma levels of VEGF were measured using a commercially available ELISA (R&D Systems, Minneapolis, USA) as described (12). Concomitant treatment of SSc patients included angiotensin-converting enzyme inhibitors, calcium channel blockers, proton-pump inhibitors, clebopride and topical glyceryl trinitrate. All
patients had received therapy with intravenous prostanoids (alprostadil and iloprost). Blood for this study was drawn after a wash-out period of at least 30 days after the last prostanoid infusion. None of the study patients received corticosteroids, methotrexate, cyclophosphamide, D-penicillamine or other potentially disease-modifying drugs.

**Isolation of microparticles by differential centrifugation**

Blood was obtained using a 19 gauge needle and collected into citrated tubes. Immediately after blood drawing, plasma was centrifuged at 1500 g for 10 min, supernatant was collected and divided into aliquots. Aliquots were stored in liquid nitrogen till used for the analysis.

The method for the isolation of microparticles by differential centrifugation has been established previously for various cell types (4). Four milliliters of plasma were centrifuged at 1500 g for 5 min to remove suspended cells. Afterwards, the cell-free supernatants were centrifuged at 100000 g for 20 min using a Centrikon T-1065 centrifuge with a TST28.38 head (Kotron Instruments, Munich, Germany) and 16 x 76 mm centrifugation tubes (Beckman instruments, Fullerton, CA, USA). The supernatant was removed and the pellet was washed twice with 10 ml apop buffer (5 mM KCl, 1 mM MgCl₂ and 136 mM NaCl, pH 7.4). The microparticles were then quantified and characterized by flow cytometry.

**Labeling of microparticles and flow cytometry analysis (FACS)**

For the differentiation and quantification of microparticles derived from platelets, erythrocytes, granulocytes, monocytes, T cells, B cells and endothelial cells by FACS, microparticles were resuspended in apop buffer containing 2.5 mM CaCl₂ and 1 % microparticle-free FCS to a final volume of 500 µl as described recently (4,
7). Microparticles were incubated for 20 min at room temperature in the dark with CD42 antibodies for the detection of platelet derived microparticles, 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 labeled with FITC in concentrations recommended by the manufacturer. Unbound antibodies were removed by two washing steps at 100000 g for 20 min. Stainings with isotype-matched irrelevant antibodies at the same concentration and under the same conditions were used as controls. After the final washing step, microparticles were resuspended in 500 µl apop buffer, 2.5 mM CaCl$_2$ and 1 % microparticle-free FCS. Microparticles were counted by measuring 1 min at the “hi-flow” modus at the FACS Calibur flow cytometer (Becton Dickinson, Mansfield, MA), and the data were evaluated with CellQuest software (Becton Dickinson Immunocytometry System, San Jose, CA). The total number of microparticles was calculated by multiplication with the ratio of total volume to measured volume. For calculation of the number of microparticles per milliliter plasma the total number was divided by four.

**Statistical analysis**

Statistical analysis was performed by the biostatistician of the group (DH). Data are expressed as mean ± standard error of the mean, if not indicated otherwise. Differences in microparticle counts were tested between groups composed by categorical parameters to assess association of these disease parameters with microparticle counts. Thereby, the Kruskal-Wallis test was used for analysis of more than two groups, and the Mann Whitney U-test for analysis of two groups. To assess
correlation of continuous variables with microparticle counts, Spearman’s rank correlation coefficients were calculated. P-values < 0.05 were considered statistically significant.

Multivariate linear regression models were applied in a stepwise forward and stepwise backward manner to determine an optimal combination of variables predicting the microparticle count. Since case numbers were low relative to the parameters to examine, an inclusion criteria of $p \leq 0.1$ was used in the stepwise forward procedure. In the stepwise backward procedure, an inclusion criteria of $p \leq 0.05$ was applied. The model was calculated including a constant term.
Results

Cellular origin of microparticles in the blood of SSc patients and controls

The origin of microparticles in the peripheral blood was identified by staining of microparticles with cell-type specific surface markers. In the blood of patients with SSc and healthy donors, platelets were the most common source of microparticles. The percentage of CD42 positive microparticles was $66.9 \pm 5.2\%$ in patients with SSc and $70.2 \pm 3.9\%$ in healthy donors (see absolute numbers below). Endothelial cells were the second most common source of microparticles with CD144 positive microparticles accounting for $8.8 \pm 0.9$ (SSc) and $7.6 \pm 1.2\%$ (healthy donors) of the total microparticles in the blood. Lower percentages of less than 5\% were found for microparticles from other cellular origin such as erythrocytes, granulocytes, monocytes, T cells and B cells.

Increased numbers of microparticles in the blood of SSc patients

Since platelet and endothelial cell microparticles were the most common source of microparticles in the blood, and since T cells and monocytes have been implicated in the pathogenesis of SSc, these microparticles were selected for further analysis. The absolute number of microparticles of total, platelet, endothelial cell, T cell and monocytes origin in patients with SSc and healthy controls are summarized in Figures 1A-1E.

Patients with SSc showed $88.0 \pm 4.8 \times 10^5$ microparticles/ml plasma. This total number of microparticles was strongly increased compared to the values that were found in the plasma of healthy controls ($42.3 \pm 9.4 \times 10^5$ microparticles/ml plasma; p < 0.001, Figure 1A). Elevated total numbers of microparticles per milliliter plasma were found in both patients with limited and diffuse systemic sclerosis ($87.6 \pm 4.5 \times$
10^5 and 89.2 ± 13.4 x 10^5 microparticles/ml plasma respectively, each p < 0.05 compared to healthy controls, Figure 1A).

Along with the higher total number of microparticles, the number of microparticles derived from platelets was significantly increased in patients with systemic sclerosis. There were 58.8 ± 4.0 x 10^5 CD42 positive microparticles/ml detectable in the plasma of SSc patients as compared to 30.4 ± 5.2 x 10^5 in healthy controls (Figure 1B). No differences in the number of platelet derived microparticles were found between patients with limited and diffuse SSc (59.0 ± 4.2 x 10^5 and 58.6 ± 9.7 x 10^5 respectively, each p < 0.001 compared to controls).

Similarly, the total number of microparticles derived from endothelial cells was increased compared to controls. The mean number of CD144 positive microparticles was 7.6 ± 0.6 x 10^5 for all patients with SSc, 8.2 ± 1.8 x 10^5 for patients with diffuse SSc, 7.3 ± 0.6 x 10^5 for patients with limited SSc and 3.0 ± 0.6 x 10^5 for healthy controls (Figure 1C). The differences between the subgroups of SSc patients were statistically significant compared to healthy controls (p < 0.001 for all SSc patients, p < 0.001 for diffuse SSc patients and p < 0.002 for limited SSc patients).

Along with increased microparticles from endothelial cell and platelet origin, the number of microparticles derived from T cells and monocytes was significantly elevated, reflecting the activation of these cells in SSc. The mean number of CD3 positive and CD14 positive microparticles was 4.1 ± 0.4 x 10^5 and 3.1 ± 0.3 x 10^5 respectively compared to 1.9 ± 0.3 x 10^5 (p < 0.02) and 1.4 ± 0.2 x 10^5 (p < 0.001) in healthy controls (Figures 1D and 1E). The number of T cell and monocytes-derived microparticles was significantly elevated in both patients with limited and diffuse SSc without differences between these subgroups.
Notably, there was no difference in the platelet, erythrocyte, monocytes and T cell counts between SSc patients and controls, indicating that the increased number of microparticles was not simply caused by increased cell numbers.

Concomitant diseases do not contribute to the increased number of microparticles in SSc patients

Since vascular diseases such as coronary artery disease and thrombotic diseases such as the antiphospholipid antibody syndrome are associated with increased numbers of microparticles (13, 14), we next searched for confounding clinical parameters that could have influenced the results. Arterial hypertension was found in 4/37 patients with SSc (11%), and in none of the controls. All other established risk factors for atherosclerosis such as smoking, diabetes, hypercholesterolemia and hyperlipidemia were equally distributed between patients and controls (Table 1).

In addition, all patients with known coronary heart disease and clinical manifestations of atherosclerosis such as stroke or peripheral obliterative arterial disease were excluded from our study.

Similarly, there was no evidence for thrombotic diseases in the past medical history of patients and controls. Increased levels of antiphospholipid-antibodies were not detectable in patients and controls (Table 1).

Moreover, patients and controls with systemic infections, which are also associated with increased numbers of microparticles, were not included in the study. Taken together, these data indicate that the increased numbers of microparticles were not caused by confounding vascular, infectious or thrombotic diseases in our patient population.
Correlation of microparticles with clinical manifestations of SSc

The increased number of microparticles from endothelial cells, inflammatory cells and platelets likely reflects their cellular activation in the pathogenesis of SSc (4). Based on these findings, we hypothesized that the number of microparticles could be associated with clinical vascular and fibrotic manifestations of the disease.

In fact, in univariate analysis, the number of total microparticles correlated significantly with the modified Rodnan Skin Score (p < 0.05, r = -0.333). As shown in Figure 2A, patients with a modified Rodnan Skin Score of ≥ 10 had significantly lower numbers of microparticles than patients with a Skin Score of < 10 (75.9 ± 7.4 x 10^5 versus 97.3 ± 5.6 x 10^5, p < 0.05). This association was further confirmed by the analysis of microparticles derived from platelets. Similar to the total number of microparticles, the number of CD42 positive microparticles correlated significantly with the modified Rodnan Skin Score (p < 0.05, r = -0.337), and patients with a modified Rodnan Skin Score of ≥ 10 showed significantly lower levels of platelet microparticles than patients with a Skin Score of < 10 (51.2 ± 5.9 x 10^5 versus 65.3 ± 5.2 x 10^5, p < 0.05, Figure 2B). There was no correlation detectable between the modified Rodnan Skin Score and the number of microparticles from other cell types.

There was also an association between the total number of microparticles in the blood of SSc patients and the presence of cutaneous ulcers (Figure 2C). Patients with cutaneous ulcers (80.1 ± 4.9 x 10^5) had lower numbers of microparticles than patients without cutaneous ulcers (106.8 ± 9.1 x 10^5, p < 0.05). This was again accompanied by the same pattern in the number of platelet-derived microparticles (cutaneous ulcers: 52.4 ± 3.8 x 10^5, without cutaneous ulcers: 74.9 ± 8.7 x 10^5, p < 0.05). No significant association of the total number of microparticles were observed
by univariate analysis with any other clinical parameter. Older patients tended to have lower number of microparticles, but this difference was not statistically significant \((\text{age} > 60\,\text{years}: 83.6 \pm 5.8 \times 10^5, \text{age} \leq 60\,\text{years}: 93.8 \pm 7.9 \times 10^5, \ p > 0.05)\). There was only one patient with elevated CRP values. This patient had the highest number of total microparticles of all patients and an increased disease activity score of 3.5.

Although many univariate analyses did not show associations of disease parameters with microparticle counts, a combination of them might still predict the increased levels in SSc patients. We checked a multivariate linear regression model on the total number of microparticles for age, sex, subtype of SSc, disease activity, CRP, duration of disease, number of platelets, modified Rodnan skin score, levels of VEGF, changes observed by nailfold capillaroscopy, presence of cutaneous ulcers, lung fibrosis, elevated sPAP, fingertip ulcers, teleangiectasias and Raynaud’s phenomenon. Platelet numbers were missing for four patients. For the 33 patients with valid numbers, neither the forward nor the backward regression process selected platelets into the model. Hence we recalculated the models for all 37 SSc patients omitting platelets from the variable list. The forward selection method led to a model \((\text{constant} = 197.071)\) consisting of age \((b = -0.683)\), modified Rodnan skin score \((b = -2.571)\), limited type of SSc \((b = -35.309)\), CRP \((b = 2.538)\) and presence of cutaneous ulcers \((b = -12.739)\), with a correlation coefficient of \(r = 0.764\). The backward selection method led to a model \((\text{constant} = 187.279)\) consisting of age \((b = -0.599)\), modified Rodnan skin score \((b = -2.826)\), limited type of SSc \((b = -37.390)\) and CRP \((b = 2.905)\), with a correlation coefficient of \(r = 0.744\). Thus, both selection procedures resulted in the same variables except for the presence of cutaneous ulcers. Since this parameter did not show significant influence \((p = 0.139)\) in the final model of the forward selection procedure, we focused on the four parameters
selected from both models. The respective correlation coefficient of $r = 0.744$ of this model results in a coefficient of determination $R^2$ of 0.55. Taken together, these results show that as much as 55% of the variability of the total number of microparticles can be explained by a combination of the four parameters modified Rodnan skin score, limited subtype of SSc, CRP and age.
Discussion

Microparticles represent a novel element in the communication between cells in biology and disease (15). Interactions between membrane molecules usually require direct cell-cell contact, and long-distance interactions between cells are mediated by soluble mediators such as cytokines. Microparticles are released from cellular plasma membranes and circulate as small membrane-coated vesicles in the peripheral blood. Thus, microparticles can mediate the communication between cells in that they allow membrane interactions between cells at long distances (15). The present study is the first report that the levels of microparticles are elevated in the blood of patients with SSc, suggesting that this novel mechanism of intercellular interaction can contribute to disease pathogenesis.

Increased levels of microparticles have been detected in diseases with vascular damage such as atherosclerosis and coronary artery disease (7, 14, 16-18). To avoid confounding effects related to differences in the prevalence of these diseases, all patients with clinical evidence of coronary artery disease were excluded from the study. In addition, established risk factors for atherosclerosis such as smoking, diabetes, hypercholesterolemia and hyperlipidemia were not present in both SSc patients and controls (except one SSc patient with diabetes). Patients suffering from other diseases known to increase the numbers of microparticles in the blood such as infectious diseases (5, 19), vasculitis and antiphospholipid syndrome (7, 13) were excluded from the study. Thus, the increased number of microparticles in the blood of SSc patients is not a secondary phenomenon due to comorbidities, but rather reflects the disease process in SSc itself.

In this regard, the release of microparticles from their parental cells is known to be enhanced in activated cells, e.g. by stimulation with cytokines (4). In fact,
increased levels of different cytokines are a main feature of the pathogenesis of SSc and circulating cells such as platelets, monocytes and T cells as well as endothelial cells are activated in SSc as indicated, for instance, by the expression of activation markers (20). Thus, the enhanced levels of microparticles found in SSc patients in our study are likely caused by an increased release of microparticles from their (cytokine)-activated parental cells.

We observed a significant inverse correlation of the total number of microparticles and of the number of microparticles derived from platelets with the modified Rodnan Skin Score. These results from the univariate analysis were further confirmed by the multivariate analysis, in which the modified Rodnan skin score and the limited subtype of SSc together CRP and age accounted for as much as 55% of the variability of microparticles counts. These results show that the increase in microparticle counts in SSc patients observed in this study is largely driven by SSc associated features (and age). The Rodnan skin score is the most commonly used and best validated outcome measure for skin fibrosis in SSc. Thus, high numbers of microparticles are associated with milder dermal fibrosis in SSc. Consistent with this finding, we previously demonstrated that microparticles potently induce the expression of matrix metalloproteinase (MMP)-1, MMP-3, MMP-9 and MMP-13 in a dose-dependent manner. In contrast, no counter-regulatory induction of the expression of tissue inhibitors of MMPs (TIMPs) was observed (4). Microparticles might, therefore, exert direct anti-fibrotic effects on fibroblasts by promoting the degradation of extracellular matrix. However, it has to be emphasized that functional experiments with SSc-derived microparticles were not part of the present study. Thus, the functional role of microparticles in the pathogenesis of SSc needs to be addressed in additional studies using SSc specific biomaterial. In addition, the results
of the present study are limited by the rather low number of patients that were examined.

In summary, in the present study, we have demonstrated that microparticles derived from different cell types are significantly elevated in SSc patients. The number of microparticles correlated inversely with the modified Rodnan skin score. Thus, microparticles may contribute to the pathogenesis of SSc as a novel intercellular communication pathway that may attenuate certain disease manifestations.
References


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</tr>
<tr>
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<td>3/12</td>
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| Disease subsets
  • limited                   | 27/37        | ---      |
  • diffuse                    | 10/37        | ---      |
| mean age (years)              | 63 ± 12 (range 29-82) | 56 ± 14 (range 42-70) |
| diabetes                      | 1/37         | 0/15     |
| smoking                       | 0/37         | 0/15     |
| arterial hypertension         | 4/37         | 0/15     |
| hypercholesterolemia          | 0/37         | 0/15     |
| hyperlipidemia                | 0/37         | 0/15     |
| disease duration (years)      | 13 ± 10 (range 3-44) | --- |
| Pulmonary involvement
  • HRCT                        | 18/37        | ---      |
  • FVC                         | 98.5 ± 20.7 %| ---      |
  • DLCO                        | 66.0 ± 23.0 %| ---      |
  • Elevated sPAP by ECHO       | 10/37        | 0/15     |
<p>| DMARDs                        | 0/37         | 0/15     |
| Modified Rodnan skin score    | 13.6 ± 11.5  | ---      |
| Disease activity              | 1.58 ± 1.40 (range 0-5.5) | --- |
| Finger tip ulcers             | 22/37        | 0/15     |
| Cutaneous ulcers              | 26/37        | 0/15     |</p>
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<td>• ACAs</td>
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ANAs: anti-nuclear antibodies, ACAs: anti-centromere antibodies, Scl-70: Scl-70 autoantibodies, HRCT: high resolution CT, DLCO: diffusion capacity for carbon monoxide, FVC: forced vital capacity. Smoking: Never smokers based on past medical history. Arterial hypertension: Defined according to WHO criteria ≥ class I. Hypercholesterolemia: Total cholesterol measured with standard methods above normal limits (normal < 220 mg/dl). Hyperlipidemia: Fasting triglycerides measured with standard methods above normal limits (normal < 170 mg/dl). HRCT: normal was defined as no evidence for fibrosis or alveolitis as judged by an experienced radiologist. Elevated sPAP by ECHO: estimated systolic pulmonary artery pressure (sPAP) > 35 mmHg by echocardiogram performed by the same experienced cardiologist in all patients. Data are shown as mean ± standard deviation.
Figure legends

Figure 1: Significant increase in the total microparticle count in patients with limited and diffuse SSc compared to healthy individuals (p < 0.05, Figure 1A). Similarly, the number of microparticles derived from platelets (p < 0.05, Figure 1B), endothelial cells (p < 0.05, Figure 1C), monocytes (p < 0.05, Figure 1D), and T cells (p < 0.05, Figure 1E) was significantly elevated in patients with limited and diffuse SSc compared to healthy individuals. Black rhombi represent single individuals, mean values are indicated by red bars.

Figure 2: Association of microparticles with clinical manifestations of SSc. The total number of microparticles (Figure 2A) as well as the number of platelet (CD42 positive) microparticles (Figure 2B) was significantly higher in patients with a modified Rodnan skin score of <10/51 compared to patients with a modified Rodnan skin score of ≥10/51. In addition, the total number of microparticles were significantly lower in patients with cutaneous ulcers (e.g. at the lower extremities, elbows, forearms, Figure 2C) compared to patients without ulcers. Box plots with 25% percentiles and medians. # indicates significance at p < 0.05.