

## **Coccidioidomycosis as a Cause of Sarcoid in Arizona**

A thesis submitted to the University of Arizona College of Medicine – Phoenix  
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## ABSTRACT

**BACKGROUND AND SIGNIFICANCE:** Sarcoidosis is a granulomatous disease of unknown etiology. Coccidioidomycosis is a granulomatous fungal infection due to *Coccidioides immitis* and *Coccidioides Posadasii* endemic to the Southwestern United States and the majority of the cases are reported from Arizona. The cause of sarcoidosis has been studied for over a hundred years without establishing an etiology. Establishing the cause of sarcoid would be a significant contribution to the understanding of an important multisystem disease.

**RESEARCH QUESTIONS:** Based on clinical observations a group of patients with two granulomatous diseases – sarcoidosis and coccidioidomycosis led to the hypothesis for this Scholarly Project – can sarcoidosis be caused by the fungus *Coccidioides*?

**METHODS:** A literature review was performed which resulted in 5 patient case reports, a medical record review was conducted of patients with sarcoidosis between 2004-2014 at Maricopa Medical Center with a case-control comparison to 68 matched patients, and PCR analysis of 34 sarcoid biopsy specimens from the 68 sarcoid patients identified from the medical record.

Also, two main patients with sarcoidosis were studied, one prospectively and the other retrospectively, both patients had their diagnosis of sarcoidosis made in Arizona and both develop sarcoidosis. There was no evidence of an etiology for their sarcoidosis at the time of diagnosis, specifically no evidence of coccidioidomycosis. The prospective patient was followed for eight years before he developed coccidioidomycosis. Predicting correctly that a patient diagnosed with sarcoid in Arizona would eventually develop coccidioidomycosis provides strong evidence for an etiologic relationship between *Coccidioides* and sarcoidosis.

**INCOMPLETE STUDIES:** There is one major study for this Project that has not been completed:

1. Genetic studies on patients with both sarcoidosis and coccidioidomycosis to determine if there is a genetic predisposition to disseminated coccidioidomycosis

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## INTRODUCTION AND SIGNIFICANCE

Sarcoid was first described in 1899 one hundred and seventeen years ago.<sup>1</sup> It is a multisystem disease characterized histologically by the presence of non-caseating epithelioid granulomas. Despite multiple investigations since its description, the cause of sarcoid has not been identified.<sup>2</sup> This research was undertaken to help define the etiology of sarcoid.

## BACKGROUND

The first author of this thesis is a medical student at the University of Arizona College of Medicine in Phoenix, Arizona. The School requires all their medical students to do a Scholarly Project over the four years of their training. Based on clinical observations made by the second author over thirteen years ago (2002) a hypothesis was formulated about a potential relationship between coccidioidomycosis (cocci) and sarcoidosis (sarcoid).<sup>3</sup> The authors have collaborated on investigations to determine if there is an etiologic association between cocci and sarcoid.

We believe we can make a credible case that there is a relationship between an infection with the fungus *Coccidioides* and sarcoid.<sup>4</sup> The most obvious problem with our research hypothesis is that sarcoid is known to occur worldwide, but cocci is found only in a localized area of the southwestern United States which would appear to make establishing a relationship between cocci and sarcoid very daunting. In doing a literature review for the Project we found a number of case reports of sarcoid being associated with cocci<sup>5-9</sup> which also support a potential relationship and encouraged us to pursue further research. The objective of this research was to investigate our clinical observations and based on those findings, see if we can prove an association between the two entities.

## HYPOTHESIS/RESEARCH QUESTION

The impetus for this study is based on clinical observations made starting about sixteen years ago when a series of four patients in Arizona were noted to have had two granulomatous diseases simultaneously, both sarcoid and a *Coccidioides* infection.<sup>3</sup> Since sarcoid is a relatively uncommon condition, the possibility of a relationship between the two was raised. The usual assumption made was that patients with sarcoid in Arizona were treated with steroids, became immunosuppressed and then they were exposed to *Coccidioides* and developed cocci. However, we postulate an alternative possibility **that certain patients can manifest their cocci infection as clinical sarcoid**. The patient would then receive steroids for the sarcoid and eventually the immunosuppression would result in the expression of the underlying cocci. The latter scenario between cocci and sarcoid was suggested by the authors at the beginning of this research in 2013.<sup>4</sup>

## CLINICAL OBSERVATIONS

We present two case studies which support our hypothesis. One of the approaches to substantiating our postulate would be to follow prospectively a newly diagnosed sarcoid patient in Arizona, anticipating that if the hypothesis was correct, the patient would eventually express an underlying infection with *Coccidioides*. The initial effort for this Project was to document the clinical course of a patient diagnosed with sarcoid in Arizona to see if *Coccidioides* played any role in his sarcoid. The following Case 1 reports on an Arizona patient diagnosed with sarcoid in 2000 who was followed for eight years before he developed disseminated cocci. This following case was presented at the Coccidioidomycosis Study Group Meeting in 2013<sup>3</sup>, to get feedback on the patient observations. It has not been reported in the medical literature.

## CASE ONE

A 50 year old White male policeman with a history of ankylosing spondylitis presented in November 2000 with hypercalcemia, thrombocytopenia and splenomegaly. He was eventually hospitalized in December 2000 and underwent a splenectomy mainly because of a persistent thrombocytopenia. The resected spleen histopathology was consistent with non-necrotizing granulomatous inflammation typical of sarcoid and three pathologists (two second opinion pathologists) were in agreement with the diagnostic histopathology. Work up for a granulomatous process, including a *Coccidioides* infection, was done in the hospital and the results were negative. No spherules were observed on histopathology and *Coccidioides* serology was negative.

His subsequent clinical course is outlined in Table 1 (Page 5). Postoperatively he was started on prednisone for the sarcoid and followed as an outpatient. At an outpatient visit in June, 2001 (approximately 7 months after the splenectomy) the patient's *Coccidioides* complement fixation (CF) titer had seroconverted from negative to positive at 1:8 with a positive IgM. All *Coccidioides* serologies on this patient were performed by the University of California at Davis, Coccidioidomycosis Serology Laboratory in Davis, California. At the time of the seroconversion the patient was asymptomatic, feeling better, back to work and being tapered off steroids. Because the patient had seroconverted from negative to positive he was transiently placed on fluconazole even though he was asymptomatic. The patient returned to work and eventually was taken off the steroids in October, 2001.

After being taken off the steroids, he declined to continue to take fluconazole. Over the next two years the patient's cocci CF titers decreased to 1:2 in March, 2002. He was working without missing any work, had no significant fevers or evidence of symptomatic cocci. Between October, 2001 and June, 2002 (roughly 6 months) the patient was off steroids, but was placed back on steroids again by a rheumatologist because of an acute polyarticular arthritis. The patient had no significant fevers with the onset of the arthritis, however while on steroids his cocci CF titers had increased to a high of 1:32. Titers  $\geq 1:32$  are generally only seen in patients with disseminated cocci.<sup>10</sup> Aside from his arthritis, he was otherwise asymptomatic for cocci,

specifically denying any fever, chills or sweats. His arthritis gradually improved and the steroids were tapered and discontinued eighteen months later in December, 2003. His cocci CF titer was still elevated at 1:32, but gradually decreased to a low of 1:4 in March, 2006. Notably his angiotensin-converting enzyme (ACE) levels were highest when his cocci CF titers were the highest. He was asymptomatic relative to cocci between June, 2006 and June, 2008. However, in June, 2008 the patient developed swelling of his right sternoclavicular joint, he did not complain of any fever, chills or sweats. Aspiration of that joint grew *Coccidioides*; his cocci CF titer then was 1:8. He was placed on oral fluconazole 400 mg daily which appeared to control the cocci. Between June, 2008 and September, 2009 his cocci CF titer decreased from 1:8 to 1:2, the right sternoclavicular joint improved and he was able to return to work. The patient was being followed infrequently because his cocci was controlled. He missed an appointment and upon inquiring, he had died late in 2009 because of “cancer of the brain”. No significant investigations were done in retrospect until the initiation of the Don Quixote Project.

Our Case 1 patient was followed prospectively for seven years before he developed disseminated cocci complicating his sarcoid. The findings in this patient supported our hypothesis that sarcoid could be caused by *Coccidioides*.

**TABLE 1. CLINICAL COURSE FOR CASE ONE¶**

DATE →	CLINICAL OBSERVATIONS →	COCCI-CF* →	ACE**¶
11/00 →	Splenomegaly and hypercalcemia →	Neg →	37¶
12/00 →	Splenectomy; DX: sarcoid¶		
01/01 →	Started steroids¶		
03/01 →	Transient eosinophilia (10%) →	Neg →	-¶
06/01 →	Asymptomatic; started fluconazole →	1:8; IgM+ →	83¶
08/01 →	Asymptomatic →	1:8 →	-¶
10/01 →	Asymptomatic; stopped steroids →	1:16 →	-¶
12/01 →	Asymptomatic; stopped fluconazole →	1:4 →	108¶
03/02 →	Asymptomatic →	1:2 →	-¶
06/02 →	Arthritis; had been started on steroids →	1:32 →	44¶
08/02 →	Arthritis →	1:32 →	63¶
10/02 →	Arthritis →	1:32 →	75¶
05/03 →	Arthritis →	1:32 →	109 (highest)¶
12/03 →	Arthritis resolved; stopped steroids →	1:32 →	90¶
06/04 →	Asymptomatic →	1:32 →	84¶
01/05 →	Asymptomatic →	1:16 →	89¶
08/05 →	Asymptomatic →	1:8 →	61¶
03/06 →	Asymptomatic →	1:4 →	-¶
10/06 →	Asymptomatic →	1:16 →	54¶
12/06 →	Melanoma diagnosed →	1:8 →	70¶
04/07 →	Asymptomatic →	1:8 →	73¶
12/07 →	Asymptomatic →	1:8 →	-¶
06/08 →	<i>Coccidioides</i> recovered on culture from the sternoclavicular joint; started fluconazole →	1:8 →	-¶
12/08 →	Cocci controlled →	1:8 →	52¶
05/09 →	Cocci controlled →	1:8 →	-¶
09/09 →	Cocci controlled →	1:2; IgM+ →	-¶
12/09 →	Expired "brain cancer"¶		

\*. Complement fixation titer to *Coccidioides* (normal = negative)¶

\*\*. Angiotension-converting enzyme level (normal 9-67)¶

## CASE TWO

A 34 year old African American man was diagnosed with disseminated cocci in 2009. He was born in California, but moved to Arizona in 1996. He was incarcerated in Arizona but transferred to a prison in Oklahoma City. Shortly after arriving in Oklahoma, he presented to the jail infirmary in 2009 with myalgias, weight loss, night sweats with pain and swelling of his left elbow. He was hospitalized and his left elbow was aspirated. Cultures of the elbow aspiration grew *Coccidioides*. His *Coccidioides* complement fixation serology was positive at 1:8. While in jail he was started on oral fluconazole. He was released from jail and returned to Arizona in 2009. Initially his clinical course was complicated by presumed non-compliance with oral antifungal agents. However, despite becoming more compliant, his clinical course between 2009 and 2012 was suggestive of refractory disease. He had been treated with the oral antifungals fluconazole and voriconazole, followed by intravenous amphotericin B as an outpatient over those years. He progressively had become more compliant with his antifungal medications, but they did not seem to control or resolve his symptoms. In 2012, despite amphotericin B as an outpatient, he became seriously ill with fevers and respiratory failure which required hospitalization and mechanical ventilation. During that hospitalization he underwent a lung biopsy. The histopathology from the biopsy revealed non-caseating granulomas consistent with sarcoid. Acid fast bacillus (AFB) and Gomori methenamin silver (GMS) stains were negative. There was no histopathologic or other evidence of an infection, specifically no evidence of *Coccidioides*. His *Coccidioides* serology had become negative and multiple cultures for *Coccidioides* were also negative. The histopathologic changes and clinical findings were consistent with sarcoid without evidence of an active *Coccidioides* infection. The patient was started on corticosteroids and maintained on amphotericin B. He clinically improved, was extubated and eventually taken off amphotericin B and switched to oral voriconazole. He was discharged on supplemental oxygen and oral voriconazole. Between 2012 and 2015 he has clinically improved and his "sarcoid/cocci" is controlled. He is off oxygen supplementation and is on maintenance voriconazole with progressively lower doses of corticosteroids.

This patient had well documented disseminated cocci in 2009, proven by positive cultures from his elbow and a cocci CF titer of 1:8. He was subsequently diagnosed with pulmonary sarcoid by a lung biopsy three years later in 2012. At the time of the sarcoid diagnosis there was no culture or serologic evidence for cocci.

## RESEARCH GOALS

Our preliminary studies documented two patients with confirmed cocci and sarcoid. The first case was documented prospectively and the second case retrospectively. The clinical findings and the epidemiologic evidence support an etiologic association between cocci and sarcoid consistent with our hypothesis. The observations confirm that the histopathology seen in these two patients was indistinguishable from the histopathology of sarcoid outside the *Coccidioides* endemic area, and by definition, the findings in these two cases are the same as patients with sarcoid in the non-endemic areas of *Coccidioides*. These observations imply that the etiology of sarcoid is likely diverse, and in Arizona, a *Coccidioides* infection can mimic (or cause) sarcoid. The fact that both of these sarcoid/cocci patients responded favorably to corticosteroids is paradoxical for the usual patients with cocci where the infection would be expected to worsen because of the immunosuppression. These unusual circumstances suggest that there is a unique immune-mediated process playing a role in these two patients with sarcoid. The implications of these two patients suggests that our hypothesis warrants additional investigations into the pathogenesis of sarcoid in the *Coccidioides* endemic area attempting to define the mechanisms of how *Coccidioides* is associated with sarcoid.

## LITERATURE REVIEW

Based on the observations made in our two patients we felt there may be reports in the literature of similar cases of cocci and sarcoid. We reviewed the medical literature and found five cases which dealt with the interaction of cocci and sarcoid. These cases provide insight into the pathogenesis of these two entities in various ways and generally support our hypothesis on how cocci can be etiologic for sarcoid. We have summarized each case and analyzed the information in light of our hypothesis.

### CASE REPORT ONE:<sup>5</sup>

Ellis, FW. Coexistent arrested disseminated coccidioidomycosis and Boeck's sarcoid. Calif Med. **1955**; 82: 400-404.

Summary: A 29 year old Black male was diagnosed with Coccidioidomycosis in 1950. He had a history of doing farm work in Bakersfield, CA. Coccidioides was cultured from a posterior neck lymph node and the right tibia. No amphotericin was given. Cultures were positive and the CF titer to Coccidioides was positive at 1:128. His infection eventually improved without treatment. Three years later in 1953, an enlarged cervical lymph node was biopsied and was found to be consistent with sarcoid. The authors thought the observations of the Coccidioides infection and sarcoid were not related and incidental to his sarcoid.

Comment: This case is interesting in that the patient had well documented disseminated cocci which went untreated in the 1950's. He was given no amphotericin and improved. Three years later he was diagnosed as having sarcoid by lymph node biopsy, presumably without any evidence of evidence of active cocci. This patient is very similar to our Case 2. Our patient had well-documented disseminated cocci and three years later he had a lung biopsy consistent with sarcoid. At the time of his sarcoid diagnosis there was no evidence of cocci. These patients support the observation a cocci infection can develop as a clinical disease indistinguishable from sarcoid.

## CASE REPORT TWO:<sup>6</sup>

Bacharach T, Zalis, EG. Sarcoid syndrome associated with coccidioidomycosis. *Am Rev Respir Dis.* **1963**; 88:248-251.

Summary: A 23 year old Black male who had a history of driving through the San Joaquin valley in California in December 1960. He subsequently developed significant epididymitis six months later in June 1961. His course was also associated with transient left ankle arthritis, cough, pleuritic chest pain and weight loss over those six months. He had resection of his epididymis, which on pathology showed non-caseating granulomas, no acid fast organisms or spherules. He was diagnosed with cocci based on a CF titer positive at 1:8. No organisms were cultured or seen. A year later in 1962, he developed right paratracheal and hilar lymphadenopathy. A lymph node biopsy showed epithelioid granulomas consistent with sarcoid. There were no spherules. His cocci skin test was negative and his serology had increased from 1:8 to 1:128. Other than the elevated CF titer 1:128 (suggestive of dissemination), there was no other documentation of cocci. Notably the patient then developed uveitis and polyarthritis. He was empirically treated with prednisone and amphotericin and responded.

Comment: The authors thought their patient had a 'sarcoid syndrome' associated with his *Coccidioides* infection, and he was treated with both steroids and amphotericin with improvement. This report is similar to our Case 2. Our patient had serologic evidence of having cocci, but would have been unlikely to be diagnosed with having cocci as a cause of his sarcoid unless he was followed prospectively.

### CASE REPORT THREE:<sup>7</sup>

Lipschultz BM, Liston HE. Steroid induced disseminated coccidioidomycosis. *Dis Chest*. **1964**; 46:355-359.

Summary: A 30 year old white male was admitted in 1951 for fever and lymphadenopathy. A lymph node biopsy was consistent with sarcoid and he was treated with steroids. In 1964 he became febrile with a cough, right chest pain and bilateral infiltrates on chest X-ray. Cultures for *Coccidioides* were negative, but his CF titer was positive at 1:8. He was treated with amphotericin and improved.

Comment: The authors thought the patient had cocci related to the immunosuppression associated with treating his sarcoid. This interpretation was the conventional association between sarcoid and cocci. This patient is similar to our case 1. Only by prospectively following him would there be any relationship.

#### CASE REPORT FOUR:<sup>8</sup>

Lord, GP. Pulmonary sarcoidosis complicated by cryptococcus and coccidioidomycosis. J Maine Med Assoc. 1974; 65(10):236-240.

Summary: A 27 year old (no race given) male medical student from Maine was admitted in 1956 for cough and fatigue associated with bilateral hilar lymphadenopathy. A lymph node biopsy was consistent with sarcoid. At that time a CF for Histoplasmosis was positive at 1:64. Serologies for Blastomycosis and Coccidioidomycosis were negative. After graduation from medical school the physician went into practice in Arizona in 1961 and developed an infection with Coccidioides which resolved spontaneously. In 1967 he had a persistently abnormal chest X-ray, prompting a lymph node biopsy, which was consistent with sarcoid. He returned to Maine in 1971. In 1972 he was admitted to the hospital for fever, headaches and malaise. Complement fixation titers for cocci were negative. There was evidence of pulmonary fibrosis and he was started on corticosteroid therapy. The patient subsequently experienced a septic syndrome with progressive altered mental status. A workup for an infectious process, including a lumbar puncture was negative. The process progressed and patient died. There was no evidence in the course of his extensive workup that he had a Coccidioides infection. At post-mortem there was Coccidioides organisms in his lungs and central nervous system. Cerebrospinal fluid tested post-mortem revealed a CF titer of 1:128 consistent with disseminated cocci with involvement of the central nervous system.

Comment: The findings in this patient are interesting. A diagnosis of sarcoid was made in Maine in 1972. There is no Coccidioides in Maine and his cocci CF test at that time was negative. However, his histoplasmosis CF was positive. By our observations this patient may have been predisposed to sarcoid which in this case may have been triggered by histoplasmosis. His first diagnosis of sarcoid was in 1956, before he moved to Arizona in 1961, where cocci is endemic. Soon after arriving in Arizona, he and his daughter developed acute Coccidioides infections which resolved without obvious problems. While in Arizona he was diagnosed again with sarcoidosis. He returned to Maine in 1971 where he subsequently died. At post-mortem he was found to have wide-spread cocci (lungs and central nervous system). This course supports the

possibility that there are persons who are genetically predisposed to having a fungal agent trigger a sarcoid response. When he was diagnosed with sarcoid in Maine, his CF titer was positive to histoplasmosis and negative for cocci. After moving to Arizona, he was documented to have a cocci infection which resolved and was then diagnosed again with sarcoidosis in 1967. He then left Arizona was returned to Maine, and was put on steroids for his sarcoid and eventually died of disseminated cocci.

## CASE REPORT FIVE:<sup>9</sup>

Sharma OP, Arora A. Coccidioidomycosis and sarcoidosis: multiple recurrences. West J Med. **1997**;166(5):345-347.

Summary: A 52 year old (race not given) male was admitted in 1985 for fevers. A chest X-ray showed hilar adenopathy and a right mid-lung infiltrate. Bronchoscopy cultures grew *Coccidioides*; a CF titer was positive at 1:256. He was treated with amphotericin B with improvement of his symptoms. Five years later in 1990, he developed skin lesions on his back, which were biopsied and felt to be consistent with sarcoid. He was treated with prednisone and ketoconazole, and the skin lesions improved. In 1992, the lesions recurred on his back, now with similar lesions now on his legs. A chest X-ray showed interstitial infiltrates and hilar adenopathy. Cultures were negative for cocci and no organisms were seen with biopsy of the skin lesions. He was started again on prednisone. In 1995 the patient developed fevers and fatigue. His CF titer to cocci was positive at 1:128. He was treated with fluconazole, which initially was associated with improvement, but then the skin lesions returned. In 1996, the skin lesions returned and he was retreated with steroids and this time fluconazole. The authors did not believe that the sarcoid skin lesions were associated with *Coccidioides*.

Comment: This patient had well documented cocci in 1985 with both positive culture and elevated serology, CF titer 1:256. Five years later in 1990 he develops skin lesions on his back which were biopsied and the pathology was consistent with sarcoid. The skin lesions were initially treated with steroids and ketoconazole. Ketoconazole has poor activity against *Coccidioides* and likely the steroids were more responsible for the improvement. The skin lesions then recurred in 1992, with an abnormal chest X-ray with interstitial infiltrates and hilar adenopathy. Workup for cocci was negative. Three years later in 1995 he developed fevers and fatigue. He was found to have cocci and was treated with fluconazole and no steroids. He initially improved, but the skin lesions recurred in 1996. This time he was treated with both steroids and fluconazole and improved. The authors did not feel there was a relationship between his sarcoid and cocci.

## RESEARCH

### **1.MEDICAL RECORD REVIEW**

Purpose: To determine if there is a relationship between sarcoid and cocci, medical records were reviewed on sarcoid patients admitted to Maricopa Medical Center in Phoenix, Arizona between the years 2004-2014.

Methods: The ICD-9 code 135.0 was used to identify and review the medical records of patients with sarcoidosis. Records which had insufficient information were excluded. Information collected per our data template included; age, sex, ethnicity, length of Arizona residence, date of sarcoid diagnosis, sarcoid tissue biopsy status, angiotensin-converting enzyme result, *Coccidioides* infection – date and location of onset, type of cocci involvement, cocci serology (complement fixation test). In addition, information on the use of corticosteroid treatment, history of active tuberculosis and presence of a malignancy were also documented.

Results: One hundred and forty-five medical records were identified with a diagnosis of sarcoid. Seventy-five medical records were excluded, mainly because of insufficient information due to outpatient and emergency room visits. Sixty-eight records were reviewed and the results are summarized in Table 2 (Page 16).

Discussion: Sixty-eight of the 145 (47%) medical records were reviewed. There were more than twice as many females (69%) compared to males (31%). There were similar findings for ethnicity, black (69%) compared to Caucasian (31%). Of the 68 sarcoid patients, 4 had cocci (6%). We also assessed the presence of malignancy and active tuberculosis to provide information on the potential role of the cell mediated immune (CMI) system in individuals with sarcoid. By medical record review we also learned there were potentially 34 tissue biopsy specimens that might be available for analysis. Some of the data from this medical record review were used in additional studies to follow.

**TABLE 2: RESULTS OF MEDICAL RECORD REVIEW  
ON 68 PATIENTS WITH SARCOIDOSIS**

Age Range (years)	26-64
Mean Age	45.5 years
Gender	47 Females (69%); 21 Males (31%)
Ethnicity	47 Black (69%); 21 Caucasian (31%)
Average ACE Level (normal 9-67)	85
Cocci Diagnosis	4
Active Tuberculosis (TB)	0
Malignancy (any type)	9
Sarcoid Tissue Biopsy Specimens	34

## **2. CASE CONTROL STUDY**

**Purpose:** To assess the rate of *Coccidioides* infections in the non-sarcoid hospital population a case control study was performed by reviewing the medical records of 68 patients matched for age, sex, ethnicity and year of admission.

**Methods:** Inclusion criteria; admission to Maricopa Medical Center between 2004-2014 without a diagnosis of sarcoid. A factor of +/- 3 years was used to match both age and year of admission. Exclusion criteria - evidence of sarcoid and incomplete records. The Fisher's exact test was used to assess the significance between the number of patients with cocci in the sarcoid group compared to the patients with cocci in the non-sarcoid control group.

**Results:** Sixty-eight patients were matched for the case control study. In the 68 non-sarcoid patients, there were 2 cases of cocci, compared to 4 cases in the sarcoid group. There were 7 cases of malignancy, compared to 9 cases in the sarcoid group. There was no significant statistical difference between the two groups for either cocci or malignancy. Unless stated otherwise, p-values, <0.05 are considered significant. Analyses were conducted using Stata, Data Analysis and Statistical Software (Version 13, 2013, Statacorp, College Station, Texas).

**Discussion:** The number of sarcoid patients with cocci was twice as high in the non-sarcoid patients (4 cases compared to 2), however the difference was not found to be statistically significant. Presumably a larger sample size might have resulted in statistical significance. The rate of malignancies in the sarcoid group was higher than controls, but also not statistically different. However, it might suggest the cell mediated immune (CMI) system that monitors for malignancies may be less effective in the sarcoid group. Our Case 1 patient died of a malignancy shortly after his *Coccidioides* infection expressed itself, which we equate to poor CMI. These events may support the importance of CMI in controlling the expression of cocci in sarcoid. If the overall CMI was waning it might also be applied to malignancies, as well as, cocci. There were no cases of active tuberculosis in either group.

### **3.POLYMERASE CHAIN REACTION (PCR) TESTING FOR COCCIDIOIDES DNA**

Purpose: PCR testing for *Coccidioides* DNA has recently become commercially available. The purpose of using this test is to detect *Coccidioides* DNA in patients with sarcoid at the time of their tissue diagnosis. At the time when the tissue diagnosis was made there was no evidence for a sarcoid etiology using conventional studies. The presence of *Coccidioides* DNA in the biopsied tissue at the time of diagnosis, would be substantial evidence that *Coccidioides* can be etiologic in sarcoid.

Methods: Standard PCR testing will be used to detect *Coccidioides* DNA. The DNA will be extracted from the formalin fixed paraffin embedded (FFPE) tissues from sarcoid patients. Specific *Coccidioides* DNA can be amplified using *Coccidioides* primers, the resultant DNA can be quantified and documented to be specific for *Coccidioides*.

Results: At the time of this writing, the *Coccidioides* DNA testing has not been completed.

Discussion: The *Coccidioides* DNA PCR testing is very specific and sensitive in many types of samples. In FFPE tissue the sensitivity can be reduced somewhat to about 70%. This should be sufficient to identify the presence of *Coccidioides* DNA. Identifying *Coccidioides* DNA in the tissues of individuals with sarcoid would significantly confirm a relationship between cocci and sarcoid.

## DISCUSSION

The title of this Project, “Coccidioidomycosis as a Cause of Sarcoidosis in Arizona” was crafted to illustrate why we believe our research has been able to demonstrate an etiologic relationship between cocci and sarcoid. The hypothesis for this Scholarly Project was that an infection by the Arizona endemic fungus, *Coccidioides*, can cause sarcoid. We presented prospective and retrospective sarcoid patient studies, as well as studies from the literature which supports the hypothesis. To explain why we believe our hypothesis to be correct we need to clarify some of the mechanisms and postulate why the cause of sarcoid has not been identified by others over the past hundred years.

Early in the Project we came to the conclusion that one of the reasons the etiology of sarcoid has been so elusive is that it is not caused by a single etiologic agent. If only *Coccidioides* causes sarcoid we would have to postulate multiple etiologies for sarcoid because this fungus has a very restricted area of endemicity, mainly found in the Southwestern United States. Therefore, we could not account for the majority of sarcoid cases around the world. However, around the world there are many systemic fungal agents, similar to *Coccidioides*, which are capable of causing a granulomatous immune response with infection and stimulating the non-caseating granulomas seen in sarcoid. Systemic fungi such as *Blastomyces*<sup>12</sup>, *Cryptococcus*<sup>13</sup> and *Histoplasma*<sup>14</sup> have all been postulated to be associated with sarcoid, however none have been proven to be causative.

There has been speculation that sarcoid could be caused by a fungus<sup>15</sup>, however after a review of 650 cases of various pulmonary fungal infections it was determined by the authors that pulmonary sarcoid and pulmonary fungal infections were clinically distinguishable. The difficulty with that conclusion based on our hypothesis, is that there is no difference between sarcoid and the cocci infection because the onset of sarcoid is due to a *Coccidioides* infection, and they are clinically indistinguishable. By definition, there can be no evidence of a *Coccidioides* infection to make the diagnosis of sarcoid. What we plan to do is to explain the pathogenesis of why this can happen. It is easy to distinguish between sarcoid and a cocci infection when immunosuppression is given for the treatment of sarcoid and the patient

develops a cocci infection following exposure to *Coccidioides*. Theoretically patients with *Coccidioides*-induced sarcoid would not be susceptible to a *Coccidioides* super-infection. However, when a sarcoid patient comes from a non-endemic area for *Coccidioides* and enters into the *Coccidioides* endemic area of Arizona, they become at risk for a *Coccidioides* infection. If a fungal infection occurs in a patient with sarcoid, theoretically the fungal infection would have to be an agent other than the one causing the sarcoid.

Our Case 1 was followed prospectively – He had sarcoid proven by the typical granulomatous pathological findings in his spleen which was confirmed by three different pathologists. By definition, he had no clinical, pathological or serologic evidence of cocci at the time of the sarcoid diagnosis. The patient was then placed on steroids shortly after his diagnosis to treat his sarcoid. After a few months on the steroids the patient clinically improved, but at the same time his *Coccidioides* blood tests seroconverted from negative to positive. He was asymptomatic at the time of the seroconversion and the presumption was made that there was a protective effect afforded by his sarcoid induced immune response to *Coccidioides* which prevented dissemination of the fungus. Initially his immune response was strong, sufficient that he was asymptomatic. However, we believe the use of steroids and passage of time adversely impacted his immune response allowing the cocci serology tests to convert from negative to positive subclinically. Ordinarily, patients with acute cocci, who are being treated with steroids, would become symptomatic and clinically worse. However, this patient was completely asymptomatic, feeling well and able to return to work. We believed this early symbiotic relationship with *Coccidioides* was the result of an uncharacterized sarcoid immune response with the ability to suppress any evidence of cocci. An immune response which completely masks the presence of a fungal infection has to be a major factors in why the etiology of sarcoid has been so difficult to document.

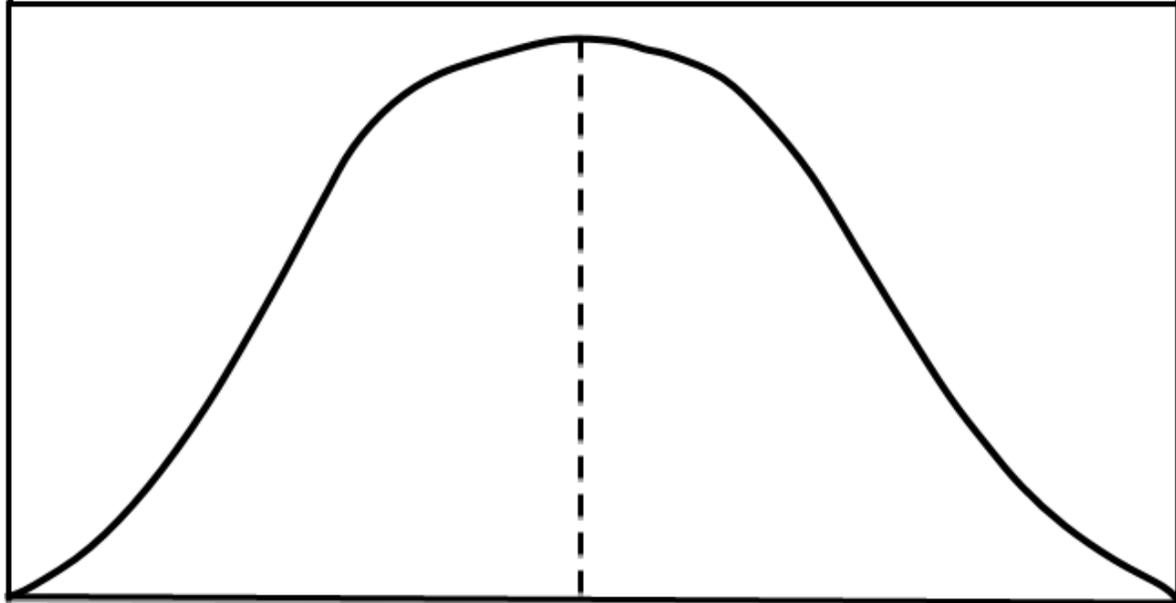
To explain why *Coccidioides* is able to cause sarcoid we hypothesize that there is a unique immune response, most likely genetically determined, that is responsible for the changes that result in the clinical picture of sarcoid. A genetic predisposition to developing sarcoid has been observed between certain families<sup>16</sup> and racial groups.<sup>17</sup> However, we have not been able to

find evidence that a genetically determined sarcoid immune response has been well characterized. In future studies we anticipate that if we can collect swab specimens from additional patients with both cocci and sarcoid, we might be able to document a genetic predisposition to developing sarcoid in that setting.

The hallmark of sarcoid is non-caseating epithelioid granulomas and these granulomas are key to defining sarcoid. Within sarcoid granulomas, the lymphocytes tend to be CD<sub>4</sub>+ T helper cells and in the periphery, the lymphocytes tend to be both CD<sub>8</sub>+ T cells and B cell lymphocytes. Since, by definition, evidence of *Coccidioides* cannot be demonstrated in patients diagnosed with sarcoid we hypothesize that certain antigens of *Coccidioides* (and perhaps other fungi) are able to trigger the sarcoid response in the appropriate individuals without evidence of the fungal infection being present. We presume that there are certain common fungal antigens that stimulate the sarcoid response. In order to account for sarcoid cases outside the endemic area of *Coccidioides*, we postulate that other fungi are capable of causing sarcoid in their respective endemic regions. We suspect that there are other causes of sarcoid, but the fungi might be the best potential etiologic agents to study.

We believe what happens to patients with “*Coccidioides*-induced” sarcoid is related to their cell-mediated immune (CMI) response. Figure 1 (Page 21) illustrates a bell-shaped curve that represents the range of the human CMI response to certain infectious agents. Leprosy is the prototypical infectious disease that best illustrates the classic human CMI response. In Figure 1 we have substituted *Coccidioides* for leprosy to illustrate the range of the CMI responses of individuals responding to a *Coccidioides* infection. Basically, the spectrum of patients who have a poor CMI response to cocci will develop disseminated cocci and those who have exceptionally strong CMI response will develop cocci according to our hypothesis. The majority of *Coccidioides* infected patients fall between the two ends of the CMI response curve and these patients tend to resolve their *Coccidioides* infection without residual. However, the sarcoid/cocci patients are those who are in the extreme tuberculoid leprosy end of the bell-shaped curve. In comparison, those on the lepromatous end of the curve are those patients who have severe disease with disseminated cocci, consistent with a poor CMI response.

FIGURE 1. UNIQUE CELL MEDIATED IMMUNE RESPONSE IN COCCIDIOIDOMYCOSIS = SARCOIDOSIS



Lepromatous

Normal CMI

Tuberculoid

||

||

Disseminated

Sarcoidosis

Coccidioidomycosis

To characterize the immune response of patients in the cocci/tuberculoid end of the CMI curve there are factors which occur with the human CMI response to leprosy that can be applied to the CMI response to cocci. There are four major factors which are shown on Table 3 (Page 24). Patients with cocci on the lepromatous end of the CMI do poorly and develop disseminated cocci. In these patients, the infecting *Coccidioides* organisms are present in large numbers and their *Coccidioides* skin test response is poor. In addition, they tend to have poor granulomas which, in turn, result in a compensatory humoral antibody response, which in cocci, is manifest by the development of positive complement fixing (CF) antibodies. The presence of CF antibodies are used clinically to gauge the severity of a *Coccidioides* infection reflecting a poor CMI response.

Those individuals who have a *Coccidioides* infection on the far left tuberculoid end of the CMI spectrum (Figure 1) represent those individuals who develop sarcoid. These patients have a very good CMI response. In contrast to “lepromatous cocci”, the patients who have “tuberculoid cocci” have no organisms present and the *Coccidioides* skin test is positive. Their granuloma formation is good and there is no compensatory humoral antibody production. These latter four observations explain why a diagnosis of cocci may not be made at the time of the sarcoid diagnosis even though the sarcoid is due to *Coccidioides*. We suggest that patients with sarcoid due to *Coccidioides*, like our Case 1, have a “tuberculoid cocci” CMI response, analogous to tuberculoid leprosy. Patients with disseminated cocci receive the most attention clinically and *Coccidioides* are not considered in the etiology of sarcoid even though there are reports of an association.

**TABLE 3. Prototypical Cell Mediated Immune Response as seen in Leprosy**

<b>Findings in Benign Disease</b> <u>Tuberculoid Leprosy</u>	<b>Findings in Severe Disease</b> <b>Lepromatous Leprosy</b>
<ol style="list-style-type: none"> <li>1. Good cell mediated immune response (skin test +)</li> <li>2. No antibody response</li> <li>3. Good granuloma formation</li> <li>4. No organisms present</li> </ol>	<ol style="list-style-type: none"> <li>1. Poor cell mediated immune response (skin test -)</li> <li>2. High antibody response</li> <li>3. Poor granuloma formation</li> <li>4. Many organisms present</li> </ol>

In a much different clinical situation, our Case 2 patient had well-documented disseminated cocci, but over three years he developed respiratory failure requiring the ICU and a ventilator. During that time he had a lung biopsy which was found to be typical for sarcoid. At that time there was no evidence of *Coccidioides* on pathology and cultures were negative. Also his cocci complement fixation (CF) serology had gone from positive 1:8 to negative. The findings were consistent with the diagnosis of sarcoid. However, the clinical setting suggests that a “tuberculoid cocci” response can occur in patients who have documented cocci who can then have no obvious evidence of *Coccidioides* infection. The mechanisms for this are not clear but presumptively they are related to changes in the patient’s CMI moving toward the tuberculoid end of the spectrum.

Both of our patients with sarcoid and cocci had a systemic infection due to *Coccidioides* which we believe resulted in sarcoid pathology. It appears that a *Coccidioides* infection can produce clinical sarcoid depending on the CMI response of the host. The immune response to *Coccidioides* in these two patients is presumed to be the same because their response resulted in histopathology consistent with sarcoid. We are hopeful that we will be able to demonstrate *Coccidioides* DNA in the biopsy tissues specimens obtained at the time of the sarcoid diagnosis. If *Coccidioides* DNA is present in the tissues of Arizona sarcoid patients it would substantiate that *Coccidioides* is etiologic. However, in tuberculoid leprosy due to *Mycobacterium leprae* finding its DNA is often difficult to detect so we anticipate that similar things may occur with “tuberculoid cocci”.

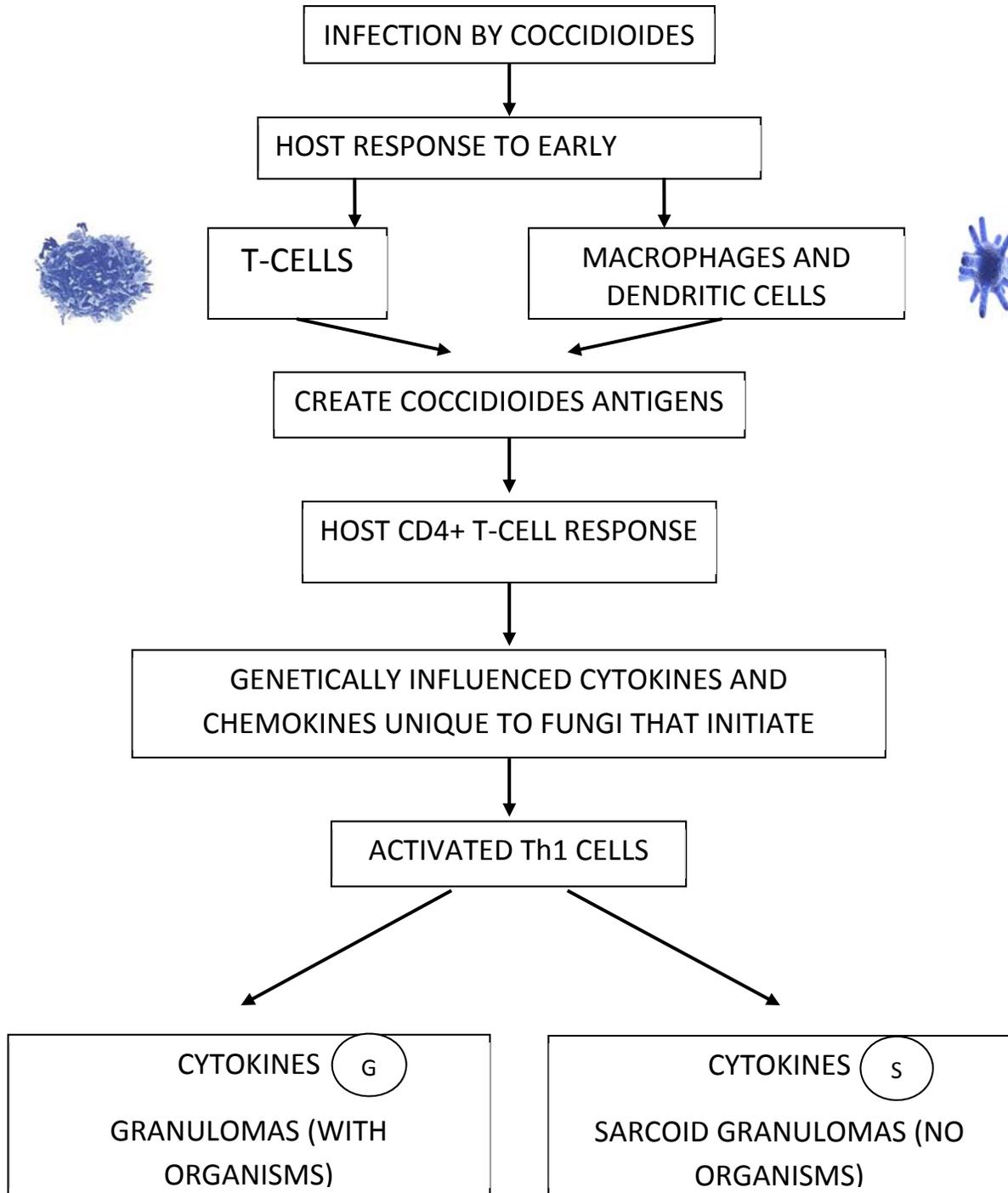
It is speculative as to how the human CMI response allows sarcoid granulomas to evolve following a *Coccidioides* infection. T cells (T lymphocytes) play a central role in the CMI response to fungi, like *Coccidioides*. Our studies suggest that sarcoid and *Coccidioides* are associated in the endemic region of the Southwestern United States. Since sarcoid is found elsewhere, we presume the same CMI response is triggered by other fungi, such as *Histoplasma*. Most likely the initial human immune response to a systemic fungal infection is the same for a number of fungal agents, all of which are capable of triggering the pathway of a granulomatous response with infection.

The human immune response associated with the development of sarcoid has been postulated by Iannuzzi.<sup>18</sup> In Figure 2 (Page 28) we have simplified the postulated sarcoid cascade to emphasize the likely areas that might be important in the interaction between an infection by *Coccidioides* and the development of sarcoid. The hallmark of sarcoid is the formation of non-caseating granulomas and, in Arizona, we believe the pathogenesis of sarcoid begins with an infection by *Coccidioides*. The *Coccidioides* antigens associated with infection are recognized as foreign by the host immune system. These antigens are generally protein fragments, but can be comprised of as yet uncharacterized chemicals which are responsible for the T cell granulomatous reaction. These T cells recruit and activate both macrophages and dendritic cells which are important in controlling the infection. The interaction between T cells, macrophages and dendritic cells hypothetically result in the release of cytokines and chemokines that are unique to the human host response to fungal antigens. This creates a cascade of chemokines and cytokines responsible for initiating the pathways which result in the formation of granulomas. Eventually the host CD<sub>4</sub><sup>+</sup> T cells are stimulated to differentiate into Th1 cells. The Th1 cells are then activated to cause the formation of granulomas. The granulomas are eventually formed when multiple macrophages are activated and able to sequester the *Coccidioides* fungal antigens. Early granulomas begin with activated macrophages coming together and eventually differentiating into epithelioid cells. This immune response prevents the fungal antigens from spreading throughout the host and at the same time stimulates the granulomatous process. Histologically the final granulomas consist of a core of epithelioid macrophages which are then surrounded by a cuff of CD<sub>4</sub><sup>+</sup>, CD<sub>8</sub><sup>+</sup> and CD<sub>20</sub><sup>+</sup> T cells which are all enclosed by an edge of fibrous tissue.

We hypothesize that the factors that influence the host sarcoid granuloma response depends on the chemical make-up of the initial fungal antigens which interact with the host triggering the granuloma cascade. Which direction the cascade progresses, either to plain granulomas or sarcoid granulomas, is presumed genetically determined. This hypothesis originated because we know that fungi are capable of causing a granulomatous immune response. However, we believe those patients who develop the *Coccidioides*-induced sarcoid granulomas are unique individuals who are genetically able to cause sarcoid granulomas instead of non-sarcoid

granulomas. The sarcoid granulomas are capable of eradicating any organisms with no histologic or serologic evidence of a fungal infection. We postulate that there are cytokines created by *Coccidioides* that are specifically capable of stimulating sarcoid granulomas without any evidence of the pathogen. We have arbitrarily labeled it in Figure 2 as cytokine SG which is capable of stimulating the sarcoid granulomas. The pathway that results in “non-sarcoid” granulomas occurs the majority of the time and is triggered into the pathway designated as cytokine G. Based on our clinical observations on Case 1 the influence of the SG immune response wanes over time (years). This can also be compounded by immunosuppression (i.e., steroids for sarcoid) which eventually allows viable *Coccidioides* organisms (presumably endospores) to overcome the immune response resulting in the expression of the *Coccidioides* infection.

**FIGURE 2. HYPOTHETICAL CELL MEDIATED IMMUNE RESPONSE TO COCCIDIOIDES IN SARCOIDOSIS**



The laboratory test most often associated with sarcoid is the angiotensin-converting enzyme (ACE) level. ACE is presumed to be produced by epithelioid cells of sarcoid granulomas.<sup>19</sup> The association between elevated serum ACE in sarcoid has been well documented. ACE levels are often used to support the diagnosis of sarcoid and used to follow the response to therapy. In the course of evaluating ACE levels in other diseases, about 70% of patients with leprosy were found to have elevated ACE levels.<sup>20</sup> In the latter study, 13 sera from patients with disseminated cocci were also tested. One of the 13 sera tested was markedly elevated at 98.9 U/ml. The reason for the elevated level in that one patient was unknown to the authors, however the possibility of concomitant sarcoid and *Coccidioides* was suggested. The highest level in our Case 1 patient was 101 U/ml (see Table 1) which was associated with his highest cocci CF titer at 1:32 which occurred while he was immunosuppressed on steroids. Notably when that patient had *Coccidioides* cultured from his sternoclavicular joint at the same time his cocci CF titer had dropped to 1:8 and the ACE fell to 52 U/ml suggesting his sarcoid immune response was waning and allowed the underlying cocci to flourish. The mechanism for why this might happen is unknown although there has been a suggestion that as the ACE secreting cells mature they produce lower ACE levels which correlates with a weakening CMI response.<sup>21</sup>

Have we established a cause and effect between *Coccidioides* and sarcoid? It appears from clinical observations that sarcoid pathology can occur with a *Coccidioides* infection. Sarcoid purists would say we have not confirmed a cause and effect. However, by definition there is no etiology for sarcoid. However, our prospectively studied patient resolves that issue. Even though he had no evidence of cocci at the diagnosis of sarcoid he developed an asymptomatic acute cocci shortly after his diagnosis. Theoretically he could have been infected with cocci in the few weeks between the sarcoid diagnosis and when the evidence for acute cocci appeared. However, the beauty of prospective studies is that we were predicting he would have evidence of cocci at some point in his sarcoid course. The onset of his cocci was essentially asymptomatic which is very unusual in the clinical setting of steroid treatment. The situation would not likely have been detected if the patient were not being prospectively followed. Documentation of his clinical course was quite revealing in what can happen in a patient with sarcoid due to cocci. It helps explain why finding the cause of sarcoid is so difficult. The

information from this Project provides the basis for further studies. A prospective study of sarcoid patients diagnosed in Arizona would be justified based on the observations of this Project.

## FUTURE DIRECTIONS

Based on our observations, we have postulated that individuals who develop both sarcoid and cocci are genetically predisposed to develop sarcoid in response to a *Coccidioides* infection. We cite the findings of GP Lord<sup>8</sup> in particular, who reported a patient who had two episodes of sarcoid. He developed the first episode in 1956 while living in Maine. At that time his sarcoid was associated with a *Histoplasma* infection. Then while in Arizona in 1967 the same patient developed sarcoid associated with an infection by *Coccidioides*. That report, and our case reports, suggests that there are certain individuals who are genetically predisposed to respond to a certain fungal infections, like cocci, with the ability to trigger the development of a sarcoid granuloma response.

Currently there is an ongoing NIH study in Arizona on the “Pathogenesis and Genetics of Disseminated Coccidioidomycosis”. The study is designed to determine how human genetics are associated with the dissemination of a *Coccidioides* infection. We see this as an opportunity for a similar genetic analysis for our sarcoid/cocci patients. We have identified other patients with a history of having both sarcoid and cocci which we plan to enter into the study. The two case studies described in this Project both had disseminated cocci which is a criteria to be entered into the NIH study. We have been in touch with the Principle Investigator who has encouraged us to submit specimens into the study. Our patients would be a subset of the patients with disseminated cocci. We are aware of other cocci/sarcoid patients and hopefully we will

## CONCLUSIONS

The findings of this scholarly project can conclude that *Coccidioides* can be a cause of sarcoidosis in Arizona. An extensive literature review revealed many cases of sarcoidosis are associated with *Coccidioides*. In a retrospective chart review, the cases of *Coccidioides* were twice as high among patients with sarcoidosis, when compared to age and sex matched controls. Although it was not statistically significant in this study, a larger sample size could presumably show more of an effect. The historical difficulty in finding etiologic organisms from sarcoid patients is probably a testament to the efficacy of the patient's cell mediated immune system in obliterating pathogens within granulomas. Prospective studies on sarcoid patients in Arizona could potentially characterize the immune response to *Coccidioides* in sarcoidosis. If *Coccidioides* can be a cause of sarcoidosis in Arizona, we suspect similar fungi from different endemic areas of the United States where they can also cause sarcoid. This line of reasoning opens up areas of future research on fungi as etiologic agents of sarcoidosis. Since one of the treatments of sarcoidosis is corticosteroids, this could theoretically lead to complications of disseminated Coccidioidomycosis from immunosuppression. A prospective study to follow patients in Arizona might be a way of establishing the pathophysiology of the interaction between fungi like sarcoid and *Coccidioides*. Future studies on the genetics of patients with both *Coccidioides* and sarcoidosis will also help to further elucidate the mechanism of the immune response of patients who have *Coccidioides* as an etiology.

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