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# Potential Usefulness of Inflammatory Markers to Monitor Respiratory Functional Impairment in Sarcoidosis

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***Background:*** **Sarcoidosis is a multiorgan inflammatory granulomatous disorder of unknown origin for which adequate markers to monitor disease severity are lacking. The aim of this study was to evaluate the potential clinical usefulness of serologic markers of inflammation [high-sensitivity C-reactive protein (hs-CRP) and serum amyloid A (SAA)], T-cell activation [soluble interleukin-2 receptor (sIL2R)], and granuloma formation [angiotensin-converting enzyme (ACE)] for monitoring of sarcoidosis.**

***Methods:*** **Of the 185 sarcoidosis patients who visited the Sarcoidosis Management Center between 1999 and 2002, we selected 144 nonsmoking patients: 73 untreated (group I) and 71 treated (group II). Subgroups of the untreated patients [group Ia (nonchronic group with time since diagnosis ≤2 years) and group Ib (chronic group with time since diagnosis >2 years)] were evaluated separately. ROC curves and logistic regression analyses were used to compare the diagnostic accuracy of different markers to assess disease severity. Pulmonary disease severity was defined by lung function test results.**

***Results:*** **In untreated subgroup Ia and the total untreated group (group I), sIL2R had the largest areas under the curves (AUCs; 0.891 and 0.799, respectively) and the highest sensitivity (82% and 64%), specificity (94% and 88%), and positive (82% and 70%) and negative (94% and 88%) predictive values among the evaluated markers in both untreated groups. Nevertheless, the confidence intervals for sIL2R AUC, sensitivity, and specificity were broad and partly overlapped those of ACE, hs-CRP, and SAA. In the treated group (group II), all four markers appeared to have comparable AUCs, ranging from 0.645 for SAA to 0.711 for sIL2R.**

***Conclusion:*** **sIL2R appears to be useful for monitoring respiratory disease severity in sarcoidosis. We recommend sIL2R measurement in the follow-up of patients with sarcoidosis.**

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In young adults, pulmonary sarcoidosis is the second most common respiratory disease after asthma. Sarcoidosis is a systemic granulomatous inflammatory disease that primarily affects the lungs and lymphatic system of the body *(1, 2)*.

Sarcoidosis is characterized by a hyperimmune response to an unknown agent at the lesion sites *(1, 3)*. In sarcoidosis, inflammatory stimuli generally lead to activation of monocyte-macrophages, which in turn produce cytokines, e.g., tumor necrosis factor-$\alpha$, and interleukins, e.g., interleukin-1 (IL1)[5] and IL6 *(4, 5)*. As a consequence, IL1 and IL6 concentrations increase and stimulate hepatic production of acute-phase proteins such as C-reactive protein (CRP) and serum amyloid A (SAA) *(6)*. CRP has been shown to be a rather stable marker of systemic inflammation *(7)*. Recently, Drent et al. *(8)*, using a traditional, less sensitive CRP method, demonstrated that a moderate increase in serum CRP is implicated in sarcoidosis. High-sensitivity CRP (hs-CRP) methods have

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[5] Nonstandard abbreviations: IL, interleukin; hs-CRP, high-sensitivity C-reactive protein; SAA, serum amyloid A; sIL2R, soluble interleukin-2-receptor; ACE, angiotensin-converting enzyme; RFI, respiratory functional impairment; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide; and AUC, area under the curve.

recently been introduced to accurately monitor minor increases in serum CRP *(9)*, but no studies evaluating hs-CRP in sarcoidosis have been reported. Increased SAA has also been shown to be independently associated with sarcoidosis activity *(10)*. SAA appears to be less sensitive to immunosuppressive drugs, such as corticosteroids, and therefore has been recommended in the follow-up of patients to whom such drugs have been administered *(6)*.

Cytokines produced by activated monocytes-macrophages, mainly IL1 and IL6, also stimulate the production of IL2. Production of IL2 leads to T-cell activation *(11)*. Activated T cells express an IL2 receptor (55-kDa/75-kDa heterodimer) on their cell surface and release a soluble form of the 55-kDa chain, the so-called soluble IL2 receptor (sIL2R) *(12)*. sIL2R was found to be increased in patients with active sarcoidosis *(13, 14)*.

In sarcoidosis, inflammation does not resolve, but leads to granuloma formation. Angiotensin-converting enzyme (ACE) is a product of granuloma (of epithelioid cells that are derivatives of the activated macrophages). Despite its shortcomings, ACE is mostly used in the assessment and follow-up of sarcoidosis *(15)*.

All of the above markers have been shown to be related to sarcoidosis activity, but their relationships with the severity of this disease have not yet been fully established. A relationship between sIL2R and severity of sarcoidosis has recently been suggested *(16, 17)*, but ROC curve analysis was lacking in both studies.

From a clinical point of view it is even more important to know whether sarcoidosis is severe, rather than active. Lung function tests provide information about the presence of respiratory functional impairment (RFI), which is one of the indicators of disease severity *(1, 18)*. Furthermore, RFI is one of the reasons to initiate treatment, which is aimed at preventing irreversible fibrotic changes *(1)*.

The aim of the present study was to determine the diagnostic accuracy of sIL2R, ACE, hs-CRP, and SAA to predict the severity of pulmonary sarcoidosis, as indicated by RFI.

## Materials and Methods

### STUDY POPULATION

Between 1999 and 2002, 185 sarcoidosis patients visited the Sarcoidosis Management Centre of the University Hospital Maastricht, a Dutch referral center for sarcoidosis. Of these patients, 144 nonsmoking patients were prospectively included in this study. The diagnosis of sarcoidosis was based on consistent clinical features and bronchoalveolar lavage fluid analysis, according to the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders (ATS/ERS/WASOG) guidelines *(1, 19)*. The diagnosis was confirmed histologically in 85% of the cases. No comorbidity was present in any of these patients. Informed consent was obtained from all participants.

In sarcoidosis, spontaneous remissions without treatment can occur. For that reason, a period of observation of 2 years is justified if a patient is relatively asymptomatic. Hence, sarcoidosis is generally considered chronic if present for more than 2 years *(20)*. Accordingly, subgroup analyses were performed with the untreated group divided in two groups: a subgroup with a time since diagnosis $\leq$2 years (group Ia; nonchronic group) and a subgroup with a time since diagnosis $>$2 years (group Ib; chronic group).

To determine reference values, we collected venous blood samples (serum) from 282 ostensibly healthy blood donors presenting at the Sanguin Blood Bank in Maastricht. The procedure followed was approved by the Medical Ethical Committee of the Hospital.

### SEROLOGIC MEASUREMENTS

Simultaneously with the lung function tests, blood samples were taken, and serum was stored at −20 °C until actual measurement, which occurred for all samples within 2 months after storage. In addition, frozen aliquots of control sera were always checked. No influence on the stability of the evaluated markers was found for the samples that were treated in this way.

Serum sIL2R was determined by a two-site chemiluminescent enzyme immunometric assay (cat. no. LKIP1; Diagnostic Product Corporation) on the IMMULITE Automated Analyzer. The detection limit of the assay is 50 kU/L, and the measuring range is 50–7500 kU/L. The within- and between-run imprecision of the assay was $<$7.2%, and the reference interval for sIL2R was 241–846 kU/L.

hs-CRP and SAA were measured by particle enhanced immunonephelometry on the BN Prospec (Dade Behring). The detection limit for hs-CRP is 0.175 mg/L, and the measuring range is 0.175–1100 mg/L, depending on dilution (N Hs CRP; cat. no. OQIY 13; supplement reagent OUMU; Dade Behring). The detection limit for SAA is 3 mg/L, with a measuring range of 3–1000 mg/L, depending on dilution (N SAA reagent; cat. no. OQMP 11; Dade Behring). The imprecision of the SAA BN ProSpec method was $<$11%, and the reference interval was 0.90–10.22 mg/L.

Evaluation of the hs-CRP assay on the BN ProSpec has been reported previously *(21)*. The reference interval was 0.26–7.24 mg/L.

Serum ACE was measured by colorimetric method (cat. no. FU 116; Fujirebio Inc.). ACE acts on the substrate *p*-hydroxybenzoyl-glycyl-L-hystidyl-L-leucine and separates *p*-hydroxybenzoylglycine, which is converted in two subsequent reactions into quinoneimine dye. The absorbance of the quinoneimine dye is measured at 505 nm to evaluate ACE activity. The imprecision of the ACE assay was $<$5.6%, and the reference interval for ACE was 9–25 U/L.

The tests and the measurements in sarcoidosis patients were evaluated by one professional analyst or a PhD student trained by this analyst; both were blinded to the patients' histories.

### EVALUATION OF SEVERITY OF SARCOIDOSIS PULMONARY DISEASE

Chest radiographs were graded according to the radiographic staging of DeRemee (0 to III), with stage IV, the end stage of lung fibrosis, added *(1, 22)*.

Lung function indices, including the forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC), were measured with a pneumotachograph. The diffusing capacity for carbon monoxide (DLCO) was measured by the single-breath method. Both measurements were performed on a Masterlab (Jaeger). The intrasession CV for DLCO was 4–6%, and the intersession CV was 9%. Values were expressed as a percentage of those predicted *(23)*.

Both the radiographic staging and pneumotachography tests were performed and interpreted by two professionals who were blinded to the patients' histories.

### STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS 10.0 for Windows (SPSS). For all selected patients, nonmissing and interpretable results were obtained for both the laboratory tests and the lung function tests. The distributions of the explanatory variables CRP, SAA, ACE, and sIL2R were positively skewed; therefore, the data are presented as medians and interquartile ranges. A log transformation was applied to normalize the data before further analysis, if appropriate. Logistic regression was used to test the discriminatory effect of the (log-transformed) explanatory variables simultaneously by use of likelihood ratio tests. A weighted sum of explanatory variables with the estimated log odds ratios as weights served as linear predictor score in a ROC analysis. Areas under the ROC curves were compared using the paired nonparametric test described by DeLong et al. *(24)*. The optimal cutoff point (for the predictor) coincided with the point on the ROC curve where the sum of sensitivity and specificity was maximal. At this point the slope to the ROC curve equals unity, which is under certain conditions the result of minimization of the total costs attributable to false-positive and false-negative outcomes *(25)*. All $P$ values were two-tailed, and $P < 0.05$ was considered statistically significant.

## Results

### DEFINITION OF SEVERITY CRITERIA AND GROUP DESCRIPTION

The clinical characteristics of the study group are presented in Table 1. Only nonsmoking patients were included in the study because smoking can cause, or at least worsen, lung inflammation. Sarcoidosis patients were divided in two groups, untreated (group I) and treated (group II).

RFI was defined as DLCO <80%, FVC <80%, or FEV1 <80% (percentage of predicted). Patients without RFI were those for whom all three indices were ≥80%, according to standard recommendations *(1)*. A cross-tabulation of the test results with respect to the reference standard (RFI) are presented in Fig. 1. Treated patients appeared to have significantly lower sIL2R concentrations compared with the untreated patients ($P < 0.05$). The differences in sIL2R between the treated and untreated group also remained significant after correction for pulmonary function tests, i.e., presence of RFI ($P < 0.05$). Moreover, 55% of the treated patients presented with RFI compared with only 30% of untreated patients ($P < 0.01$).

**Table 1. Clinical characteristics of the studied sarcoidosis population (n = 144).[a]**

| | Group I | Group II |
|---|---|---|
| n | 73 | 71 |
| Gender,[b] M/F | 33/40 | 46/25 |
| Mean (SD) age,[d] years | 40.8 (11.0) | 43.8 (10.4) |
| Serologic markers, median (range[e]) | | |
| sIL2R,[b] kU/L | 733 (464–1244) | 584 (400–832) |
| ACE, U/L | 20.0 (16.0–27.0) | 20.0 (16.0–27.0) |
| hs-CRP, mg/L | 3.60 (1.68–8.36) | 3.03 (1.28–6.79) |
| SAA, mg/L | 5.33 (2.28–9.13) | 5.90 (3.14–11.70) |
| Lung function tests | | |
| RFI,[c] n (%) | 22 (30%) | 39 (55%) |
| Mean (SD) DLCO, % | 87.5 (18.6) | 82.3 (18.0) |
| Mean (SD) FVC, % | 99.2 (21.5) | 91.0 (20.4) |
| Mean (SD) FEV1, % | 92.2 (23.2) | 81.7 (23.1) |
| Chest radiographic staging, n (%) | | |
| Stage <II | 33 (45%) | 22 (31%) |
| Stage ≥II | 40 (55%) | 49 (69%) |

[a] Patients were selected according to the criteria described in *Materials and Methods*. Group I, all untreated patients; group II, all treated patients.
[b,c] Group I vs group II: [b] $P < 0.05$; [c] $P < 0.01$.
[d] Variables presented as mean (SD) follow a gaussian distribution.
[e] Range is 25th–75th percentiles.

The untreated group was further divided into two subgroups: a subgroup with time since diagnosis ≤2 years (group Ia; nonchronic group) and a subgroup with time since diagnosis >2 years (group Ib; chronic group). The clinical characteristics of the two subgroups are presented in Table 2. There was no relationship of inflammatory markers with time since diagnosis, but we observed significant differences in DLCO and FVC between the two groups ($P < 0.05$).

### DIAGNOSTIC ACCURACY OF EVALUATED INFLAMMATORY MARKERS

The ROC analysis results for the untreated and treated groups are presented in Fig. 2. Overall, the areas under the curves (AUCs) were significantly different from the null-hypothesis, true area = 0.5 (meaning no discrimination). In the group of untreated patients (group I; n = 73), the AUC for sIL2R was significantly larger than the AUC for ACE ($P = 0.033$), but we found no significant difference for sIL2R compared with hs-CRP ($P = 0.236$) or SAA ($P = 0.180$). The AUCs for hs-CRP and SAA were com-

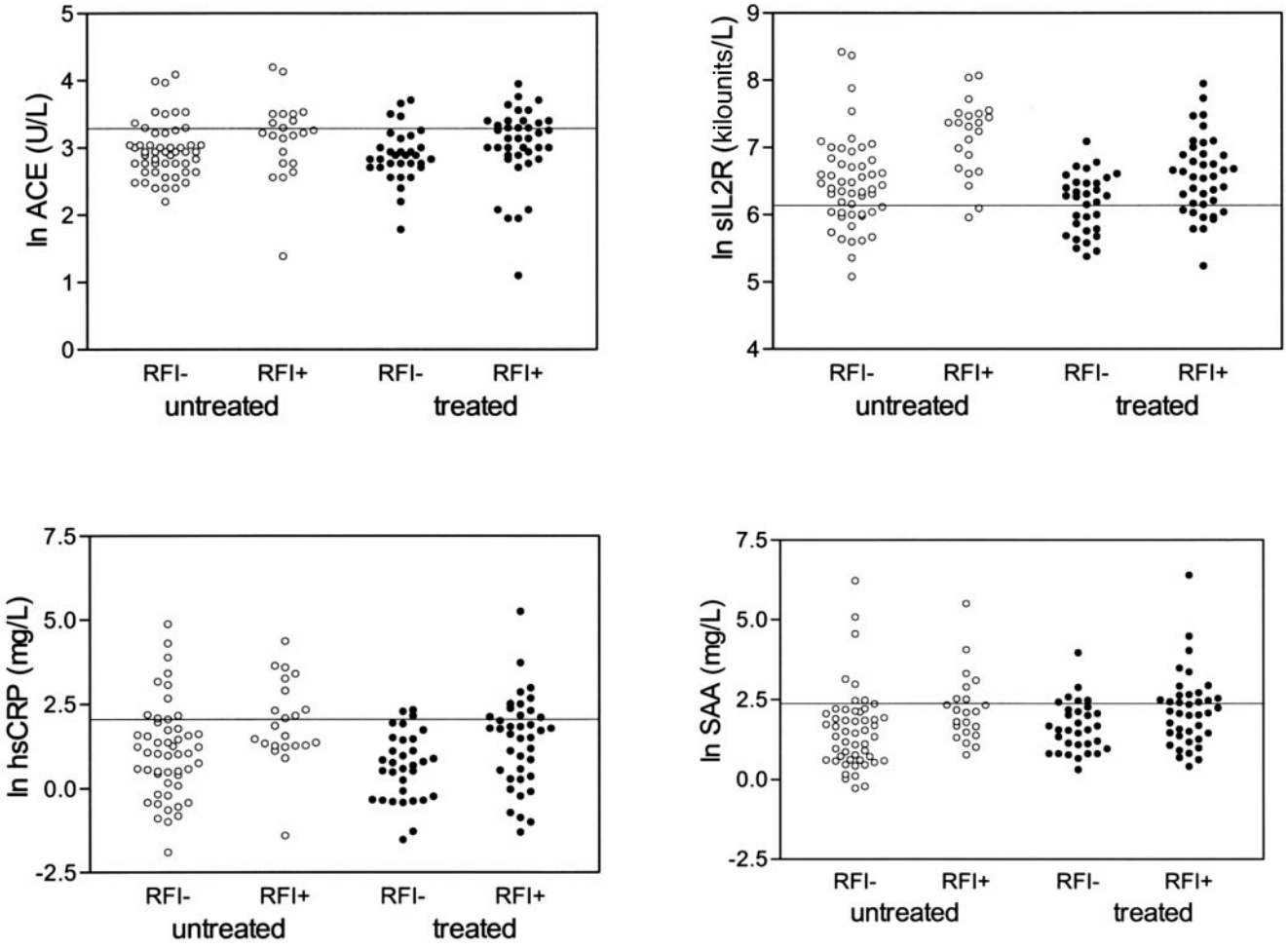


Fig. 1. Distribution of ln-transformed test results with respect to the reference standard.

RFI was used as a reference standard (*RFI*$^+$, RFI present; *RFI*$^-$, RFI absent). RFI was defined as present if DLCO was <80%, FEV1 was <80%, or FVC was <80% of the predicted value and as absent if DLCO was ≥80%, FEV1 was ≥80%, or FVC was ≥80% of the predicted value. ○, untreated patients; ●, treated patients. In each group, the *horizontal line* represents the upper limit of the reference interval (97.5th percentile).

parable ($P > 0.005$, not significant), and although smaller, both were not significantly different from the AUC for ACE (Fig. 2A).

In treated patients (group II; n = 71), the evaluated inflammatory markers had comparable AUCs, which were all <0.720 (Fig. 2B). However, in all three tested groups, the AUC for sIL2R was the largest. All ROC curves were significantly different from the AUC of 0.5.

In addition, logistic regression was used to test the discriminatory effect of explanatory variables simultaneously. The various combinations of markers yielded different logistic regression models giving different linear prediction scores for the construction of AUCs.

The linear prediction score based on the combination of all four markers yielded AUCs (SE) of 0.812 (0.055) for group I and 0.744 (0.058) for group II. The combinations of sIL2R and hs-CRP, sIL2R and SAA, and sIL2R and ACE yielded AUCs (SE) of 0.812 (0.054), 0.803 (0.056), and 0.803 (0.057), respectively, for group I and 0.733 (0.059), 0.732 (0.059), and 0.708 (0.061) for group II. However, none of these models appeared to be significantly different from the AUCs for sIL2R alone [0.799 (0.058) for group I and 0.711 (0.061) for group II].

### SUBGROUP ANALYSIS

The ROC results for the subgroups of untreated patients are presented in Fig. 3. In group Ia (nonchronic group; n = 42), only sIL2R ($P < 0.0001$) and ACE ($P < 0.04$) had an AUC significantly different from the null hypothesis; the *P* values for the AUCs for hs-CRP and SAA were 0.141 and 0.074, respectively. In group Ia, the AUC for sIL2R did not differ significantly from the AUC for ACE ($P = 0.111$).

In group Ib (chronic group; n = 31), the AUCs for sIL2R ($P = 0.043$), hs-CRP ($P = 0.019$), and SAA ($P = 0.035$) all were significantly different from the AUC of 0.5 ($P < 0.05$), in contrast to ACE ($P = 0.536$). The data for group Ib are presented in Fig. 3B.

The linear prediction score based on a combination of all four markers yielded AUCs (SE) of 0.886 (0.056) for

**Table 2. Clinical characteristics of the subgroups of untreated sarcoidosis patients (n = 73).[a]**

| | Group Ia | Group Ib |
|---|---|---|
| n | 42 | 31 |
| Gender, M/F | 21/21 | 12/19 |
| Mean (SD) age,[b] years | 41.1 (11.8) | 40.3 (10.1) |
| Serologic markers, median (range[c]) | | |
| sIL2R, kU/L | 825 (520–1437) | 618 (454–1203) |
| ACE, U/L | 19.5 (16.0–27.0) | 20 (14–27) |
| hs-CRP, mg/L | 3.55 (1.62–7.29) | 3.90 (1.68–8.68) |
| SAA, mg/L | 5.50 (2.33–9.29) | 4.67 (2.16–9.16) |
| Lung function tests[d] | | |
| RFI, n (%) | 11 (26%) | 11 (36%) |
| Mean (SD) DLCO,[d] % | 89.6 (14.7) | 84.8 (22.7) |
| Mean (SD) FVC,[d] % | 101.4 (16.5) | 96.2 (26.9) |
| Mean (SD) FEV1, % | 94.7 (19.1) | 88.8 (27.8) |
| Chest radiographic stage, n (%) | | |
| Stage <II | 22 (53%) | 11 (35%) |
| Stage ≥II | 20 (48%) | 20 (65%) |

[a] Patients were selected according to the criteria described in *Materials and Methods*. Group Ia, untreated patients with time since diagnosis ≤2 years; group Ib, untreated patients with time since diagnosis >2 years.

[b] Variables presented as mean (SD) follow a gaussian distribution.

[c] Range is 25th–75th percentiles.

[d] $P < 0.05$ for group Ia vs group Ib.

group Ia and 0.773 (0.084) for group Ib. The combinations of sIL2R and hs-CRP, sIL2R and SAA, and sIL2R and ACE yielded AUCs (SE) of 0.812 (0.067), 0.889 (0.055), and 0.880 (0.058), respectively, for group Ia and 0.777 (0.083), 0.723 (0.093), and 0.723 (0.043) for group Ib. However, they also were not significantly different from the AUCs for sIL2R alone [0.891 (0.054) for group Ia and 0.723 (0.043) for group Ib].

For the untreated group of patients, the optimal cutoff points were defined, as were their sensitivity/specificity pairs and predictive values. The combined results are presented in Table 3. Somewhat overlapping sensitivity and specificity confidence intervals were observed, but sIL2R had the highest combination of positive and negative predictive values among the markers: 70% and 85%, respectively, for group I; 82% and 94% for group Ia; and 67% and 84% for group Ib. In group Ib, however, the negative predictive value for both SAA and hs-CRP was 85% and the positive predictive value was only 50% for the chosen cutoffs.

### PROGNOSTIC VALUE OF sIL2R FOR UNTREATED PATIENTS

Although determining the prognostic value of sIL2R in untreated patients was beyond the scope of this study and should be addressed in a prospective study, some follow-up data were available. One of the questions raised was how many patients in the nonchronic untreated group, which is the most interesting to predict outcome, were finally treated with respect to the sIL2R values. Only 7 of 31 patients with low sIL2R values (≤1300 kU/L) compared with 8 of 11 patients with high sIL2R values (>1300 kU/L) needed treatment. This indicates that 73% of the cases with high values had a less favorable outcome compared with only 23% of cases with low sIL2R.

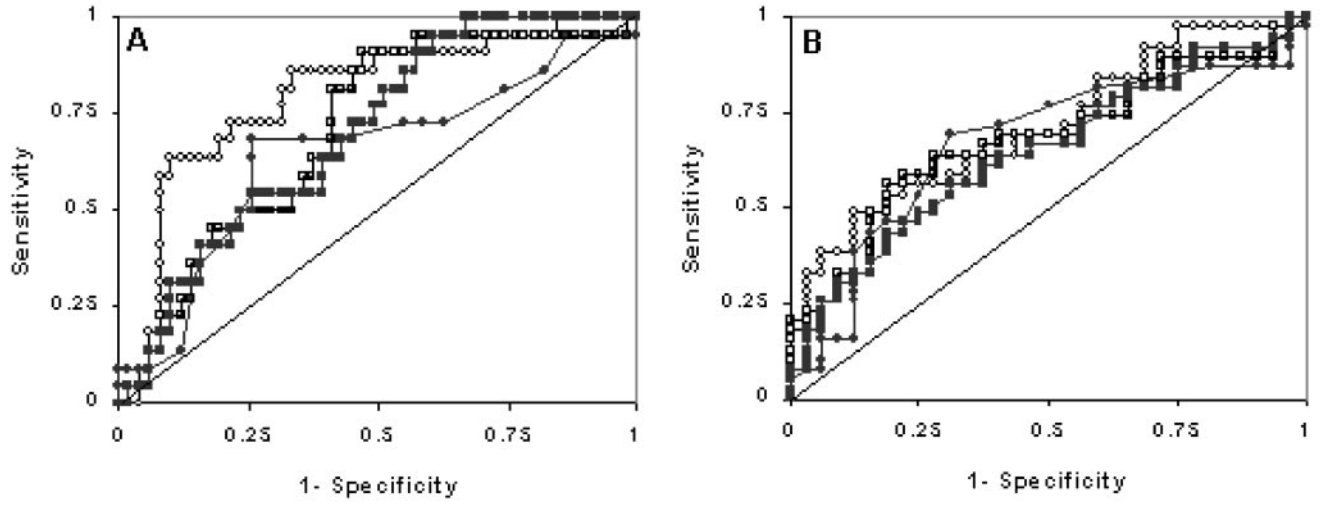


Fig. 2. ROC curves for the inflammatory markers to determine RFI in untreated (*A*) and treated (*B*) patients.

●, ACE; ○, sIL2R; ■, SAA; □, hs-CRP. The *diagonal line* indicates an AUC of 0.5 (no discrimination between the two states). RFI was defined as present if DLCO was <80%, FEV1 was <80%, or FVC was <80% of the predicted value and as absent if DLCO was ≥80%, FEV1 was ≥80%, or FVC was ≥80% of the predicted value. For group I (all untreated patients; *A*), the AUCs (95% confidence intervals) were 0.799 (0.686–0.913) for sIL2R, 0.650 (0.504–0.795) for ACE, 0.708 (0.583–0.832) for hs-CRP, and 0.701 (0.580–0.821) for SAA. For group II (all treated patients; *B*), the AUCs (95% confidence intervals) were 0.711 (0.592–0.829) for sIL2R, 0.671 (0.541–0.801) for ACE, 0.681 (0.556–0.806) for hs-CRP, and 0.645 (0.518–0.773) for SAA.

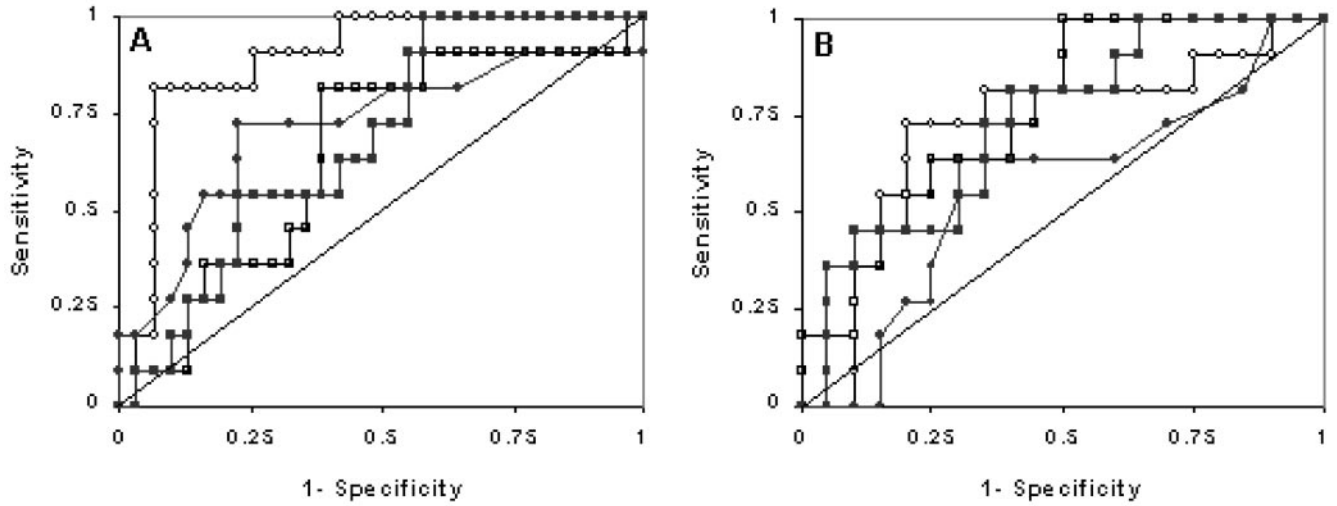


Fig. 3. ROC curves of the inflammatory markers in the untreated subgroups of patients.

●, ACE; ○, sIL2R; ■, SAA; □, hs-CRP. The *diagonal line* indicates an AUC of 0.5 (no discrimination between the two states). RFI was defined as present if DLCO was <80%, FEV1 was <80%, or FVC was <80% of the predicted value and as absent if DLCO was ≥80%, FEV1 was ≥80%, or FVC was ≥80% of the predicted value. (*A*), group Ia (nonchronic group). AUCs (95% confidence intervals) were 0.891 (0.786–0.997) for sIL2R, 0.720 (0.523–0.917) for ACE, 0.651 (0.466–0.836) for hs-CRP, and 0.683 (0.518–0.848) for SAA. (*B*), group Ib (chronic group). AUCs (95% confidence intervals) were 0.723 (0.521–0.924) for sIL2R, 0.568 (0.351–0.786) for ACE, 0.759 (0.591–0.927) for hs-CRP, and 0.732 (0.552–0.912) for SAA.

## Discussion

### DIAGNOSTIC PERFORMANCE OF THE EVALUATED MARKERS

This study evaluated the diagnostic accuracy of inflammatory markers to predict respiratory severity (RFI) in sarcoidosis. The present study provided a clear definition of the reference standard and used ROC curves in the assessment of the test performance as proposed by Zweig et al. *(25)*. The respiratory severity was assessed by lung function test results. In the whole untreated sarcoidosis patient group as well as in the subgroups of untreated group divided according to the time since diagnosis (group Ia, the nonchronic group, and group Ib, the chronic group), ROC curves and logistic regression analysis indicated that sIL2R has the highest ability to determine pulmonary severity. Comparable ROC curves for ACE, SAA, and hs-CRP were found in both untreated groups, independent of time since diagnosis. In the treated group (group II), all markers showed the same, weak ability to predict severity in sarcoidosis. Furthermore, their lines were far from the ideal ROC shape, giving several possible (sub)optimal cutoff points. Logistic regression analysis yielded linear predictor scores based on different combinations of markers, which were

**Table 3. ROC curve analysis results for the inflammatory markers in relation to RFI in the untreated (sub)groups.[a]**

| | Selected cutoff | Sensitivity (95% CI),[b] % | Specificity (95% CI), % | PPV, % | NPV, % |
|---|---|---|---|---|---|
| Group I (n = 73) | | | | | |
| ACE | 21 U/L | 68 (45–86) | 75 (60–86) | 54 | 84 |
| sIL2R | 1200 kU/L | 64 (41–83) | 88 (76–96) | 70 | 85 |
| hs-CRP | 3.0 mg/L | 91 (71–99) | 53 (39–67) | 46 | 93 |
| SAA | 2.5 mg/L | 96 (77–99) | 37 (24–52) | 40 | 95 |
| Group Ia (n = 42) | | | | | |
| ACE | 21 U/L | 73 (39–94) | 77 (59–90) | 53 | 89 |
| sIL2R | 1300 kU/L | 82 (48–98) | 94 (79–99) | 82 | 94 |
| hs-CRP | 3.5 mg/L[c] | 82 (48–98) | 58 (39–76) | 41 | 90 |
| SAA | 8.0 mg/L[c] | 55 (23–83) | 77 (59–90) | 46 | 83 |
| Group Ib (n = 31) | | | | | |
| ACE | 21 U/L[c] | 64 (30–89) | 70 (46–88) | 54 | 78 |
| sIL2R | 750 kU/L | 73 (39–94) | 80 (57–94) | 67 | 84 |
| hs-CRP | 3.5 mg/L | 82 (48–98) | 55 (32–77) | 50 | 85 |
| SAA | 4.0 mg/L | 82 (48–98) | 55 (32–77) | 50 | 85 |

[a] Group I, all untreated patients; group Ia, nonchronic untreated patients (time since diagnosis ≤2 years); group Ib, chronic untreated patients (time since diagnosis >2 years).

[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

[c] The AUCs of the ROC curves had wide confidence intervals (see legends of Figs. 2 and 3) and were not significantly different from 0.5.

used to construct the ROC curves in the various groups, but the ROC curves of the obtained models were comparable to the sIL2R ROC curve.

Although the sensitivity confidence intervals for ACE and sIL2R were largely overlapping, the positive predictive values were higher for sIL2R than for ACE. In addition, the specificity confidence intervals for sIL2R and ACE only partly overlapped (Table 2). Because these markers were correlated with each other, the DeLong method (nonparametric method) was used to avoid overemphasizing the differences between the AUCs *(24)*. It could definitely be confirmed that sIL2R was the strongest predictor of RFI in both untreated groups by means of logistic regression analysis (parametric method). These results are in agreement with results reported for previous clinical studies with comparable numbers of patients *(16, 17)*. The study of Grutters et al. *(17)* also suggested that extrapulmonary manifestations are accompanied by increased sIL2R values. Although extrapulmonary manifestations of sarcoidosis were beyond the scope of this study, this underlines the importance of sIL2R in sarcoidosis.

In line with results reported by others, this study demonstrated that ACE concentrations have poor predictive value in sarcoidosis *(16, 26–28)*. The reason for its poorer performance compared with sIL2R might, at least in part, may be explained by the fact that ACE concentrations can be influenced by an ACE polymorphism (I/D polymorphism in intron 16 of the *ACE* gene) *(29, 30)*. Therefore, adjustment of the reference values for the ACE polymorphism has been suggested *(30, 31)*. Nevertheless, with respect to the ACE polymorphism and susceptibility to disease progression, inconclusive data have been reported *(32, 33)*.

In the present study, the potential usefulness of hs-CRP and SAA to predict RFI in sarcoidosis was evaluated. The confidence intervals for hs-CRP and SAA sensitivity and specificity were broad and only partly overlapped with the confidence interval for sIL2R. In addition, their positive predictive values were much lower than those of sIL2R. These results are in agreement with a previous study, which found that the mean CRP concentrations in patients with stable or progressing disease (indicating severe disease) did not differ significantly from those in controls, in contrast to sIL2R *(16)*. It appears that the acute-phase response, reflected through increased CRP and SAA concentrations, can be expected only in patients with active disease, including Löfgren syndrome *(8, 10, 16)*.

SAA has been shown to be less sensitive to immunosuppressive drugs (i.e., corticosteroids) and has been recommended for monitoring of patients to whom such drugs have been administered *(6)*. However, this could not be confirmed by our study. Indeed, in treated patients, all four markers had comparable, rather low, AUCs. Because corticosteroids might affect the concentrations of the markers differently, we selected only those patients who had been on treatment for at least several months. Presumably this is the only way to gather information about the potential usefulness of the evaluated markers to reflect RFI in patients with sarcoidosis under treatment in general. To date, ACE has not appeared to be useful in the follow-up of sarcoidosis patients during corticosteroid treatment *(34)*. Similar results were demonstrated for sIL2R, CRP, and SAA in the present study.

### DEFINITION OF SEVERITY

The recommendations of the STARD group for the evaluation of diagnostic accuracy studies *(35)* were followed as far as possible in the present study. Pulmonary disease severity is usually evaluated by lung function tests and chest radiography *(1, 18)*, but there is no gold standard. In addition, there is only a weak correlation between lung function tests and chest radiographic stage *(1, 36)*. The most common indicators of RFI are DLCO and FVC *(1)*, which give information on the actual state of the lungs. Both indicate mutually restrictive and/or obstructive pulmonary function abnormalities in sarcoidosis *(1)*. Abnormal FVC, DLCO, and FEV1 values are traditionally used as indicators for treatment in case of pulmonary involvement *(36)*.

In conclusion, to initiate treatment it is crucial to know whether sarcoidosis is severe, rather than active. Hence, in this study, we examined whether the evaluated markers were able to predict sarcoidosis severity. Sarcoidosis severity was defined through RFI. In the untreated group of patients, sIL2R appeared to be the best marker for predicting disease severity, whereas the traditionally used ACE appeared comparable to hs-CRP and SAA. We therefore recommend the measurement of sIL2R, in addition to the standard measurement of ACE, to monitor disease severity and follow-up in sarcoidosis.

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