Present Status of Laboratory Diagnosis of Sarcoidosis

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ABSTRACT

The clinical pathologist is involved in the diagnosis of sarcoidosis in a variety of ways. Many of the traditional hemotologic and biochemical studies performed in study of patients suspected of having sarcoidosis are, however, of little diagnostic assistance. Hypercalcemia is now an infrequent manifestation of sarcoidosis and routine study of calcium metabolism in asymptomatic patients with early sarcoidosis is rarely rewarding. Serum globulin fractions are increased particularly in black patients with sarcoidosis but the changes are non-specific and like serum immunoglobulin level measurements are likewise of little diagnostic help. The Kveim test has not found wide use because of the lack of standardized and stable testing materials. Biopsy methods remain the best method of demonstrating the typical epithelioid granulomas characteristic of sarcoidosis. Mediastinoscopy and lung biopsy have the highest yield and should be utilized when readily accessible lesions such as skin, palpable lymph nodes and subcutaneous nodules are not available. The development of in vitro techniques for measurement of delayed hypersensitivity should permit an in vitro diagnostic test for sarcoidosis if a specific antigen can be obtained from splenic or lymph node suspensions. An important responsibility of the laboratory is the detection of mycobacterial or fungal diseases which may simulate or superinfect sarcoidosis. Mycobacterial infection has been encountered rarely in recent years, but aspergillosis and cryptococcus have proven to be common problems in patients with sarcoidosis. Serologic tests should be widely available for the prompt diagnosis of these important infections.

Sarcoidosis is a multi-system disorder of as yet unknown cause, commonly involving mediastinal and peripheral lymph nodes, lungs, liver, eyes and skin, with less frequent involvement of many other organs and tissues. The characteristic histopathologic feature is multiple epithelioid cell granulomas without caseation. The epithelioid cell granulomas may resolve completely or proceed to fibrosis. Depression of delayed-type hypersensitivity and a Kveim reaction are often demonstrable. Local sarcoid reactions, limited to a single organ as well as granulomas due to demonstrable
infection and hypersensitivity, must be excluded by clinical, microbiologic and immunologic study. In questionable cases, histologic changes should be demonstrated in several sites.

The clinical pathologist is involved in the diagnosis of sarcoidosis in a variety of ways. Many of the studies he is called upon to carry out in cases of suspected sarcoidosis are, however, of little diagnostic assistance and are ordered on the basis of habit rather than demonstrated worth.

**Hematology**

White blood cell counts below 5,000 occur in one-third of the cases. When splenomegaly is present, purpura and thrombocytopenia are frequently encountered. Hemolytic anemia is a rare manifestation of sarcoidal splenomegaly, and its occurrence in sarcoidosis may be fortuitous. Eosinophilia of a mild degree (4 to 6 percent) is another common feature of sarcoidosis, but a marked eosinophilia is rarely encountered. Anemia is uncommon, but it should be noted that hemoglobinopathies are found twice as often in sarcoidosis cases as in other black patients.

Studies of blood groups indicate an increased frequency of group A in patients with sarcoidosis. Bone marrow aspiration biopsy is infrequently of diagnostic assistance, granulomas being demonstrable in less than 15 percent of patients.

**Biochemistry**

**Disordered Calcium Metabolism**

Hypercalcemia and hypercalcuria were reported in the past to be common manifestations of sarcoidosis. Serum calcium elevation was noted in as high as 40 percent of patients, with hypercalcuria in an even higher proportion.

Recent studies, however, indicate that hypercalcemia is much less frequently encountered at the present time. Persistent elevation has been observed in only 1 or 2 percent in recent prospective studies with no relationship to season or exposure to sun demonstrable. The reported high frequency of hypercalcuria has been based on studies of ambulatory patients without adequate dietary control. A recent metabolic study of 18 patients with subacute or chronic active sarcoidosis showed no significant increase in calcium urinary excretion on a normal calcium intake, on a low calcium intake or on a high calcium intake. It appears that disordered calcium metabolism is not a subclinical feature of most patients with sarcoidosis. Rather, it is a reflection of severe and extensive sarcoidosis with widespread osseous and renal granulomatosis. It is probable that the decline in prevalence of hypercalcemia in recent years is due to the fact that patients with severe and widespread sarcoidosis as a rule receive prednisone therapy because of respiratory or other symptoms. It is patients with involvement of this marked severity who would have developed hypercalcemia prior to the general use of corticosteroid therapy.

In view of the infrequency of hypercalcemia in sarcoidosis at the present time, serum calcium and urinary calcium measurements are rarely of diagnostic assistance. These tests should, of course, be made in patients with sarcoidosis who have symptoms that could be related to hypercalcemia.

**Serum Proteins**

It has long been known that American patients with sarcoidosis frequently have increased globulin and reversal of the A/G ratio. Recent studies indicate that hypergammaglobulinemia is a significant feature chiefly in black patients with chronic sarcoidosis. Serum immunoglobulin levels have also been studied employing commercially available plates. IgA and IgM levels were significantly elevated in black patients with chronic and advanced disease, but the levels were not elevated in white patients. Levels of IgM were frequently
Present Status of Laboratory Diagnosis of Sarcoidosis

Elevated with little relationship to race or character of disease. IgD levels are normal in sarcoidosis. Studies of the serum proteins have thus proven to be of no diagnostic value although it should be kept in mind that sarcoidosis is one of the causes of hyperproteinemia.

Uric acid levels are also occasionally elevated in sarcoidosis but gout is infrequent.

Endocrine Function

Attention has been called to an increased frequency in sarcoidosis of both hyperthyroidism and Hashimoto’s thyroiditis. Granulomatous involvement of the thyroid gland is infrequent and the reason for the increased frequency of thyroid disorders is not clear. It was reported a few years ago that abnormal responses to metyrapone were a characteristic and diagnostic feature in sarcoidosis, but subsequent studies failed to support this. It is likely that the occasionally abnormal response to metyrapone is the result of hepatic involvement and altered metyrapone metabolism.

Renal Function

Impaired renal function is an uncommon manifestation of sarcoidosis at the present time. Nephrocalcinosis secondary to hypercalcemia was, in the past, a frequent finding at autopsy, but the smaller number of cases of hypercalcemia encountered in recent years has resulted in less renal damage in cases of sarcoidosis.

Liver Function Abnormalities

Epithelioid granulomas are demonstrable on aspiration needle biopsy of the liver in approximately 70 percent of cases of sarcoidosis, but palpable enlargement and disordered liver function are considerably less frequent. The liver function tests most often abnormal are alkaline phosphatase and SGOT. Serum albumin is rarely depressed and bilirubin elevation occurs in less than 1 percent of cases. Clinical disease due to hepatic sarcoidosis is quite infrequent, but in rare instances sarcoidosis may be responsible for hepatic failure and portal hypertension.

A problem of special interest is that of hepatic granulomatosis in patients without thoracic or other evidence of sarcoidosis. These patients present, as a rule, with fever or fatigue and aspiration biopsy of liver is often performed because of these symptoms. When epithelioid granulomas are found, it may be difficult to determine whether the disease is primarily hepatic or whether the hepatic findings are part of a systemic granulomatosis. It has been found that the Kveim test is usually negative in these circumstances and cannot be relied upon for exclusion of sarcoidosis.

The best means of determining whether the granulomatosis is disseminated is by mediastinoscopy which demonstrates granulomatous involvement of mediastinal nodes in 95 to 100 percent of patients with sarcoidosis. It is our recommendation that whenever unexplained hepatic granulomatosis is under study, mediastinoscopy should be performed as the most definitive means of establishing the presence of systemic granulomatous disease. It is of course appropriate to use skin tests, serologic tests and cultural methods to distinguish sarcoidosis from infectious granulomatoses. An alternative approach is laparotomy, which may demonstrate granulomatous involvement of abdominal lymph nodes or spleen.

The diagnosis of hepatic granulomatosis has taken on special urgency in the past few years since laparotomy has been employed for the staging of Hodgkin’s disease. In a careful study of this problem, has demonstrated that the hepatic granulomas in some instances represent a local sarcoid reaction, but that often there is evidence of systemic granulomatosis warranting a diagnosis of sarcoidosis. The association of these two diseases may reflect
increased susceptibility to Hodgkin’s disease among patients with sarcoidosis.

**IMMUNOLOGIC FEATURES**

Delayed hypersensitivity is one of the cardinal features of sarcoidosis. The tuberculin reaction depression observed in patients who develop sarcoidosis persists even after recovery. In recent years, *in vitro* techniques have been developed for measuring lymphocyte sensitization. Measurement of lymphocyte transformation and migration inhibition factor (M.I.F.) have been used to demonstrate the sensitization of cultured lymphocytes to PPD and Kveim antigens. Most observers have found that the results of *in vitro* tests parallel those of *in vivo* tests, but Caspary and Field have recently reported that the lymphocytes of anergic sarcoidosis patients remain sensitive to tuberculin when measured with M.I.F. test. These authors postulate that circulating blocking antibodies are responsible for the depression of delayed hypersensitivity in this disease.

**CIRCULATING ANTIBODIES**

Patients with sarcoidosis have recently been shown to have increased titres of antibodies to E. B. virus and to a lesser degree to measles, herpes and influenza viruses. It has not been possible to demonstrate rising titres of these antibodies and although some investigators attribute an etiologic role to viral infection in sarcoidosis, an alternate explanation is that the increased titres merely reflect the adenopathy and splenomegaly of sarcoidosis.

**THE KVEIM TEST**

For many years the Kveim reaction, a torpid granulomatous response to the intracutaneous injection of sarcoideal lymph node or splenic suspensions, was regarded as a specific test for sarcoidosis. Unfortunately, commercial production of a standardized test material never proved feasible and import or interstate distribution of Kveim test materials has not been approved by the FDA. As a consequence, Kveim antigens have been available to few American physicians. Recent studies with the two generally available antigens, one produced by the Commonwealth Serum Laboratory of Australia and the other produced by the National Laboratories in Colingdale, England have shown unequivocal reactions in patients with other diseases. Although the Kveim test is negative in normal persons and patients with degenerative pulmonary disease, chronic tuberculosis and neoplasms, there are now reports of frequent reactions in chronic lymphatic leukemia, disseminated lupus, florid tuberculosis, regional ileitis and brucellosis. This lack of diagnostic specificity has been observed not only with the Kveim test *in vivo* but also *in vitro* studies of cultured lymphocytes. Differences in histologic interpretation play a minor role in disagreements concerning the diagnostic specificity of the Kveim reaction. Most positive tests show unequivocal epithelioid granuloma formation but in a minority of cases, the granulomas are non-specific and differences in interpretation will be encountered. It is not established whether the Kveim reaction is truly an immunologic one. The test material must be particular, antibodies have never been demonstrated and transfer studies are unconvincing. It is clear that an *in vitro* test for sarcoidosis would represent a major advance in diagnosis, eliminating a number of difficulties encountered with the intracutaneous Kveim test, such as the need for a six week delay in diagnosis and for withholding corticosteroids during this interval. Lymphocyte transformation and M.I.F. techniques are at present sufficiently accurate so that *in vitro* tests should be practical as soon as stable and reproducible Kveim antigens are available. It appears that this is our greatest need in the diagnosis of sarcoidosis and it
is hoped that the employment of modern immunochemical techniques will permit isolation of a factor which is peculiar to sarcoidosis.

Until this is accomplished or until the causative agent of sarcoidosis, conceivably a slow virus, is isolated, the diagnosis will have to continue to be made by a combination of clinical, radiologic and histologic methods with careful exclusion of the many other granulomatous diseases which occasionally simulate sarcoidosis. It is in the exclusion of these other diseases that the laboratory must, at the present time, play an important role.

**Selection of Sites for Biopsy**

Granulomatosis in sarcoidosis is so widely disseminated that every known biopsy approach has proven positive in this disease. However, the safest and most rewarding sites for tissue biopsy are palpable lymph nodes, subcutaneous nodules and cutaneous lesions. When careful physical examination reveals none of these abnormalities, the procedures of choice are mediastinoscopy or lung biopsy (positive in 98 percent of cases) or aspiration biopsy of the liver (positive in 70 percent). Scalene node biopsy has been superseded by mediastinoscopy which affords a much higher yield. Biopsy of muscle is useful in patients presenting with erythema nodosum. Other tissues, such as bone marrow and normal appearing bronchial, palatal or conjunctival mucosa, occasionally demonstrate granulomas but the yield is too low to encourage routine use of these procedures.

Facilities should be available for serologic tests to measure antibodies against the fungal infections which patients with sarcoidosis are so prone to get, namely, aspergillosis and cryptococcosis as well as other fungal diseases which may simulate sarcoidosis, namely, histoplasmosis and coccidiodomycosis. The use of special stains in all biopsies in an effort to demonstrate fungi is an essential routine.

Tuberculosis has, in recent years in the United States, been an infrequent sequel to sarcoidosis and studies indicate that the second strength tuberculin test is reliable in excluding tuberculosis even in patients with sarcoidosis. Accordingly, costly cultural studies of tuberculosis are unnecessary in patients who fail to react to second strength tuberculin.

It is evident that the diagnosis of sarcoidosis will, in the future, be concerned with detection of complicating Hodgkin's disease and fungal infection more than mycobacterial disorders.

**References**


"Die when I may, I always want it said of me by those who know me best, that I always plucked a thistle, and planted a flower where I thought a flower would grow."

Abraham Lincoln