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Increased serum YKL-40 in patients with pulmonary sarcoidosis—a potential marker of disease activity?☆

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Summary *Background:* YKL-40, a growth factor for fibroblasts and vascular endothelial cells, is secreted by macrophages and neutrophils. Elevated serum YKL-40 is found in patients with diseases characterized by inflammation, tissue remodelling and ongoing fibrosis. The aim was to evaluate whether macrophages and giant cells in the granulomatous sarcoid lesions of patients with pulmonary sarcoidosis produce YKL-40 and to determine whether serum YKL-40 in these patients were associated with disease activity.

Methods: Serum YKL-40 was determined by radioimmunoassay in 27 patients with a histological diagnosis of pulmonal sarcoidosis. Immunohistochemical staining for YKL-40 antigen was performed in five biopsies with pulmonary sarcoid lesions. Serum YKL-40 was likewise measured in 173 healthy age-matched control subjects.

Results: Mononuclear cells/macrophages and giant cells in pulmonary sarcoid granulomas expressed YKL-40 protein. Serum YKL-40 was higher in patients with pulmonary sarcoidosis compared to controls ($P < 0.001$) and 63% had elevated serum YKL-40. There was a positive correlation between serum YKL-40 and serum

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angiotensin converting enzyme (rho = 0.55, $P = 0.0053$). Patients with serum YKL-40 > median value in the patient group had lower carbon monoxide diffusion capacity corrected for alveolar volume (D_LCO/VA) than patients with serum YKL-40 ⩽ the median value ($P = 0.015$).

Conclusions: Serum YKL-40 may be a novel biomarker of sarcoid disease activity and ongoing fibrosis in patients with pulmonary sarcoidosis.

Introduction

YKL-40*, a member of family 18 glycosyl hydrolases,[1–5] is a heparin and chitin-binding lectin[4,5] without chitinase activity.[1,5] The gene for YKL-40 is known[6] and located on chromosome 1, and its crystal three-dimensional structure has been described.[7] However, the site and mode of binding of YKL-40 to cell surface receptors is still unknown. The biological function of YKL-40 is not known in detail. One study has shown that YKL-40 is a growth factor for fibroblasts and acts synergistically with insulin-like growth factor 1 in stimulating the growth of fibroblasts.[8] YKL-40 is also a growth factor for chondrocytes and synovial cells,[9] is a chemo-attractant for endothelial cells and stimulates migration of these cells at a level comparable to basic fibroblast growth factor.[10] Furthermore, YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating that YKL-40 may play a role in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells.[10]

YKL-40 is secreted in vitro by activated neutrophils,[11] macrophages during late state of differentiation,[6,12,13] arthritic chondrocytes[1] and cancer cells.[14,15] In vivo YKL-40 is expressed by macrophages in inflamed synovial membrane[13,16,17] and atheromatous plagues[18] and by macrophages and giant cells in the media of arteritic vessels of patients with giant cell arteritis.[19] The pattern of YKL-40 expression in normal and disease states suggests that the protein plays a role in inflammatory processes, remodelling of the extracellular matrix and development of fibrosis. Elevated serum concentrations of YKL-40 are found in patients with rheumatoid arthritis,[2,17,20,21] giant cell arteritis,[19] inflammatory bowel disease,[22,23] bacterial infections,[24,25] liver fibrosis[26–28] and metastatic cancer.[29–32]

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology characterized by the formation of noncaseating epithelioid cell granulomas.[33] Although essentially all organs of the body may be affected by sarcoidosis, the lungs are the most commonly involved.[34] Disease activity is accompanied by chronic inflammation resulting in mononuclear cell infiltrates and formation of granulomas. Even in the early phase of granuloma formation, a fibrotic response may be observed. In some patients with sarcoidosis, the fibrotic response can produce substantial and irreversible organ dysfunction and remodelling. A significant fraction of patients with chronic active pulmonary sarcoidosis succumb to respiratory failure.

The natural course of sarcoidosis is unpredictable in the individual patient. In most cases pulmonary involvement clears or stabilizes in more than 80% of affected patients.[35] However, permanent severe pulmonary dysfunction may occur and accounts for most morbidity and mortality.[35] Many attempts have been made to find serological biomarkers of disease activity in pulmonary sarcoidosis, which could help identify patients at risk for irreversible organ damage, e.g. lung fibrosis. Since the early 1980's, measurement of serum peptidyl-dipeptidase A also known as serum angiotensin-converting enzyme (SACE) has been used routinely to monitor disease activity in patients with sarcoidosis.[36–38] In patients with sarcoidosis, ACE is produced predominantly in the epithelioid cells/macrophages in sarcoid granulomas.[39] Therefore, the SACE concentration reflects the total granuloma mass,[40] but is not useful as a prognostic parameter of significant irreversible organ dysfunction.[38]

The aim of the present study was threefold: Firstly, to evaluate whether mononuclear cells/macrophages and giant cells in sarcoid granulomas of patients with pulmonary sarcoidosis produce YKL-40. Secondly, to evaluate the distribution pattern of serum YKL-40 levels in patients with sarcoidosis. Thirdly, to search for an association between serum YLK-40 and disease activity.

Methods

Patients: The hospital records of 222 patients with a diagnosis of sarcoidosis who had been admitted to

*YKL-40 is also named: human cartilage glycoprotein-39 (HC gp-39),[1] 38-kDa heparin binding glycoprotein (Gp38k),[4] Chitinase 3-like protein (CHI3L1),[6,8] Breast regressing protein 39 kDa (brp-39),[15] and Chondrex.[20]

the Department of pulmonary medicine, Gentofte Hospital in the period 1978–1995 were retrospectively investigated and data from the time of diagnosis and at succeeding follow-up investigations were recorded. In the period 1985–1995 the patients were systematically investigated with pulmonary function tests and blood tests at diagnosis and at out-patients clinic follow-up investigations after 1,3,6, 9 and 12 months and then once per year. This was not done according to a research protocol, but as a part of the clinical routine. In 27 of these patients blood specimens were available. Consequently we used this pre-existing material in the present study, which should be considered as a hypothesis generating pilot study. The study included 27 patients (22 men) with a median age of 41 years (range 24–64) with a diagnosis of pulmonary sarcoidosis confirmed by histology. Eight patients had manifestations of extrapulmonary sarcoidosis. The median disease duration was 12 months (range 0–198). During the observation period 12 patients were treated with prednisolone, and six of these were treated at the time of blood sampling with doses of 2.5–10 mg/daily. These patients had been tapered of from doses of 30–40 mg/daily over a period of 3 months to 7 years. None of the patients had cancer, liver disease, or symptoms/signs of non-sarcoid inflammatory joint disease.

Control subjects: Serum YLK-40 levels were determined in 173 healthy age-matched subjects (median age 34 years, range 24–64).[41] SACE was measured in 74 healthy adult subjects, members of the hospital staff and blood donors aged 20–60 years.

Biochemical analysis: Blood samples were allowed to clot at room temperature and centrifuged at 2000 g for 10 min. Serum samples were stored within 3 h at −80 °C. Plasma immunoglobulins (IgG, IgA, IgM), plasma calcium and erythrocyte sedimentation rate (ESR) were analyzed by routine methods. Serum YKL-40 was measured by an in-house radioimmunoassay (RIA) using rabbit antibody against human YKL-40.[2] SACE was analyzed by a spectrophotometric method.[42]

Lung function tests: Spirometry, carbon monoxide diffusion capacity (D_LCO) and alveolar volume (VA) were measured on VMAX 6209 (Sensor Medics Corporation, Yorba Linda, California, USA). D_LCO was measured by the single-breath method and VA by single-breath dilution of helium. The results were expressed as D_LCO corrected for VA (D_LCO/VA). Chest X-ray was not available in synchrony with blood sampling and lung function test.

Immunohistochemical staining for YKL-40: By flexible bronchoscopy, transbronchial lung biopsy specimens were obtained from five patients with pulmonary sarcoidosis in the initial diagnostic evaluation. The specimens were fixed in formaldehyde and stained using a conventional peroxidase staining technique for monoclonal antibodies (DAKO Envision System/HR, DK-1309, Copenhagen). A monoclonal antibody against human YKL-40 was used in an IgG concentration of 7 μg/l. A 3,3′-diamino-benzidine chromogen solution was used as colour substrate and recognized as a brown colour. Counterstaining was performed with Mayer haematoxylin.

Statistical analysis: Statistical analysis was performed with the Statview 5.0 for the MacIntosh using non-parametric statistics. The significance of differences was assessed by Mann–Whitney's test for unpaired differences correlations by Spearman's rho test. P-values $\leqslant 0.05$ were considered to be significant.

Results

In lung biopsy specimens from all five patients with pulmonary sarcoidosis, positive staining for YKL-40 antigen was found in macrophages and giant cells in sarcoid granulomas and in macrophages and neutrophils in areas with inflammation (Fig. 1). There was no YKL-40 staining in lymphocytes or fibroblasts.

Median serum YKL-40 concentration in sarcoidosis patients was 420 μg/l (range 106–1000), significantly ($P < 0.001$) higher compared to controls (median 100 μg/l; 5–95 percentile: 60–214 μg/l). Serum YKL-40 was elevated in 17/27 (63%) of the sarcoidosis patients (i.e. above the 95% percentile in controls). All patients had normal haemoglobin levels (mean 9.3 mmol/l (range 7.8–10.5).

The 95% CI for SACE in healthy adult subjects was 30–115 U/l. Median SACE level in sarcoidosis patients was 109 U/l (range 48–448). SACE was elevated (i.e. above the 95% percentile in controls) in 13/27 (48%) of sarcoidosis patients. Serum YKL-40 concentrations displayed a positive correlation to SACE ($\text{rho} = 0.55$, $P = 0.0053$) (Fig. 2) and an inverse correlation to D_LCO/VA ($\text{rho} = -0.40$, $P = 0.04$). Serum YKL-40 and SACE were not correlated to ESR, haemoglobin, plasma aspartate aminotransferase, plasma alkaline phosphatase, plasma calcium, plasma phosphate, plasma immunoglobulins, FEV_1, FVC and TLC.

Patients with serum YKL-40 concentrations of >420 μg/l (i.e. above the median serum YKL-40 level of all the patients) had significantly lower D_LCO/VA than patients with serum YKL-40 below or

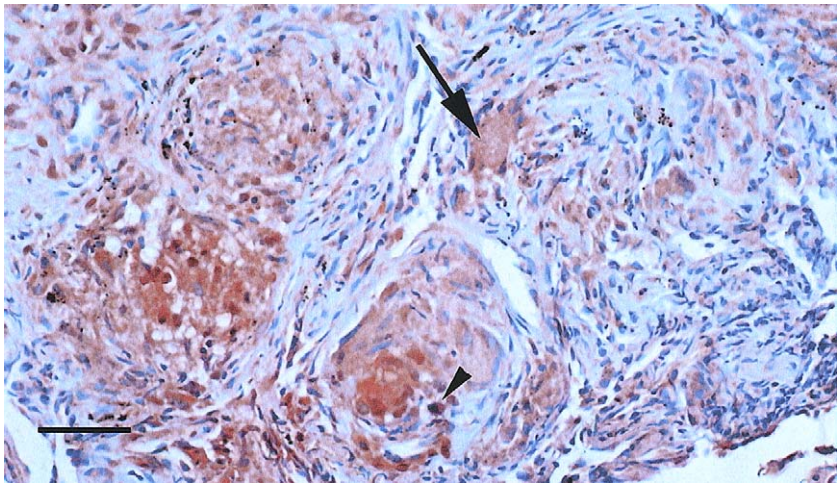

Figure 1 Light micrograph of immunohistochemical staining for YKL-40 in a lung biopsy specimen from a patient with pulmonary sarcoidosis (magnification × 20). Bar=50 μm. Arrow=YKL-40 positive giant cell. Arrow head=YKL-40 positive mononuclear cell.

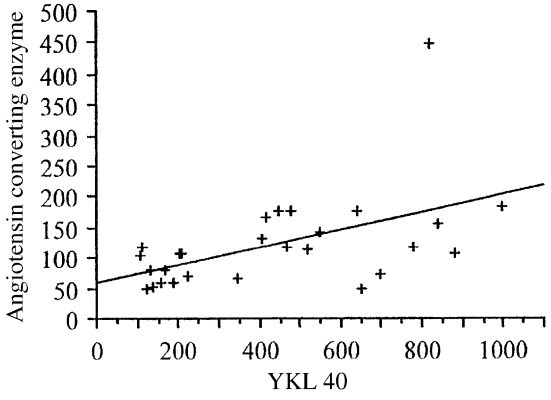

Figure 2 Correlation (Spearman's rho) between serum YKL-40 (μg/l) and serum angiotensin converting enzyme (SACE, U/l) in 27 patients with pulmonary sarcoidosis.

equal to the median (4.37 ml/min mmHg/l (range 3.54–5.73) vs. 5.24 (3.70–6.62), $P = 0.015$) (Fig. 3, right). No difference in D_LCO/VA was found in patients with SACE levels >109 U/l (i.e. above the median SACE level of all the patients) compared to patients with SACE ⩽109 U/l (Fig. 3, left). If the patients were divided according to D_LCO/VA, patients with a D_LCO/VA below the median level of all patients (i.e. <4.83 ml/min mmHg/l) had significantly higher median serum YKL-40 level (535 μg/l (range 112–1000)) compared to median serum YKL-40 level in patients with a high D_LCO/VA (204 μg/l (106–880), $P = 0.026$). The corresponding median values for serum SACE was 131 U/l (50–448 U/l) vs. 103 (48–176), $P = 0.04$. Serum YKL-40 was not related to the radiographic stages of the Chest X-ray that was not available in synchrony with blood sampling and lung function test.

Patients treated with prednisolone during the observation period had a trend towards higher serum YKL-40 levels than untreated patients ($P = 0.14$), and there was a borderline correlation between the cumulated dose of prednisolone and serum YKL-40 (rho = 0.38, $P = 0.053$). There was no difference in serum YKL-40 between smokers (495 μg/l (166–780), $N = 8$) and non-smokers (345 μg/l (106–1000), $N = 19$, $P = 0.33$).

Discussion

This is the first study of YKL-40 in patients with sarcoidosis. Using immunohistochemical staining of lung biopsies from patients with pulmonary sarcoidosis we found YKL-40 expression by macrophages and Langhans' giant cells in the sarcoid granulomas and by macrophages and neutrophils in areas with inflammation. We then determined serum YKL-40 in a small series of patients with pulmonary sarcoidosis and found elevated serum levels in 2/3 of the patients. Interestingly, the highest serum YKL-40 concentrations were found in patients with low D_LCO/VA. In contrast, SACE, which is used as routine follow-up parameter, could not discriminate between patients with low or high D_LCO/VA.

The definite biological function of YKL-40 is unclarified. YKL-40 probably has a function in both acute and chronic inflammatory processes and YKL-40 initiates a signalling cascade in fibroblasts that lead to increased cell proliferation,[8] suggesting that YKL-40 also has a role in conditions leading to tissue fibrosis. In patients with liver diseases of different aetiology elevated serum YKL-40 reflects

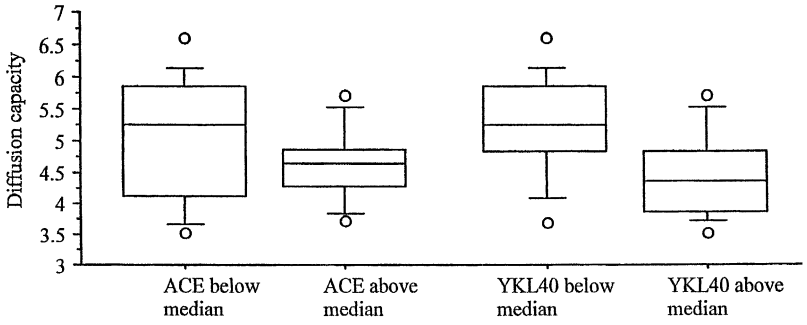

Figure 3 Box plots of diffusion capacity corrected for alveolar volume (D_LCO/VA, ml/min mmHg/l) in 27 patients with pulmonary sarcoidosis with SACE below and above the median value (109 U/l) in the entire series (right) and with serum YKL-40 below and above the median value (420 μg/l) in the entire series (left). The 5th, 25th, 50th, 75th and 95th percentiles are shown.

liver fibrosis,[26–28] and high serum YKL-40 in patients with alcoholic liver disease predicts short survival.[28] Whether YKL-40 is involved in the pathogenesis of sarcoidosis and in progression of organ fibrosis in patients with sarcoidosis remains to be clarified. Sarcoidosis is associated with up-regulated local and systemic inflammatory immune responses.[33] No specific common antigen has been identified in patients with sarcoidosis, and it is possible that more than one antigen may be involved in the T-cell response. YKL-40 has been demonstrated to be an autoantigen in patients with rheumatoid arthritis. YKL-40 derived peptides, which bind with high affinity to the rheumatoid arthritis associated HLA-DR1 and DR4, are recognized by peripheral T cells from patients with rheumatoid arthritis, and these T cells show a proliferative response to YKL-40 peptides.[43–45] In BALB/c mice, YKL-40 induces a chronic relapsing arthritis, which can be delayed and suppressed by inducing tolerance through nasal administration of YKL-40.[43,44]

Future studies should verify whether the YKL-40+ macrophages in patients with pulmonary sarcoidosis belong to the CD14+, CD16+ types. YKL-40+ monocytes/macrophages in peripheral blood and synovium from patients with rheumatoid arthritis are different from "classic" monocytes/macrophages and circulating dendritic precursors. The YKL-40+ cells are CD16+, have a dim expression of CD14 and resemble the CD14+, CD16+ monocyte population described by Ziegler-Heitbrock.[46] The role of the CD14+, CD16+ cell type remains to be determined. They are believed to be of pro-inflammatory type and a more mature version of monocytes with properties of tissue macrophages. Interestingly, the CD14+, CD16+ monocytes are increased in numbers in patients with rheumatoid arthritis,[16] sepsis,[47] tuberculosis[48] and solid tumours.[49]

The result of the present study of YKL-40 in patients with pulmonary sarcoidosis suggests that it would be interesting to study changes in serum YKL-40 concentrations in a longitudinal setting of patients with sarcoidosis. It needs to be clarified whether the serum YKL-40 level in combination with lung function tests and other potential serological parameters of disease activity (e.g. SACE, sIL-2R, TNFα, sTNF-RII) could be useful to monitor in order to identify at an early stage patients with sarcoidosis at high risk of irreversible lung fibrosis.

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