SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2012; 29; 34-40 © Mattioli 1885

Contribution of T cell subset analysis in induced sputum in diagnosing ocular sarcoidosis

Amit Meshi[1], Meira Neudorfer[2]*, Elizabeth Fireman[3]*

[1] Department of Ophthalmology, Meir Medical Center, Kfar Saba; [2] Department of Ophthalmology and the [3] Institute of Pulmonary Diseases, National Service for Interstitial Lung Diseases, Tel Aviv Sourasky Medical Center, both affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel

Abstract. *Objective:* The aim of this study was to establish a correlation between the diagnosis of ocular sarcoidosis and the presence of an elevated CD4/CD8 ratio in the induced sputum(IS) of patients with uveitis and no other systemic symptoms. *Methods:* This retrospective chart review study included all newly diagnosed uveitis patients treated between 1998-2006. IS examinations and determination of angiotensin-converting enzyme (ACE) levels were carried out. A CD4/CD8 ratio >2.5 and an ACE level >145 μl/ml/min were considered abnormal. The etiology of uveitis was retrieved from the medical records. *Results:* Twenty males and 26 females (mean age 47±16.1 years) were enrolled. The CD4/CD8 ratio was elevated in 26 (56.5%) patients, and five (10.9%) were diagnosed as having sarcoidosis by the end of follow-up. The sensitivity and specificity of the T lymphocytes CD4/CD8 ratio in diagnosing sarcoidosis were 100% and 48.8%, respectively. CD4/CD8 ratios were not significantly different between the sarcoid and non-sarcoid groups ($p>0.05$), but the former tended to have higher levels ($p=0.0991$). The mean ACE level of the sarcoid patients was significantly higher than that of the non-sarcoid patients ($p<0.001$). *Conclusion:* CD4/CD8 lymphocytes ratios obtained by IS were sensitive in uveitis patients with concomitant sarcoidosis, suggesting that analysis of T cells subsets in IS may rule out an etiology of sarcoidosis in newly diagnosed uveitis patients. *(Sarcoidosis Vasc Diffuse Lung Dis 2012; 29: 34-40)*

Key words: ocular sarcoidosis, induced sputum, CD4/CD8 lymphocytes ratio, angiotensin-converting enzyme.

Introduction

Sarcoidosis is a systemic disorder of unknown origin that is characterized by the pathological hallmark of noncaseating granuloma (1). The most commonly affected organs are the lungs, lymph nodes, skin and eyes (1-3). Ocular involvement occurs in 25%-60% of patients with systemic sarcoidosis (4), and the most common ocular manifestation is uveitis (30%–70%), preceding the non-ocular signs of sarcoidosis in about 30% of the cases (3). The visual impact of sarcoid-associated uveitis can be severe, with about 10% of patients losing vision in at least one eye (3). The diagnosis of ocular sarcoidosis is challenging since the clinical presentation of sarcoid-associated uveitis is not pathognomonic and sometime cannot be differentiated from uveitis related to other etiologies. In addition, there are no definitive diagnostic blood, skin or radiologic imaging tests specific for the disorder (1). As such, sarcoidosis is usu-

Received:
Accepted after Revision:
Correspondence: Amit Meshi MD,
Department of Ophthalmology, Meir Medical Center,
59 Tchernichovsky St., Kfar Saba, 44281 Israel.
Tel. +97297472154
Fax +97297472427;
E-mail: amitmeshi@gmail.com

* Equal contribution.

The research was performed in partial fulfillment of the MD thesis requirements of Amit Meshi, MD, in the Sackler Faculty of Medicine, TelAviv

ally diagnosed on the basis of compatible clinical, radiologic and histologic findings (5).

The use of the CD4 (helper T lymphocytes)/CD8 (suppressor-cytotoxic T lymphocytes) ratio as a diagnostic tool is based on the finding that helper T lymphocytes (CD4+) accumulate in target organs and function as key effectors in the formation of sarcoid granulomas (6, 7). Obtaining cells and secretions from the respiratory tract was solely through bronchoalveolar lavage (BAL) until the introduction of the induced sputum (IS) technique. The noninvasive feature of IS made it the preferred method of studying inflammatory processes in the lungs (asthma and chronic obstructive pulmonary disease [COPD]), cancer, lung infections and interstitial lung diseases (8, 9). Moreover, a significantly higher percentage of neutrophils and a lower percentage of macrophages were found in IS samples compared to BAL samples (9), while T cell subpopulations were found to correlate well in both methods (9, 10). This latter finding suggested the possible use of CD4/CD8 ratio obtained by IS in the diagnosis of sarcoidosis as was previously done with BAL.

We had reported a correlation between elevated angiotensin-converting enzyme (ACE) levels and increased CD4/CD8 ratios obtained by the IS of uveitis patients (11). This correlation was found both in established sarcoidosis patients as well as in uveitis patients without any systemic symptoms, suggesting that sarcoidosis or some other sarcoid-like disorder was the cause of uveitis in the latter patients. The aim of the current retrospective study was to establish a correlation between the diagnosis of sarcoidosis and the finding of elevated CD4/CD8 ratio obtained by IS in uveitis patients without other systemic symptoms.

Materials and Methods

The study was approved by the Institutional Review Board of the Tel-Aviv Sourasky Medical Center.

Patients

We retrospectively reviewed the medical records of all new uveitis patients referred to the Ophthalmology Department (Tel-Aviv Medical Center, Israel) for evaluation between 1998-2006 (Table 1). They were then referred to the Institute of Pulmonary Diseases (Tel Aviv Medical Center, Israel) to undergo an IS examination and for the determination of their ACE levels. The medical records were reviewed for information on whether an etiology had been determined for the uveitis or if it was diagnosed as being idiopathic, as well as the patient's medical status at follow-up (i.e., diagnosed as having sarcoidosis or some other systemic disease). In addition, the presence of an inflammatory disease was noted when there was any evidence of a condition associated with a systemic immunological reaction (autoimmune disorders or chronic infectious diseases). The date of the IS examination was taken as the beginning of the follow-up, and the date the etiology of the uveitis was established or the date of the last clinic visit was considered as the end of the follow-up.

Table 1. Demographic and clinical data of the study population (n=46)

Characteristic	Number	(%)
Gender	20/26 (M/F)	43.5/56.7
Age, yrs (mean ±STD)	47±16.1	
Final diagnosis		
Sarcoidosis (Group I)	5	10.8
Ocular disease (Group II)	7	15.2
Combined systemic and ocular disease (Group III)	12	26.1
Idiopathic (Group IV)	22	47.8

M, male; F, female; STD, standard deviation

IS Induction and Processing

A modified method of Popov et al (12) was used to induce and process IS. All patients were able to expectorate an adequate sputum sample. Briefly, sputum was processed as soon as possible within 2 hours of collection. Dithiothreitol (Sputalysin, Calbiochem Corp., San Diego, CA, USA) was added and mixed mechanically with the sputum in a shaking water bath at 37°C for complete homogenization. The cell suspension containing phosphate-buffered solution was filtered through a 52-μm nylon gauze (BNSH Thompson, Scarborough, Ontario, Canada) diluted with RPMI supplemented with fetal calf serum (FCS) to achieve a concentration of 10^3/μl. One drop was placed in each cytocentrifuge cup already in place in a Shandon III cytocentrifuge (Shandon Southern Instruments, Sewickley, PA, USA), and cytospins were prepared at 1000

RPMI supplemented with 10% FCS (Biological Industries, Beit Haemek, Israel) for 5 minutes. Separate cytospin slides were stained by Giemsa. Three-hundred nonsquamous cells were counted and the results were expressed as a percentage of the total nonsquamous cell count. Only samples containing ≤20% squamous cells were used.

Evaluation of the Phenotype of Sputum Cells

Flow cytometric analysis was performed on a dual FACS 440 equipped with an Ar and Kr laser (Becton-Dickinson). The information was collected on a logarithmic scale. The selection of the lymphocyte population was based on side scatter and expression of CD45. Lymphocyte subsets were identified by monoclonal antibodies as follows: CD4 = T helper cells and CD8 = T suppressor-cytotoxic cells. Monoclonal antibodies were directly conjugated to either phycoerythrin or fluorescent isothiocyanate. Cells were incubated for 10 minutes with Epics Coulter Q-Preoperative preparation (Immunoprep Reagent Q Prep, Beckman Coulter, Int SA Nyon Switzerland) and read either immediately or after 24 hours.

ACE Assay

ACE was assayed using a spectrometric method as previously described (13). In this assay, furyl-acryloyl-phenylalanilyl-glycylglycine is hydrolyzed to furyl-acryloyl-phenylalanine and glycylglycine. Hydrolysis of furyl-acryloyl-phenylalanilyl-glycyl-glycine results in a decreased absorbance at 340 nm. The ACE activity is determined spectrophotometrically by comparing the sample reaction rate with that obtained with a provided calibrator.

Statistical Analysis

All statistical analyses were performed using both parametric and non-parametric tests due to the small number of patients in some of the groups. Comparison between the 4 diagnosis groups for all parameters was performed using a one-way analysis of variance and the Kruskal-Wallis test. Both the t test and the Mann-Whitney test were performed whenever 2 groups were compared. Statistical analysis was performed using the SAS for Windows version 9.1.3

Funding

Funding was by internal departmental sources that had no role in this study.

Results

Forty-six patients newly diagnosed as having uveitis of unknown etiology were included in this study. Their mean age was 47±16.1 years (Table 1). The CD4/CD8 ratio was found to be elevated (>2.5) in 26 (56.5%) of them. At the end of the follow-up, 5 (10.9%) patients were diagnosed as having sarcoidosis (Group I), 7 (15.2%) patients as having an isolated ocular disease (Group II), 12 (26.1%) as having combined ocular and systemic disease (Group III), and 22 (47.83%) as having idiopathic uveitis (Group IV) (Table 1). The mean follow-up time was 3.5±2.2 years. The mean time until diagnosis of sarcoidosis was 10.4±8.5 months. The demographic and clinical data of the patients categorized according to their final diagnosis is shown in Tables 2-5, including gender, age, type of uveitis, CD4/CD8 ratio, ACE levels and type of systemic

Table 2. Demographic and clinical parameters of the sarcoidosis patients (Group I)

No.	Gender	Age, y	Uveitis	CD4/CD8	ACE Level	Follow-up, mo	Diagnostic Procedures for SA	SA Stage
1	F	55	A/BL	3.04	131	2	TB biopsy	II-III
2	M	54	A/BL	4.45	160	13	TB biopsy, CT	II-III
3	M	40	A/UL	5.16	219	20	CT	0
4	M	31	P/BL	3.2	260	16	Skin biopsy, CT Gallium-67 scan	I
5	F	63		3.17	192	1	TB biopsy	II-III
Mean±STD		48.6±12.9		3.8±0.95	192.4±50.2*	10.4±8.5		

F, female; M, male; A, anterior, BL, bilateral; P, posterior; UL, unilateral; CD4, cluster of differentiation 4 (helper T lymphocytes); CD8, cluster of differentiation 8 (suppressor-cytotoxic T lymphocytes); ACE, angiotensin-converting enzyme (μl/ml/min); SA, sarcoidosis; TB, transbronchial; CT, computed tomography; STD, standard deviation.

* p<0.001 of ACE levels of sarcoid patients vs. non sarcoid patients (Table 3, Table 4 and Table 5)

Table 3. Demographic and clinical parameters of patients with ocular disease (Group II)

No.	Gender	Age, y	Uveitis	CD4/CD8	ACE Level	IFD (+/-)	Follow-up, mo	Final diagnosis
1	F	38	A/UL	2.8	81	-	45	PSS
2	M	48	A/UL	3.2	106	-	45	FHU
3	M	41	A/UL	1.21	67	-	57	HKU
4	F	51	A	1.26	126	-	65	MFC
5	F	85	P/BL	0.81		-	70	HKU
6	M	50	P/UL	1.3	108	-	26	VZU
7	M	43	P	2.21	91	-	22	BRC
Mean± STD		50.9±15.8		1.83±0.91	96.5±21.1		47.1± 18.4	

F, female; M, male; A, anterior; BL, bilateral; P, posterior; UL, unilateral; CD4, cluster of differentiation 4 (helper T lymphocytes); CD8, cluster of differentiation 8 (suppressor-cytotoxic T lymphocytes); ACE, angiotensin-converting enzyme (μl/ml/min); IFD, inflammatory disease; PSS, Posner-Schlossmann syndrome; FHU, Fuchs' heterochromic uveitis; HKU, herpetic kerato-uveitis; MFC, multifocal choroiditis; VZU, varicella zoster uveitis; BRC, birdshot retino-choroidopathy; STD, standard deviation

Table 4. Demographic and clinical parameters of patients with combined systemic and ocular disease (Group III)

No.	Gender	Age, y	Uveitis	CD4/CD8	ACE Level	IFD (+/-)	Follow-up, mo	Final diagnosis
1	M	35	A/UL	3.1		+	16	SSA
2	M	45	A/UL	0.5	130	+	3	CSD
3	M	52	A/UL	2		+	89	SSA
4	M	34	A/UL	4.3	99	+	40	BD
5	F	29	A/UL	1	56	+	27	SSA
6	M	62	A/UL	3.73	104	+	87	TINU
7	M	59	P/BL	2.54	166	+	91	SSA
8	M	33	P/UL	2.8	168	+	66	BD
9	M	60	P/UL	2.02	142	+	35	SSA
10	M	31	P/UL	2.2	73	+	26	BD
11	F	32	P/BL	1.23	135	+	19	BD
12	F	60	-	6.9	94	+	71	Vasculitis
Mean± STD		44.3±13.4		2.69±1.72	116.7±37.7		47.5±31.5	

F, female; M, male; A, anterior; BL, bilateral; P, posterior; UL,unilateral; CD4, cluster of differentiation 4 (helper T lymphocytes); CD8, cluster of differentiation 8 (suppressor-cytotoxic T lymphocytes); ACE, angiotensin-converting enzyme (μl/ml/min); IFD, inflammatory disease; SSA, seronegative spondyloarthropathies, CSD, cat-scratch disease; BD, Behçet's disease; TINU, tubulointerstitial nephritis and uveitis; STD, standard deviation

disease. CD4/CD8 ratios in IS test were not significantly different between the groups (3.8±0.95, 1.83±0.91, 2.69±1.72 and 2.66±1.79 in Groups I, II, III and IV, respectively, p>0.05), although there was a trend towards higher values among patients with sarcoidosis (Group I) (3.8±0.95) compared to all the others combined (2.53±1.65, p=0.0991). When the mean values of ACE levels were compared between these same groups, however, the Group I patients had a significantly higher mean ACE level (192.4±50.24 μl/ml/min) than all other patients combined (121.92±38.69 μl/ml/min, p<0.001).

The presence of an inflammatory disease had no significant influence on ACE levels. There was a correlation of borderline significance between an elevated CD4/CD8 ratio and an increased ACE level in all 41 tested individuals (p=0.056). The sensitivity and specificity of a CD4/CD8 ratio >2.5 as obtained by IS in the diagnosis of sarcoidosis were 100% and 48.78%, respectively (Table 6). The sensitivity and specificity of an ACE level >145 μl/ml/min in the diagnosis of sarcoidosis were 80% and 75%, respectively.

Discussion

This study was designed to establish the etiology of uveitis by means of IS evaluations of newly diagnosed patients without any other systemic symptoms, and to determine whether there is a correlation between an elevated CD4/CD8 lymphocyte ratio and the diagnosis of sarcoidosis. The working hypothesis was that an elevated CD4/CD8 lymphocyte

Table 5. Demographic and clinical parameters of patients with idiopathic uveitis (Group IV)

No.	Gender	Age, y	Uveitis	CD4/CD8	ACE level	IFD (+/-)	Follow-up, mo
1	F	81	A/BL	3.6		-	44
2	F	30	A/BL	2.6	97	-	59
3	F	62	A/BL	6.2	124	-	34
4	F	62	A/UL	0.6	96	-	27
5	F	40	A/UL	1		-	103
6	F	43	A/UL	3.3	89	+	35
7	F	25	A/UL	0.68	196	+	12
8	M	53	A/UL	1.1	94	-	89
9	F	53	A/UL	3.73	134	+	36
10	M	37	A/UL	4.53	232	-	42
11	F	37	A/UL	2.7	166	-	37
12	F	60	P/BL	6.5	91	-	22
13	F	75	P/BL	4.7	193	-	34
14	F	78	P/BL	1.56	149	-	55
15	M	44	P/BL	2.6	152	-	30
16	F	18	P/BL	3.8	100	-	21
17	F	55	P/UL	3.3	126	+	55
18	F	41	P/UL	1	151	-	41
19	F	31	P/UL	2.7	124	-	66
20	F	22	P/BL	0.8	110	-	24
21	M	21		0.57	131	-	78
22	F	62		1	88	-	78
Mean±STD		46.8±18.7		2.66±1.79	132.1±40.5		46.5±23.9

F, female, M, male; A, anterior, BL, bilateral, P, posterior, UL, unilateral; CD4, cluster of differentiation 4 (helper T lymphocytes); CD8, cluster of differentiation 8 (suppressor-cytotoxic T lymphocytes); ACE, angiotensin-converting enzyme (μl/ml/min); IFD, inflammatory disease; STD, standard deviation

Table 6. Sensitivity* and specificity** of a CD4/CD8 ratio >2.5 in the diagnosis of sarcoidosis

	Sarcoidosis Patients (%)	Non-sarcoidosis Patients (%)	Total N, (%)
CD4/CD8 >2.5	5 (10.87)	21 (45.65)	26 (56.52)
CD4/CD8 ≤2.5	0	20 (43.48)	20 (43.48)
Total (%)	5 (10.87)	41 (89.13)	46 (100)

CD4, cluster of differentiation 4 (helper T lymphocytes); CD8, cluster of differentiation 8 (suppressor-cytotoxic T lymphocytes).
* 100%
** 48.78%
Positive Predictive Value = 19.23%
Negative Predictive Value = 100%

ratio in IS samples would differentiate between uveitis caused by sarcoidosis and uveitis with some other etiology. Analysis of our findings revealed that the 5 patients who were ultimately diagnosed as having sarcoidosis did have an elevated CD4/CD8 lymphocytes ratio, while the remaining 21 who were non-sarcoid also had an elevated ratio as well. In this context, we propose that an IS CD4/CD8 ratio within normal values can exclude the presence of ocular sarcoidosis.

The lymphocytes ratios were not significantly different between the patient groups, although there was a borderline correlation between an elevated CD4/CD8 lymphocyte ratio and an increased ACE level which we interpreted as being indicative for the use of both of these elevated parameters to diagnose sarcoidosis as we had previously shown in an earlier study (11).

By the end of the follow-up period, sarcoidosis was diagnosed as being the etiology of the uveitis of 5 of our 46 (11%) newly diagnosed uveitis patients. Smith et al. (4) reviewed the data on uveitis patients described in the literature and reported a range of 3%-10%. All of our current patients whose uveitis was caused by sarcoidosis had an elevated CD4/CD8 lymphocyte ratio in their IS. Consequently, the sensitivity and the negative predictive value (NPV) of the CD4/CD8 ratio >2.5 were 100%. We had previously showed a sensitivity of 100% of this lymphocyte ratio in distinguishing sarcoidosis from non-granulomatous interstitial lung diseases (8). We also found that all sarcoid patients who underwent IS testing had an elevated CD4/CD8 lymphocytes ratio (9). These findings

suggest that analysis of T cells subsets in IS may be a valuable tool in establishing or ruling out sarcoidosis as the etiology of a given patient's uveitis.

The specificity and positive predictive value (PPV) of an elevated lymphocyte ratio, on the other hand, were low, i.e., 48.78% and 19.23%, respectively. Almost one-half of our non-sarcoid patients (n=21, 45.6%) had a CD4/CD8 ratio >2.5. One possible explanation for the large number of positive IS tests despite the low number of sarcoidosis cases may be under-diagnosis of sarcoidosis which may be due to its variable clinical course and the difficulty in diagnosing sarcoidosis by the currently available methods. A positive diagnosis of sarcoidosis requires a biopsy specimen from an involved organ (1, 5). Since our patients presented with the sole finding of uveitis and had no other organ involvement, there was no medical or ethical justification to proceed with a more invasive procedure, such as transbronchial lung biopsy.

With no histologic findings to confirm or rule out the diagnosis of sarcoidosis, some sarcoid patients might have been missed in our group. In their review article, Iannuzzi et al. (5) noted that several reports suggested the possible usefulness of 18-F-fluorodeoxyglucose positron-emission tomography (18-FDG PET) in diagnosing patients without apparent lung involvement in order to identify organs that may be candidates for diagnostic biopsy.

A low specificity and PPV may be expected when the etiology of uveitis is something other than sarcoidosis and there is an elevation of the CD4/CD8 ratio. Systemic diseases, such as Crohn's disease and seronegative arthritis, may cause elevation of T-helper lymphocytes in the lungs, even without respiratory symptoms (14, 15). For example, there is a migration of intestinal lymphocytes through the general circulation to the common mucosal immune system of the lung in Crohn's disease (14). Moreover, Winterbauer et al (16) analyzed BAL fluid of different lung diseases, such as infections and bronchial hyper-reactivity, and showed that they were associated with elevated CD4/CD8 ratios which were not significantly different from those in sarcoidosis.

In the current work, we found an elevated CD4/CD8 ratio (2.69±1.72) in the group of patients with combined systemic and ocular diseases, mainly Behçet's disease and seronegative spondyloarthropathies. An increase of T lymphocytes in the lungs in these inflammatory conditions may be due to a dynamic balance between entry and exit of lymphocytes from the intravascular pool and other compartments of the lung to the bronchoalveolar space (17). "Homing" of lymphocytes in the lung in various systemic or local inflammatory processes may explain the low specificity and PPV of T cell subsets in the IS of patients with sarcoidosis.

The value of the serum ACE level in diagnosing and managing sarcoidosis remains controversial. Iannuzzi et al. (5) claimed that the measurement of the serum ACE level is an insensitive and nonspecific diagnostic test as well as a poor therapeutic guide. In the current study, however, the sarcoid group had higher ACE levels compared to the non-sarcoid group ($p<0.001$). We had previously found that 80% of sarcoid patients with no uveitis and 71% of patients with uveitis, all of whom had a CD4/CD8 ratio >2.5, had increased ACE levels as well (11). Power et al. (18) demonstrated that the sensitivity of an elevated ACE level in diagnosing ocular sarcoidosis was 73% and the specificity was 83%. We calculated a sensitivity and specificity of 80% and 75%, respectively, in the current study, thus supporting a correlation between elevated ACE levels and the diagnosis of sarcoidosis (19).

The main limitations of this paper are first the lack of histological findings to confirm or rule out the diagnosis of sarcoidosis in some sarcoid patients which might have been missed in our group and secondly the limited follow up of the patients over the years.

In conclusion, we found that the CD4/CD8 lymphocyte ratio obtained by the IS technique was sensitive in differentiating newly diagnosed uveitis patients whose ophthalmic pathology was due to sarcoidosis. This finding suggests that analysis of T cells subsets in IS may be a valuable tool in ruling out sarcoidosis in newly diagnosed uveitis patients. The lymphocyte ratio did not prove to be specific enough in diagnosing a sarcoid source in these patients. The low specificity may suggest that other conditions (probably non-infectious inflammatory ones) might have caused the elevation of their CD4/CD8 ratios in IS. The results of this work support the measurement of serum ACE levels in the diagnosis of sarcoidosis, although it is not currently recommended (5). The clinical implications of this work are that in

the absence of definitive laboratory tests for ocular sarcoidosis, the IS methodology for detecting subpopulations of T lymphocytes in the sputum may provide a noninvasive diagnostic tool to be combined with other clinical parameters to reach the correct final diagnosis.

Acknowledgment

Esther Eshkol is thanked for editorial assistance.

References

1. Newman LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med 1997; 336: 1224-34. Review.
2. Deremee RA. Sarcoidosis. Mayo Clin Proc 1995; 70: 177-81.
3. Rothova A. Ocular involvement in sarcoidosis. Br J Ophthalmol 2000; 84: 110-6.
4. Smith JA, Foster CS. Sarcoidosis and its ocular manifestations. Int Ophthalmol Clin 1996; 36: 109-25.
5. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med 2007; 357: 2153-65.
6. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med 1999; 160: 736-55.
7. Hunninghake GW, Crystal RG. Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. N Engl J Med 1981; 305: 429-34.
8. Fireman E. Induced sputum: opening a new window to the lung. Sarcoidosis Vasc Diffuse Lung Dis 2001; 18: 263-71.
9. Fireman E, Topilsky I, Greif J, et al. Induced sputum compared to bronchoalveolar lavage for evaluating patients with sarcoidosis and non-granulomatous interstitial lung disease. Respir Med 1999; 93: 827-34.
10. Maestrelli P, Saetta M, Di Stefano A, et al. Comparison of leukocyte counts in sputum, bronchial biopsies, and bronchoalveolar lavage. Am J Respir Crit Care Med 1995; 152: 1926-31.
11. Neudorfer M, Leibovitch I, Onn A, Loewenstein A, Fireman E. Induced sputum for identifying sarcoidosis in patients with uveitis. Ophthalmology 2002; 109: 858-61.
12. Popov T, Gottschalk R, Kolendowicz R, Dolovich J, Powers P, Hargreave FE. The evaluation of a cell dispersion method of sputum examination. Clin Exp Allergy 1994; 24: 778-83.
13. Holmquist B, Bunning P, Riordan JF. A continuous spectrophotometric assay for angiotensin converting enzyme. Anal Biochem 1979; 95:540-8.
14. Fireman Z, Osipov A, Kivity S, et al. The use of induced sputum in the assessment of pulmonary involvement in Crohn's disease. Am J Gastroenterol 2000; 95: 730-4.
15. Scherak O, Kolarz G, Popp W, Wottawa A, Ritschka L, Braun O. Lung involvement in rheumatoid factor-negative arthritis. Scand J Rheumatol 1993; 22: 225-8.
16. Winterbauer RH, Lammert J, Selland M, Wu R, Corley D, Springmeyer SC. Bronchoalveolar lavage cell populations in the diagnosis of sarcoidosis. Chest 1993; 104: 352-61.
17. Pabst R, Tschernig T. Lymphocyte dynamics: caution in interpreting BAL numbers. Thorax 1997; 52: 1078-80.
18. Power WJ, Neves RA, Rodriguez A, Pedroza-Seres M, Foster CS. The value of combined serum angiotensin-converting enzyme and gallium scan in diagnosing ocular sarcoidosis. Ophthalmology 1995; 102: 2007-11.
19. Ashutosh K, Keighley JF. Diagnostic value of serum angiotensin converting enzyme activity in lung diseases. Thorax 1976; 31: 552-7.