



# Design of A Case Control Etiologic Study of Sarcoidosis (ACCESS)

ACCESS Research Group\*

**ABSTRACT.** Sarcoidosis is a chronic granulomatous disorder of unknown cause, characterized by activation of T-lymphocytes and macrophages. A Case Control Etiologic Study of Sarcoidosis (ACCESS) is a multicenter study designed to determine the etiology of sarcoidosis. The study organization includes 10 Clinical Centers, a Clinical Coordinating Center, specialized Core Laboratories, a Central Specimen Repository, and a Project Office at the National Heart, Lung, and Blood Institute. In addition to etiology, ACCESS will examine the socioeconomic status and clinical course of patients with sarcoidosis. We propose to enroll 720 newly diagnosed cases of sarcoidosis and compare them to 720 age, sex, and race matched controls and follow the first 240 cases for two years.

Leads to the etiology of sarcoidosis have come from diverse sources: in clinical laboratory investigations, alveolitis has been found to precede granulomatous inflammation; in case control studies, familial aggregation has been identified; and in case reports, recurrence of granulomatous inflammation has been observed after lung transplantation. We describe the rationale for the study design based on genetic, environmental, infectious, and immune dysregulation hypotheses and the methods used for selecting controls.

The cause may not prove to be a single, known exposure. Interactions of exposures with genetic predispositions would have important implications for our understanding of immune responses as well as the pathogenesis of sarcoidosis. J CLIN EPIDEMIOL 52;12:1173–1186, 1999. © 1999 Elsevier Science Inc.

**KEY WORDS.** Sarcoidosis, etiology, case-control

## INTRODUCTION

Sarcoidosis is a chronic granulomatous disorder of unknown cause that is characterized by activation of T-lymphocytes and macrophages [1–3]. For many years, sarcoidosis was presumed to be an atypical manifestation of tuberculosis because of the similarity between the inflammatory responses of the two diseases. However, as culture techniques became more widely employed to diagnose tuberculosis and tuberculosis became less common, it became clear that sarcoidosis was not simply a variation of tuberculosis.

When the diagnosis of sarcoidosis became more specific, studies were performed to determine the incidence and prognosis of the disease [4–6]. These studies provided more information on sarcoidosis, but they provided a biased view. Studies of the age, race, and residence of Americans with

sarcoidosis emphasized the predominance of this disease in African-Americans over Caucasians in the United States. These studies also suggested that a rural background was quite common in this disease. A sarcoid belt was defined in the Central-Atlantic states. However, many of these observations were based on a selected population, United States veterans admitted to the Veterans Administration hospitals. These were men who had previously been healthy enough to serve in the military. International epidemiologic studies have focused on the differences among countries in the incidence and manifestations of sarcoidosis [7,8].

The definitive diagnosis of sarcoidosis requires tissue confirmation of granuloma, with no evidence of mycobacterial or fungal infections [9,10], but many epidemiologic studies did not require tissue diagnosis. Instead they relied on chest roentgenograms which are not specific. Studies based on autopsy may not be representative of patients with sarcoidosis, since the disease may totally resolve and the percentage of patients dying of sarcoidosis is less than 5% [11]. Also, the data obtained from autopsy studies are limited since work and social histories are usually not available. Complete and valid case ascertainment as well as accurate medical, occupational, and social histories are important in the search for specific etiologies.

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A Case Control Etiologic Study of Sarcoidosis (ACCESS) is a multicenter study designed to determine the etiology of sarcoidosis. The study organization includes ten Clinical Centers, a Clinical Coordinating Center, specialized Core Laboratories, a Central Specimen Repository, and a Project Office at the National Heart, Lung, and Blood Institute. In addition to etiology, ACCESS will examine the socioeconomic status and clinical course of patients with sarcoidosis. ACCESS proposes to enroll 720 newly diagnosed cases of sarcoidosis and compare them to 720 age, sex, and race matched controls and follow the clinical course of 240 cases for two years. This report describes the rationale for the study design, the methods used for selecting controls, and four major categories of etiologic hypotheses—genetic, environmental, infectious, and immune dysregulation.

## METHODS

### *Case and Control Selection*

A case control study is the most appropriate design to assess possible etiologic factors of a relatively low incidence disease such as sarcoidosis [12]. In ACCESS, incident cases and matched controls will be compared on the prevalence of various exposures prior to the date of diagnosis of sarcoidosis. Information on exposures will be obtained through interview.

Some earlier case control studies had a large proportion of cases that may have had sarcoidosis for years prior to enrollment which increases the likelihood of errors in reporting exposures. ACCESS will include only cases of sarcoidosis with recent tissue confirmation of granuloma and a compatible clinical course. A recently diagnosed case is defined as a patient who had tissue confirmation less than six months prior to enrollment. A multicenter design was selected to ensure broad geographic distribution of cases, and a large enough sample size for adequate power in assessing *a priori* hypotheses.

Tissue samples are considered positive for sarcoidosis if they demonstrate non-caseating granuloma and are read as being compatible with a diagnosis of sarcoidosis, without other possible causes such as tuberculosis or histoplasmosis. All biopsies will be reviewed by designated pathologists at each Clinical Center for quality control purposes. Kveim agent (from one source) may be used to confirm a diagnosis of sarcoidosis in patients with erythema nodosum.

Each Clinical Center will enroll, interview, examine, and collect blood specimens from 72 incident cases with sarcoidosis and 72 matched controls during the recruitment period (1 November 1996 through 30 June 1999). Cases may be recruited from a variety of clinical settings including inpatient, hospital-based outpatient, and non hospital-based outpatient. Each Clinical Center has defined, in advance, the geographic area from which cases are recruited. There is one population-based control group selected for

the study. Controls matched to cases on age (within five years), gender and self-designated race (black, white, other) are recruited through random digit dialing. Random digit dialing was chosen as the method to select controls to approximate a probability sample of the population [13].

Only patients 18 years of age or older are eligible for the study. Neither cases nor controls can have active tuberculosis or be taking anti-tuberculosis therapy. Potential controls with a past history of sarcoidosis, chronic beryllium disease, fungal diseases treated with systemic chemotherapy, granulomatous hepatitis, primary biliary cirrhosis, Bell's palsy, uveitis, Crohn's disease, or erythema nodosum of unknown etiology are not eligible.

All cases and controls complete similar evaluations including collection of demographic information (age, gender, race, residence, marital status, etc.), medical history, environmental, and occupational exposure history, information about first degree relatives, health related quality of life, and medical care usage. The same personnel interview both the cases and the controls. For patients with sarcoidosis, the extent of organ involvement is assessed in a standard fashion. Cases will have a physical examination at the time of the interview. Chest roentgenogram readings, pulmonary function studies, complete blood count, and blood chemistries including serum calcium and liver function studies are recorded for all cases.

### *Statistical Methods*

The goal of 720 cases and 720 controls was determined by the ACCESS investigators so that the proposed study would have sufficient power (80–90%) to identify associations between exposure and case status with odds ratios  $\geq 2.0$  even when the prevalence of the exposure is small. There will be at least 90% power to identify associations between exposures and case status with odds ratios of  $\geq 2.0$  when the proportion of exposed controls is 0.05 and 78% power to detect odds ratios  $\geq 1.8$  with the same frequency of exposed controls. It was decided that any exposure linked to the occurrence of sarcoidosis with an odds ratio of  $\geq 2.0$  would be an important association to detect even though odds ratios  $\geq 3.0$  are frequently required to establish that an association exists [14].

This large number of cases and controls also provides adequate power to investigate associations for subgroups and to identify interactions between genetic markers and environmental exposures. For instance blacks may have a different set of risk factors than whites. There will be at least 88% power to detect odds ratios  $\geq 2.0$  and 82% to detect odds ratios  $\geq 1.8$  in a selected subset representing half of study population (360 cases and 360 controls) if the prevalence of the exposure in the control population is 0.1. The efficiency of an interaction test between a genetic factor and an exposure with one half of the patients in each of two genetic groups is approximately one half the efficiency of

the test for the exposure in all cases and controls [15]. The test for interaction will have 80–90% power to detect changes in the ratio of the exposure-sarcoidosis odds ratios  $\geq 2.0$  when comparing the two genetic groups.

The presentation of the matched case-control data will follow the methods outlined by Breslow and Day [16]. Categorical data will be presented using cross tabulations of the number of cases exposed versus the number of controls exposed. The methods for estimating the odds ratios using matched pair contingency tables McNemar's test will be the basis of inferences on the measure of association. Ninety-five percent confidence limits will be calculated using the binomial approximation. For continuous variables the means of controls versus the means of cases will be compared using paired *t*-tests. Point estimates of the differences in case and control means will be calculated as well as 95% confidence intervals of the differences. Analysis using one or more exposure variables will be performed using conditional logistic regression methods. This method will also be used to estimate the odds ratios and 95% confidence limits.

It will not be feasible to estimate odds ratios for age, gender, race or geographic area (controls are chosen from the same telephone area code or within the same postal zip code as cases), since these are matching variables, however, interaction terms between age, gender, race and geographic area and other exposure variables will be estimated and statistical tests will be performed to determine if these odds ratio estimates are homogenous across the different matching strata. An analysis to determine whether an exposure is associated with a genetic predisposition to sarcoidosis will be performed using interaction terms in conditional logistic regression models.

A systematic approach to the analysis of all of the variables collected in ACCESS has been developed by the ACCESS investigators. Each exposure as well as combinations of exposures will be analyzed. Data Analysis Working Groups have discussed how infectious agents, environmental exposures, and genetic factors might interact to cause sarcoidosis and have identified specific hypotheses to be tested. This was done without investigator review of the preliminary data. These hypotheses will be tested at the 0.05 alpha level. It is possible that certain associations may become apparent after the data are reviewed by the investigators. These results will be distinguished from those that were developed before the data were reviewed. In recognition of the large number of hypotheses being considered in this study, the investigators have specified that associations identified after examination of the data will not be considered statistically significant unless the *P*-value for the odds ratio is less than 0.01. Even with this restriction, some spurious associations may be identified. The strength of the association and its clinical plausibility in the light of the other associations found in ACCESS will be used to determine whether the association is spurious or not. It is expected that a reasonable subset of exposure variables will be

significantly associated with sarcoidosis and that will lead to further investigation into the etiology of sarcoidosis.

A clinical course study will be conducted on the first 252 cases (goal was 240) enrolled in ACCESS; each Clinical Center is expected to contribute at least 20 cases. The total number of cases is sufficient to detect moderate changes in means (three quarters of standard deviation) among defined subgroups and moderate changes (odds ratio of three) for categorical outcomes. The analytic techniques for the clinical course study will include analysis of variance, regression, life table analysis and standard analyses for categorical variables.

These analysis techniques will be used to analyze the following aspects of the possible etiology of sarcoidosis: genetics, environmental exposure, infection, and immunologic response as a cause of sarcoidosis.

## DISCUSSION

### *Genetic Aspects of Sarcoidosis*

Genetic susceptibility to sarcoidosis is suggested by ethnic variation, a greater concordance of disease in monozygotic than dizygotic twins and by reports of familial aggregation [2, 17, 18]. Over four hundred kindreds with more than one member affected with sarcoidosis have been reported [19]. Moreover, one study found that 19% of African-American patients and 6% of Caucasians report a positive family history of sarcoidosis [20].

In the context of a case control study design, three aspects of the genetic epidemiology of sarcoidosis can be addressed: familial risk can be quantitated; genetic mechanisms can be tested as the cause of familial aggregation; and candidate susceptibility genes can be assessed [21]. Familial risk can be quantitated by counting the relatives of cases and controls, confirming whether the relatives have sarcoidosis and calculating an odds ratio.

Segregation analysis can be performed in a case control study to test whether genetic mechanisms explain familial aggregation [22]. Segregation analysis tests whether the observed mixture of phenotypes among offspring is compatible with simple Mendelian inheritance. Over the years, segregation analysis has been broadened to fit general models of inheritance in pedigrees, but the ultimate goal is the same: to test for compatibility with Mendelian expectations by estimating parameters of a given inheritance model. Statistical compatibility between the disease pattern among family members and Mendelian inheritance does not prove a susceptibility gene exists. Proof must rely on more definitive biologic data.

Candidate susceptibility genes can be tested using association studies which seek evidence for differences in the frequency of occurrence of specific alleles comparing patients to unrelated affected and unaffected individuals from a population in a case control study [21,22]. Relationships between alleles and clinical status can be further evaluated

within categories of specific environmental exposures and according to extent of disease among cases.

A major limitation of association studies is that differences in the frequency of alleles may occur if cases and controls are drawn from genetically different populations even though no disease genes exist. Any allele frequency differences found in ACCESS should be verified in other populations. Differences in frequency of alleles found between distinctly different populations but not between cases and controls within one population suggest gene admixture instead of causation.

HLA genes are the most notable candidate susceptibility genes in sarcoidosis. In previous studies, HLA Class I and II genes have been associated with different categories of sarcoidosis characterized by specific presenting features, i.e., age, extent of disease, ethnic groups, and by prognosis [23–25]. Importantly, HLA gene products play a direct role in the immune response. CD4+ and CD8+ T lymphocytes respond to antigenic peptides that are bound to HLA Class I and II proteins. [26]. Thus the ability to develop a CD4+ or CD8+ T cell immune response to specific antigens is dependent upon specific immunogenic peptide sequences binding to HLA Class I or II molecules prior to being presented to T lymphocytes.

Sarcoidosis has been observed to be associated with an increased number of CD4+ T lymphocytes at the site of disease activity [1]. CD4+ T lymphocytes respond to antigenic peptides that are bound to HLA Class II molecules. Thus the ability of specific HLA Class II molecules to bind antigenic peptides, may determine antigens (i.e., environmental agents) associated with sarcoidosis. Therefore, this study will investigate HLA Class II associations with sarcoidosis and correlate any associations with environmental history. Since the major heterogeneity of HLA Class II molecules resides in the  $\beta$  chains, the molecular sequence of HLA Class II DP, DQ and DR beta chains will be determined by polymerase chain reaction (PCR) amplification of genomic DNA and hybridization with sequence specific oligonucleotide probes (SSOP). If any molecular sequence is not definitively identified with SSOP, the allele will be sequenced [27]. Case and control frequencies will be compared not only for specific HLA Class II alleles, but also for specific hypervariable amino acids that are crucial for peptide binding. This study will evaluate associations between HLA Class II alleles, hypervariable amino acid positions, and detailed, environmental histories.

### ***Environmental and Occupational Exposures***

Several lines of evidence support the hypothesis that the cause or causes of sarcoidosis are environmental or occupational exposures. The lungs and skin—two common target organs for sarcoidosis—are regularly in contact with environmental agents. Several occupational and environmental exposures include many antigens that can induce sensitiza-

tion—a cell-mediated immune response responsible for the development of granulomas [28,29]. Several occupational and environmental agents are known to cause granulomatous disease resembling sarcoidosis. For example, chronic beryllium disease, due to inhalation and sensitization by beryllium, and other metal-induced granulomatous lung diseases due to aluminum, titanium, and zirconium, mimic sarcoidosis [30–33]. Similarly, hypersensitivity pneumonitis due to inhalation of organic or inorganic antigens is easily misdiagnosed as intrathoracic sarcoidosis [34]. A variety of antigens—including mycobacterial extract, avian proteins, fungal spores, schistosome eggs, carrageenan, and many infectious agents—induce granulomatous responses in experimental animals [28].

Despite individual study flaws, published epidemiologic investigations of sarcoidosis suggest an environmental clustering of disease. A marked predilection for sarcoidosis to develop in early adulthood, and disease onset is infrequently observed in children or the elderly, suggesting that either infectious or non-infectious causative exposures occur in working-age individuals as they enter the workforce [5,35,36]. The tendency for the disease to become clinically apparent in cold months—winter and early spring—may suggest that exposure occurs when people spend time in closed, confined spaces at work or at home during these months [2, 37–39]. Geographic clustering of disease in the southern United States and in other parts of the world has promoted much speculation concerning weather, soil, and foliage [6,40–44]. Past studies have noted high prevalence of sarcoidosis where there is lumbering activity and exposure to farm animals and pets [41,43–45]. Rural residence, birthplace, or time spent in rural regions have been associated with sarcoidosis [5,6,41,42,46].

Several studies suggest that exposures to granuloma-inducing antigens at work may cause sarcoidosis [4,40,45, 47]. Cummings et al. observed clustering in communities with lumbering or wood milling as the principal local industry [44]. Others have found clustering among mechanics, postal workers [45], and firefighters [48]. Particularly interesting have been at least two studies which suggest that health care workers are at increased risk [49,50]. In a well documented clustering that has occurred in the relatively isolated population on the Isle of Man, individuals with sarcoidosis were more likely to have had prior contact with an individual who had sarcoidosis compared to controls (39.6% versus 1.1%). These contacts included neighbors, unrelated cohabitants, and co-workers [40,49,51]. Others have noted disease clustering in time and place, often with coworkers [48, 52]. Similarly, there has been a cluster of cases among sisters and unrelated social contacts, including one sister's employer [53]. Together these data strongly suggest either person-to-person transmission or shared exposure to an environmental agent. Although familiar clustering may be explained on the basis of shared genes, shared common environmental exposures must be considered as well.

Detailed occupational and environmental exposures ever and in the three years prior to tissue diagnosis are obtained for each case and control. Exposure to various agents will be assessed not only by work type, but also a detailed list of possible agents will be investigated. Since all patients will have been diagnosed within six months of the questionnaire, we hope to reduce recall bias for exposure to agents with structured inquiries (example form pages in Appendix 2).

### *Infectious Agents in Sarcoidosis*

Several observations suggest sarcoidosis may be caused by an infectious agent. Clusters of cases in unrelated patients suggest an environmental agent, such as an infectious organism. Granuloma formation can be induced in mice by injected human sarcoidosis tissue. This can be prevented by pretreating with autoclaving, storage at  $-20^{\circ}\text{C}$  for one week, or irradiation [54]. In addition there are case reports of possible transmission of sarcoidosis via allogenic bone marrow transplantation [55] and by cardiac transplantation [56]. Also, sarcoidosis has occurred in the lungs that have been transplanted into patients with the disease [57,58].

There are many known infectious agents that can cause granulomas that resemble those of sarcoidosis, including mycobacteria, herpes viruses, histoplasmosis, treponematosi, sporotrichosis, coccidiomycosis, schistosomiasis, listeria, the agent of Whipple's disease and *Rhodococcus* sp [59–67]. Because sarcoidosis has a world-wide distribution, it is necessary to postulate that the putative agent that causes sarcoidosis is also widely distributed, if there is indeed a single infectious disease as a cause. It is also possible that sarcoidosis may be the result of several different infections.

Although granulomas may occur in direct response to the presence of an intact, infecting organism, they may also occur as a response to an infectious agent product such as the cell wall. This appears to be the case in some instances of herpes zoster related cutaneous granulomas [62,68] in which viral nucleic acid is not found in the granuloma. Alternatively, the granulomas may be a response to a host antigen that has been altered by the infection. Thus, it is conceivable that the sarcoidosis granuloma, if related to an infection, does not contain infectious organism DNA or viable organisms.

In searching for an infectious etiology for sarcoidosis there have been attempts to identify unusual objects (either foreign body or infectious agents) using light and electron microscopy to study granulomas. Structures resembling leptospiral or large mycobacteriophage microorganisms have been identified in bronchoalveolar lavage (BAL) fluid from patients with sarcoidosis [69] and from the center of the sarcoidosis granuloma [70]. Similar structures have also been identified in the newly recognized syndrome of familial granulomatous disease [71]. While these unusual bodies may be infectious agents, one study has identified them as likely damaged platelets [69], raising the intriguing possibil-

ity of platelet alterations associated with an infectious agent.

Clinically, the granulomas of sarcoidosis are diffusely spread throughout the body, implying strongly that an etiologic agent is distributed through the blood stream, at least at some point in the disease. Indeed many infectious agents that appear organ specific, can now be detected in blood specimens using molecular techniques [72–75]—this list includes many of the mycobacterial species, as well as viruses and fungi. Therefore, blood samples will be examined for any putative infectious agent(s) in sarcoidosis. Infectious organism DNA may be present in the blood, most likely in acute cases in the early stages of disease.

We will examine blood specimens for the presence of DNA coding for micro-organism related ribosomal DNA (r-DNA). Micro-organisms have been classified, into three main domains—Bacteria, Archaea and, Eucarya [76]. The domain Archaea has not been found to have any human pathogens at this time, [77] whereas human pathogens do come from the Bacteria and Eucarya domains. The approach of examining for 16S ribosomal DNA has recently been applied to examine for the putative agent of Kawasaki's disease [78] and to demonstrate the causative organisms in Whipple's disease [79].

Another technique for investigating possible etiologic agent(s) in sarcoidosis involves the use of differential display polymerase chain reaction (DD-PCR) technology [80, 81]. The unique advantage of this approach is that DD-PCR could detect any type of infectious agent that contains DNA, including agents that lack r-DNA or that do not have known sequences to allow designing of specific PCR primers. Thus, this approach could detect viral as well as bacterial and eukaryotic agents. DD-PCR involves the use of arbitrary oligonucleotide primers to amplify most mRNAs found in the cell using reverse transcription PCR, with modifications to cDNA coding regions. Once such genes are identified, they can be eluted from the gel, reamplified, cloned, and characterized.

One possible clue to the etiology of sarcoidosis is the Kveim agent. Following studies by Williams [82] and Nickerson [83], Kveim found that 12 of 13 patients with sarcoidosis exhibited slowly maturing epithelioid granulomas at the intracutaneous injection sites of sarcoidal lymph node homogenates [84]. Larger series of patients [85–87] confirmed Kveim's findings as sensitive for sarcoidosis. In a report of a study of 750 patients with presumed sarcoidosis [88], patients with other granulomatous disease [89] and an international study including 3,244 subjects in 37 countries throughout the world [90], Siltzbach established the Kveim test as a sensitive and specific diagnostic procedure. Acceptance of the Kveim test has been impeded by reports of false positive tests. Almost all reports of false-positive Kveim reactions in non-sarcoidosis adenopathy [91], Crohn's disease, ulcerative colitis [92], and in collagen vascular disorders [93] have been traced to a faulty batch of non-validated

Kveim suspensions manufactured in 1968. In ACCESS the lots of Kveim suspension that will be used in the tissue diagnosis of some patients have high specificity [94–96].

Kveim test suspension induces immunogenic granulomas with distinct characteristics consistent with a T helper type 1 (Th1) host response. The inciting agent in Kveim must be particulate, because it persists in tissues for a relatively protracted period of time [97–99]. Kveim test material is a suspension, not a solution. After high speed, ultracentrifugation, the supernate is inactive [100]. Activity resides in a membrane bound protein. Kveim suspensions retain diagnostic efficacy when exposed to heat, cold, acid, alkali and other chemicals [101,102]. Comparison of the positive Kveim granulomas with the dermal lesions occurring in sarcoidosis patients reveals the same histologic pattern, the same array of CD8+ and CD4+ T cells and macrophages, maturing in an orderly sequence over a four-week period of incubation, with similar elaboration of lysozyme, angiotensin converting enzyme and endopeptidase [95,103–106]. Thus, Kveim suspensions contain an active granulomagenic agent. The suspensions elicit a granulomatous response that is indistinguishable from disease induced granulomas. Our study includes tests of the *in vitro* response to Kveim agent which may identify the active agent(s) of Kveim test material and its role in initiating the granulomatous response. Also, evidence for any putative infectious agent discovered by molecular biology techniques is sought in the Kveim test material.

### **Immunology of Sarcoidosis**

Defining the etiology of sarcoidosis will require not only identifying the initiating agent(s) of the disease, but demonstrating that candidate agents induce immunologic responses characteristic of the disease. For many years, sarcoidosis was conceptualized as a disease of down-regulated immunity with peripheral lymphopenia and cutaneous anergy [107]. The application of bronchoalveolar lavage led to the observation that pulmonary sarcoidosis is characterized by increased numbers of CD4+ T cells in the lung of patients with active pulmonary sarcoidosis [1,108] leading to a fundamental reappraisal of sarcoidosis as a disease characterized by enhanced immune activity at sites of granulomatous inflammation.

Recent studies support the concept that sarcoidosis is a disease of immune dysregulation, likely the result of persistent antigenic stimulation at sites of inflammation. Sarcoid lung T cells are characterized by a down-regulation of CD3 T-cell receptor surface density. The reduced receptor density is consistent with prior activation of these T cells through their T-cell receptors [109]. Some patients have biased expression of specific V $\beta$ , V $\alpha$ , V $\gamma$ , or V $\delta$  T-cell receptor genes from lung or blood T cells [110–116]. Furthermore, biased T-cell subsets were found to be decreased in number in response to treatment or with spontaneous re-

mission of the disease, consistent with the hypothesis that these cells play a critical role in the pathogenesis of sarcoidosis [111,117] and with the hypothesis that the immune response of sarcoidosis is oligoclonal [115,116,118].

Studies in the 1980s demonstrated that active pulmonary sarcoidosis is characterized by enhanced expression of cytokines such as Interleukin 2 (IL2), Interferon  $\gamma$  (IFN $\gamma$ ), and tumor necrosis factor alpha (TNF(x) in the lung [119]. More recently, active pulmonary sarcoidosis has been shown to be characterized by a striking polarization of cytokine expression towards a type I (T helper 1) cytokine profile with enhanced expression of Interleukin 12 (IL12) as well as IFN $\gamma$  and IL2, and little or no detectable expression of the type 2 cytokines, Interleukin 4 (IL4) and Interleukin 5 (IL5) [120]. Interestingly, IL12 production is strongly stimulated by bacteria, bacterial products, mycobacteria, intracellular parasites, and some viruses. The observation that sarcoid lung macrophages (but not control lung macrophages) produce IL12 suggests that a key stimulus for IL12 production in sarcoidosis resides in the lung environment [120].

### **Study of the Clinical Course of Sarcoidosis**

Although the primary objective of this epidemiologic study is to explore the etiology of sarcoidosis, it provides a unique opportunity to clarify the longitudinal course of the disease. This prospective study can provide some insight into the disease course over the first two to three years.

Other descriptive studies of the outcome of sarcoidosis have been reported, and most of these have attempted to evaluate corticosteroid treatment. In a report from one center which used a standardized approach to initiating and terminating corticosteroids, the investigators reported that treated patients could usually have therapy withdrawn after one year and the rate of relapse was less than 15%. This study consisted of Caucasians only. Others have reported a much higher incidence of relapse and need for chronic therapy when treating patients seen at urban centers. Most of these patients are African-American. ACCESS will accumulate data in a standardized manner to estimate frequencies of disease resolution and progression.

The clinical data from the time of enrollment in the study and follow-up at 24 months include physical examination, laboratory data, spirometry, chest radiography, medical history, case follow-up, and diagnostic specimen report. Availability of, use of, and adherence with medical care, and use, duration, frequency, dose and response to sarcoidosis medications are recorded on study forms. Follow-up medical history including other health problems, pregnancy, hospitalizations, work status, smoking history and income can be compared with the original baseline data. Organ involvement is determined as definite or probable according to previously established criteria for each organ.

One advantage of ACCESS is that the study will include only patients who have initial, tissue diagnoses within six months of enrollment. However, the limited period of follow up and the lack of prescribed management regimens, will produce a great variety of treatment experiences. Patients may be treated or untreated for varying periods. Indeterminate outcomes may be observed with periods of less than a year on or off treatment. Nevertheless, it should be possible to categorize the clinical state at follow-up according to whether the sarcoidosis has resolved, remained stable, or worsened, and whether pharmaceutical treatments were prescribed. These categories can be cross-classified according to pulmonary and extra-pulmonary disease activity.. Patterns of organ involvement can be reviewed in relation to outcomes, with the documentation of organ systems involved initially and on follow-up 24 months later.

### Socioeconomic and Psychosocial Aspects

In the United States, sarcoidosis has been reported to be predominantly a disease of young adult black, [121, 122], particularly women, who also are more likely to have severe disease [123,124]. In the United States, blacks are more likely than whites to have low socioeconomic status (SES), defined by low levels of income and education, and are more likely to have financial barriers to health care than persons of higher SES. Regardless of race, persons of low SES are less likely to have health insurance, to receive medical care, and to be adherent consumers of medical services and therapy [125–127].

ACCESS will investigate the relationship of sarcoidosis status and clinical severity at presentation and at two years, according to race, SES, insurance coverage, use of health care services, and perceived access to care. Sarcoidosis patients enrolled during the first year of the study will be re-interviewed two years later using the standardized questions from the initial enrollment evaluation.

The relationship between sarcoidosis and psychosocial characteristics and quality of life is unexplored. It is likely that sarcoidosis, a potentially progressive disease with uncertain outcomes that may cause temporary and permanent impairment, affects the quality of life of both acute and chronically ill patients [128–130]. Both the symptoms of the disease as well as the consequences of steroid therapy could affect patient functional status, mood, self esteem, and interpersonal relations [131]. The ACCESS questionnaires content includes measures of: generic Quality of Life, using the Medical Outcomes Study (MOS) Short Form 36 (SF-36); depression, using the Center for Epidemiologic Studies Depression Scale (CES-D Scale); social support and isolation using the Medical Outcomes Study social support scale (MOS-SSS scale); and an optimism measure called the Life Orientation Test (LOT). The questionnaire will be given to all participants at the time of enrollment into the study. Sarcoidosis patients enrolled during the first year of

recruitment will be given the questionnaire again two years later. Questionnaire data will be analyzed in association with demographic, social, medical, and environmental characteristics as well as with physical and laboratory characteristics to determine the psychosocial and health related quality of life features associated with sarcoidosis.

### Status of Study

Cases and controls are being recruited on schedule according to the ACCESS protocol described above. Controls are matched to cases for age (no more than five years younger or older), gender, race/ethnicity, and geographical location as required by study design. As of December 3, 1998, the ACCESS investigators had collected data on 536 (74%) pairs of cases with sarcoidosis and matched controls of the planned 720 cases and controls. The 536 pairs included 356 (66%) female case and control pairs and 180 (34%) male pairs. Table 1 summarizes the cases and controls by gender and ethnic origin. Three controls enrolled in the study identified themselves as “black” on initial telephone contact but as “other” at the time of the data collection interview. More cases (34%) and controls (33%) were in the 30–39 year old age group than any other age group. Almost as many were in the 40–49 year old age group (31% cases and 32% controls). The age range 20–29 years old accounted for 11% of cases and 12% of controls (Figure 1). This distribution is similar to that reported for other studies of sarcoidosis. Our goal was to have as wide a range of initial presentations as possible. Forty-four (8%) of the cases had erythema nodosum. Extra thoracic disease was evident among 321 (60%) of cases. Pulmonary parenchymal involvement was reported on the basis of chest roentgenogram findings for 282 (53%).

### CONCLUSION

Sarcoidosis has been well characterized pathologically, and the most interesting research results in recent years have

**TABLE 1. Distribution of cases and controls by gender and ethnic origin**

	Ethnic origin			Total	%
	White	Black	Other		
Sarcoidosis cases					
Female	172	176	8	356	66.4
Male	122	54	4	180	33.6
Total	294	230	12	536	100
Controls					
Female	172	173	11	356	66.4
Male	122	54	4	180	33.6
Total	294	227 <sup>a</sup>	15	536	100

<sup>a</sup>Three controls described themselves as “black” at the time of random digit dialing telephone recruitment, but as “other” at the time of data collection interview.

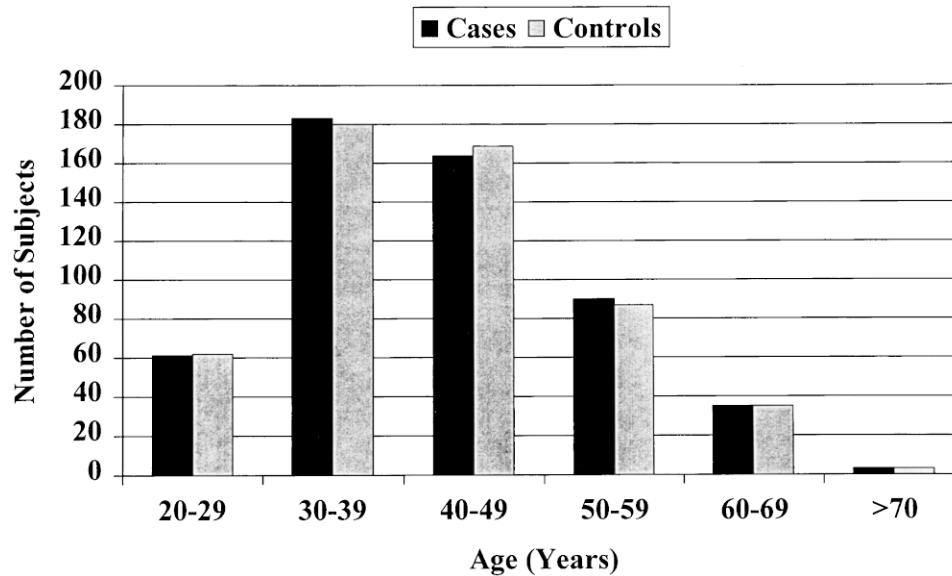


FIGURE 1. The number of sarcoidosis cases (solid bars) and their controls (shaded bars) for each of the age groups is shown.

addressed the cellular mechanisms of disease activity. Conclusions about etiologic factors which would be of use in treatment or primary prevention of sarcoidosis have been difficult to reach. Previous studies of the etiology and clinical course of sarcoidosis in the United States, especially in black patients in the United States, have been limited to relatively small numbers of patients.

ACCESS investigators will identify 720 patients with sarcoidosis, establish disease stage using standardized criteria, select an appropriate control for each case, and use case control methods to search for exposures or genetic predispositions which could cause sarcoidosis. Recent advances in laboratory methods (e.g., polymerase chain reaction technology) will be used in ACCESS to address the etiology of sarcoidosis. The etiology may not prove to be a single, known exposure. An interaction of exposures with genetic predisposition would have important implications for our understanding of immune responses as well as the pathogenesis of sarcoidosis. The clinical course of sarcoidosis will be evaluated in a diverse patient population. Psychosocial and quality of life contributions to and effects of sarcoidosis will also be studied.

ACCESS study design is sufficiently robust to study several etiologic hypotheses at the same time, but this study may only generate hypotheses for definitive testing.

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## APPENDIX 1.

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\*Principal Investigator(s) for ACCESS Research Group.

Appendix 2  
 Example of ACCESS forms.

**OCCUPATIONAL AND RECREATIONAL QUESTIONNAIRE**

ID No.				-				
Form Type	O	R	0	1				

1. **SUBJECT'S INITIALS:** \_\_\_\_\_

2. **DATE OF INTERVIEW:** \_\_\_\_\_ - \_\_\_\_\_ - \_\_\_\_\_  
 Month Day Year

A. **REFERENCE DATE:** \_\_\_\_\_ - \_\_\_\_\_ - \_\_\_\_\_  
 (COMPLETE PRIOR TO INTERVIEW) Month Day Year

B. **REFERENCE PERIOD:** (1) \_\_\_\_\_ - \_\_\_\_\_ - \_\_\_\_\_  
 (COMPLETE PRIOR TO INTERVIEW) Month Day Year  
 to

(2) \_\_\_\_\_ - \_\_\_\_\_ - \_\_\_\_\_  
 Month Day Year

3. **WAS PARTICIPANT UNEMPLOYED WITH NO WORK EXPERIENCE OR DISABLED WITHOUT PREVIOUS WORK EXPERIENCE? (SEE FORM 11, QUESTION 3.)** Yes No  
 ( 1 ) ( 2 )

**IF YES, GO TO QUESTION 80.  
 IF NO, CONTINUE WITH SCRIPT.**

**ACTIVITIES ON THE JOB**

Now I would like to ask you some questions about specific job related activities. I will read slowly from a long list and ask you whether you have ever had a job — even if the job lasted less than six months and was before [reference period] — that involved any of the following activities. Please tell me if you have worked in any of them and if you worked on the job for more or less than one year. I will also be asking if the activity occurred during the reference period. As you think about this, please feel free to use the anchor dates we discussed to help you determine if the activity was near one of the special dates.

**USE THE ANCHOR DATES TO ESTABLISH IF THE EVENT HAPPENED IN THE REFERENCE PERIOD. IF PARTICIPANT ANSWERS “NEVER”, GO TO THE NEXT ACTIVITY.**

**ASK EACH ACTIVITY IN TURN AND PAUSE BRIEFLY. FOR EACH ACTIVITY, IF PATIENT DOESN'T ANSWER, CHECK NEVER AND GO TO THE NEXT ACTIVITY.**

		<b>A</b>			<b>B</b>	
		<b>Employment</b>			<b>More Than</b>	
		<b>Never</b>	<b>Ended Job Before Reference Period</b>	<b>Current Employment or Ended in the Reference Period</b>	<b>One Year</b>	<b>No</b>
		( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
4.	Aircraft manufacturing	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
5.	U.S. Army	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
6.	U.S. Navy	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
7.	U.S. Air Force	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
8.	U.S. Marines	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
9.	Other branch of armed forces	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
10.	Nuclear worker	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
11.	Animal laboratory worker	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
12.	Assembling or fabricating	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
13.	Auto or truck repair	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
14.	Automotive manufacturing	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
15.	Bank teller	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
16.	Raising birds	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
17.	Carpentry or woodworking	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
18.	Cashier	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
19.	Child care worker (i.e., children under the age of 18)	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
20.	Cleaning private household, domestic worker (Do not include cleaning your own house.)	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
21.	Clerical or office work	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )
22.	Construction	( 1 )	( 2 )	( 3 )	( 1 )	( 2 )