



Coccidioidomycosis

S T U D Y G R O U P

**PROCEEDINGS OF THE
THIRTY SEVENTH ANNUAL
COCCIDIOIDOMYCOSIS STUDY GROUP MEETING**

Meeting Number 37

April 3, 1993

Tucson, Arizona

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Abstract 1: Tumor Necrosis Factor production by human mononuclear cells exposed to killed arthroconidia and spherules of *Coccidioides immitis*

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The in vitro production of tumor necrosis factor (TNF) by adherent human peripheral blood mononuclear cells (MNL) incubated with arthroconidia or spherules derived from the dimorphic fungus *Coccidioides immitis* were examined. Using a bioassay measuring percent cytotoxicity of L929 cells, arthroconidia and spherules induced the production of measurable amounts of TNF by MNL. The arthroconidial and spherule preparations both contained < 0.01 ng/mL of endotoxin, below that needed to induce cytotoxicity in the bioassay. There was a significant difference between the amount of TNF produced, as measured by bioassay, by MNL incubated with spherules based on whether the donor demonstrated dermal hypersensitivity to spherulin or not. Specifically, MNL from skin-test positive donors induced cytotoxicity of 54.4 ± 9.7 compared to 8.9 ± 5.6 for MNL from skin-test negative donors ($p = 0.008$). Based on ELISA, the vast majority of TNF induced by arthroconidia or spherules was TNF-alpha, with minimal production of TNF-beta. These are the first data to show the production of TNF in human coccidioidomycosis.

Abstract 2: Amino Terminal Protein sequence analysis of *C. immitis* chitinase

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A chitinase, isolated and affinity purified from *Coccidioides immitis* spherule-endospore culture filtrate, was subjected to amino terminal protein sequence analysis using automated Edman degradation. The resulting 18 amino acid sequence was compared with the previously reported IDCf amino acid sequence. The two sequences were homologous, supporting the previous identification of the IDCf antigen as a chitinase. Additional amino acid residues observed at the N-terminus of purified chitinase suggest that the IDCf sample had undergone proteolytic processing.

The availability of the N-terminal portion of the chitinase/IDCF antigen will provide a basis for the synthesis of corresponding oligonucleotide probes that can be used for screening genomic and cDNA libraries of *C. immitis*. These clones containing the chitinase gene will allow further investigation of the chitinase temporal expression and regulation and may permit the production of a recombinant protein.

**Abstract 3: Developing nucleic acid markers for population genetic study of
Coccidioides immitis and *Histoplasma Capsulatum***

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Population genetic studies of nucleic acid variation can be used to (1) infer the relative frequency of sexual and asexual reproduction, (2) improve molecular-genetic detection methods for allowing for intraspecific variation, and (3) search for correlations between genotype (or clone) and phenotype (e.g., pathogenicity). For human pathogens, such information might improve diagnosis and treatment. Previous studies of molecular phylogenetics placed *Coccidioides immitis* and *Histoplasma capsulatum* (= *Ajellomyces capsulatus*) in the Onygenales (Ascomycota). Oligonucleotide probes have been made for their identification via PCR. To continue these studies in populations, we are (1) developing safe and rapid methods for nucleic acid extraction from pathogenic fungi and (2) searching for nuclear markers to study intraspecific variation. Our strategy for safe DNA extraction relies on first killing the fungus. With polymorphic loci, we want to know the basis for variation in all individuals. Candidate sequences, therefore, are generated by PCR amplification with random primers, and sequence variation in regions that amplify from all individuals is revealed by single-strand conformation polymorphism (SSCP) electrophoresis. Polymorphism revealed by SSCP is confirmed by sequence analysis. This research is supported by NIH, NIAID.

Abstract 4: Coccidioidomycosis in Northeast Brazil; adding a new area to the map of endemic Coccidioidomycosis

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Three recent cases of coccidioidomycosis establish the state of Piaui in northeastern Brazil as an endemic focus. On August 31, 1991, a 38-year-old Brazilian man, his 11-year-old son, and a 24-year-old male cousin, all lifelong residents of Oeiras, Piaui, in northeastern Brazil, went to dig and hunt for armadillo in the hills near their home. Nine days later, all three became ill with symptoms including high fever, weakness, myalgias, cough, and dyspnea. Chest x-rays revealed pulmonary infiltrates in all three. They were treated with antibiotics for presumed bacterial pneumonia at a local hospital. Complement fixation and immunodiffusion tests showed serologic evidence of CC in all three. Immunodiffusion tests for paracoccidioidomycosis and histoplasmosis, both endemic there, were negative. After several days, the 11-year-old recovered spontaneously. The other two continued to have severe pneumonia with bilateral pulmonary infiltrates; they both recovered after receiving amphotericin, although the 38-year-old later developed a lesion of disseminated CC on his face. *Coccidioides immitis* was isolated from the sputum of one of the patients after mice were inoculated intraperitoneally with a specimen, and spherules were observed in mice liver, spleen, and lungs. Subsequently, *C. immitis* was isolated from 3 of 24 soil samples collected near the place where the three patients were presumably exposed. One of 20 relatives and neighbors of the three patients had a positive spherulin skin test. Although the Brazilian medical literature includes two case reports published in the late 1970s of CC diagnoses based on histopathologic evidence, this report of culture-confirmed cases and isolation of *C. immitis* from the environment establishes Oeiras in northeast Brazil as an endemic focus of CC. Further studies are required to determine the extent of CC in Piaui and the magnitude of the public health problem it poses there.

Abstract 5: Identification methods for *Coccidioides immitis* developed in Japan

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Due to Japanese economic development, the number of Japanese overseas tourists has rapidly increased, resulting in an increase in the number of Japanese suffering from coccidioidomycosis. *Coccidioides immitis* is very dangerous to work with. To identify the fungus, we have to verify 'spherules' using both in vivo or in vitro methods. However, these methods are attended with danger. To avoid these dangerous procedures, we have developed three methods as follows:

1.) A method using the 'agar-implantation method': This method has been reported in 'Mycopathologia' 89: 51-57, 1985

2.) A method using the ubiquinone (Coenzyme Q) Systems: *C. immitis* and *Malbranchea* species closely resemble each other in taxonom. The spherule is the only morphologically different characteristic from the genus *Malbranchea*. We have applied ubiquinone system to differentiate *C. immitis* from the genus *Malbranchea*. All eleven strains of *C. immitis* evidenced ubiquinone-10 (Q-10) as the major isoprenologue without any exception, accompanied by ubiquinone-9 (Q-9) as a minor component. In the *Malbranchea* species two kinds of isoprenologues are established to be major ubiquinones. Eleven species produced dihydrogenated ubiquinone-10(O-10(H₂)) and four species produced Q-10 as the major ubiquinone, respectively. However, these four species were differentiated from *C. immitis* by using the following method.

3. A method using 'Bacto Yeast Nitrogen Base (Difco)': First, thirty grams of dextrose and 1.2% agar are added to 'Bacto Yeast Nitrogen Base' presented by Difco. *C. immitis* is inoculated at the center of a plate, and then the plate is incubated at 37°C. The plate turned upside down is observed at adequate intervals under a light microscope. Approximately 14 days after incubation, terminal and intercalary chlamydospore-like cells which look like 'racket hyphae' of dermatophytes are formed and one month later, chains of spherical cells are produced. These spherical cells swell and some of them are divided into several compartments by septa. They multiply by fission. However, it is difficult to find out endospores in them. This method is very simple and safe for identification of *C. immitis*.

Abstract 6: Coccidioidomycosis Outbreak on the Salt River Indian Reservation

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Coccidioidomycosis is typically thought to be acquired by the inhalation of arthrospores which become airborne when the soil in which they are growing is disturbed. Most cases of cocci cannot be traced to a specific time or site of spore inhalation. We report an outbreak of cocci in a Salt River Pima Indian family following the excavation of a large pit in their yard.

In mid June 1992, five male members of an extended Indian family dug a 5 ft. x 5 ft. x 6 ft. pit in the desert yard of their residence. One other adult and two children were closely involved observers of the excavation. All were members of the Salt River Pima Indian tribe and resided on the tribal reservation, which is located just east of Scottsdale, Arizona. The inhabited areas are typically undeveloped desert very characteristic of the Lower Sonoran life zone. Within four (4) days of completing the excavation, four of the five participants developed an acute illness characterized by cough, fever, chest pain, dyspnea¹ and a macular rash. Two of the symptomatic individuals required hospitalization with one requiring intensive care management of hypoxemia and acute pneumonia. All eventually recovered from their illness.

Abstract 7: A Transplanted Outbreak of Coccidioidomycosis in Tennessee

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Persons not living in areas endemic for coccidioidomycosis may become infected when visiting these areas but may not experience symptoms until after their return home. We report an outbreak of coccidioidomycosis that occurred in a Marine Reserve unit of 27 men based in Nashville, Tennessee after their return from a week training exercise in southern California during the recent epidemic there. They conducted maneuvers in the soil, including digging and sleeping in shallow trenches. The index case occurred in a black man who developed skin dissemination of *Coccidioides immitis*. Serum was collected on three occasions over 4 months and Spherulin skin tests were performed at 6 months on all men. Ten (37%) had acute respiratory symptoms within 4 to 6 weeks of their return. Of these, seven reacted positively to the skin-test, including six who also had serologic evidence of coccidioidomycosis infection. None of the asymptomatic men had evidence of infection. The proportion of persons infected in this outbreak who were symptomatic (7/7) was significantly higher than expected, since only 40% of infected persons develop symptoms. This finding along with a substantial attack rate was likely due to intense soil exposure. This report underscores the need to consider coccidioidomycosis in patients who have traveled to areas endemic for this disease. Similar outbreaks probably occur regularly, especially during epidemics, and are only recognized in the minority of cases when the disease is severe.

Abstract 8: An Epidemic of Coccidioidomycosis in South San Joaquin Valley

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In 1991 an outbreak of coccidioidomycosis of epidemic proportions occurred in Kern County California. This five-fold increase in cases presented a unique opportunity to evaluate this disease. Data was collected from the records of 400 cases to describe the clinical, laboratory and radiographic features. Patients were followed for one year to document treatment and outcome.

Sixty percent of the cases were less than 40 years old. Fever (75%), cough (72%), chest pain (42%), fatigue (34%), and erythema nodosum (26%) were the most prevalent symptoms. Headache (20%) without meningitis was also noted. Chest radiographs revealed infiltrates alone in 76% with the left lower lobe being most commonly involved (30.4%). Effusions with infiltrates (11%) and adenopathy with infiltrates (3.4%) were additional observations. Initial skin tests were negative in 53%, which was associated with high complement fixation titers and dissemination. Eosinophilia was present in 25% of cases. Dissemination occurred in 5% and in 20.5% of blacks.

Treatment with azoles or amphotericin was utilized in 46% of cases. Therapy was started within 30 days for 66% of those treated. Ketoconazole was used primarily for milder cases with amphotericin and fluconazole utilized for more serious presentations. Hospital days totaled 1470 with costs estimated at 1.5 to 2.0 million.

Dissemination, death, or chronic symptoms requiring treatment occurred in 9% of cases. Significant odds ratios for a poor outcome included a negative skin test (21.4), black race (8.6), male sex (3.1) and a CF titer greater than 1:32 (4.3). Mortality was 3.6% with the cause of death being progressive pulmonary in 71.4% and disseminated in 28.6%.

This data base should be useful for validation, quantification, and future research in coccidioidomycosis.

Abstract 9: Coccidioidomycosis in Tulare County, California 1991

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Background: In 1991, 1208 cases of coccidioidomycosis (CC) were reported to the CDHS, compared with an annual average of 450 cases during 1986-1990. Of the 1991 cases, 74% were reported during 09/91 through 12/91 (the epidemic period).

Methods: We conducted our study in Tulare County (TC), a county in the Central Valley, where CC is endemic. We identified cases, defined by serologic evidence of acute infection during the epidemic period, using data from the CC serology laboratory at UC Davis and other reference laboratories, and the-TCHD CC reporting system. We studied risk factors for severe disease by comparing patients who were hospitalized with those who were not, using review of medical records and telephone interviews of case-patients. We evaluated the sensitivity of the TCHD CC reporting system by comparing the number of CC cases reported to the system in 1991 with the number of CC cases diagnosed at the UC Davis laboratory.

Results: During the epidemic period, 128 cases were identified. Attack rates were highest among residents of three contiguous towns in southwestern TC (relative risk [RR] =5.2, 95% confidence interval [CI] =3.7-7.5) and among Asians (RR=4.4, CI=2.4-8.7). None of the Asians were lifetime residents of the Central Valley, compared with 47% of Hispanics and 58% of whites. Thirty-five (27%) case-patients were hospitalized, 30 with severe primary pulmonary disease and 5 with disseminated CC. Male sex and nonwhite race were risk factors for hospitalization (RR, males=2.5, CI=1.3-5.2; RR, nonwhites=2.3, CI 1.1-4.6). Twenty-six (13%) of 202 CC cases diagnosed at UC Davis during 1991 were reported to TCHD.

Conclusions: The high attack rate among Asians could be explained by their recent immigration to the Central Valley. This study extends previous observations of more severe CC in males and nonwhites by demonstrating increased rate of hospitalization among these groups because of severe primary pulmonary disease as well as dissemination. Though CC is a reportable disease, few practitioners reported newly identified cases to TCHD. This outbreak would not have been recognized using the TCHD CC reporting system alone.

Abstract 10: Tumor necrosis factor A in the Cerebrospinal fluid of patients with Coccidioidal meningitis

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In this preliminary study, we examined the levels of the cytokine tumor necrosis factor- α (TNF) in the cerebrospinal fluid (CSF) from patients diagnosed with coccidioidal meningitis. TNF levels were determined by ELISA. CSF from 17 subjects was assayed; 6 were HIV-infected; 2 had multiple samples measured. TNF was detected in the CSF of 8 of 17 subjects with a mean \pm SEM of 15.8 ± 4.5 pg/ml (Range: 3.9 - 37.8) for those samples with detectable levels. There were no differences between CSF from HIV-infected and non-infected patients. Multifactorial regression analysis showed no significant correlation between CSF white cell count, glucose, protein, or complement-fixing antibody titer. However, there was a significant correlation to clinical score ($p = 0.013$). TNF levels in CSF were measured over time in two subjects. In both instances, there was an initial increase after fluconazole therapy was started, with a subsequent decrease. These data suggest that TNF can be detected in the CSF in about half of patients with coccidioidal meningitis and there is no correlation to standard markers of meningeal inflammation. Measurement of TNF and other inflammatory cytokines in the CSF of patients with coccidioidal meningitis may be useful in the future. A more complete study is contemplated.

Abstract 11: Identification of *Coccidioides immitis* DNA using the Polymerase Chain Reaction

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Coccidioidomycosis is a disease of particular importance in endemic areas and even more so in the area of acquired immunodeficiency syndrome. The laboratory diagnosis of infection caused by *Coccidioides immitis* is based in the traditional methods of staining and culture of the fungi from clinical specimens. In an effort to improve the diagnostic technique for *C. immitis* we have begun to develop a rapid and sensitive diagnostic test employing the polymerase chain reaction (PCR). Using the nucleotide sequence for a *C. immitis* cell wall-associated proteinase gene (Cole et al, Infect.Immun. 1992; 60:416) we developed oligonucleotide primers for PCR. Two sets of nested oligonucleotide primers (18-mers) were synthesized and used in PCR amplifications. DNA was extracted from *C. immitis* mycelial culture by using standard techniques of DNA isolation for filamentous fungi. Amplification reactions were performed in 100 ul volumes using recombinant Taq DNA polymerase. After amplification the product was detected by agarose gel electrophoresis and ethidium bromide staining. Two distinct bands (386 and 234 bp in length) were detected corresponding to the predicted size of the amplified fragments. Southern blot hybridization with an internal ³²P-labelled oligonucleotide probe confirmed specificity of the amplified fragment. We conclude that this technique may be useful for the detection of *C. immitis* in clinical specimens.

Abstract 12: Diagnosis of Pulmonary Coccidioidomycosis in patients with and without HIV infection. Comparison of Bronchoalveolar lavage, culture and transbronchial biopsy

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The results of all fiberoptic bronchoscopic examinations which detected *Coccidioides immitis* at two medical centers in an area endemic for coccidioidomycosis were retrospectively reviewed. *C. immitis* was detected by cytological examination of fluid from either bronchial wash or bronchoalveolar lavage (BAL) fluid in 8 of 19 HIV-infected patients (42%) and in 11 of 35 patients without HIV infection (31%). In all cases, the fluid samples grew *C. immitis*. The median time to positive identification of the fungus was 25 days. Transbronchial biopsy was performed simultaneously in eight cases and *C. immitis* was identified by morphologic examination in all eight. These results indicate that cytologic examination of bronchial wash or BAL fluid from patients with and without HIV infection is diagnostic in less than half of cases of pulmonary coccidioidomycosis. Culture of the same fluid appears to be more sensitive than cytological examination in establishing this diagnosis.

Abstract 13: Increased delivery of Amphotericin B to the central nervous system by increasing the permeability of the blood brain barrier with peptide RMP-7

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RMP-7 is a nonapeptide analog of bradykinin which increases the uptake into the brain of compounds of 2000 daltons or less when administered intravenously. We studied the effect of RMP-7 at a dose of 5 ng/kg iv daily for 8 days on the cerebrospinal fluid (CSF) levels of amphotericin B in dogs (n=5 to 6 per group) receiving amphotericin B 0.3 mg/kg daily. Amphotericin B levels in CSF were measured by HPLC on the first and eighth day of treatment.

On Day 1, the levels of amphotericin B in CSF were only slightly higher in the RMP-7 treated dogs than in the controls. On Day 8, trough levels of amphotericin B in the RMP-7 treated dogs (10 ng/ml) were twice those in controls. The AUC for the period 1 to 12 hours after drug administration on Day 8 was 160 ng x hr/mL for the RMP-7 treated dogs and 78 ng x hr/mL for the control dogs.

RMP-7 is currently in clinical trials as an adjuvant to amphotericin B for the treatment of cryptococcal meningitis in patients with AIDS.

Abstract 14: Efficacy of the Triazole D0870 against systemic murine Coccidioidomycosis

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A systemic model of murine coccidioidomycosis was established to test the therapeutic efficacy of D0870. Female CD-1 mice were infected i.v. with 50 arthroconidia of *Coccidioides immitis* and oral therapy initiated 4 days later. Mice were treated through day 24 postinfection with either various doses of D0870 on a QD or QOD schedule, QD fluconazole (FLU), D0870 diluent alone or were given no Rx. All mice given 100 mg/kg of FLU, 10 mg/kg QD or 10 or 100 mg/kg QOD of D0870 survived through the end of the experiment; only 30 to 90% of mice in other regimens survived. D0870 showed dose-responsive reduction of *C. immitis*. FLU at 1 or 100 mg/kg was equivalent in clearance of burden; 100 mg/kg of FLU was superior to controls ($P < 0.05-0.001$). All regimens of D0870, except 1 mg/kg QOD, were superior to controls ($P < 0.001$). Spleen burdens were readily cleared by both FLU at 100 mg/kg (50% cured) and D0870 at 10 mg/kg QD (60% cured) or 10 or 100 mg/kg QOD, which cured 80 and 70%, respectively. FLU at 100 mg/kg cured no mice of liver or lung infection, whereas 10 mg/kg of D0870 QD or 100 mg/kg QOD cured 30 to 60% of mice of infections in these organs; D0870 at 10 mg/kg QD or 10 or 100 mg/kg QOD were superior to FLU at 100 mg/kg ($P < 0.001$). Furthermore, D0870 at 10 mg/kg QD cured 2 mice of infection and at 100 mg/kg QOD cured 3 mice; no FLU treated mice were free of infection. Based on these results D0870 given QOD or QD is estimated to be 10 or 50-fold, respectively, more efficacious than FLU in the treatment of coccidioidomycosis. D0870 shows promise as a therapeutic agent and should be further tested.

Abstract 15: natural History of Disseminated Coccidioidomycosis. Analysis of Armed Forces - VA Collaborative Study Group Records, 1955-1958

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DC, a serious complication of *Coccidioides immitis* infection, is always treated with antifungal drugs. However, detailed quantitative information about its manifestations and untreated course is lacking. We are reviewing a VA/military database, compiled before the availability of effective antifungal drugs, which contains records for 699 patients (pts) with coccidioidal infection, of whom 94 had extra-meningeal DC. 97% of the 699 pts and 100% of DC pts were male the proportion of pts who had DC varied significantly by race ($p < 0.001$); DC occurred in 36 (39%) of 92 blacks, 9 (38%) of 24 Filipinos, and 5 (16%) of 31 Hispanic pts, but only 42 (8%) of 530 white pts. DC lesions were the first manifestation of infection in 52% of the first 31 pts with DC analyzed, and the first DC site was skin in 17 (55%), lymph nodes in 5 (16%), skeleton in 3 (10%), meninges in 2 (6%), and other sites in 4 (13%). Eleven pts (35%) died of DC; 10 survived less than 6 months (median = 60 days) after the first signs of DC. Eleven of the 19 subjects who had DC at multiple sites died, compared to 0 of 9 who had DC at a single site ($p < 0.005$) All 6 pts with meningitis died, compared to 5 of 25 pts without meningitis ($p < 0.001$). Our findings should provide an historical basis to evaluate contemporary therapies for disseminated infections.

Abstract 16: Coccidioidomycosis in Transplant recipients

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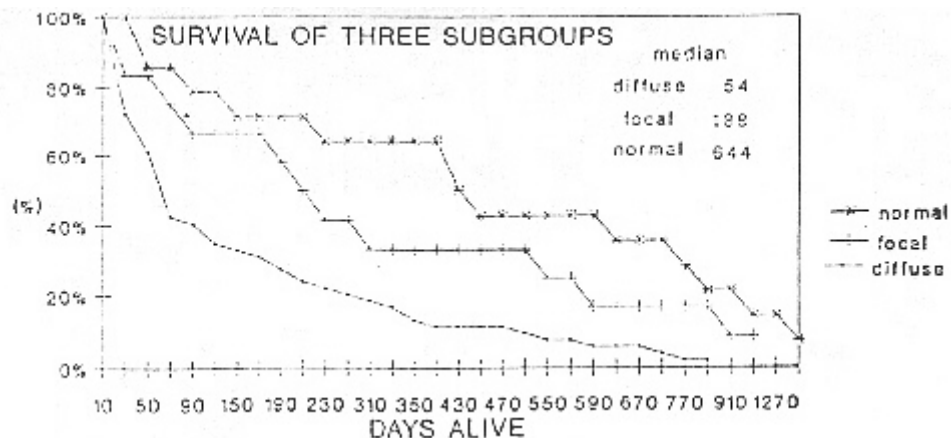
Coccidioidomycosis has been reported in cardiac, liver, and renal transplant recipients. To date, coccidioidomycosis has not been reported in bone marrow transplant (BMT) recipients. We report three cases of coccidioidomycosis in allogeneic BMT recipients; one case of pulmonary coccidioidomycosis and two cases of disseminated coccidioidomycosis. The two patients with disseminated disease died, whereas the patient with localized pulmonary coccidioidomycosis survived. Diagnosis and treatment of coccidioidomycosis was difficult in these patients. The diagnosis was made pre-mortem by visualization of *Coccidioides immitis* spherules along with culture of the organism from lung specimens obtained by bronchoscopy with transbronchial biopsy in two of the patients. The third patient was diagnosed with disseminated coccidioidomycosis at autopsy. Sterile body sites, such as blood and cerebrospinal spinal fluid, grew *C. immitis* post mortem. Two of the patients had coccidioidal serologies performed and were negative. Treatment consisted of amphotericin B in two of the three patients. The third patient died after receiving only 15 mg of amphotericin B. Coccidioidomycosis in BMT recipients can result in life-threatening complications. Aggressive diagnostic tests, such as transbronchial biopsy and open lung biopsy, should be pursued in patients at risk for coccidioidomycosis.

Abstract 17: Coccidioidomycosis in HIV-infected patients: review of 91 cases.

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We performed a retrospective study of 91 HIV-infected patients diagnosed with *coccidioides immitis* between January 1982 and February 1993 at Maricopa Medical Center, Phoenix, AZ. Eighty-five (93%) patients were male and six (7%) were female. The major risk factors for acquisition of HIV infection were: homosexuality, 46%; intravenous drug use (IVDU), 24%; multiple sexual partners, 10%. Fifty one percent were Caucasians, 22% African Americans, 22% Hispanic and in 5% race was not known. Fever and chills, cough, weight loss and night sweats were the most frequent presenting symptoms and palpable lymphnodes and splenomegaly were the most frequent physical findings. Lung was involved in 80%, followed by meninges in 15%. Fifty nine patients had diffuse pulmonary involvement, 13 had focal pulmonary disease, 15 had a normal chest radiograph and in 4 the chest radiograph was not available. Fourteen patients also had meningitis. The mean CD4 cell counts were 55, 127, 95 cells/UL in the diffuse, focal and normal group respectively. Bronchoalveolar lavage was frequently performed and had a yield of 67%. At last follow up fifty five (60%) patients had died, 27 were alive and 9 were lost to follow up. The median duration of survival was 54 days for the diffuse pulmonary group, 188 days for the focal pulmonary group and 644 days in patient with normal chest radiographs ($P < .05$). Thirteen of 14 patients with meningitis died. Therapy consisted primarily of intravenous amphotericin B and/or azole. Patients who received more than 1500 mg of amphotericin B survived longer than those who received less than 1500 mg of amphotericin B



Abstract 18: Prediction of Relapse after treatment for Coccidioidomycosis

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Relapse has been a problem in the treatment of Cocci with both amphotericin B (AMB) and the azoles. We reviewed the records of 34 patients who required therapy for Cocci between 1973 and 1993, 10 relapsed (R) and 25 did not relapse (NR) during follow-up (one patient had 2 courses of therapy). Duration of followup was a mean of 9.4 yr in R and 3.5 yr in NR. Total dose of AMB for R was a mean of 3.8g and two patients received additional ketoconazole for 17m each. Total AMB for NR was a mean of 2.1g and 5 patients received additional azoles for a mean of 15.4m. Ten NR received azoles only for a mean of 10.7m. Time to relapse was 7.3m (1-21m).

All 34 patients had a clinical response to therapy. A four-fold or greater fall in CF during therapy was seen in 7/9 (78%) of R and 17/20 (85%) NR ($p = 0.956$). Using a nonparametric test (Wilcoxon Rank Sums), there was no significant difference between R and NR for the lowest complement fixation (CF) titer during therapy, the end of therapy CF or the peak CF. A peak CF $\geq 1:256$ was seen in 5/10 who relapsed and 3/25 (12%) NR ($p=0.048$). For serial coccidioidin skin tests (SCST), 2/19 (11%) who had consecutive positive skin tests during therapy relapsed as compared to 7/14 who had negative skin tests ($p=0.034$). Univariate logistic regression modelling revealed an odds ratio (OR) for relapse of 8.5 (95% CI 1.4-51.5) and 7.3 (95% CI 1.3-41.4) for negative SCST and a peak CF $\geq 1:256$, respectively. Stepwise-logistic regression determined that negative SCST and peak CF $\geq 1:256$ were independently predictive with an OR of 5.7 (90% CI 1.1-28) and 5.4 (90% CI 1.1-27.1), respectively.

We conclude that clinical response, lowest CF, end of therapy CF, and decrease of CF ≥ 4 fold are not predictive of relapse. The best predictors of relapse are negative serial coccidioidin skin tests and a peak CF titer $\geq 1:256$.

The opinions expressed in this article are the views of the authors and do not represent the position or views of the Department of the Navy, the Department of Defense, or the US Government.

Abstract 18: The relationship of serum and cerebrospinal fluid fluconazole levels to treatment outcome in thirty cases

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During the past two years thirty cases of coccidioidal meningitis (CM) presented as part of an unprecedented epidemic. Previous fluconazole experience at Kern Medical Center and studies reviewed by others led to therapy being offered as an alternative to IV and IT Amphotericin B for CM.

The data to be discussed is an evaluation of patients treated with oral fluconazole at doses ranging from 400 to 1400 mg/day. The demographics, presenting symptoms, and laboratory findings will be summarized and an analysis of the clinical and cerebral spinal fluid parameters will be reviewed.

The relationship of serum and cerebral spinal fluid fluconazole levels to clinical and laboratory response will be presented and the therapeutic implications discussed.

Abstract 19: 54 Years of Cocci skin testing in Kern County (11,500 skin tests revisited)

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We have previously reported here ('61, '64, '79 and '86) the 50% drop in Cocci skin test reactivity in our students from '39 to '59, presumed due to major changes in irrigation and cultivation, with another 50% drop by 1965 (less well explained). It then tended lower, with a small rise in 5/92, early in the recent Cocci epidemic.

The annual attack rate (gross figures aren't much use unless you factor in how long your subjects have been exposed) had gone from about 10% in 1939 to around 1% in 1-8th graders. Adult figures have consistently shown a higher attack rate of 2-4% per annum (a rough figure) before the current epidemic. The attack rate now seems headed back toward where it was before.

