LACTATE DEHYDROGENASE A POSSIBLE MARKER OF PROGRESSIVE MICROVASCULOPATHY AND INTERSTITIAL LUNG DISEASE IN SYSTEMIC SCLEROSIS

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ABSTRACT

Introduction: Systemic sclerosis (SSc) is associated with excessive inflammation, fibrosis and vasculopathy. Early endothelial dysfunction inducing widespread microvasculopathy represent also a trigger for fibrotic remodeling. SSc patients with progressive severe fibrotic remodeling present increased levels of serum lactate dehydrogenase (LDH), resulted from hypoxia, oxidative stress, cellular lysis and platelets activation.

We try to asses serum LDH activity in SSc patients in order to use it as a possible marker of early vasculopathy and pulmonary interstitial involvement, in relation with clinical and biological markers of disease activity.

Materials and methods: We prospectively studied 40 patients with SSc, diagnosed according to the American College of Rheumatology (ACR) criteria. Laboratory tests included routine tests and immunological determinations for SSc, serum LDH, platelets activation, mean platelet volume and sP-Selectin level. All patients were evaluated for peripheral vascular, cardiac, pulmonary and renal involvement.

Results: Serum LDH was increased in about half of patients (53.33%), being positively correlated with Rodnan score and negatively correlated with flow mediated dilatation (FMD), right ventricle diastolic dysfunction and transfer factor of the lung for carbon monoxide (TlCO). A good correlation was observed between LDH activity and the other markers of platelet activation - sP-selectine levels and mean platelet volume.

Conclusion: In SSc patients increased serum activity of LDH may represent a marker of early endothelial dysfunction associated with progressive vasculopathy and fibrotic remodeling with pulmonary involvement. The values of serum LDH can be used for monitoring patients during their treatments and also as an initial step for further investigation procedures. Prognosis is determined by the degree of internal organ involvement and this can be lessened by early detection and prompt intervention for organ-specific manifestations.

Key words: endothelial dysfunction, lactate dehydrogenase (LDH), sP-Selectin, microvascular disturbances, transfer factor of the lung for carbon monoxide (TlCO).

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Introduction

Systemic sclerosis (SSc) is a clinically heterogeneous and often lethal disorder of the connective tissue of unknown etiology characterized by micro and macrovascular disturbances, deposition of collagen in the skin and internal organs, Raynaud phenomenon and scleroderma. Renal crises, pulmonary fibrosis and vascular remodeling - leading to pulmonary hypertension have been shown to be related with severe outcome of the disease(1). Patients with SSc according to the LeRoy-Medsger system are further divided into SSc subsets - limited SSc cutaneous SSc and diffuse cutaneous SSc with different patterns of skin and internal organ involvement autoantibodies and outcome(2).

SSc is a rare disease reported incidence and prevalence estimates vary greatly according to geographic location and methods of case ascertainment. Using ACR (American College of Rheumatology) classification criteria and the revised LeRoy-Medsger criteria in Europe (Italian study) the annual incidence is 43 cases per million
and prevalence 254 cases per million. The disease most commonly occurs in women between the ages 35 and 55, but men and children can be affected as well. Women are affected 4-5 times more frequent than men. No socioeconomic variables affecting scleroderma incidence were identified and till present there is no epidemiological evidence of an infectious agent as a disease trigger.

Microvascular damage is one of the earliest events in the onset of SSc and there is an evidence of widespread endothelial dysfunction in SSc patients, responsive for pulmonary, myocardial and peripheral vascular lesions. Mechanisms of vascular damage may include oxidative stress, factors released from activated platelets, disturbed angiogenesis, resulting in dysfunctional microvascular beds and progressive hypoxia.

It was demonstrated that in patients with SSc, tissues’ hypoxia determines a metabolism shift to anaerobic glycolysis. Lactate dehydrogenase (LDH) catalyzes the reversible transformation of pyruvate to lactate, having a central position in the anaerobic cellular metabolism. Induction of LDH occurs during hypoxia, LDH transcription being regulated by the hypoxia inducible factor (HIF-1). LDH release into extracellular medium is usually used as a biochemical parameter of cell membrane damage, platelet activation, but also as marker for angiogenesis.

In present study we try to asses serum LDH activity in SSc patients in order to use it as a possible marker of early vasculopathy and pulmonary interstitial involvement, in relation with clinical and biological markers of disease activity and vascular involvement.

Material and methods

Study population

We prospectively studied 40 patients with SSc, diagnosed according to the ACR criteria. The study was approved by the local ethics committee and informed consent was obtained from all participants. Patients were classified having limited (16 patients) and diffuse (24 patients) cutaneous form according to the criteria of LeRoy. Patients were followed-up for a mean period of 3 years. 30 patients were treated according to vascular, cardiac and pulmonary involvement. The control group consisted of 10 SSc patients without pulmonary involvement, who received beside specific treatments, low doses of statins and corticosteroids for coronary heart disease and/or inflammatory joints disease.

Diagnostic tests

Laboratory tests included routine tests and immunological tests for SSc (anticentromere and antitopoisomerase-1 antibodies), and capillaroscopy.

Serum LDH activity was assessed using Roche/Hitachi c system based on the conversion of cofactor NAD+ to NADH, which was used for quantitative determination of LDH (normal values: 135-225 U/L).

In 30 SSc patients LDH activity was measured at baseline in absence of hemolysis, malignancies, myopathy or other factors inducing muscular lesions (statins, corticosteroids).

Platelets activation was assessed by morphometric methods, measuring mean platelet volume (MPV) with normal values < 10 fl and by determining serum sP-selectine level measured using enzyme-linked immunosorbent assay (ELISA) kit (normal values 41.23±7.23 ng/ml).

Pulmonary involvement was assessed by chest radiography, computed tomography and pulmonary function tests - forced vital capacity (FVC), forced expiratory volume in first second (FEV1), transfer factor of the lung for carbon monoxide (TLC), total lung capacity (TLC).

Assessment of digital vasculopathy: score of digital vasculopathy (DV): without DV (only Raynaud phenomenon)- 0, minimal skin lesion- 1, necrosis- 2, osteolysis- 3; [DV score = lesion x digits number].

Flow mediated dilatation (FMD) (measured at brachial artery): normal value > 10%.

Two dimensional and Doppler echocardiography was performed with Aloka ultrasound S400 for assessment of systolic and diastolic function of left ventricle (LV) and right ventricle (RV) and pulmonary pressure estimation attempted by Doppler echocardiographic methods (PAP).

Statistical analysis

All values were presented as mean ± standard deviation (SD). The statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) software, version 11.0 -SPSS Inc., Chicago, Illinois, USA. The significance of correlations was evaluated by determining Pearson’s rank correlation coefficients. A two-tailed p-value < 0.05 was considered significant.
Results

The clinical and biologic characteristics of patients are detailed in table 1. Rodnan score (RS) was: RS<25 (18 patients), 25≤ RS≤ 35 (10 patients) and RS>35 (12 patients).

In study group patients with pulmonary involvement (30 patients) serum LDH activity was increased in 60% patients (18 patients) (420±130 U/L), most of them (12 patients) having values between 300-500 U/L and over 500 U/L in 6 patients.

Endothelial dysfunction is present in almost all patients: decreased FMD values were present in 63% patients, being negatively correlated with digital vasculopathy score (R=-0.564; p=0.001), Rodnan score (R=-0.753; p=0.000) and serum LDH (R=-0.432; p=0.020) (Fig. 1).

Table 1: Baseline characteristics of patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>38</td>
</tr>
<tr>
<td>Male gender</td>
<td>2</td>
</tr>
<tr>
<td>Age</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Mean duration of the disease</td>
<td>6 ± 2 years</td>
</tr>
<tr>
<td>SSC limited</td>
<td>16</td>
</tr>
<tr>
<td>SSC diffuse</td>
<td>24</td>
</tr>
<tr>
<td>Capillaroscopy</td>
<td></td>
</tr>
<tr>
<td>- early</td>
<td>19</td>
</tr>
<tr>
<td>- active</td>
<td>12</td>
</tr>
<tr>
<td>- late</td>
<td>9</td>
</tr>
<tr>
<td>Rodnan score &gt; 25</td>
<td>18</td>
</tr>
<tr>
<td>39mmHg &gt; PAP &gt; 25mmHg</td>
<td>5</td>
</tr>
<tr>
<td>Systolic tricuspid flow velocity &gt; 2.9 m/s</td>
<td>3</td>
</tr>
<tr>
<td>Transmural flow (E/A) &lt; 0.9</td>
<td>16</td>
</tr>
<tr>
<td>Transtricuspid flow (Etr/Atr) &lt;0.9</td>
<td>18</td>
</tr>
<tr>
<td>TAPSE &lt; 20mm</td>
<td>7</td>
</tr>
<tr>
<td>TLCO &lt; 70%</td>
<td>14</td>
</tr>
<tr>
<td>FEV1 &lt; 70%</td>
<td>6</td>
</tr>
<tr>
<td>FMD &lt; 6%</td>
<td>21</td>
</tr>
<tr>
<td>sP-selectin &gt; 50 ng/ml</td>
<td>24</td>
</tr>
</tbody>
</table>

SSc, systemic sclerosis; PAP, pulmonary artery pressure; TAPSE, tricuspid annular plane systolic excursion; T1LCO, transfer factor of the lung for carbon monoxide; FEV1, forced expiratory volume in first second; FMD, Flow mediated dilatation.

LDH activity was correlated with Rodnan Score (R=0.481; p=0.002) and with digital vasculopathy. Beside peripheral vascular involvement, there are significant correlation between LDH and pulmonary involvement as reflected by decreased TLCO (R=-0.465; p=0.010) (Fig. 2), which may represent early pulmonary microvascular disturbances and parenchymal change.

LDH activity was negatively correlated with RV diastolic dysfunction (R=-0.48; p=0.007).

No correlation was found between LDH and transtricuspidian flow velocity and a poor correlation was observed between LDH and systolic RV
function (tricuspid annular plane systolic excursion-TAPSE). Diastolic function of LV and RV is early disturbed, possible due to microvascular myocardic involvement\(^{(21, 22)}\).

A good correlation was observed between LDH activity and the other markers of platelet activation, sP-selectine levels (\(R=0.489; p=0.006\)) (Fig. 3), and VMP (\(R=0.449; p=0.013\)).

During the follow-up period, 6 patients with improvement of pulmonary functional parameters (\(T_{LCO}, FEV1\)), presented also a decrease in serum LDH activity.

SSc patients without pulmonary involvement did not display LDH values above the upper limit.

**Discussion**

Vascular abnormalities and interstitial lung disease constitute characteristics of SSc\(^{(6, 7, 23)}\). The events and mechanisms that initiate vascular injuries or prevent its repair are not fully elucidated.

Inflammation, oxidative stress, a distinct rheological status are important risk factors for vascular damage in SSc patients\(^{(24)}\). Vascular remodeling occurring as a response to various injuries is a multicellular event and adventitial fibroblasts play an important role\(^{(41, 25)}\). The adventitial fibroblasts are regulators of vascular remodeling under hypoxic condition.

Microvascular injury and remodeling has been demonstrated as an initial event in pulmonary interstitial fibrosis, which occurs frequently in SSc patients. The lung microvessels and vascular endothelium are critical sites in interstitial fibrosis, oxidative stress and inflammation driving the initiation and progression of interstitial lung disease\(^{(20)}\).

These widespread microvascular disturbances, leading to progressive hypoxia, induce lactate production and proangiogenic signaling. The good correlation between LDH and FMD indicate the endothelial dysfunctionality as an early event in vascular remodeling\(^{(27)}\).

In SSc patients LDH may be released from activated platelets and platelet microparticles, alveolar epithelial and endothelial cells lysis\(^{(28, 29, 30)}\).

Several studies\(^{(31, 32)}\) suggest that LDH level may serve as a surrogate marker for activation of HIF-1 (hypoxia-inducible factor 1) production. Endothelial cells may respond to lactate by increasing production of VEGF (vascular endothelial growth factor)\(^{(10)}\). In SSc angiogenesis is incomplete or disturbed, despite the increased expression of a large array of pro-angiogenic factors.

Among inflammatory cells involved in processes leading to vascular dysfunctionality, platelets play an important role. Platelets derived mediators have a pleiotropic range of action that can influence the vascular tone, inflammatory processes, the fibrogenic response and angiogenesis\(^{(33, 34)}\).

Recent studies provide support for varying degrees of platelets activation and aggregation in different forms and stages of systemic sclerosis\(^{(14)}\).

Markers of platelet activation (evaluation of MPV, sP-selectin, LDH release) usually elevated in diseases characterized by microvascular involvement\(^{(34)}\) were increased in SSc patients, indicating an active role of platelets in progressive microvascular damage. Thrombin and lysophosphatidic acid released by activated platelets bind to epithelial cell surface receptors inducing cytoskeletal changes with activation of growth factors (TGF-\(\beta\))\(^{(35, 36)}\).

**Conclusions**

The increased serum activity of LDH in SSc patients and its significant correlations with parameters of vascular dysfunction (depressed FMD, decreased TLCO, ventricular dysfunction, digital vasculopathy score) and markers of platelets activation may represent a marker of early endothelial dysfunction associated with progressive vasculopathy and fibrotic remodeling with lung involvement. The values of serum LDH can be used for monitoring patients during their treatment but also as an initial step for further investigation procedures for detecting disease progression. Prognosis is determined by the degree of internal organ involvement.

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Fig. 3. Correlation between sP-selectin and LDH. LDH, Lactate dehydrogenase.
and this can be lessened by early detection and prompt intervention for organ-specific manifestations.

References


