Biomarkers in Sarcoidosis: Can microRNAs Fill the Gap?

Sarcoidosis remains an enigmatic disease. In most cases, making a correct diagnosis in the face of competing etiologies requires invasive procedures. Even when histology demonstrates classic noncaseating granulomas, clinicians must recognize that other granulomatous diseases can masquerade as sarcoidosis. Furthermore, after the diagnosis is settled upon, we continue to struggle with a fundamental question: how do we identify patients destined for progression versus regression of sarcoidosis?

These questions regarding diagnosis and prognosis could be resolved by the identification of sarcoidosis biomarkers (1). Previous efforts to define a circulating sarcoidosis biomarker revolved most prominently around angiotensin-converting enzyme; however, the current literature highlights its poor sensitivity and insufficient specificity (2). Recently, microRNA (miRNA) has emerged as a promising methodology for diagnosis and prognosis in multiple respiratory diseases, including lung cancer, chronic obstructive pulmonary disease, asthma, idiopathic pulmonary fibrosis and cystic fibrosis (3, 4). miRNAs are 18–25 nucleotide bits of nonprotein coding RNA that are termed master regulators because of their ability to posttranscriptionally silence or modulate multiple target sets of genes. The first investigation of miRNAs in sarcoidosis tissue highlighted a key scientific complexity: miRNA profiles in sarcoidosis lung and lymph node tissues did not overlap with miRNA profiles from peripheral blood mononuclear cells (PBMCs) (5). Tissue-specific miRNA profiles were again found in later studies of sarcoidosis that examined BAL versus blood lymphocyte miRNAs (6). Despite these challenges, an assessment of both peripheral and lung miRNA gene targets demonstrated convergence of the miRNA signal at the TGF-β/WNT molecular pathway (5). Subsequently, a comparison of patients with favorable signs and symptoms versus patients with radiologically advanced sarcoidosis revealed that serum miRNA also targeted the TGF-β pathway (7). Recent miRNA investigations further implicated the Jak-STAT pathway (8), angiogenesis and extracellular matrix remodeling (6), and expression of IFN-γ (9). Despite these important advances, the current body of literature regarding miRNA in sarcoidosis remains small (10), and the hope that miRNA will serve as a clinically useful sarcoidosis biomarker remains unfulfilled.

In this issue of the *Journal*, Ascoli and colleagues (pp. 40–54) vigorously pursue the challenge of defining sarcoidosis biomarkers using miRNA (11). Their receiver operating characteristic curve analysis reveals a composite eight-miRNA signature that discriminates between sarcoidosis and controls with 74.8% accuracy for all samples. Importantly, the authors extend their work by identifying miRNA signatures associated with clinical markers of poor outcome, and developing a gene network analysis that highlights future pathways for research.

The authors used PBMCs collected during the ACCESS (A Case Control Etiologic Study of Sarcoidosis) study (12) from 31 patients recently diagnosed with sarcoidosis and 25 patients without granulomatous disease. These two groups, which were well balanced in terms of demographics, were prospectively split into discovery and validation cohorts. A microarray analysis annotated against the miRBase registry identified 2,578 mature miRNA transcripts, 69 of which were differentially expressed. A machine learning algorithm then selected 54 feature miRNAs that discriminated between sarcoidosis and controls. Subsequently, logistic regression models adjusted for sex, age, smoking history, and use of immunomodulatory therapy further selected eight signature miRNAs. Four of these signature miRNAs were overexpressed and four were underexpressed in sarcoidosis. Interestingly, two of these eight miRNAs, hsa-miR-4306 and hsa-miR-6729-5p, have not been previously described. The eight-miRNA signature discriminated between sarcoidosis and controls with a sensitivity of 68%, specificity of 71%, positive predictive value of 88%, and odds ratio of 5.36.

Ascoli and colleagues then investigated the prognostic implications of the signature miRNAs. Hsa-miR-150-3p and hsa-miR-342-5p (both of which were underexpressed in sarcoidosis compared with controls) were associated with a decreased percentage of lymphocytes, percent predicted forced expiratory volume, and forced vital capacity, although most of the patients' spirometry results still fell within the normal range. Lymphopenia and airflow obstruction predict poor outcome in sarcoidosis; therefore, the authors posit that their results point to a future direction of research for identifying a prognostic biomarker of sarcoidosis. The top Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways targeted by these two miRNAs included FOXO signaling, stem cell pluripotency, proteoglycans in cancer, and MAP-kinase (MAPK) signaling. A miRNA–KEGG pathway interaction analysis revealed hsa-miR-342-5p as a major hub of interaction in addition to hsa-miR-128-3p and hsa-miR-4306. Cell-cycle/arrest and malignancy pathways have been previously identified in sarcoidosis, whereas other pathways, such as HIPPO, are novel. Taken together, these analyses reveal targets for future study that may illuminate the mechanisms of sarcoidosis.

Although this translational work provides exciting news, several points temper enthusiasm for its results. This was a small, single-center study with no representation of African-Americans. Expansion to a larger and more diverse cohort could change the accuracy of this biomarker signature. Also, given that blood was collected within 6 months of tissue diagnosis, this miRNA signature represents a snapshot taken early in the disease course, and lacks representation of any patients with pulmonary fibrosis or significantly abnormal spirometry. To further define a prognostic biomarker, longitudinal studies that include multiple tissue types and sarcoidosis stages will be instructive. Finally, although the ability to differentiate sarcoidosis from health is an important first step in biomarker development, the ability to discriminate sarcoidosis from

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other etiologies in the differential diagnosis would truly alter clinical practice.

So, can a circulating miRNA signature predict outcomes or obviate the need for diagnostic biopsies in sarcoidosis? Today, the answer is no. However, Ascoli and colleagues lay the foundations for achieving these long-term goals. Future studies in large, diverse cohorts will pave the way, as will the longitudinal collection of biological samples in conjunction with clinical data aggregation. Similar to the case with lung cancer, it is likely that a combined miRNA signature will outperform any single miRNA biomarker for sarcoidosis diagnosis or prognosis (13). At present, this study of a circulating miRNA signature affords a glimpse into key regulatory pathways that set sarcoidosis apart, and prepares the way for future biomarker studies. ■

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Catherine A. Bonham, M.D.
Section of Pulmonary and Critical Care Medicine
University of Chicago
Chicago, Illinois

ORCID ID: 0000-0001-9132-4083 (C.A.B.).

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