Circulating Dickkof-1 as a potential biomarker associated with the prognosis of patients with rheumatoid arthritis-associated interstitial lung disease

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To the Editor: Interstitial lung disease (ILD) is a common extra-articular manifestation in patients with connective tissue diseases (CTDs), including rheumatoid arthritis (RA), which contributes significantly to the disease burden and excess mortality.[1] It has been well-recognized that RA-associated ILD (RA-ILD) patients have a threefold higher risk of death than RA patients without ILD.[2] However, among approximately 30% of ILD patients, the specific diagnosis cannot be made from clinical findings and high-resolution computed tomography features, resulting in diagnostic and management uncertainty.[3] Noninvasive blood biomarkers with diagnostic and prognostic utility may, therefore, provide alternative information for identifying vulnerable patients,[4] especially in clinical settings with limited medical resources. Dickkof-1 (DKK1) protein level was found to increase in lung tissue specimens from donors and idiopathic pulmonary fibrosis (IPF) patients.[5] In this context, the DKK1 was able to alter Wnt-induced epithelial cell proliferation in a dose-dependent manner,[5] implying clinical relevance of DKK1 in both ILD and RA.

This study was approved by the Ethics Committee of the General Hospital of Ningxia Medical University, and written informed consent was received prior to examination.

In this study, 102 patients (34 males and 68 females) with RA, including 35 RA patients with ILD patients and 67 RA patients without ILD, according to the criteria's of American College of Rheumatology and 2013 idiopathic interstitial pneumonia (IIP) classification were recruited. All the patients visited the General Hospital of Ningxia Medical University, China from December 2010 to January 2019. The clinical, laboratory, radiographic, and outcome data of all enrollments were collected from medical records and the survival status was obtained from medical records and/or telephone interview.

Concentration of serum DKK1 was measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits per manufacturer's instructions. The ELISA kit for DKK1 was a product of Elsbscience Inc. (Wuhan, China). For detection of DKK1 protein, the undiluted serum was directly detected with stock suspension.

Statistical analysis of data was performed using PRISM (version 5; GraphPad Software, La Jolla, CA, USA) and/or SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). One-way analysis of variance or Kruskal–Wallis test was employed for comparing the means of more than two groups, and the *t*-test was conducted for comparison between two groups. Receiver operating characteristic (ROC) curve was used to determine the best cut-off value and validity of certain variable. The multivariate logistic regression analysis was employed with SPSS software.

Out of the 35 patients with RA-ILD, the mean age was 60.4 ± 1.6 years (range: 50.0–80.0 years), 18 (51.4%) females and 17 males (48.6%), and the average duration of diseases was 10.1 ± 1.6 years (range: 0.4–40.0 years) at the time of sample collected (mean [SEM]). The majority of

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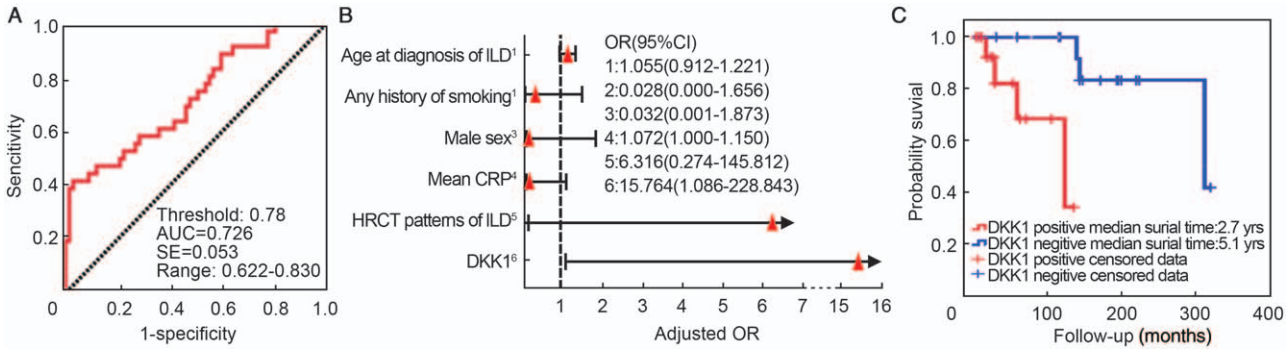

Figure 1: Risk factors for mortality in RA with ILD in uni- and multi-variate analysis. (A) Threshold and AUC values of DKK1 level from the area under the receiver-operating characteristic curves, ordered by decreasing adjusted odds ratio. (B) A forest plot showing the association of clinical risk factors and mortality in rheumatoid arthritis–interstitial lung disease (ILD) patients analyzed by binary logistic regression analysis. (C) Kaplan-Meier survival curves of DKK1 positive group (red, $n = 15$) and DKK1 negative group (blue, $n = 12$) showed that comparisons across two groups demonstrated significant differences in survival estimates between groups ($n = 27$, $P = 0.04$). Statistical differences were observed between DKK1 positive and DKK1 negative groups (2.7 *vs.* 5.1 years, $P < 0.001$. OR: odds ratio; CI: confidence interval.

ethnic population was Chinese Han (95%). Out of the patients, 22 were current smokers (55.0%), and the average 28-joint disease activity score (DAS28) was 5.9 ± 1.1 (range: 4.0–7.7). The average swollen joint count and arthralgic count were 6 (range: 3–12) and 8 (range: 4–12), respectively. At the time of the ILD diagnosed, patients were treated with conventional synthetic and biologic agents: nonsteroidal anti-inflammatory drugs (NSAIDs) ($n = 7$, 20% [7/35]), glucocorticoids ($n = 4$, 11.4% [4/35]), MTX ($n = 7$, 20.0% [7/35]), and DMARDs ($n = 17$, 48.6% [17/35]). Patients with ILD were significantly more likely to have ever smoked and/or were current smokers, regardless of gender or RA duration.

To determine whether circulating DKK1 protein was correlated with RA-ILD patients, the concentration of DKK1 protein was evaluated. DKK1 concentrations in sera of patients with RA-ILD (mean [SEM] 0.90 [0.17] ng/ml) were significantly increased as compared with RA patients without ILD (mean [SEM] 0.34 [0.03] ng/ml, $P < 0.0001$) and healthy subjects (mean [SEM] 0.28 [0.03] ng/ml, $P < 0.0001$).

The above-mentioned data showed that plasma DKK1 protein was more abundant in RA-ILD patients in comparison with that of RA patients without ILD and healthy subjects, the correlation of DKK1 protein and serologic features were analyzed. Interestingly, the DKK1 protein was positively correlated with CRP ($r = 0.4837$, $P = 0.0032$). Notably, the mean CRP level (45.5 ± 7.90 mg/L *vs.* 20.89 ± 4.03 mg/L, $P = 0.0086$, $n = 15$) in the RA with ILD group over the 9-year follow-up period was significantly higher than that in the RA control group.

An increased circulating DKK1 protein was detected in RA-ILD patients than in non-ILD RA patients and healthy subjects, suggesting DKK1 may have a clinical value in identifying ILD and monitoring ILD progression in RA patients. To evaluate the significance of serum DKK1 protein in clinical settings, we analyzed the sensitivities and specificities of DKK1 for identifying RA patients with ILD. The area under the curve (AUC) was 0.726 (SE: 0.053; range: 0.622–0.830; threshold: 0.78) for DKK1 [Figure 1A]. Interestingly, multivariate logistic regression analysis was performed to evaluate the risk factors for mortality while adjusting for confounding variables [Figure 1B]. The factors that were associated with fatal outcome were DKK1 protein (OR: 15.764; 95% CI: 1.086–228.843; $P = 0.043$) and CRP (OR: 1.072; 95% CI: 1.000–1.150; $P = 0.049$). In order to further detect the prognostic value of DKK1 in RA-ILD, a follow-up study was conducted. All of 35 RA-ILD patients were initially followed up, among them, 8 RA-ILD patients were lost and 27 RA-ILD patients were analyzed for the survival time. About 55.6% (15/27) of RA-ILD patients were positive for DKK1, were determined using a ROC threshold. The survival time was also analyzed in DKK1 positive group and DKK1 negative patients. The median survival was 5.1 years (1.3–12.0 years) in DKK1 negative patients but only 2.7 years (1.1–10.3 years) in DKK1 positive group ($P = 0.041$; Figure 1C), suggesting a worse median survival time in DKK1 positive group than DKK1 negative patients. Of note, the causes of death were available for only a small portion of the 27 RA-ILD subjects, although 8 of the known deaths, 5 were found pulmonary in nature and died from a cause at least in part related to the RA-ILD. These results imply that the DKK1 may be potential prognostic biomarker for accessing the disease progression ILD in RA patients, which needs further identification with large size of patient groups.

Collectively, this study analyzed the correlation of circulating DKK1 and clinical manifestations in 35 RA-ILD patients. These data suggest that the circulating DKK1 protein may serological biomarker with predicting values in RA-ILD, which warrants further investigation in clinical settings. However, this study has several limitations, including the small size of RA-ILD samples studied, the lack of complete follow-up data, such as pulmonary function and/or BALF testing. Additionally, most RA-ILD patients do not undergo surgical lung biopsy to confirm the pathological type. These limitations may partially explain the discrepancies between our study and other studies. Therefore, these findings require further confirmation in a larger and more selected population in the future.

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Conflicts of interest

None.

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