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Valeur diagnostique du rapport CD103+CD4+/CD4+ pour différencier la sarcoïdose d'autres causes de lymphocytose alvéolaire.

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Diagnostic value of the CD103+CD4+/CD4+ ratio to differentiate sarcoidosis from other causes of lymphocytic alveolitis

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Running head: BAL CD103+CD4+/CD4+ ratio in sarcoidosis

Key words: Sarcoidosis; CD103; Bronchoalveolar Lavage; Integrin; Lung Diseases, Interstitial; CD4-Positive T-Lymphocyte; Flow Cytometry

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Abstract

Introduction:

CD103 is a specific integrin present on some CD4+ lymphocytes of the mucosal immune system. It has been hypothesized that most CD4+ lymphocytes in pulmonary sarcoidosis do not originate from mucosal sites but from redistribution from the peripheral blood, and therefore do not bear the CD103 integrin. Several studies have suggested that a low CD103+ percentage among bronchoalveolar lavage (BAL) CD4+ lymphocytes discriminates between sarcoidosis and other causes of lymphocytic alveolitis, but contradictory data exist.

Methods:

We reviewed 1151 consecutive patients with BAL lymphocytosis >10% and flow cytometry performed between 2006 and 2014. 944 cases were excluded due to poor BAL quality (n= 97), unavailable clinical data (n= 760), or unclear diagnosis (n= 87). The remaining 207 patients were grouped into 9 diagnostic categories. To assess the discriminative value of the CD103+CD4+/CD4+ ratio to distinguish sarcoidosis from the other entities, area under ROC curves (AUC) were determined.

Results:

Sarcoidosis patients (n=53) had a lower CD103+CD4+/CD4+ ratio than the other diagnostic categories. AUC was 62% for sarcoidosis compared to all other patients and 69% for sarcoidosis compared to other interstitial lung diseases. When combining CD103+CD4+/CD4+ and CD4+/CD8+ ratios, AUC increased to 76% and 78% respectively. When applying published cut-offs from 4 previous studies to our population, AUC varied between 54 and 73%.

Conclusions:

The CD103+CD4+/CD4+ ratio does not accurately discriminate between sarcoidosis and other causes of lymphocytic alveolitis, neither alone nor in combination with CD4+/CD8+ ratio, and is not a relevant marker for the diagnosis of sarcoidosis.

Introduction

Sarcoidosis is a chronic multi-systemic disease of unknown origin characterized by granulomatous inflammation in various organs. It affects the lungs in 90 % of cases, and is the most frequent interstitial lung disease with a prevalence of 121/100 000 [1][2]. Diagnosis of pulmonary sarcoidosis is based on clinical symptoms, chest imaging, bronchoalveolar lavage (BAL), and histopathology obtained by either bronchial biopsy (BB), transbronchial lung biopsy (TBB), more recently transbronchial needle aspiration (TBNA), or rarely surgical lung biopsy [3][4][5].

Pulmonary sarcoidosis is characterized by increased T-cells in BAL fluid (BALF) compared to healthy subjects [6]. These T-cells are predominantly CD4+, with a CD4+/CD8+ ratio typically 3:1 to 5:1 compared with a ratio of 2:1 in healthy subjects [7]. However, the diagnostic value of BAL in sarcoidosis is limited by the lack of a specific and sensitive marker. BAL lymphocytosis is not specific of sarcoidosis and the use of CD4+/CD8+ ratio is controversial except when it is higher than 3.5 and only if the disease is active [8].

Several studies have investigated the CD103 integrin present on some CD4+ lymphocytes as a diagnostic marker of sarcoidosis [9][10][11][12]. CD103 is a specific molecule of the mucosal immune system [13]. It has been hypothesized that most CD4+ lymphocytes in pulmonary sarcoidosis do not originate from mucosal sites, but from redistribution from the peripheral blood, and therefore do not possess the CD103 integrin [14]. Kolopp-Sarda et al. were the first to investigate this integrin and suggest that a high CD4+/CD8+ ratio combined with a low CD103+/CD4+ ratio may allow to distinguish sarcoidosis from other interstitial lung diseases (ILDs) with a 96% sensitivity [9]. Similarly, Mota et al. found a 98% sensitivity [10]. However, two other studies showed a low performance of CD103+/CD4+ ratio as a diagnostic marker for sarcoidosis [11][12]. The goal of the present study was to further investigate the value of the CD103+CD4+/CD4+ ratio to differentiate sarcoidosis from other causes of lymphocytic alveolitis in a retrospective case series.

Materials and methods

Case selection

The case selection process is summarized in figure 1. 1151 BAL flow cytometries were performed at the Pathology Institute of the Lausanne University Hospital between January 1st, 2006 and July 18th, 2014. Flow cytometry was routinely performed on all BAL samples with >10% lymphocytes on differential cell count. 97 cases were excluded due to insufficient technical quality of the BAL sample. The electronic medical records of the remaining 1054 cases were reviewed. 760 cases were excluded due to unavailable clinical data (mainly because BAL was received from other medical facilities). Of the remaining 294 cases, 87 cases were excluded because the final diagnosis was unclear or unavailable (n=46), or because pre-defined diagnostic criteria (see below) were not met (n=41). The remaining 207 cases were included.

Pre-defined diagnostic criteria were the following. The diagnosis of sarcoidosis required compatible clinical and imaging picture and the presence of non-caseating granuloma on tissue biopsy (n = 22) or transbronchial needle aspiration cytology of mediastinal lymph nodes (n = 25). Typical stage I

sarcoidosis occurring in the context of Löfgren syndrome without biopsy or cytology was also accepted (n=6). The diagnosis of pulmonary tuberculosis required the presence of acid-fact bacilli, positive polymerase chain reaction and/or positive cultures for *M. tuberculosis* on sputum, bronchial aspiration, or BAL (n = 22). A diagnosis of infection other than tuberculosis required either identification of a virus or bacteria on sputum, bronchial aspiration, or BAL (n = 23), or marked clinical improvement with antibiotic therapy (n = 15). Hypersensitivity pneumonitis (HP) was diagnosed according to the criteria of Schuyler et al. (n = 6) [15]. Diagnosis of drug-induced pneumonitis was based on a history of exposure to a drug with known pulmonary toxicity, a temporal relationship between exposure and onset of symptoms, and improvement after drug withdrawal (n = 19). Organizing pneumonia (OP) was diagnosed on the basis of compatible clinical and imaging features, and presence of buds of granulation tissue on transbronchial lung biopsy without any feature suggesting another diagnosis (n = 5). The diagnosis of OP was also accepted in the absence of biopsy if radiation therapy for breast cancer, a classical cause of OP, occurred in the past year (n = 4). Nonspecific interstitial pneumonia (NSIP) was diagnosed on surgical lung biopsy (n = 2). A diagnosis of NSIP pattern was also accepted if the clinical and imaging findings were highly suggestive even in the absence of surgical lung biopsy (n = 12).

Cases not meeting the above diagnostic criteria were discarded. These included suspicion of sarcoidosis without histological diagnosis (n=13), clinical suspicion of tuberculosis without microbiological evidence of *M. tuberculosis* infection (n=7), clinical suspicion of infection without sufficient evidence to support this diagnosis (n=10), and clinical suspicion of OP without histological proof (n=11).

Included cases were grouped into 9 diagnostic categories: sarcoidosis, tuberculosis, non-tuberculous infections, HP, NSIP, OP, drug-induced lung diseases, other ILDs, and other diagnoses. As some categories had a small number of cases, cases were further grouped into 4 larger categories: sarcoidosis, infections (including tuberculosis and non-tuberculous infections), ILDs (including HP, NSIP, OP, drug-induced lung diseases, and other ILDs) and other diagnoses. Statistical analysis was performed for both the 9-category and 4-category distributions.

Bronchoalveolar lavage and flow cytometry

BAL fluid was collected during regular diagnostic workup in sterile bottles and filtered through gauze. 10 ml of the sample were cytocentrifugated then smeared and stained with May-Grünwald Giemsa, modified Papanicolaou and Prussian Blue for analysis. For differential cell count, a sample corresponding to 250.000-300.000 cells was passed through a multipore filter. After staining with modified Papanicolaou, a minimum of 2x100 cells were counted.

For flow cytometry, 10-30ml of native sample were centrifuged, decanted and resuspended in NH4Cl or Roswell Park Memorial Institute medium several times until reaching a cellularity of $2x10^6$. The cells were stained with fluorochrome-labelled monoclonal antibodies for CD45, CD3, CD4, CD8, and CD103 following a standard protocol and five-color flow cytometric analysis was performed (Navios Beckman Coulter flow cytometer).

Statistical analysis

Data analyses were performed using Stata software (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). Univariable analyses were performed and the results expressed as median and interquartile range (IQR) for continuous non-normally distributed variables, and as percentages for categorical variables. Bivariable analyses were done using Student's t-test and one-way ANOVA or Kruskal-Wallis tests. All hypothesis testings were 2-sided and type I error was controlled at the 0.05 level.

ROC curves were used to assess the discriminative power of the cellular marker ratios CD103+CD4+/CD4+, CD4+/CD8+ and their combination to distinguish sarcoidosis from other diagnoses. Optimal cut-off value, sensitivity, specificity and precision were determined for each ROC curve.

Results

Study population

A total of 207 patients with a confirmed diagnosis were included. The main characteristics of the study population are presented in table 1. The mean age was 54 years and 47% were women. Patients with sarcoidosis, HP and tuberculosis were significantly younger than patients of other diagnostic categories (p=0.0001). BAL neutrophils were significantly lower in sarcoidosis and higher in HP and non-tuberculous infections (p=0.0001). No significant differences were found between categories for BAL lymphocyte count.

Analysis of flow cytometry in 9 diagnostic categories

Results of flow cytometry in the 9-category distribution are presented in table 2. The CD4+/CD8+ ratio was significantly higher in sarcoidosis compared to all other diagnoses except for tuberculosis (figure 2A). The area under the ROC curve (AUC) to discriminate between sarcoidosis and all other diagnoses was 79 % (figure 3A). The cut-off value where specificity and sensitivity of CD4+/CD8+ ratio for sarcoidosis were equal (between 69% and 70%) was 3.4.

The CD103+CD4+/CD4+ ratio was significantly lower in sarcoidosis compared to drug-induced ILDs, NSIP and HP (figure 2B). The AUC to discriminate between sarcoidosis and all other diagnoses was 62% (figure 3B). A combination of CD4+/CD8+ and CD103+CD4+/CD4+ ratios resulted in an AUC of 76% (figure 3C), which was lower than the AUC of CD4+/CD8+ ratio alone.

Analysis of flow cytometry in 4 diagnostic categories

The data was also analysed after grouping cases into 4 large categories: sarcoidosis, all infections, all ILDs and others (table 3). CD4+/CD8+ ratio was significantly higher in sarcoidosis compared to each other category individually (figure 4A). The only significant difference in the CD103+CD4+/CD4+ ratio was between sarcoidosis and ILDs (figure 4B), with a higher ratio in ILD (0.14 vs 0.08, p<0.001).

The CD4+/CD8+ ratio in sarcoidosis compared to all ILDs had an AUC of 79%, with a cut-off where specificity and sensitivity are equal (69-70%) at 3.4. The AUC for the CD103+CD4+/CD4+ ratio was

69%. The combination of the CD4+/CD8+ and CD103+CD4+/CD4+ ratios resulted in an AUC of 78%, which was lower than that of the CD4+/CD8+ ratio alone (data not shown).

Validation of cut-off values from previous studies

4 studies analyzing CD103+CD4+/CD4+ ratio in sarcoidosis have been previously performed [9][10][11][12]. The cut-off values defined in these studies to discriminate between sarcoidosis and other diagnoses were applied to our study population for comparison (table 4). Sensitivity varied between 55% and 98%, and specificity between 9% and 86%. The precision of classification varied between 32% and 78%, and the AUC varied between 54% and 73%.

Discussion

In the present study, we demonstrate that the BAL lymphocyte CD103+CD4+/CD4+ ratio does not accurately discriminate between sarcoidosis and other causes of lymphocytic alveolitis, neither alone nor in combination with CD4+/CD8+ ratio, and is therefore not a relevant marker for the diagnosis of sarcoidosis. Furthermore, the CD103+CD4+/CD4+ ratio does not accurately discriminate between sarcoidosis and other ILDs. When applying the previously published CD103+CD4+/CD4+ ratio cut-offs to our population, we generally found lower sensitivity and specificity than in the original studies.

The CD4+/CD8+ ratio has been previously investigated to determine whether it could make an accurate diagnosis of sarcoidosis without lung biopsy [7][16][17][18]. In agreement with these data, we found a significant correlation between sarcoidosis and an elevated CD4+/CD8+ ratio (>3.4 in our study). However, the AUC of the CD4+/CD8+ ratio to distinguish sarcoidosis from other causes of lymphocytic alveolitis was only 79%, and it was also 79% to separate sarcoidosis from other ILDs. Thus, on its own, this marker cannot differentiate between sarcoidosis and other interstitial lung diseases with enough accuracy to be useful in daily practice.

Four previous studies have investigated the CD103 integrin on CD4+ T-lymphocytes in BAL as a putative marker for sarcoidosis [9][10][11][12], and several of them have suggested that a high CD4+/CD8+ ratio combined with a low CD103+CD4+/CD4+ ratio would allow to distinguish sarcoidosis from other causes of alveolar lymphocytosis. Kolopp-Sarda et al. first reported that the combination of a CD4+/CD8+ ratio > 2.5 and a CD103+/CD4+ ratio < 0.31 had a sensitivity of 96% to discriminate between sarcoidosis and other causes of lymphocytic alveolitis [9]. These authors concluded that the combination of both ratios would constitute a good marker for the diagnostic of sarcoidosis. Heron et al., and Mota et al. also reported a good discriminative power for the CD103+CD4+/CD4+ ratio (table 4), and agreed with Kolopp-Sarda's conclusions [10][12]. However, in another study by Hyldegaard et al., the combination of CD4+/CD8+ and CD103+CD4+/CD4+ ratios had a sensitivity of only 42% and a specificity of 91%, which led to conclude that the combination of these ratios was a poor marker for sarcoidosis. Of note, all these studies had small study populations (table 4) and two of them included less than 20 cases of sarcoidosis [9][11].

In our population, the CD103+CD4+/CD4+ ratio had a weak discriminative power for the diagnosis of sarcoidosis, with an AUC of only 62% to distinguish sarcoidosis from all other causes of lymphocytic alveolitis, and an AUC of 69% to distinguish sarcoidosis from other ILDs. The use of combined

CD4+/CD8+ and CD103+CD4+/CD4+ ratios resulted in modest increases of the AUC to 76% for sarcoidosis versus all causes of lymphocytic alveolitis, and to 78% for sarcoidosis versus other ILDs. This remained lower than the AUC of the CD4+/CD8+ ratio alone (79%), suggesting that the contribution of the CD103+CD4+/CD4+ ratio itself was low.

We also tested the cut-off values suggested by other studies on our population (table 4). The highest percentage of correctly classified patients (78%) was achieved by using Hyldegaard et al.'s cut-off, whereas with the one suggested by Mota et al., the percentage of correctly classified patients was very low (32%). Further analysis of our population using the CD103+CD4+/CD4+ ratio alone showed a high sensitivity but a very low specificity, and the precision did not exceed 37% (table 4). Altogether, this reinforces our own observations suggesting that the CD103+CD4+/CD4+ ratio, either alone or combined to the CD4+/CD8+ ratio, does not provide sufficient diagnostic precision to identify sarcoidosis among other causes of lymphocytic alveolitis.

The strong points of our study was the large size of the population (n=207), which was much higher than any other study of the CD103+CD4+/CD4+ ratio. The number of sarcoidosis cases (n=53) was also higher than most other studies, and almost equaled the 56 cases reported by Heron et al [12]. Unlike the other studies, we were able to precisely classify our population in well-defined diagnostic categories and compare sarcoidosis to each category individually, as well as to broader diagnostic groups. This allowed a more precise analysis of CD103+CD4+/CD4+ ratio for other diagnoses such as HP, NSIP, infections and other ILDs.

One limitation of our study is that we included only patients with BAL lymphocytosis, whereas there have been reports of cases of sarcoidosis without this feature [11][19][20]. However, we believe that this occurrence is rare and would have not invalidated our conclusions. Another limitation is that all diagnostic categories were not equally represented. For instance, there was only a small number of patients with HP in our population.

Altogether, the hypothesis behind CD103+CD4+/CD4+ ratio in sarcoidosis could very well be true. Indeed, all studies demonstrate that there is a lower percentage of CD4+ lymphocytes presenting the CD103 integrin in the lungs of patients with sarcoidosis as compared to patients with other illnesses causing pulmonary lymphocytosis. However, as demonstrated in the present study, the CD103+CD4+/CD4+ ratio does not have enough discriminative power to accurately differentiate between sarcoidosis and other disorders causing pulmonary lymphocytosis, and cannot replace lung biopsy or lymph node needle aspiration for the diagnosis of sarcoidosis.

Acknowledgements

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Legends to figures

Figure 1:

Case selection process including the number of excluded cases and the reason for exclusion. HP: hypersensitivity pneumonitis, NSIP: nonspecific interstitial pneumonia, OP: organising pneumonia.

Figure 2:

Box-whiskers plots of CD4+/CD8+ (A) and CD103+CD4+/CD4+ (B) ratios in 9 diagnostic categories. HP: hypersensitivity pneumonitis, NSIP: nonspecific interstitial pneumonia, OP: organising pneumonia.

Figure 3:

ROC curves for CD4+/CD8+ ratio (A), CD103+CD4+/CD4+ ratio (B) and their combination (C) to distinguish sarcoidosis from all other diagnoses.

Figure 4:

Box-whiskers plots of CD4+/CD8+ (A) and CD103+CD4+/CD4+ (B) ratios in 4 diagnostic categories.

Table 1: Demographic and BAL characteristics of the study population

Diagnostic category	n	age (yrs)		BAL lymphocytes (%)		BAL neutrophils (%)		BAL eosinophils (%)	
		median	iqr	median	iqr	median	iqr	median	iqr
Sarcoidosis	53	46	20	47	29	2	4	0	1
Tuberculosis	22	37	31	39	18	3	7	0	1
Other infections	38	61	21	43	28	9	20	0	2
HP	6	36	37	49	27	24	25	2	2
NSIP	14	67	13	39	25	4	5	4	7
OP	9	67	7	47	24	4	10	3	12
Drug-induced pneumonia	19	71	18	55	34	4	6	1	2
Other ILD	21	59	23	51	41	7	7	1	3
Other diagnoses	25	57	19	45	29	4	9	1	1
Total	207	56	28	45	29	4	9	1	2

HP: hypersensitivity pneumonitis, NSIP: nonspecific interstitial pneumonia, OP: organising pneumonia; ILD; interstitial lung disease, iqr: interquartile range.

Table 2: Flow cytometry for the 9-category distribution

	CD4+/CD8+ ratio			CD103+CD4+/CD4+ ratio		
Diagnostic category	**median**	**iqr**	**p value***	**median**	**iqr**	**p value***
Sarcoidosis	5.3	5.9	-	0.08	0.15	-
Tuberculosis	2.3	2.5	0.052	0.09	0.11	0.488
Other infections	1.7	1.9	<0.001	0.09	0.13	0.242
HP	1.8	3.5	<0.001	0.27	0.61	0.037
NSIP	0.6	1.4	<0.001	0.21	0.21	0.018
OP	2.0	2.1	<0.001	0.10	0.09	0.698
Drug-induced pneumonia	1.3	2.9	0.044	0.15	0.16	0.060
Other ILD	1.6	3.6	0.011	0.14	0.19	0.006
Other diagnoses	1.6	3.1	0.010	0.10	0.16	0.106
Total	2.4	3.6		0.10	0.17	

HP: hypersensitivity pneumonitis, NSIP: nonspecific interstitial pneumonia, OP: organising pneumonia, ILD: interstitial lung disease, iqr: interquartile range. * compared to sarcoidosis.

Table 3: Flow cytometry for the 4-category distribution

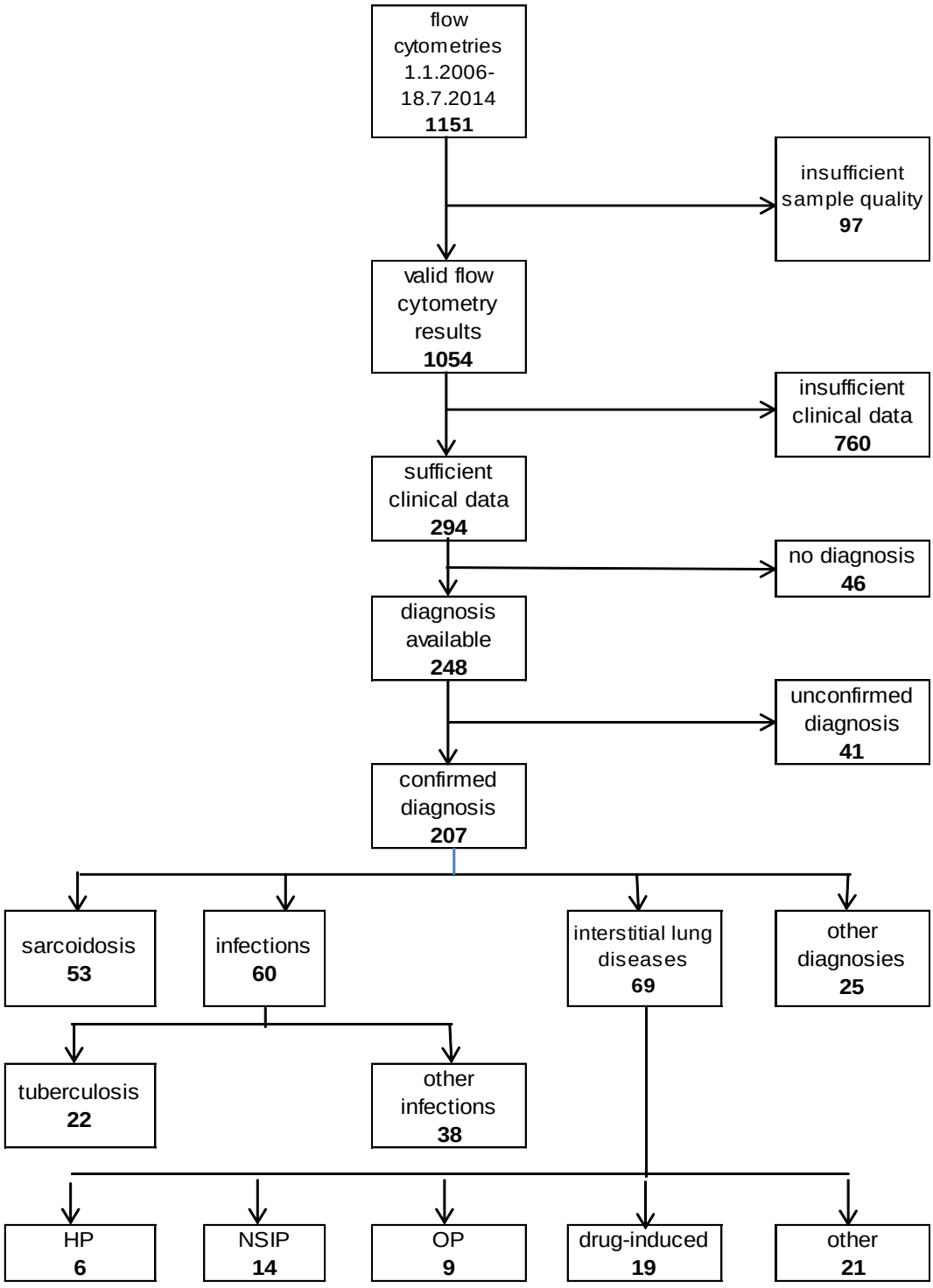
	CD4+/CD8+ ratio			CD103+CD4+/CD4+ ratio		
Diagnostic category	**median**	**iqr**	**p value***	**median**	**iqr**	**p value***
Sarcoidosis	5.3	5.9	-	0.08	0.15	-
All infections	2.0	2.7	<0.001	0.09	0.12	0.213
All ILDs	1.5	3.2	<0.001	0.14	0.19	<0.001
Other diagnoses	1.6	3.1	0.009	0.10	0.16	0.102
Total	2.4	3.6		0.10	0.17	

ILD: interstitial lung disease. iqr: interquartile range. * compared to sarcoidosis

Table 4: Comparison to other studies

Study	Cut-off	Population	Published values		Our population			
			Se (%)	Sp (%)	Se (%)	Sp (%)	Precision (%)	AUC (%)
Kolopp-Sarda [9]	CD4/CD8 > 2.5 and CD103/CD4 < 0.31	total 93, sarcoidosis 18	96%	-	75	70	72	73
	CD103/CD4 < 0.31		-	-	94	18	37	56
Heron [12]	CD4/CD8 >3 and CD103CD4/CD4 <0.2	total 119, sarcoidosis 56	66	89	58	80	74	69
	CD103CD4/CD4 < 0.2		-	-	94	18	37	56
Mota [10]	CD103/CD4 < 0.4	total 86, sarcoidosis 41	81	78	98	9	32	54
Hyldgaard [11]	CD4/CD8 > 3.8 and CD103CD4/CD4 < 0.22	total 107, sarcoidosis 19	42	91	55	86	78	70
	CD103CD4/CD4 < 0.22		-	-	85	28	43	56

Figure 1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sufficient clinical data 294
no diagnosis 46
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confirmed diagnosis 207
sarcoidosis 53
infections 60
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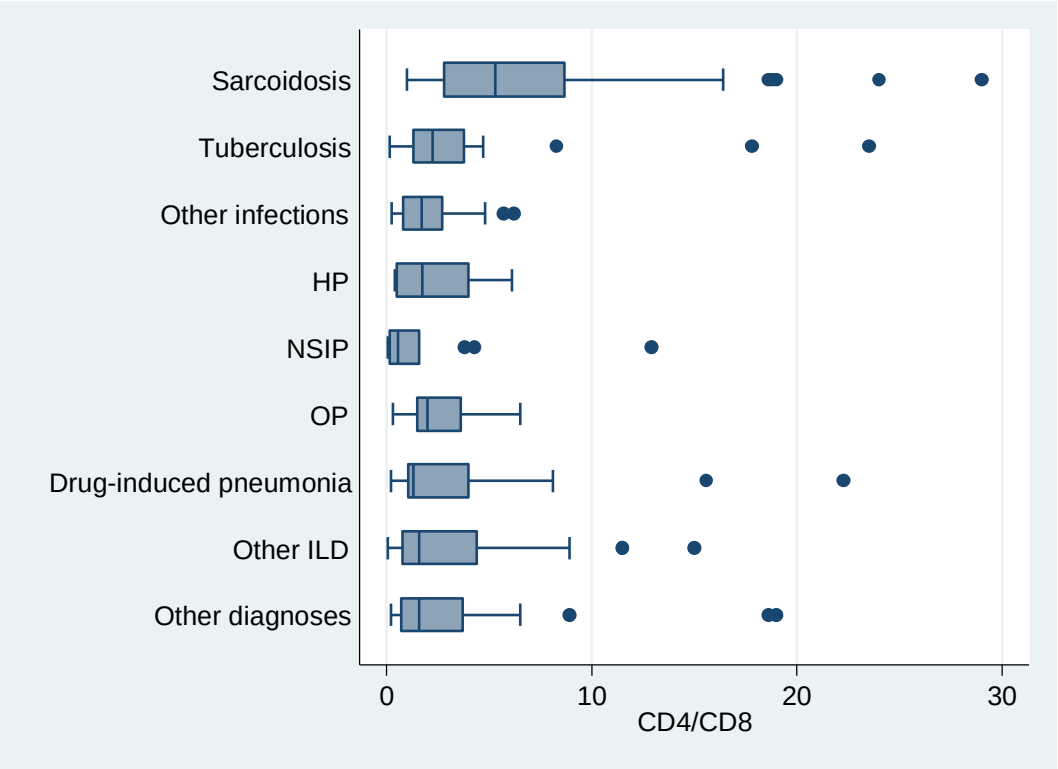
tuberculosis 22
other infections 38
HP 6
NSIP 14
OP 9
drug-induced 19
other 21

Figure 2

A:


Sarcoidosis
Tuberculosis
Other infections
HP
NSIP
OP
Drug-induced pneumonia
Other ILD
Other diagnoses
0
10
20
30
CD4/CD8

B:

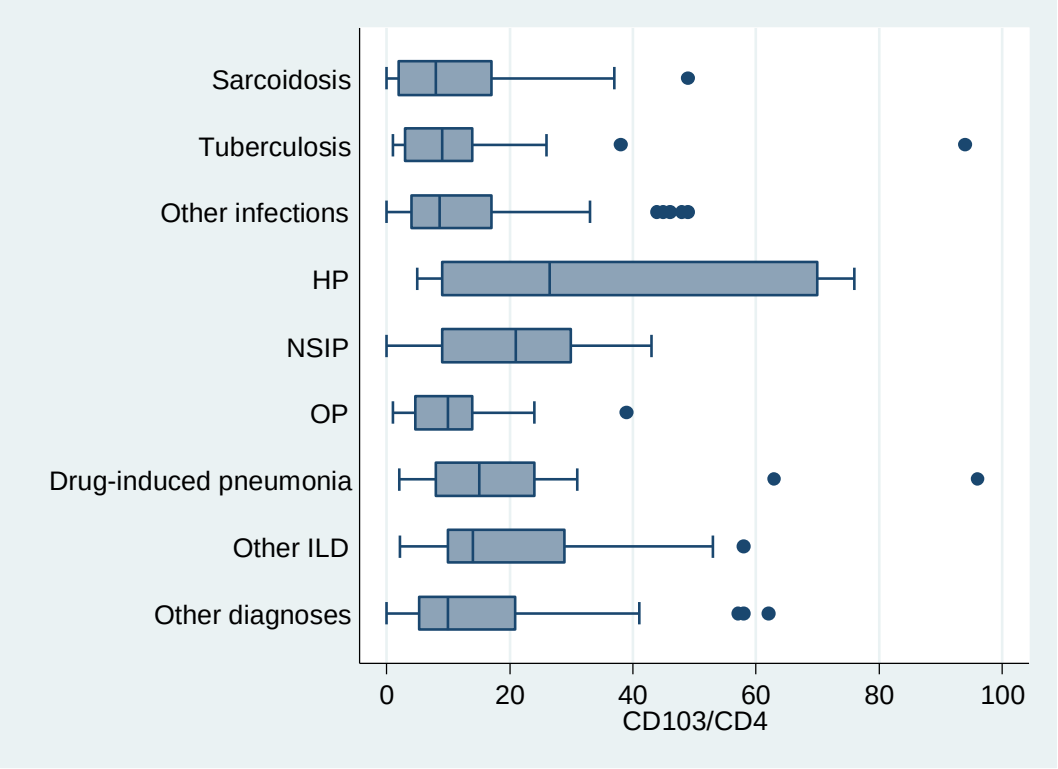
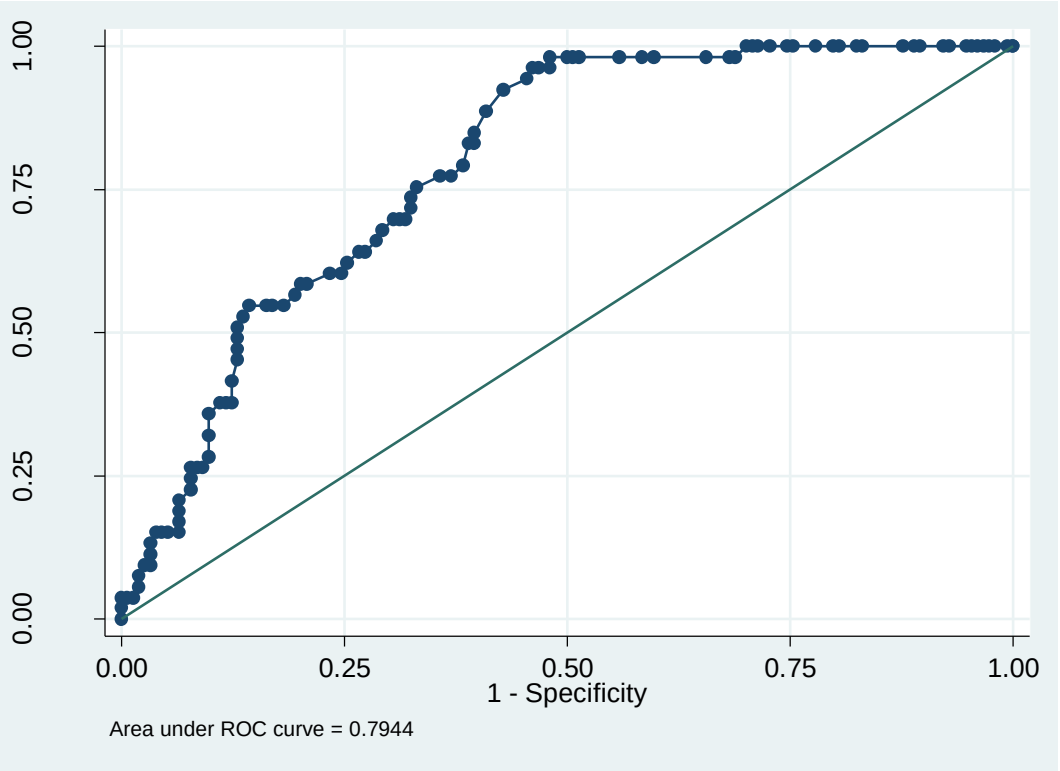

Sarcoidosis
Tuberculosis
Other infections
HP
NSIP
OP
Drug-induced pneumonia
Other ILD
Other diagnoses
0
20
40
60
80
100
CD103/CD4

Figure 3

A: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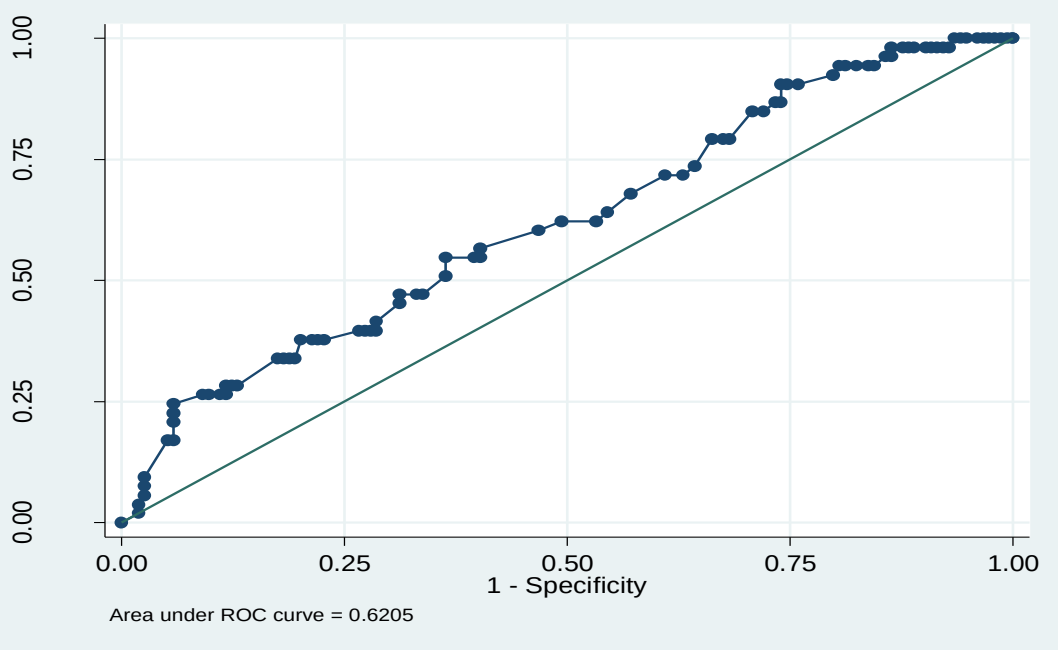
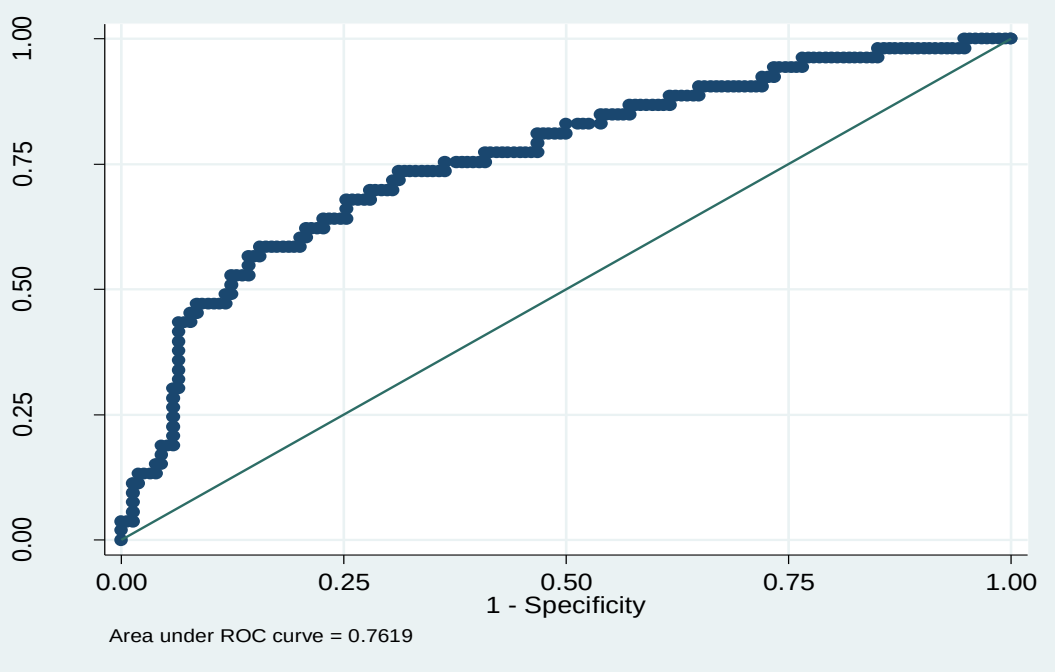
0.25
0.50
0.75
1.00
1 - Specificity
Area under ROC curve = 0.7944

B:


0.00
0.25
0.50
0.75
1.00
1 - Specificity
Area under ROC curve = 0.6205

C:


1.00
0.75
0.50
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0.00
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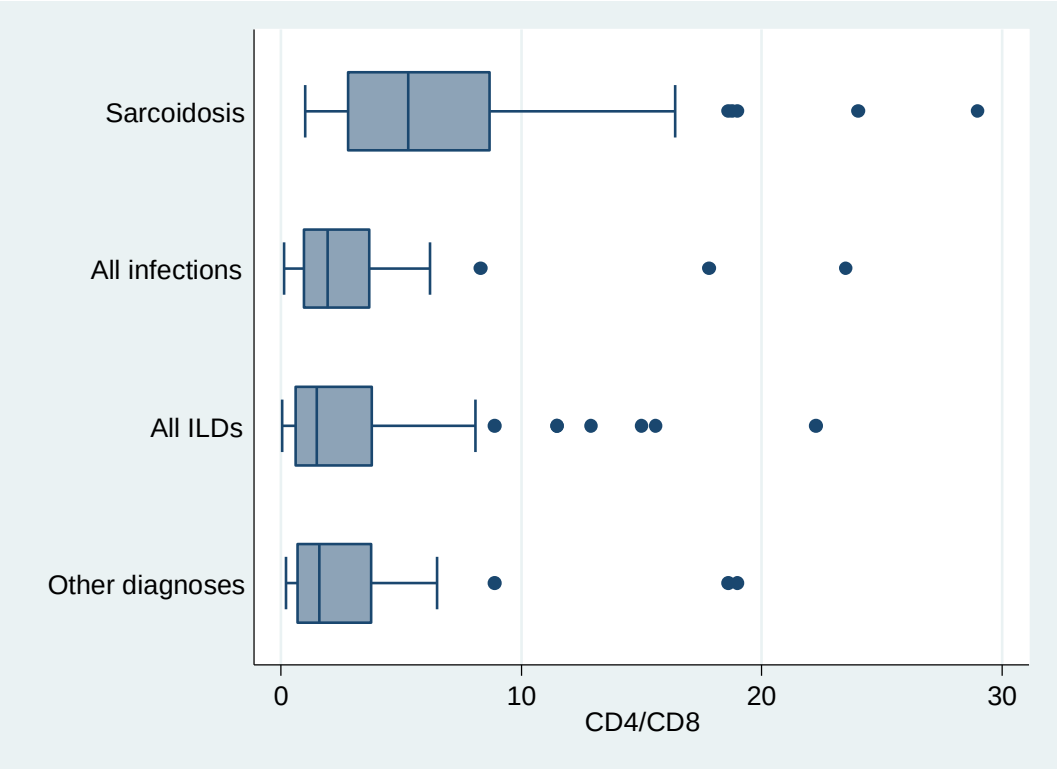
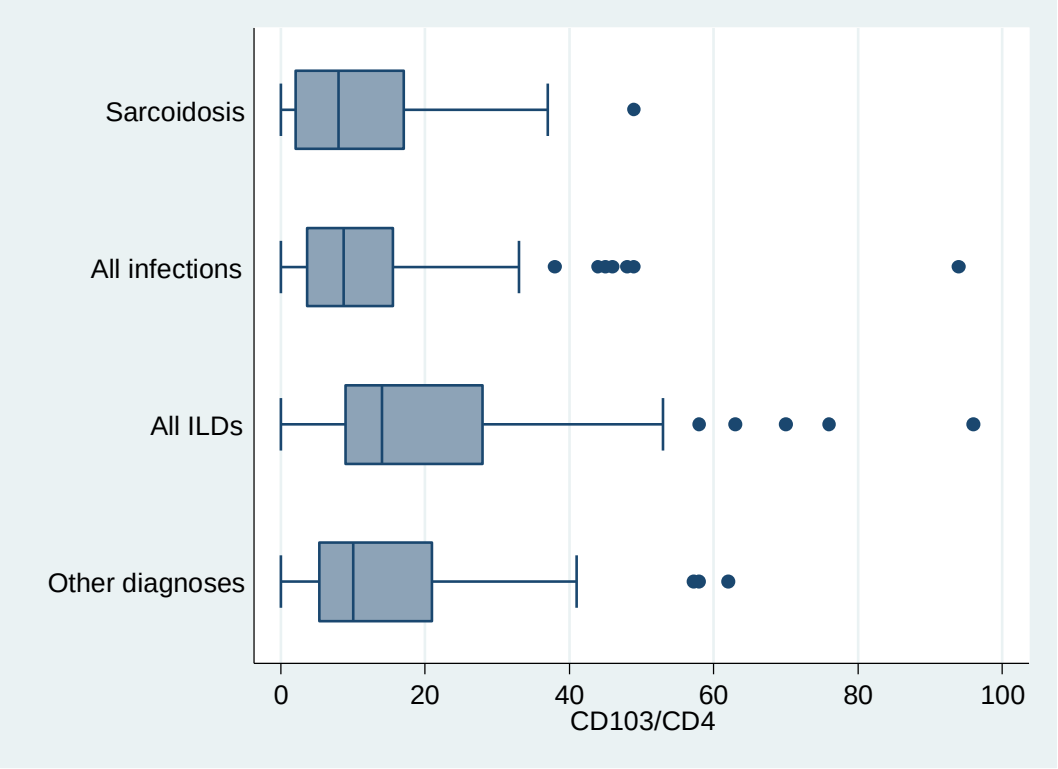
0.25
0.50
0.75
1.00
1 - Specificity
Area under ROC curve = 0.7619

Figure 4

A:


Sarcoidosis
All infections
All ILDs
Other diagnoses
0
10
20
30
CD4/CD8

B:


Sarcoidosis
All infections
All ILDs
Other diagnoses
0
20
40
60
80
100
CD103/CD4

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