identification of cardiac involvement is therefore of paramount clinical importance.

The concentration of ischemia-modified albumin (IMA), as measured by the albumin cobalt binding test (Ischemia Technologies, Inc.), is a new marker to rule out transient myocardial ischemia (1, 9). This test measures the binding of exogenous cobalt to the NH₂ terminus of human albumin. In the presence of myocardial ischemia, structural changes occur in the NH₂ terminus of albumin, rapidly reducing its capacity to bind transition metal ions after an ischemic event (10).

We assessed the accuracy of the albumin cobalt binding test for detecting ischemia in SSc patients and investigated the role of myocardial ischemia and peripheral oxidative stress in this condition. We also considered carbonyl residues and advanced oxidation protein products (AOPP) as factors indicative of protein oxidation.

We included consecutive patients hospitalized for systemic follow-up who fulfilled the American Rheumatism Association preliminary criteria for SSc. The exclusion criteria were pregnancy; symptoms of heart failure, including class III or IV dyspnea (New York Heart Association); venous distension and recent major lower limb edema; pulmonary arterial hypertension (systolic arterial pressure >40 mmHg and/or mean artery pressure >25 mmHg, determined by echocardiography); severe pulmonary involvement (forced vital capacity or carbon monoxide diffusing capacity <50% of the predicted normal value); renal involvement (creatinine concentration >16 µmol/L); or severe disease complications such as cancer or gangrene. At the time of the study, none of the patients was taking medication for cardiac or vascular disease. If previously treated with vasodilators, patients were asked to stop taking these drugs 3 days before admission. This interruption period corresponds to five times the half-life of calcium channel blockers and angiotensin-converting enzyme. All patients gave informed consent for all procedures, and the study was approved by the local ethics committee (Paris, Cochin).

We assessed the following in all patients: blood cell count, Westergren erythrocyte sedimentation rate, serum creatinine concentration, and anti-centromere and antitopoisomerase I antibody concentrations. The concentration of high-sensitivity C-reactive protein was measured by immunoturbidimetry on a Roche modular PP instrument using the CRP latex Tina-quant® assay (Roche Diagnostics). Pulmonary involvement was assessed by computed tomography scan, forced vital capacity, and the ratio of carbon monoxide diffusion capacity to hemoglobin concentration. Pulmonary arterial systolic pressure was determined by Doppler echocardiography at rest. The thickness of the skin was quantified on a scale of 0–3, by use of the modified Rodman skin scoring technique, for each of 17 body surface areas (11, 12).

All patients underwent thallium-201 myocardial single-photon-emission computerized tomography at rest, using a gamma camera (Starport 400AT; General Electric) interfaced with an ADAC computer (DPS 3300). Myocardial perfusion was assessed semiquantitatively by two expe-
rienced practitioners, using a blind protocol and a 17-
segment model as follows: score 4 for normal, 3 for mild
reduction (not definitely abnormal), 2 for moderate reduc-
tion (definitely abnormal), 1 for severe reduction, and 0
for absence of uptake (13). We determined both a “global
perfusion score” (sum of the mean perfusion scores for
each of the 17 segments) and the “number of perfusion
defects” (with a perfusion defect defined as a score ≤2).
The controls were healthy laboratory staff who agreed to
provide blood. Blood samples (10 mL) were collected in
tubes without anticoagulant. The samples were centri-
fuged at 3000g for 10 min within 1 h of collection, and the
resulting sera were stored at −80 °C until use. The inter-
val between sample collection and analysis was <10
weeks.

Cardiac troponin I (cTnI) was measured by an immu-
nochemiluminescent assay on an ACS180 analyzer (Bayer
Diagnostics). In accordance with the manufacturer’s data,
the lower limit detection of this assay was 0.03 μg/L; the
total imprecision (CV) was 10% and 20%, respectively, at
0.4 and 0.1 μg/L. Serum IMA was measured on a Roche
Modular PP instrument. Because no cutoff value was
validated for this analyzer, 85 kilounits/L, the upper limit of
the range of IMA concentrations for a reference popu-
lation (95th percentile of a population of 283 apparently
healthy individuals), was used as a cutoff point for ischemia, in accordance with data reported by the manu-
facturer and determined with the Roche Modular P ana-
lyzer. The inter- and intraassay CVs were <3.7% and
4.3%, respectively, at IMA concentrations of 106 and 60
kilounits/L (n = 20). Protein carbonyl groups were deter-
mined by ELISA (14), and the AOPP concentrations were
determined as described by Witko-Sarsat et al. (15). The
lowest concentrations determined in our laboratory with
CVs of 10% and 20% were, respectively, 0.20 and 0.12
μmol/g for protein carbonyl groups and 25 and 17
μmol/L for AOPP. Concentrations of IMA and markers of
oxidative stress are expressed as medians with ranges.
Data were analyzed by the Mann-Whitney test for group
comparisons and the Spearman rank correlation test for
assessment of the relationship between quantitative vari-
ables. P values <0.05 were considered significant.

We investigated 32 consecutive SSc patients [mean (SD)
age, 54.1 (11.6) years], including 26 women. The clinical
and laboratory data for these patients are presented in
Table 1. None of the following variables was correlated
with IMA values: age, pulmonary fibrosis, carbon mon-
oxide diffusion, autoantibody status, high-sensitivity C-
reactive protein, and erythrocyte sedimentation rate.

In SSc patients, the median global myocardial perfusion
score was 37 (range, 8–47; scores <41 were considered
abnormal), and the median of number of perfusion de-
defects was 11 (range, 5–17). All patients had a cTnI concen-
tration <0.4 μg/L (10% CV); two patients had a cTnI value
>0.1 μg/L (20% CV). The median (range) IMA concentra-
tion was 87 kilounits/L (55–115 kilounits/L; Fig. 1). Nineteen patients (59%) had IMA concentrations ≥85
kilounits/L, exceeding the 95th percentile for a popu-
lation of 283 apparently healthy individuals in ac-

Table 1. Clinical and biological characteristics of patients
with SSc.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SSc patients (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) disease duration [range], years</td>
<td>6.5 (4.8) [1–20]</td>
</tr>
<tr>
<td>Cutaneous form, limited/diffuse</td>
<td>15/17</td>
</tr>
<tr>
<td>Mean (SD) skin score (Rodman’s score)</td>
<td>12 (7.3)</td>
</tr>
<tr>
<td>Raynaud syndrome, n (%)</td>
<td>32 (100)</td>
</tr>
<tr>
<td>Lung fibrosis on computed tomography scan, n (%)</td>
<td>16 (50)</td>
</tr>
<tr>
<td>Forced vital capacity &lt;75% of predicted value, n (%)</td>
<td>8 (25)</td>
</tr>
<tr>
<td>Ratio of carbon monoxide diffusion capacity to hemoglobin &lt;80% of predicted value, n (%)</td>
<td>8 (25)</td>
</tr>
<tr>
<td>Pulmonary hypertension (systolic pulmonary arterial pressure &gt;40 mmHg), n (%)</td>
<td>23 (72)</td>
</tr>
<tr>
<td>Positive for anti-topoisomerase I antibodies, n (%)</td>
<td>15 (47)</td>
</tr>
<tr>
<td>Positive for anti-centromere antibodies, n (%)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Mean (SD) serum creatinine, μmol/L</td>
<td>78.8 (13.1)</td>
</tr>
<tr>
<td>Mean (SD) erythrocyte sedimentation rate, mm/h</td>
<td>20.2 (6)</td>
</tr>
<tr>
<td>Mean (SD) C-reactive protein, mg/L</td>
<td>8.7 (6.6)</td>
</tr>
<tr>
<td>Mean (SD) cTnI, μg/L</td>
<td>0.07 (0.06)</td>
</tr>
<tr>
<td>Mean (SD) IMA, kilounits/L</td>
<td>89 (13)</td>
</tr>
<tr>
<td>Mean (SD) serum albumin, g/L</td>
<td>39.2 (4.3)</td>
</tr>
<tr>
<td>Patients receiving low-dose prednisone (ongoing treatment), n (mean mg/day)</td>
<td>9 (7.4)</td>
</tr>
<tr>
<td>Patients receiving angiotensin-converting enzyme inhibitor, n (%)</td>
<td>9 (21)</td>
</tr>
<tr>
<td>Patients receiving o-penicillamine, n (%)</td>
<td>8 (19)</td>
</tr>
<tr>
<td>Patients receiving omeprazole, n (%)</td>
<td>32 (100)</td>
</tr>
</tbody>
</table>

cordance with the manufacturer’s data determined with the
Roche Modular P analyzer.

The IMA concentration was not correlated with the
global myocardial perfusion score (r = 0.13; P = 0.48) or
the number of perfusion defects (r = 0.23; P = 0.2). SSc
patients diagnosed less than 5 years previously had
higher median IMA concentrations [93 (74–115) kilo-
units/L] than did patients with longer disease durations
[83 (55–106) kilounits/L; P <0.05; Fig. 1]. IMA concentra-
tions were inversely correlated with disease duration (r =
−0.48; P <0.01) and positively correlated with skin score
(r = 0.54; P = 0.002).

Concentrations of serum markers of oxidative stress
were significantly higher in SSc patients than in controls:
carbonyl residues, 0.82 (0.37–1.09) μmol/g vs 0.34 (0.26–
0.64) μmol/g (P <0.001); AOPP, 95.1 (36.6–280) μmol/L
vs 78.2 (43.2–129) μmol/L (P <0.05). The IMA concentra-
tion was correlated with carbonyl residue concentration
(r = 0.59; P = 0.002) but not with AOPP concentrations.
However, neither carbonyl residues nor AOPP were
related with disease duration.

SSc patients had high IMA concentrations, but the IMA
concentration was not correlated with global myocardial
perfusion score or the number of perfusion defects, de-
spite functional impairment of the coronary microvascu-
lature (16). The IMA concentration was associated with
ischemia itself may damage serum albumin as much as, if not more than, the liver. Whether reperfusion after an ischemic event is unclear, but reperfusion after an ischemic event is reversible or lead to preferential degradation by proteolytic systems, as reported for other oxidized protein. Nevertheless, the high values obtained in the albumin cobalt binding test for SSc patients reflect a succession of ischemic-reperfusion episodes, given the short half-life of IMA.

**Fig. 1.** Comparison of IMA values (kilounits/L) as a function of SSc disease duration (n = 32): recent (<5 years) or longer duration (>5 years). Horizontal bars indicate median values. The dashed line indicates the 95th percentile for a population of 283 apparently healthy individuals.

**disease duration and skin score in SSc patients, reflecting the strong dependence of activity on the intensity of free radical reactions in the first few years of the disease, especially in patients with diffuse forms, who have high skin scores early in their disease.**

Free radicals generated by reperfusion injury and the inflammatory process may be of major importance in SSc patients. This study confirms that SSc is associated with excessive protein oxidative stress, as reflected by the high concentrations of carbonyl groups and AOPP. These results suggest that protein oxidation may occur early in the pathogenesis of SSc and may indicate underlying subclinical disease (oxidative stress or vascular dysfunction).

IMA has been studied primarily in selected populations thought to display myocardial involvement only in the absence of confounding clinical conditions. However, other organs seem to be responsible for the increase in IMA. For example, the study of recent (5 years) or longer duration (>5 years) increases in IMA concentration were closely related to the concentration of carbonyl groups but not AOPP concentrations. Reduced albumin-cobalt binding with transient ischemia induced during elective percutaneous transluminal angioplasty. This result is not surprising because these markers do not provide the same information concerning the extent of oxidative damage to proteins (15), the half-lives of these damaged proteins, and/or their clearance rate. Albumin is the most abundant serum protein, with a mean concentration of 0.63 mmol/L, and is a powerful extracellular antioxidant. The biochemical mechanism modifying the N-terminal region of albumin during ischemia is unclear, but reperfusion after an ischemic event may damage serum albumin as much as, if not more than, ischemia itself (21). These modifications to albumin may involve hypoxia, acidosis, or free radical damage, most of which occur within minutes. IMA seems to have a short half-life, returning to baseline values in 6–12 h, as shown recently in patients with stable angina pectoris after transient ischemia induced during elective percutaneous transluminal angioplasty (9). However, it is unclear whether the changes in the N-terminal region of albumin are reversible or lead to preferential degradation by proteolytic systems, as reported for other oxidized proteins (22). Nevertheless, the high values obtained in the albumin cobalt binding test for SSc patients reflect a succession of ischemic-reperfusion episodes, given the short half-life of IMA.

**References**

Evaluation of Imprecision for Analysis of Short Tandem Repeats by Use of Mixed Blood Cells in Variable Concentrations, Sun-Young Kang,1,2 Chang-Seok Ki,1 Hee-Jin Kim,3 Ki-o Lee,1 Jae-chun Bae,1 Sun-Hee Kim,1 and Jong-Won Kim,1* (1 Department of Laboratory Medicine, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea; 2Department of Diagnostic Laboratory, Center for Clinical Services, National Cancer Center, Goyang-si, Gyeonggi-do, Republic of Korea; * address correspondence to this author at: Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwondong, Gangnam-gu, Seoul 135-710, Korea; fax 82-2-3410-2719, e-mail jwonk@smc.samsung.co.kr)

Monitoring of chimerism after allogeneic stem cell transplantation is important for the early diagnosis of graft failure or disease relapse. Two main approaches used for monitoring chimerism are fluorescence in situ hybridization and PCR of short tandem repeats (STRs) expressing high degrees of polymorphism (1). Although both methods are useful, the STR assay has been increasingly used because fluorescence in situ hybridization can be used only in cases with specific genetic aberrations or a sex-mismatched donor (2–4). The STR assay, which produces quantitative results within 1 day, uses fluorescence-labeled primers and a capillary electrophoresis system (5, 6). However, the precision of the assay’s performance with respect to the chimeric stages of hematopoietic cells has not been fully investigated. We therefore aimed to evaluate the assay imprecision (CV) for seven STRs, D7S820, D8S1179, D165539, D18S51, D21S11, TH01, and TPOX, to determine whether the precision changes according to the degree of chimerism. We used cell mixtures at various concentrations to simulate hematopoietic chimerism, and we also determined the detection limit.

In an initial screening to find adequate samples for evaluation, we obtained peripheral blood specimens from 96 volunteer donors. Genomic DNA was isolated by use of Wizard Genomic DNA purification reagents (Promega) and was assayed for allele determination for seven different STRs. PCRs were set up in a final volume of 25 μL containing 10 × buffer, 200 μM deoxynucleotide triphosphates, 5 pmol of each primer labeled with a fluorescence dye, 1 U of Taq polymerase (Roche), and template DNA. PCR was carried out in a GeneAmp PCR System 9600 (Applied Biosystems), and PCR products were analyzed by capillary electrophoresis on an ABI 3100 (Applied Biosystems) with performance-optimized polymer 4 (POP-4), a 47-cm capillary, and GA buffer plus EDTA. We mixed 1 μL of PCR product with 12 μL of deionized formamide containing 0.3 μL of GeneScan-500 ROX Size Standard (Applied Biosystems). Each sample was heated at 93 °C for 3 min to denature the DNA, chilled for 3 min at 4 °C, and then separated on ABI 3100. The sizes of the PCR products were determined by use of GeneScan software (Applied Biosystems).

After we determined the alleles for the 96 donors based on PCR product size, we calculated the number of alleles, the heterozygosity, and useful statistical values for application to STR analysis, by use of the PowerStat program (Promega).

On the basis of the allele data, we chose two unrelated individuals among volunteers who shared only one allele for each STR. We excluded individuals who had stutter bands because interpretation could be difficult when stutter bands were present. We drew whole blood from the selected volunteers and determined leukocyte counts on the XE-2100 automated hematology analyzer (Sysmex). To simulate mixed chimerism, we calculated the volumes required to achieve a constant 10⁷ leukocytes with a targeted proportion of each sample. For example, when A and B had 5000 and 8000 leukocytes/μL, respectively and we had planned to make 1:1 mixtures of A and B, we took 1000 μL of the well-mixed whole blood from A and 625 μL from B. The mixture of the two would then contain 5 × 10⁶ leukocytes from A and 5 × 10⁶ leukocytes from B. After mixtures had been prepared, DNA extraction was performed as described above.

Samples targeting five different concentrations (1%, 5%, 50%, 95%, and 99% of one selected donor) were used to calculate the precision of each STR assay. Each sample was processed separately, and the measurement protocol consisted of two runs per day for 7 days. The results are presented as the ratio of the donor peaks area, which was calculated as follows: ratio = area of donor peaks/area of donor and recipient peaks (7). To determine the detection limit, we prepared 17 samples with concentrations ranging from 0% to 100% and assayed them twice. The detection limit was defined as the lowest dilution concentration at which the peak-area ratio of the minor cell population was >0.01 in each of two estimations.

The information for the observed alleles and statistical values for the seven STRs are represented in Table 1. The detection limits of the seven STR assays were between 0.5% and 5%, and the imprecision results are shown in Fig. 1. The imprecision ranged from 5.4% for TPOX to 12% for D7S820, on average, for all concentrations, and was inversely related to the proportion to the concentration of cell mixtures.

This study shows that the precision differed among STR assays and, for each marker, was related to the concentration of cell mixtures simulating chimeric stages of hematopoietic cells. This was especially evident at low-concentration <5%, where the imprecision of the