# Serum Levels of Ficolin-3 (Hakata Antigen) in Patients with Systemic Lupus Erythematosus

TRINE ANDERSEN, LEA MUNTHE-FOG, PETER GARRED, and SØREN JACOBSEN

*ABSTRACT.* ***Objective.*** Ficolin-3 is a serum protein of putative importance in autoimmunity. Our objective was to investigate any differential expression of ficolin-3 in patients with systemic lupus erythematosus (SLE) or its clinical subsets.

***Methods.*** Serum levels of ficolin-3 (S-ficolin-3) were determined in 95 patients with SLE and 103 healthy controls using an ELISA.

***Results***. Median S-ficolin-3 was 56.1 μg/ml (range 0 to ≥ 87.3) and 32.4 μg/ml (10 to ≥ 87.3) in patients and controls, respectively ($p < 0.001$). Increased S-ficolin-3 was associated with hemolysis, positive Coombs test, and lymphopenia, but not with SLE Disease Activity Index scores or C-reactive protein. In one patient without detectable S-ficolin-3, the *FCN3* gene appeared normal.

***Conclusion***. The elevation of S-ficolin-3 and its association with specific manifestations in SLE may indicate a pathogenetic role of ficolin-3 in SLE. (First Release Feb 1 2009; J Rheumatol 2009; 36:757–9; doi:10.3899/ jrheum.080361)

*Key Indexing Terms:*

SYSTEMIC LUPUS ERYTHEMATOSUS — COMPLICATIONS — ETIOLOGY
HUMAN FCN3 PROTEIN — FICOLIN — IMMUNOLOGY

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease[1] characterized by a humoral response against nuclear components, which may be triggered by insufficiently cleared apoptotic cells undergoing secondary necrosis and thereby releasing intracellular antigens[2]. Early complement pathway components C1q and mannose-binding lectin (MBL) serve as intermediates between apoptotic cells and macrophages, facilitating opsonization[3,4]. Besides C1q and MBL, ficolins constitute a new class of complement system initiators.

The ficolins consists of ficolin-1, ficolin-2, and ficolin-3 (Hakata antigen/H-ficolin)[5]. Ficolin-3 is a serum protein that may associate with MBL-associated serine proteases to activate the complement system[6]. Ficolin-3 binds to and sequesters late apoptotic cells[7].

Because ficolin-3 activates the complement system and mediates the clearance of late apoptotic cells we investigated differential expression of ficolin-3 in patients with SLE and its clinical subsets.

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## MATERIALS AND METHODS

*Patients and controls.* Peripheral venous blood samples were obtained from 95 consecutive Danish patients with SLE[1] (Table 1) and from 103 blood donors with similar distribution of age and sex as the patients. Samples were drawn in a vacutainer system, clotted for 30 min, and centrifuged at 3000 rpm for 15 min. Serum was stored at –20°C. Informed consent was obtained and the study was approved by the local medical ethics committee.

*Clinical data and disease activity measures.* Clinical data were systematically retrieved. The cumulative presence of typical SLE related manifestations including classification criteria as described[8] and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score[9] were recorded before determination of ficolin-3 levels. Serum concentration of C-reactive protein (CRP) was measured using a highly sensitive assay [CRP (Latex) HS, Roche Diagnostics, Mannheim, Germany; detection limit 0.1 mg/l; upper normal limit 5.0 mg/l)].

*Ficolin-3 in serum.* Serum levels of ficolin-3 (S-ficolin-3) were determined using a sandwich enzyme linked immunosorbent assay (ELISA)[10]. Because of the possible interference of heterophilic antibodies, every run of the assay was made in parallel with a plate coated with mouse antibodies without specificities against human proteins. To sera positive for interfering heterophilic antibodies, mouse antibodies were added, which removed the false-positive signal completely when reanalyzed.

*Detection of ficolin-3 autoantibodies and sequencing of FCN3.* In one patient with no detectable ficolin-3 in serum the possible presence of autoantibodies against ficolin-3 was determined by a previously described ELISA (32/10). The promoter region and the coding region of *FCN3* gene were sequenced as described[10].

*Statistics*. Mann-Whitney test and Spearman correlation analysis were used for group comparison of S-ficolin-3 and correlation analysis, respectively. p values < 0.05 were considered to indicate statistical significance.

## RESULTS

*Serum levels of ficolin-3.* S-ficolin-3 concentration was significantly higher in patients with SLE compared to healthy blood donors ($p < 0.0001$; Figure 1). One patient with SLE had extremely low amounts of measurable ficolin-3, with

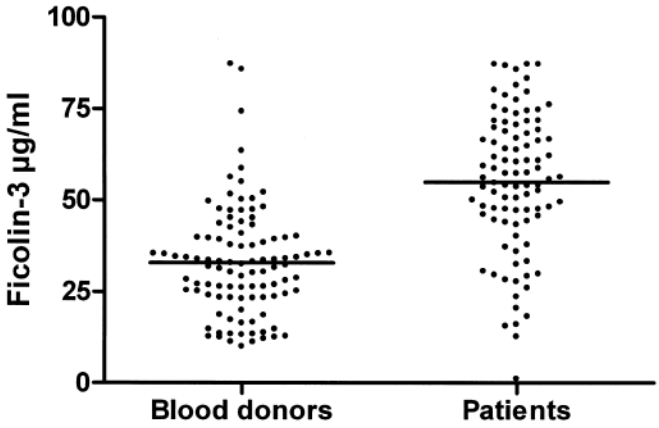


*Figure 1.* Ficolin-3 concentrations in 95 patients with systemic lupus erythematosus (SLE) and 103 healthy blood donors. Horizontal lines in each group indicate the median values, which were 56.1 µg/ml (range 1.1 to > 87.3 µg/ml) and 32.4 µg/ml (range 10 to > 87.3 µg/ml), respectively.

*Table 1.* Demographic and clinical characteristics of 95 patients with systemic lupus erythematosus (SLE). Median and range are given for continuous variables.

| Characteristic | |
|---|---|
| Males, n | 9 |
| Females, n | 86 |
| Age of first symptom, yrs (range) | 26 (6.0–71) |
| Age of onset, yrs (range) | 30 (13–72) |
| Age at inclusion, yrs (range) | 38 (15–73) |
| Disease duration, yrs (range) | 5.2 (0–37) |
| SLE disease activity score (range) | 2.0 (0–19) |

serum levels on repeat determinations ranging from 0 µg/ml to 1.1 µg/ml. This patient had also delivered DNA to a parallel project, allowing us to sequence the promoter and coding parts of *FCN3*. However, no mutations were found. In addition we looked for anti-ficolin-3 antibodies in this patient, but such antibodies were not detected.

*Associations to clinical features and measures of disease activity.* Measures of S-ficolin-3 stratified according to the cumulative presence of clinical and serological manifestations of SLE are shown in Table 2. Hemolysis, positive Coombs test, and lymphopenia were associated with increased S-ficolin-3. In 7 patients with peripheral neuropathy, S-ficolin-3 was lower than in the rest of the patients. S-ficolin-3 was not associated with alopecia, subacute LE, cutaneous vasculitis, signs of secondary Sjögren's syndrome, pulmonary fibrosis, myocarditis, endocarditis, lupus headache, myelopathy, myositis, noninfectious fever, thrombotic episodes, or infections requiring hospitalization (data not shown). S-ficolin-3 did not correlate to the SLEDAI scores at the time of blood sampling (Spearman's rho = –0.07, p = 0.54). Serum CRP in the SLE group was determined to range from 0.1 to 90.1 mg/l with a median value of 2.2 mg/l. Serum levels of CRP and ficolin-3 were not correlated (Spearman's rho = –0.05, p = 0.64).

## DISCUSSION

A novel finding of our study was the increased serum levels of ficolin-3 in patients with SLE. As ficolin-3 has functional similarities to C1q and MBL, the described association between SLE and deficiency states of C1q and MBL[11] could

*Table 2.* Serum levels of ficolin-3 in various clinical subgroups of 95 patients with systemic lupus erythematosus (SLE) according to the classification criteria SLE and selected other manifestations.

| Criterion | Patients with Manifestation | | Patients without Manifestation | | p |
|---|---|---|---|---|---|
| | No. of Patients | Ficolin-3 Levels, median (range) µg/ml | No. of Patients | Ficolin-3 Levels, median (range) µg/ml | |
| Classification criteria | | | | | |
| Malar rash | 43 | 60.9 (12.7 to ≥ 87.3) | 52 | 53.6 (1.1 to ≥ 87.3) | 0.24 |
| Discoid rash | 24 | 59.6 (15.6 to ≥ 87.3) | 71 | 55.7 (1.1 to 86.9) | 0.12 |
| Photosensitivity | 50 | 57.5 (12.7 to ≥ 87.3) | 45 | 54.1 (1.1 to ≥ 87.3) | 0.61 |
| Mucosal ulcers | 19 | 57.4 (12.7 to ≥ 87.3) | 76 | 55.9 (1.1 to ≥ 87.3) | 0.91 |
| Arthritis | 63 | 56.1 (1.1 to ≥ 87.3) | 32 | 55.6 (12.7 to ≥ 87.3) | 0.93 |
| Serositis | 38 | 60.2 (15.6 to ≥ 87.3) | 57 | 53.7 (1.1 to ≥ 87.3) | 0.19 |
| Proteinuria/nephropathy | 59 | 54.8 (1.1 to ≥ 87.3) | 36 | 57.4 (15.6 to ≥ 87.3) | 0.93 |
| Epilepsy/psychosis | 11 | 66.5 (23.6 to ≥ 87.3) | 84 | 55.2 (1.1 to ≥ 87.3) | 0.22 |
| Hemolysis | 11 | 73.8 (1.1 to ≥ 87.3) | 84 | 54.1 (12.7 to ≥ 87.3) | 0.007 |
| Thrombocytopenia | 32 | 55.6 (12.7 to 80.2) | 63 | 56.1 (1.1 to ≥ 87.3) | 0.74 |
| Leukopenia | 38 | 56.9 (12.7 to 85.9) | 57 | 55.7 (1.1 to ≥ 87.3) | 0.69 |
| Lymphopenia | 44 | 60.7 (18.2 to ≥ 87.3) | 51 | 53.6 (1.1 to 85.9) | 0.04 |
| Anti-DNA antibodies | 78 | 54.5 (1.1 to ≥ 87.3) | 16 | 59.2 (12.7 to ≥ 87.3) | 0.54 |
| Antiphospholipid antibodies | 23 | 53.7 (27.8 to ≥ 87.3) | 65 | 55.7 (1.1 to ≥ 87.3) | 0.70 |
| Anti-Smith antibodies | 11 | 57.4 (12.7 to ≥ 87.3) | 79 | 54.8 (1.1 to ≥ 87.3) | 0.93 |
| Antinuclear antibodies | 91 | 56.4 (1.1 to ≥ 87.3) | 4 | 41.5 (27.8 to 71.7) | 0.34 |
| Other manifestations | | | | | |
| Coombs test | 22 | 68.1 (29.3 to ≥ 87.3) | 73 | 53.7 (1.1 to ≥ 87.3) | 0.003 |
| Peripheral neuropathy | 7 | 29.6 (16.0–71.7) | 88 | 57.4 (1.1 to ≥ 87.3) | 0.02 |

be expected also to apply for ficolin-3. Further, ficolin-3 might play a protective role against development of autoimmunity[7]. On the other hand, serum amyloid protein P (SAP), which has similar characteristics with regard to clearance of late apoptotic cells as ficolin-3[12], has not been found to be decreased in SLE[13]. Even though not pronounced in SLE, innate defense proteins, for example CRP[14], may be upregulated in SLE. By still unknown mechanisms this may also apply for ficolin-3. As S-ficolin-3 was not correlated to SLEDAI scores or S-CRP, an upregulation of ficolin-3 may relate to features of early pathogenesis. The association of S-ficolin-3 with lymphopenia, hemolysis, and positive Coombs test in our study may indicate a yet unexplained role in antibody mediated activity against cellular components of the peripheral blood. The finding of low S-ficolin-3 in patients with peripheral neuropathy is unexplained and does not fit with the other findings of our study. The possibility of a spurious finding remains.

However, one patient had extremely low serum concentrations of ficolin-3 on repeated detections, which could be explained by the presence of anti-ficolin-3 antibodies[15], but such antibodies were not detected in this patient. As well we detected no mutations of the *FCN3* gene by DNA sequencing. This does not exclude splice defects in introns or epigenetic phenomena, which may explain the deficiency state of the patient.

Patients with SLE in our study had either an upregulated expression or altered catabolism of ficolin-3, and the association of these with certain hematolic manifestations of SLE may indicate a pathogenetic role of ficolin-3 in SLE.

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