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CA 15-3 as an alternative marker for KL-6 in fibrotic lung diseases

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Abstract. *Background:* KL-6 is a mucin that is increased in interstitial lung diseases (ILD), and in some malignancies. CA 15-3, a tumor marker for breast cancer, refers to the same mucin but utilizes antibodies against different epitopes. *Objective:* The aim of our study was to evaluate CA 15-3 as a viable alternative to KL-6 as a marker for ILDs with and without fibrosis. *Design:* Serum from 242 patients with ILDs and from 327 healthy controls were included and KL-6 and CA 15-3 were measured in all subjects. Regression analyses and ROC curves were used to compare the performances of both markers. *Results:* KL-6 and CA 15-3 levels were both significantly higher in the ILD patients compared to the controls ($p < 0.0001$). A weak yet significant correlation was found between serum KL-6 and CA 15-3 levels in the controls (R=0.39, $p<0.0001$), but showed a much higher correlation in the patient group (R=0.85, $p<0.0001$). CA 15-3 correlated best with KL-6 in patients with fibrotic ILDs (R=0.83, $p<0.0001$). KL-6 performed better as a marker compared to CA 15-3 in most ILDs. Both markers performed best in identifying idiopathic pulmonary fibrosis (IPF) and were equally able to differentiate between ILDs with and without fibrosis: (sensitivity and specificity %): 100/97, 95/92, and 90/72, respectively. *Conclusion:* CA 15-3 and KL-6 are equally sensitive and specific in terms of differentiating between ILDs with and without fibrosis. The wide availability, ease of use, and cost effectiveness, make CA 15-3 a viable alternative for KL-6 as a possible marker for pulmonary fibrosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2010; 27: 138-146)*

Key words: interstitial lung diseases, fibrotic lung diseases, biomarkers, CA 15-3, KL-6

Introduction

Krebs von den Lungen-6 (KL-6), named after its associated malignant pulmonary disease in which this mucin was found in elevated concentrations, has been heralded as a promising marker for various lung diseases associated with pneumonitis (1). In particular, KL-6 is associated with the presence of fibrosis including idiopathic pulmonary fibrosis (IPF) (2, 3).

KL-6 is a commercially available monoclonal antibody raised against a specific epitope of the heavily glycosylated mucin 1 protein (4). In the literature, however, the term KL-6 is commonly used to indicate the protein, rather than the antibody.

Mucin 1, encoded by the MUC1 gene, is identical to the target molecule to which antibodies have been developed collectively known as CA 15-3. The CA 15-3 assay utilizes a couple of antibodies which are directed against a unique variable-number tandem repeat on the protein backbone (DF3) and a

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carbohydrate epitope on that repeat (115D8) (5). CA 15-3 and commercial kits alike are currently being used as markers for monitoring early recurrence of breast cancer (6), while KL-6 has been claimed to be specific to MUC1 expression by the lung tissue. The latter is supported by studies reporting that KL-6 specifically recognizes mucin 1 that is derived from type II pneumocytes following injury and subsequent regeneration (7, 8).

The observation that KL-6 is elevated in various malignancies including breast cancer (9) and that CA 15-3 levels are also increased in interstitial lung disease (ILD) associated with polymyositis/dermatomyositis (10), suggests that perhaps CA 15-3 and KL-6 may be used interchangeably as markers for both cancer and ILDs associated with pulmonary fibrosis. In fact, CA 15-3 was recently found to be an excellent marker for disease progression in pulmonary fibrosis (11).

CA 15-3 is widely available, fully automated on an immunochemistry analyzer with robust quality control and monitoring, and is less costly than the KL-6 ELISA kit. Thus, the option to expand the utility of CA 15-3 into the diagnostic workup and/or monitoring of interstitial lung diseases would be beneficial in many ways. The aim of our study was to evaluate CA 15-3 as a viable alternative to KL-6 as a marker for specific ILDs, including IPF, sarcoidosis, and extrinsic allergic alveolitis (EAA).

Methods

Patients

Serum from 242 unrelated Dutch patients with interstitial lung diseases (ILDs) (147 males/95 females; age at diagnosis and/or phlebotomy (years ± SD): 48.8 ± 16.5, were included in this retrospective study. At the time of this study, none of the included patients was diagnosed with cancer.

Of this group, 179 patients (48 IPF/usual interstitial pneumonia (UIP); 15 nonspecific interstitial pneumonia (NSIP); 11 extrinsic allergic alveolitis (EAA), 92 sarcoidosis (stage 0/I (n = 48); stage II (n = 13); stage III (n = 14); stage IV (n= 17)); 9 Löfgren's syndrome; 4 various other ILDs) were diagnosed with an ILD at the time blood was collected in which KL-6 and CA 15-3 were measured. These patients were not on any treatment regimen for their ILD as they presented for the first time for diagnostic work-up. In the remaining patients (n=63), KL-6 and CA 15-3 were measured in blood samples, which were collected at later stages during follow-up. These included patients (n) with sarcoidosis (both stage II) (2), EAA (15), desquamative interstitial pneumonia (7); Churg-Strauss (1); CREST (4); iatrogenic pneumonitis (6); pulmonary alveolar proteinosis (1); Arcwelder's disease (1); asbestosis (1); COPD (1); chronic eosinophilic pneumonia (1); idiopathic hemosiderosis (1); NSIP (2); Langerhans cell histiocytosis (4); lymphangioleiomyomatosis (1); lymphoid interstitial pneumonia (2); Wegener's granulomatosis (1); mixed connective tissue disease (2); non-classifiable interstitial pneumonia (2); pulmonary veno-occlusive disease (1); respiratory bronchiolitis-interstitial lung disease (1); rheumatoid arthritis (2); scleroderma (3); vasculitis (1). The treatment status of these 63 patients was unknown at the time of phlebotomy.

The diagnosis of sarcoidosis was established in 100 patients when clinical findings were supported by histological evidence and after exclusion of other known causes of granulomatosis in accordance with the consensus of the ATS/ERS/WASOG statement on sarcoidosis (12). IPF/UIP (n=48) was diagnosed in accordance with the ATS/ESR international consensus statement (13) and NSIP (n=15) was diagnosed by means of multidisciplinary assessment of the patient using radiographic data (chest X-rays and HRCT) and/or biopsy of the lung. All other ILDs were diagnosed either by experienced chest physicians who are specialized in ILDs, or by multidisciplinary assessment using radiography and clinical signs and symptoms.

Controls

Venous blood samples were obtained from 327 (117 males/210 females) ostensibly healthy employees of the St Antonius Hospital (years ± SD): 40.2 ± 11.5. By completing a questionnaire, relevant background information was provided by these volunteers, which included medication and hereditary diseases. None of the volunteers were diagnosed with any malignancy at the time of phlebotomy.

The predominantly female workforce at this hospital explains the overrepresentation of women

who participated in this study. Fifty-five individuals (33 women and 22 men) smoked for at least five pack-years.

The medical ethical committee of the St Antonius Hospital approved the study conducted and all subjects gave formal written consent.

Measurement of serum markers

Serum CA 15-3 was measured with the Roche CA 15-3 II immunoassay (Roche Diagnostics GmbH, Mannheim, Germany (www.roche.com)), according to the manufacturer's instructions. The assay was run on a Roche Cobas 6000 (601E module) analyzer.

KL-6 concentrations in serum were measured by an ELISA technique using a specific KL-6 antibody kit (ED046; Eisai Co., Tokyo, Japan (www.eisai.co.jp)) as described previously (14). All samples were run in duplicate and mean values were used for analysis.

Statistical Analysis

Descriptive data for continuous variables are reported as median with 25th and 75th percentiles (unless otherwise stated). Non-parametric tests (Mann-Whitney) were used to examine differences in continuous variables between groups. Linear regression analyses were performed on KL-6 and CA 15-3 data sets to examine correlations expressed as Spearman's rho *(R)*. The sensitivity and the specificity were established using the receiver operating characteristic (ROC) curve by plotting the sensitivity against the reverse specificity (1 minus specificity) at each value. Comparison of ROC curves between KL-6 and CA15-3 was performed by using the approach described by Hanley et al. (15). This calculation accounts for the correlation that exists between two (or more) markers that are measured in a paired population.

The statistical evaluation of our data was performed using SPSS 16 (SPSS Inc., Chicago, IL, USA (www.spss.com)) and Graphpad Prism version 5 (Graphpad Software, Inc., San Diego, CA, USA (www.graphpad.com)) software packages.

Statistical significance was denoted by a value of $p < 0.05$ for all tests performed.

Results

Table 1 summarizes the observed serum KL-6 and CA 15-3 levels in healthy controls and patients with ILDs. The median (25th - 75th percentiles) levels of KL-6 and CA 15-3 in the 327 healthy individuals were 231 (178-294) U/ml and 14.9 (11.0-19.4) U/ml, respectively. In the ILD patients group as a whole, the

Table 1. Serum KL-6 and CA 15-3 values in ILD patients and controls

	Controls	Total ILD	Sarcoidosis	Sarcoidosis (stages 0-I)	Sarcoidosis (stages II-IV)	IPF/UIP	EAA*	NSIP
N	327	242	94	48	46	48	26	15
Male/Female	117/210	147/95	52/42	22/26	30/16	37/11	13/13	7/8
Age, mean (range)	40.2 (18-68)	48.8 (14-87)	38.5 (15-75)	37.4 (15-69)	39.6 (20-75)	64.0 (29-87)	53.4 (33-72)	57.7 (37-80)
KL-6 (U/mL) median (25th - 75th percentiles)	231 (178-294)	804† (459-1689)	547§ (374-784)	432¶ (352-602)	762¶ (534-931)	1656†† (1128-2592)	1759§§ (763-4292)	2212¶¶ (1049-3717)
CA 15-3 (U/mL), median (25th - 75th percentiles)	14.9 (11.0-19.4)	28.3‡ (18.6-49.3)	22.1‖ (16.4-32.5)	18.6** (14.9-27.5)	26.3** (20.8-34.7)	59.6‡‡ (40.4-103.6)	29.6‖‖ (21.7-88.6)	41.9*** (22.9-89.5)

ILD: interstitial lung disease; IPF: idiopathic pulmonary fibrosis; UIP: usual interstitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia

* Two patients were also diagnosed with pulmonary hypertension, and one patient was also diagnosed with scleroderma.

† controls vs. ILD, $p < 0.0001$; ‡ controls vs. ILD, $p < 0.0001$; § controls vs. sarcoidosis, $p < 0.0001$; ‖ controls vs. sarcoidosis, $p < 0.0001$; ¶ sarcoidosis parenchymal vs. non-parenchymal, $p < 0.0001$; ** sarcoidosis parenchymal vs. non-parenchymal, $p = 0.002$; †† controls vs. IPF/UIP, $p < 0.0001$; ‡‡ controls vs. IPF/UIP, $p < 0.0001$; §§ controls vs. EAA, $p < 0.0001$; ‖‖ controls vs. EAA, $p < 0.0001$; ¶¶ controls vs. NSIP, $p < 0.0001$; *** controls vs. NSIP, $p < 0.0001$

median (25th - 75th percentiles) KL-6 level was 804 (459-1689) U/ml and 28.3 (18.6-49.3) U/ml for CA 15-3. These KL-6 and CA 15-3 levels were both significantly higher in the ILD patients compared to the controls (Mann-Whitney test, p<0.0001 for both markers) (table 1 and figure 1A, 1B).

A modest but significant correlation was found between serum KL-6 and CA 15-3 levels in the control group (R = 0.39, p<0.0001; figure 2A). The two markers showed a much higher correlation in the patient group (R = 0.85, p<0.0001; figure 2B).

When the ILD patients were further divided into specific diagnoses, CA 15-3 correlated best with KL-6 in patients with fibrotic ILDs. More specifically, IPF/UIP patients showed high median levels of both KL-6 (1656 U/ml) and CA 15-3 (59.6 U/ml) that were closely correlated (R = 0.94; p < 0.0001).

KL-6 and CA 15-3 levels strongly correlated with each other in EAA patients (R = 0.89, p<0.0001), but compared to their respective controls, the median KL-6 value (1759 vs. 231 U/ml) was increased by more than 7-fold while the median CA 15-3 (29.6 vs. 14.9 U/ml) showed a 2-fold increase (table 1).

KL-6 and CA 15-3 also correlated with each other in sarcoidosis patients (R = 0.74, p<0.0001), albeit to a lesser extent when compared to the other ILDs. Sarcoidosis patients with parenchymal involvement of the lungs (stages II, III, IV) and those without (stages 0-I) parenchymal involvement, showed comparable correlations between KL-6 and CA 15-3 in both groups: R = 0.80, p<0.0001 and R = 0.74, p<0.0001 respectively.

Sarcoidosis patients with stages 0/I/II had higher KL-6 levels (596 U/ml) and CA 15-3 levels (23.4 U/ml) than KL-6 (231 U/ml) and CA 15-3 (14.9 U/ml) in controls, p < 0.0001 for both. KL-6 was significantly higher in patients with parenchymal involvement (stages II-IV) compared to patients without parenchymal involvement (stages 0-I) of the lungs (table 1): 762 vs. 432 U/ml respectively, p < 0.0001. Similar results were found for CA 15-3: 26.3 (parenchymal) vs. 18.6 U/ml (non-parenchymal), p = 0.002.

A comparison between the patients with stage III and stage IV showed no significant differences for either marker, KL-6: 790 (stage III) vs. 659 U/ml (stage IV), p = 0.79 and CA 15-3: 28.0 (stage III) vs. 21.3 (stage IV), p = 0.51.

When patients were grouped according to ILD with evident pulmonary fibrosis according to HRCT and/or biopsy (IPF/UIP (n=48) + DIP (n=4) + NSIP (n=13) + EAA (n=10) + nonclassifiable inter-

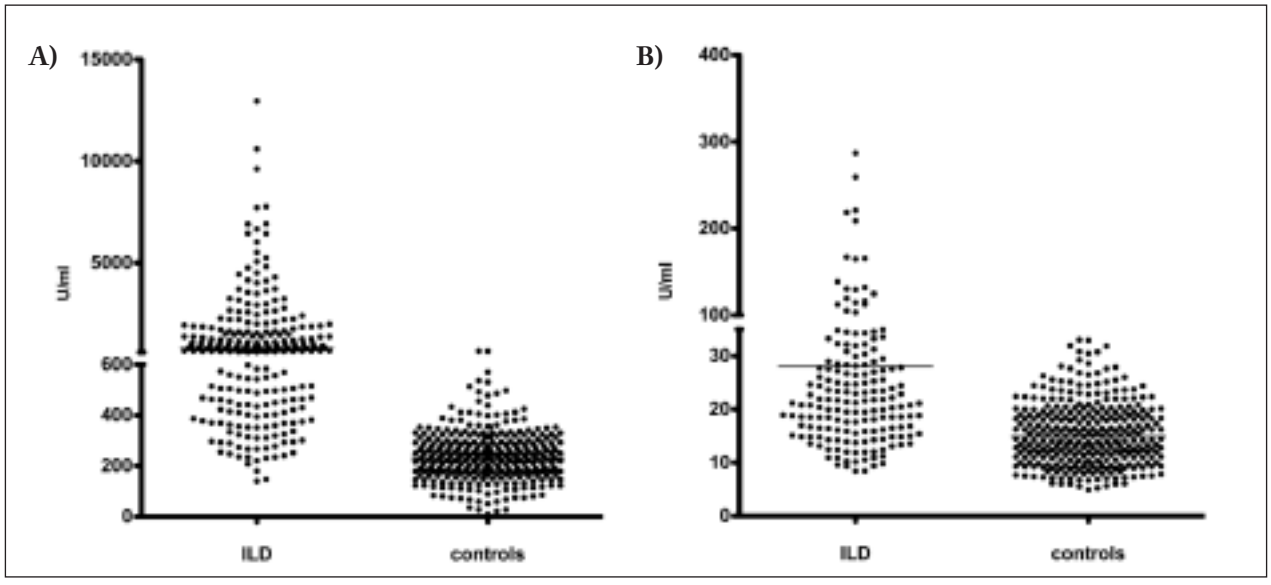

Fig. 1. Serum KL-6 (A) and CA 15-3 values (B) in patients with interstitial lung disease (n = 248) and healthy controls (n = 327). The horizontal line in the clusters represents the median value. **A** p < 0.0001 (Mann-Whitney); **B**: p < 0.0001 (Mann-Whitney).

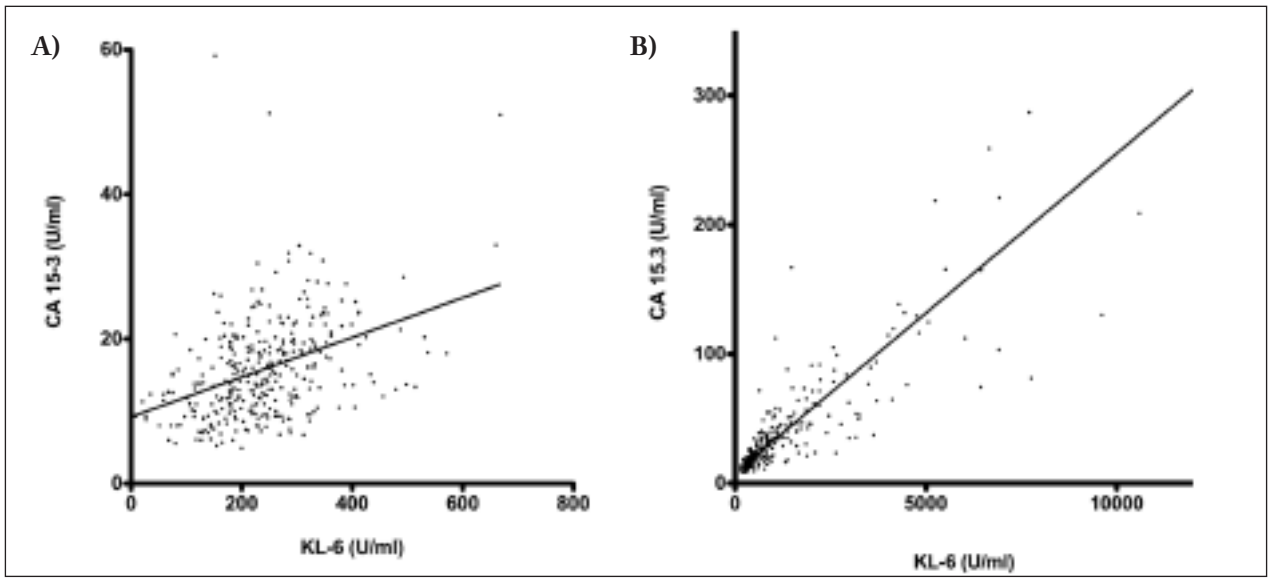

Fig. 2. Correlation between serum KL-6 and CA 15-3 levels in **(A)** healthy controls (n = 327) and **(B)** in patients with interstitial lung disease (n = 242). **A** R = 0.39; p < 0.0001; **B** R = 0.85, p<0.0001.

stitial pneumonia (NCIP) (n=2), the correlation between KL-6 and CA 15-3 was also significant (R = 0.83, p<0.0001). Stage IV sarcoidosis patients were not included in the fibrosis group, since the evidence of fibrosis was only established according to chest radiography.

ROC curves of KL-6 and CA 15-3

To assess the abilities of KL-6 and CA 15-3 to identify various disease states, ROC curves were made (figure 3). Cut-off levels were set as the closest point to 100% sensitivity and 100% specificity. Table 2 lists the results of KL-6 and CA 15-3 as markers for the total ILD group and separately for sarcoidosis, IPF, EAA, NSIP, and a selection of ILDs with fibrosis, including a subset of EAA and NSIP. The highest sensitivities and specificities were observed for KL-6 in all categories compared to CA 15-3 (table 2). Among the different ILDs, the best performance of both KL-6 and CA 15-3 was observed in IPF/UIP (sensitivity/specificity% (area under the curve (AUC) [95%CI]): 100/97 (0.999 [0.997-1.000]) and 95/92 (0.981 [0.964-0.998]), respectively. The area under the curves were significantly different between the two (p < 0.001).

Both markers showed the poorest performance for sarcoidosis patients sensitivity/specificity% (AUC [95% CI]): KL-6: 81/92 (0.903 [0.866-0.942]) and CA 15-3: 50/88 (0.768 [0.718-0.820]). The diagnostic performance of KL-6 was better for sarcoidosis patients with parenchymal involvement (versus controls) than for those without parenchymal involvement of the lungs: KL-6: 89/92 (0.933 [0.877-0.989]) (parenchymal) vs. 81/74 (0.889 [0.843-0.935]) (non-parenchymal). The areas under the curves were not significantly different (p = 0.1).

CA 15-3 also performed better for sarcoidosis patients with parenchymal involvement, but the optimal sensitivity, specificity and AUC were less than was found for KL-6: CA 15-3: 76/81 (0.837 [0.769-0.905]) (parenchymal) vs. 46/75 (0.710 [0.639-0.782]) (non-parenchymal). The areas under the curves of parenchymal vs. controls and non-parenchymal involvement vs. controls were significantly different (p < 0.01).

In the group with fibrotic lung diseases with various etiologies (n=77), not including sarcoidosis because the presence (or absence) of fibrosis was not established with either HRCT or biopsy, KL-6 showed a slightly higher sensitivity/specificity% (AUC [95% CI]) of 98/97 (0.995 [0.987-1.004]) compared to CA 15-3: 91/95 (0.975 [0.955-0.995]), (p < 0.001).

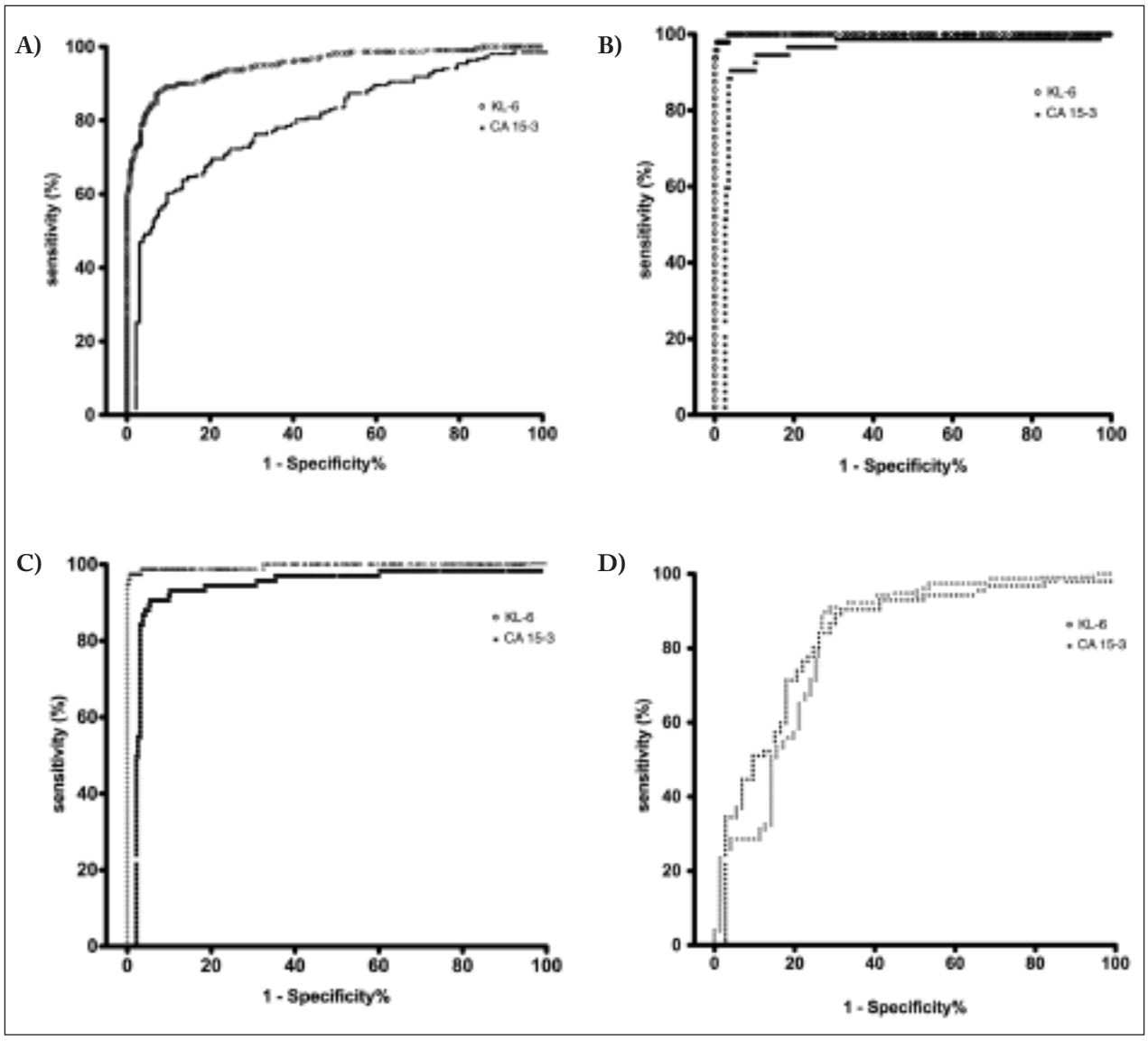

Fig. 3. Receiver operator characteristics curves of serum KL-6 and CA 15-3 levels in patients with interstitial lung diseases.
A interstitial lung diseases vs controls; **B** idiopathic pulmonary fibrosis/usual interstitial pneumonia vs controls;
C interstitial lung diseases with fibrosis vs controls; **D** interstitial lung diseases with fibrosis vs interstitial lung diseases without fibrosis.

Finally, when ROC curves were made of KL-6 and CA 15-3 levels in ILD patients with fibrosis (n=77) versus ILD patients without fibrosis (n=71), the markers performed similarly in terms of identifying the presence of pulmonary fibrosis (sens/spec% (AUC [95%CI]): 90/72 (0.820 [0.750-0.891]) (CA 15-3) vs 90/72 (0.872 [0.815-0.928]) (KL-6) (p = 0.064).

Discussion

Our data shows that KL-6 outperformed CA 15-3 in terms of its ability to differentiate between healthy individuals and ILD patients. The correlation between the two was much higher in ILD patients than in controls, which is probably inherent to the increased robustness of both tests at much high-

Table 2. Diagnostic performance of serum KL-6 and CA 15-3 in interstitial lung diseases.

	ILD*	Sarcoidosis	IPF/UIP	EAA	NSIP	Pulmonary fibrosis[II]
n	242 sens/spec (%)	94 sens/spec (%)	48 sens/spec (%)	27 sens/spec (%)	15 sens/spec (%)	77 sens/spec (%)
KL-6						
Optimal cut-off[†]	86/91	81/92	100/97	96/94	92/98	98/97
> URL[‡]	78/96	64/96	97/99	88/96	92/96	97/98
AUC	0.946	0.903	0.999	0.989	0.973	0.995
95% CI	0.927-0.964	0.866-0.942	0.997-1.000	0.997-1.001	0.926-1.002	0.987-1.004
CA 15-3						
Optimal cut-off[†]	63/88	50/88	95/92	73/89	77/88	91/95
> URL[§]	47/97	26/97	90/97	46/96	70/97	87/97
AUC	0.826	0.768	0.981	0.883	0.892	0.975
95% CI	0.791-0.861	0.718-0.820	0.964-0.998	0.811-0.955	0.793-0.991	0.955-0.995

ILD: interstitial lung disease; IPF: idiopathic pulmonary fibrosis; UIP: usual intersitial pneumonia; EAA: extrinsic allergic alveolitis; NSIP: nonspecific interstitial pneumonia; UAC: area under the curve, 95% CI = 95% confidence interval.

* Includes (n): sarcoidosis (94), IPF/UIP (48), EAA (27), NSIP (15), desquamative interstitial pneumonia (7); Churg-Strauss (1); CREST (4); iatrogenic pneumonitis (6); pulmonary alveolar proteinosis (1); Arcwelder's disease (1); asbestosis (1); COPD (1); chronic eosinophilic pneumonia (1); idiopathic hemosiderosis (1); Langerhans cell histiocytosis (4); Löfgren's disease (9); lymphangioleiomyomatosis (1); lymphoid interstitial pneumonia (2); Wegener's granulomatosis (1); mixed connective tissue disease (3); non-classifiable interstitial pneumonia (2); pulmonary veno-occlusive Disease (1); respiratory bronchiolitis-interstitial lung disease (2); rheumatoid arthritis (2); sclerodermia (4); vasculitis (1).

† Concentration at closest point to 100% sensitivity and 100% specificity.

‡ URL (upper reference limit) of KL-6 reference interval, determined in healthy controls.

§ URL of CA 15-3 reference interval, determined in healthy controls.

II Includes (n): IPF/UIP (48), desquamative interstitial pneumonia (4), nonspecific interstitial pneumonia (13), extrinsic allergic alveolitis (10), non-classifiable interstitial pneumonia (2).

er overall values as found in the ILD group. It should be noted, however, that extreme values in the patient population drove up the correlation coefficient significantly. But it demonstrates that KL-6 and CA 15-3 show a very good correlation in affected individuals in particular, which concludes that one or the other marker can be used for disease follow-up.

CA 15-3 and KL-6 performed on par in terms of the ability to identify IPF/UIP and ILDs with pulmonary fibrosis. It seems therefore that the two markers agree best in pulmonary diseases that are hallmarked by fibrosis. This observation is in accordance with an earlier preliminary study which showed that KL-6 and CA 15-3 correlated well in patients with interstitial pneumonia associated with collagen diseases (16).

In this study, the weakest performance was observed for both KL-6 and CA 15-3 as markers for sarcoidosis. The latter showed the poorest sensitivity and specificity for detecting sarcoidosis. In contrast to earlier findings by Ricci et al. (11), our data showed that sarcoidosis patients with stages 0, I and II combined, had higher CA 15-3 levels than did controls. The mean value of CA 15-3 in controls reported by Ricci was slightly higher (18.08 U/ml) than our mean value for controls (15.6 U/ml), while their stage I/II patients had mean CA 15-3 levels of 23 U/ml, compared to 23.4 U/ml in our study. In Ricci's study, however, the number of sarcoidosis patients with stage I and II was only 14, which may not have had sufficient power to identify these marginal yet significant differences in CA 15-3 levels.

We showed that both KL-6 and CA 15-3 were significantly higher in patients with parenchymal involvement compared to patients without parenchymal involvement of the lungs. A finding that was also reported by Miyoshi et al. (17) and Ricci et al. (11). This was also demonstrated by the better diagnostic performance of both markers in sarcoidosis patients with parenchymal involvement. However, neither KL-6 nor CA 15-3 was able to differentiate patients with stage III from those with stage IV. This lack of difference may be due to the presence of active fibrotic processes in some of the patients with

stage III, which cannot be identified using chest X-ray. On the other hand, although not significant, the stage IV group had slightly lower levels of both markers compared to stage III. It may be that these lower levels reflect an inactive, non-reversible fibrotic entity, while KL-6 and to a lesser extent CA 15-3, are increased in an active process involving inflammation and fibrotic formation. This notion is supported by studies which showed that KL-6 is not deemed suitable for diagnosing specific ILDs such as sarcoidosis (18) or bird fancier's lung disease, a form of EAA (19). Instead, KL-6 may be used as an indicator of disease activity and progression since KL-6 has shown strong associations with parameters of disease progression (18-20).

The largest discrepancy between the diagnostic performances of KL-6 and CA 15-3 were observed in the EAA group (table 2). At a cut-off point of the upper reference level, nearly half of these patients showed normal CA 15-3 levels with mostly elevated KL-6 values. Interestingly, the correlation between KL-6 and CA 15-3 was very strong. It shows that the dynamic changes of both markers are similar in EAA, but with overall higher KL-6 than CA 15-3 values compared to controls. Practically speaking, the strong correlation with KL-6 may still allow the use of CA 15-3 as a follow-up, rather than a diagnostic, marker for EAA after a diagnosis has been made. The optimal cut-off value at which CA 15-3 is most sensitive, is still well below the upper reference limit currently used in most laboratories (30 U/mL). Thus, a lower cut-off value for CA 15-3 may be recommended for identifying pulmonary fibrotic lesions. Although this would be at the expense of specificity, it could aid in the work-up toward diagnosing a fibrotic ILD.

Our results raise the question as to why mucin 1 is not equally picked up on by two different assays in ILDs which may or may not be associated with fibrosis. As KL-6 and CA 15-3 performed equally well as markers for ILD associated with pulmonary fibrosis, but seem discordant in ILDs which are not, it seems that KL-6 is more sensitive for both immunologic disease activity and the presence or development of fibrosis. As mentioned earlier, KL-6 is elevated in fibrotic lung diseases (2, 3), but it also marks the activity of inflammatory events in sarcoidosis and EAA (18, 19). Whether these findings are related to true "lung-specific" modifications of glycosylation of mucin 1 that makes the KL-6 assay more sensitive than CA 15-3 for identifying ILDs is unknown. The specific epitopes on the MUC1 mucin to which proprietary antibodies have been developed as CA 15-3, may be less affected in ILDs than the epitope to which the KL-6 antibody is raised. On the other hand, when KL-6 was used and compared to CA 15-3 as a marker for diagnosing breast cancer, the first was found to be much more sensitive than the latter in identifying any stage of cancer or cancer recurrence (9). It seems therefore that regardless of whether it originates from inflammation, cancer or pulmonary fibrosis, the elevations of KL-6 are more readily amplified than CA 15-3. As the TD-4 MUC1 workshop pointed out over a decade ago, the carbohydrate residues are involved in many epitopes, by regulating epitope accessibility or masking determinants, or by stabilizing preferred conformations of peptide epitopes within the MUC1 protein core (5). Thus, the ability of antibodies to recognize the carbohydrate residues on MUC1 mucin (such as KL-6), seems by no means static and may be prone to modifications of post-translational glycosylation that could take place in the increased output of cells from which mucins originate in disease states such as cancer (21, 22) and perhaps also ILDs. This may be reflected in the overall higher extent with which KL-6 is elevated compared to CA 15-3 in diseases such as ILD and cancer. On a final note, the treatment status of our patient cohort is heterogeneous because only those who presented for the first time at which a diagnosis was made were regarded as non-treated. The other 63 patients could have been on current treatment. There is little doubt that treatment with corticosteroids or other treatment regimens may influence the concentrations of circulating KL-6 and/or CA 15-3 with the improvement or worsening of the disease. However, assuming that KL-6 and CA 15-3 are modulated equally by (corticosteroid) treatment, the correlations obtained between the two markers would not be expected to change. Nevertheless, additional studies are needed to investigate the influence of different treatment modalities on differential expression of KL-6 and CA 15-3.

In conclusion, our study shows that CA 15-3 may be used for identifying and monitoring IPF and other fibrotic ILDs including EAA, NSIP, NCIP, IP, and UIP. Based on the results, which showed that

both markers are equally sensitive and specific for differentiating between ILDs with and without pulmonary fibrosis, CA 15-3 and KL-6 may be used interchangeably. Since CA 15-3 offers numerous benefits over KL-6, such as ease of use, cost effectiveness and quality control, the rather cumbersome KL-6 ELISA can be replaced by the fully automated CA 15-3 assay.

In order to determine whether CA 15-3 is able to replace KL-6 as a marker for the other ILDs, more patients with intricate clinical phenotypic descriptions are needed. In addition, systematically recorded radiographic data will also allow for better assessment of the performance of CA 15-3 as a disease markers in (fibrotic) sarcoidosis.

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References

1. Hermans C, Bernard A. Lung epithelium-specific proteins: Characteristics and potential applications as markers. Am J Respir Crit Care Med 1999; 159: 646-78.
2. Ohnishi H, Yokoyama A, Kondo K, et al. Comparative study of KL-6, Surfactant Protein-A, Surfactant Protein-D, and Monocyte chemoattractant protein-1 as serum markers for interstitial lung diseases. Am J Respir Crit Care Med 2002; 165: 378-81.
3. Kohno N, Yokoyama A, Kondo K, Nakajima M. Prognostic value of circulating KL-6 in idiopathic pulmonary fibrosis. Eur Respir J 2004; 24: A1671 (Abstract).
4. Kohno N, Akiyama M, Kyoizumi S, Hakoda M, Kobuke K, Yamakido M. Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. Jpn J Clin Oncol 1988; 18: 203-16.
5. Price MR, Rye PD, Petrakou E, et al. Summary report on the isobm td-4 workshop: Analysis of 56 monoclonal antibodies against the MUC1 mucin. San diego, calif., november 17-23, 1996. Tumour Biol 1998; 19 Suppl 1: 1-20.
6. Bast RC, Jr, Ravdin P, Hayes DF, et al. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: Clinical practice guidelines of the american society of clinical oncology. J Clin Oncol 2001; 19: 1865-78.
7. Kohno N, Kyoizumi S, Awaya Y, Fukuhara H, Yamakido M, Akiyama M. New serum indicator of interstitial pneumonitis activity. Sialylated carbohydrate antigen KL-6. Chest 1989; 96: 68-73.
8. Inoue Y, Barker E, Daniloff E, Kohno N, Hiwada K, Newman LS. Pulmonary epithelial cell injury and alveolar-capillary permeability in berylliosis. Am J Respir Crit Care Med 1997; 156: 109-15.
9. Ogawa Y, Ishikawa T, Ikeda K, et al. Evaluation of serum KL-6, a mucin-like glycoprotein, as a tumor marker for breast cancer. Clin Cancer Res 2000; 6: 4069-72.
10. Wong RC, Klingberg S, Wilson R. Ca15-3 and cancer associated serum antigen assays are alternatives to the KL-6 assay for measuring serum muc-1 levels in patients with interstitial lung disease associated with polymyositis/dermatomyositis. J Rheumatol 2002; 29: 2021-22; author reply 2022.
11. Ricci A, Mariotta S, Bronzetti E, et al. Serum CA 15-3 is increased in pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 2009; 26: 54-63.
12. Hunninghake GW, Costabel U, Ando M, et al. Ats/ers/wasog statement on sarcoidosis. American thoracic society/european respiratory society/world association of sarcoidosis and other granulomatous disorders. Sarcoidosis Vasc Diffuse Lung Dis 1999; 16: 149-73.
13. American thoracic society. Idiopathic pulmonary fibrosis: Diagnosis and treatment. International consensus statement. American thoracic society (ats), and the european respiratory society (ers). Am J Respir Crit Care Med 2000; 161: 646-64.
14. Kohno N, Awaya Y, Oyama T, et al. KL-6, a mucin-like glycoprotein, in bronchoalveolar lavage fluid from patients with interstitial lung disease. Am Rev Respir Dis 1993; 148: 637-42.
15. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 1983; 148: 839-43.
16. Okada M, Suzuki K, Nakanishi T, Nakashima M. Serum levels of KL-6 are positively correlated with those of CA15-3 in patients with interstitial pneumonia associated with collagen diseases. Respirology 2006; 11: 509-10.
17. Miyoshi S, Hamada H, Kadowaki T, et al. Comparative evaluation of serum markers in pulmonary sarcoidosis. Chest 2010.
18. Janssen R, Sato H, Grutters JC, et al. Study of Clara cell 16, KL-6, and Surfactant Protein-D in serum as disease markers in pulmonary sarcoidosis. Chest 2003; 124: 2119-25.
19. Janssen R, Grutters JC, Sato H, et al. Analysis of KL-6 and SP-D as disease markers in bird fancier's lung. Sarcoidosis Vasc Diffuse Lung Dis 2005; 22: 51-7.
20. Kobayashi J, Kitamura S. Serum KL-6 for the evaluation of active pneumonitis in pulmonary sarcoidosis. Chest 1996; 109: 1276-82.
21. Irimura T, Denda K, Iida S, Takeuchi H, Kato K. Diverse glycosylation of MUC1 and MUC2: Potential significance in tumor immunity. J Biochem 1999; 126: 975-85.
22. Kim YS, Gum J, Jr, Brockhausen I. Mucin glycoproteins in neoplasia. Glycoconj J 1996; 13: 693-707.