Serum connective tissue growth factor is aberrantly expressed in idiopathic inflammatory myopathy

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Connective tissue growth factor (CTGF) is a cysteine-rich protein secreted by fibroblasts and various other cells, playing a key role in regulating the extracellular matrix and mediating cell communication (1). As a target gene of transforming growth factor-β (TGF-β), CTGF is crucial in fibrosis, modulating TGF-β activity, and is markedly overexpressed in fibrotic lesions across multiple organs (2). Idiopathic inflammatory myopathies (IIM) comprise a heterogeneous group of autoimmune muscle disorders frequently associated with interstitial lung disease (ILD). While previous studies have linked CTGF to overall disease activity and vascular involvement in specific IIM subtypes, its role in IIM-associated ILD remains unclear (3). This cross-sectional study investigates serum CTGF levels in IIM patients, emphasising its clinical significance in ILD.

From January 2020 to February 2023, 100 adult patients with IIM and 30 healthy controls were recruited at West China Hospital, Sichuan University. Inclusion criteria were as follows: all patients met the Bohan and Peter criteria and EULAR/ACR criteria (2017), and patients with immune-mediated necrotising myopathy (IMNM) met the European Neuromuscular Centre criteria (2017). Participants <18 years, patients with other autoimmune diseases, chronic disease, tumours, or other muscle diseases, pregnant or lactating women, or patients who had engaged in strenuous physical activity within 1 week prior to recruitment were excluded. Detailed clinical information for both groups, are provided in Supplementary Table S1. Disease activity was

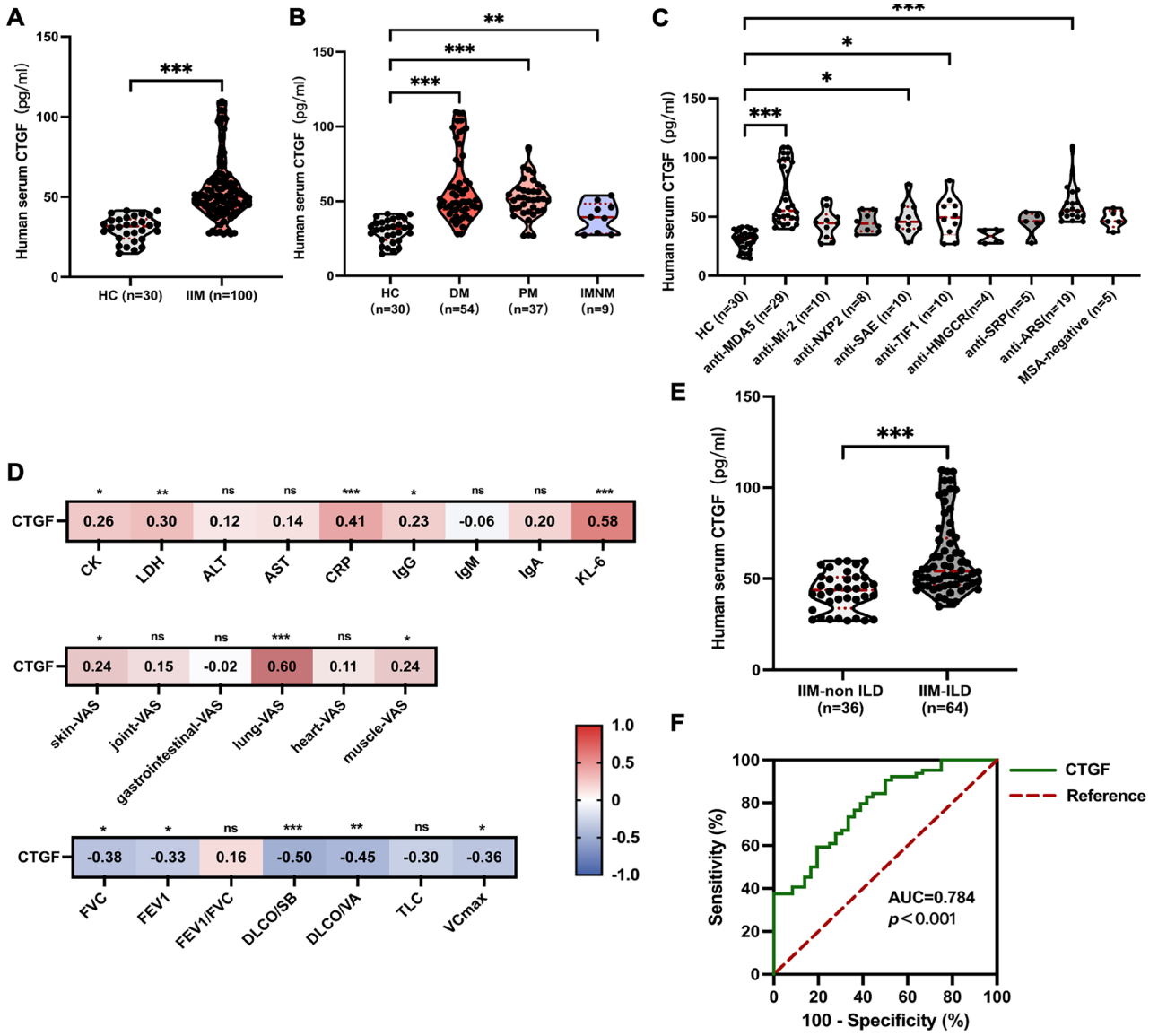

Fig. 1. Serum CTGF levels are elevated in patients with IIM and may be associated with IIM-ILD. **A**: Serum CTGF levels in HCs, patients with IIM. **B**: Serum CTGF levels in HCs and patients with DM, PM, and IMNM. **C**: Serum CTGF levels in HCs and patients with IIM classified by MSAs. **D**: Correlation analysis between serum CTGF level and clinical indicators, VAS scores for organ involvement, and pulmonary function test in patients with IIM. **E**: Serum CTGF levels in patients with IIM-ILD and IIM-non-ILD. **F**: Diagnostic value of CTGF in patients with IIM-ILD. The scatter dot plots are expressed as median with an interquartile range. Data are expressed as median (interquartile range), *p<0.05, **p<0.01, ***p<0.001. CTGF: connective tissue growth factor; HC: healthy control; IIM: idiopathic inflammatory myopathy; DM: dermatomyositis; PM: polymyositis; IMNM: immune-mediated necrotising myopathy; ILD: interstitial lung disease; MDA5: melanoma differentiation-associated gene 5; Mi-2: complex nucleosome remodelling histone deacetylase; NXP2: nuclear matrix protein 2; SAE: small ubiquitin-like modifier activating enzyme; TIF1: transcription intermediary factor 1; HMGCR: hydroxymethylglutaryl CoA reductase; SRP: signal recognition particle; ARS: aminoacyl-transfer RNA synthetase; MSA: myositis-specific autoantibody; CK: creatine kinase; LDH: lactate dehydrogenase; ALT: alanine transaminase; AST: and aspartate transaminase; CRP: C-reactive protein; KL-6: Krebs Von den Lungen-6; VAS: visual analogue score; AUC: area under the curve; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; DLCO/SB: single-breath diffusing capacity of the lungs for carbon monoxide; DLCO/VA: diffusing capacity of the lungs for carbon monoxide per alveolar ventilation; TLC: total lung capacity; VCmax: maximum vital capacity.

evaluated using the myositis activity visual analogue score (VAS), while ILD was diagnosed via high-resolution CT. Serum CTGF levels were quantified using ELISA kits (ab261851, Abcam). The relationships between serum CTGF levels, clinical indicators, and disease activity were analysed using Spearman correlation. This study adhered to the principles of the Declaration of Helsinki, was approved by the hospital's Ethics Committee (no. 246 in 2019), and obtained written informed consent from all participants prior to enrolment.

Serum CTGF levels were significantly higher in patients with IIM compared to healthy controls (55.01 *vs*. 30.23 pg/ml, $p<0.001$; Fig. 1A). Among IIM subtypes, patients with dermatomyositis (DM), polymyositis (PM), and IMNM exhibited elevated CTGF levels compared to controls [59.75 ($p<0.001$), 51.61 ($p<0.001$), and 39.70 ($p=0.005$) pg/ml, respectively] (Fig. 1B). Subgroup analysis based on autoantibody profiles revealed significantly higher CTGF levels in patients positive for anti-melanoma differentiation-associated protein 5 (anti-MDA5), anti-small ubiquitin-like modifier activating enzyme (anti-SAE), anti-transcription intermediary factor 1 (anti-TIF1), and anti-aminoacyl-transfer RNA synthetase (anti-ARS) antibodies compared to controls [68.63 ($p<0.001$), 48.48 ($p=0.02$), 49.68 ($p=0.01$), and 61.05 ($p<0.001$) pg/ml, respectively] (Fig. 1C).

The correlations between serum CTGF levels and clinical indicators, as well as VAS scores for organ involvement (skin, joints, gastrointestinal tract, lungs, heart, and muscles), were analysed in IIM patients (Fig. 1D). Serum CTGF levels showed significant positive correlations with CK (r=0.26, $p=0.010$), LDH (r=0.30, $p=0.002$), CRP (r=0.41, $p<0.001$), IgG (r=0.23, $p=0.026$), and KL-6 (r=0.58, $p<0.001$). Notably, CTGF levels were positively correlated with VAS scores for skin (r=0.24, $p=0.015$), lungs (r=0.60, $p<0.001$), and muscles (r=0.24, $p=0.016$). Furthermore, serum CTGF levels were significantly higher in IIM patients with ILD (IIM-ILD) compared to those without ILD (IIM-non-ILD) (61.83 *vs*. 42.89 pg/ml, $p<0.001$) (Fig. 1E). In 41 IIM-ILD patients who underwent pulmonary function tests, serum CTGF levels negatively correlated with multiple parameters, including forced vital capacity (r= -0.38, $p=0.014$), forced expiratory volume in one second (r= -0.33, $p=0.037$), single-breath diffusing capacity of the lungs for carbon monoxide (r=-0.50, $p<0.001$), DLCO per alveolar ventilation (r= -0.45, $p=0.004$), and maximum vital capacity (r= -0.36, $p=0.021$) (Fig. 1D). Finally, receiver operating characteristic curve analysis identified serum CTGF as a potential biomarker for distinguishing IIM-ILD from IIM-non-ILD, with a cut-off value of 45.51 pg/ml (sensitivity = 82.81%, specificity = 58.33%, area under the curve = 0.784, $p<0.001$) (Fig. 1F).

Serum CTGF levels increase in skeletal muscle following injury, driving inflammation and fibrosis through multiple signalling pathways (4, 5). In this study, serum CTGF levels showed positive correlations with CK, LDH, and muscle VAS scores, highlighting its role in muscle and microvascular inflammation central to IIM pathophysiology. As a downstream mediator of TGF-β, CTGF is a key factor in organ fibrosis (6). Previous studies have shown that conditional overexpression of CTGF in alveolar type II epithelial cells disrupts alveolarisation and vascular development, inducing vascular remodelling (7). Animal models of pulmonary fibrosis have shown that elevated CTGF expression exacerbates lung fibrosis (8). In our study, serum CTGF levels were significantly elevated in DM-ILD patients and positively correlated with KL-6 and lung VAS scores, reflecting disease severity. Negative correlations with specific pulmonary function test parameters further support its association with ILD-related ventilation impairments, suggesting the role of CTGF in IIM-associated pulmonary interstitial fibrosis.

In summary, these findings suggest that CTGF may play a role in IIM pathogenesis and serve as a potential serological marker for IIM-ILD.

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