Study Group Summary 12/11/08

Several different types of alternative assays based on ELISA or flow cytometry (also called multiplex bead) assays have been developed for detecting antinuclear and other autoantibodies in systemic rheumatic diseases. Some diagnostic laboratories have used these automated assay systems to replace "Screening ANA" tests conventionally performed by immunofluorescence on HEp-2 cells. Serious concerns have been raised for these new assays among rheumatologists and the ACR has established an ANA Task Force to address these concerns. Our study group at the 2008 annual ACR meeting in San Francisco included a presentation by Dr. John Goldman from Atlanta, Georgia entitled "Inaccurate Results for ANA" in which he presented data collected from rheumatologists in the Southeast showing the problems which have been encountered.

When ANA screening is performed by immunofluorescence on HEp-2 cells, a 'universe' of antigen-antibody systems is detected, reflecting autoantibodies reacting with nuclear components, including proteins, DNA or RNA present in nucleoplasm, nucleolus, nuclear membrane, and special nuclear bodies. In addition, autoantibodies reacting with cytoplasmic components in mitochondria, ribosomes, Golgi bodies and many other cytoplasmic structures can also be detected. This 'universe' of autoantibodies that can be detected with the conventional ANA screening test using HEp-2 cells, numbers in the hundreds.

The newer automated assays, the ELISA and flow cytometric systems, have been specifically constructed by the manufacturers to contain a limited number of cellular antigens. This is essentially due to technical constraints. At this time, this number varies from eight to thirteen or fourteen cellular antigens. Thus, these automated assays are capable of detecting the equivalent number of autoantibodies and no more. The manufacturers of these automated assays have been careful in selecting antigen-antibody systems which are present in higher frequency in diseases such as systemic lupus erythematosus, Sjogren's syndrome and scleroderma, and therefore, for these diseases, the automated assays have high sensitivity. However, even in these diseases, there are antigen-antibody systems that are present in low frequency and these autoantibodies would not be detected. Similarly, autoantibodies against dozens of other cellular antigens are not detected with the newer automated assays. However, it is important to point out that in the ELISA and multiplex bead assays, the cellular antigens used in these systems are highly purified and if the assays are done correctly, the detection of a specific autoantibody is highly accurate.

There was an extensive discussion of this issue at the meeting. The ACR Autoantibody Study Group recommends the following guidelines to rheumatologists who have had problems with laboratories using ELISA or Multiplex Bead assays as screening ANA tests:

1. When ordering a screening ANA test, it is important to very specifically state that an Immunofluorescent ANA (IF- ANA) on HEp-2 cells is requested. It is important that the diagnostic laboratory define the titer of the autoantibody detected and the titer

range of normal sera. When possible, the pattern of immunofluorescent staining should be reported and the lab should indicate which type of assay has been used to report the results.

2. If the IF-ANA is positive, further tests using ELISA or Multiplex Bead assays may be ordered to determine the specificity of the antibody. These tests would define the type of ANA and help in determining the nature of the illness.