

LABORATORY DIAGNOSIS OF AUTOIMMUNE DISEASES

Updated 2002 by Dr. Laura McCloskey and Dr. Andra Showalter)

Textbook reference:

1. Peter JB, Schoenfield Y, eds: *Autoantibodies*. Elsevier 1996.

This book is a comprehensive review of numerous autoantibodies. Each antibody is discussed in a separate chapter including nomenclature, antigenic structure, function, pathogenic role, clinical utility, methods of detection, interpretation and effects of treatment. Extensive bibliographies included with each chapter.

Anticardiolipin Antibody, antiphospholipid antibody, lupus anticoagulants and β 2 Glycoprotein I:

2. Harris EN: Diagnosis of the antiphospholipid syndrome: a proposal for use of laboratory tests. *Lupus* 7 Suppl 2:S144-148, 1998.

This review article addresses new ELISA techniques for antiphospholipid antibody testing. These new techniques employ different antigens which provide better determinations of antiphospholipid antibodies.

3. Roubey RAS, Eisenberg RA, Harper MF, Winfield JB: "Anticardiolipin" Autoantibodies Recognize β 2 Glycoprotein I in the Absence of Phospholipid. *J Immunol* 154:954-960, 1995.

This study employs binding experiments and inhibition assays to clarify the antigenic specificity of what has been termed "anticardiolipin antibody" Previously the exact role of beta-2 glycoprotein I had been unclear. "The data support the hypothesis that phospholipid bound beta-2 glycoprotein I is the physiologic target of aCl."

4. Reber G, Arvieux J, Comby E, Degenne D, de Moerloose P, Sanmarco M, Potron G: Multicenter Evaluation of Nine Commercial Kits for the Quantitation of Anticardiolipin Antibodies. *Thromb Hemost* 444-452, 1995.

This paper illustrates the lack of standardization for anticardiolipin antibody testing using commercial kits. Differences in rates of aCl positivity may be attributed to the configuration of the assay, the preparation of the reagents and the unpredictability of certain samples in any particular assay system. Standardization of this test has not yet been achieved.

5. Alving BM: Lupus Anticoagulants, anticardiolipin antibodies and the antiphospholipid syndrome. In: Loscalzo J, Schafer AI eds: *Thrombosis and Hemorrhage*. Blackwell Scientific Publications, 1994:749-766.

This chapter is a thorough and concise explanation of each of the topics in the title. It includes background information, pathophysiology, immunology, methods of testing and treatments.

6. Mackworth-Young C: Antiphospholipid antibodies: more than just a disease marker? *Immunology Today* 11:60-64, 1990.

Review of the clinical features of APS and the pertinent autoantibodies.

7. Cronin ME, Biswas RM, Van der Straten C, Fleisher TA, Klippel JA: IgG and IgM Anticardiolipin Antibodies in Patients with Lupus and Anticardiolipin Antibody Associated Clinical Syndromes. *J Rheumatol* 15:795-798, 1988.

Article investigating the clinical significance of IgG aCl and IgM aCl.

Anti dsDNA Antibody, Anti ssDNA Antibody and Antihistone Antibody:

8. Brinkman K, Termaat R, Berden JHM, Smeenk RJT: Anti-DNA antibodies and lupus nephritis: the complexity of crossreactivity. *Immunol Today* 11:232-233, 1990.

Review of DNA anti-DNA interactions. Immune complex formation and antibody avidity are addressed.

9. Wold RT, Young FE, Tan EM: Deoxyribonucleic acid antibody: A method to detect its primary interaction with deoxyribonucleic acid. *Science* 161:806-807, 1968.

This paper describes the Farr assay for detection of anti dsDNA antibody which is the gold standard.

10. Winfield JB, Faiferman I, Koffler D: Avidity of the anti-DNA antibodies in serum and IgG glomerular eluates from patients with Systemic Lupus Erythematosus. *J Clin Invest* 59:90-96, 1977.

This paper identifies different specificities and avidities of DNA antibodies in patients with SLE. The data suggests an association of high avidity dsDNA antibody with glomerulonephritis.

11. Koffler D, Miller TE, Faiferman I: Antipolynucleotide Antibodies: The Rheumatic Connection. *Human Pathol* 14:406-418, 1983.

Explanation of DNA interaction with antigens and comparison of various methods of detection. Includes discussion on anti ssDNA antibody and anti RNP antibody.

Anti Sm and RNP Antibodies

12. Tan EM, Kunkel HG: Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J Immunol* 96:464-471, 1966.

This is the original work describing the anti Smith (Sm) antibody system.

13. Mattioli M, Reichlin M: Physical association of two nuclear antigens and mutual occurrence of their antibodies: the relationship of the Sm and RNA protein (Mo) systems in SLE.

This work is the characterization of the RNP antigen/antibody system and it's relationship to the Sm antigen/antibody system.

14. Sharp GC, Irwin WS, LaRoque RL, Velez C, Daly V, Kaiser AD, Holman HR: Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. J Clin Invest 50:350-359, 1971.

This paper describes the entity known as Mixed Connective Tissue Disease and the presence of antibodies to "extractable nuclear antigen" which contains both Sm and RNP.

15. Takeda Y, Wang GS, Wang RJ, Anderson SK, Petterson I, Amaki S, Sharp GC: Enzyme-linked immunosorbent assay using isolated (U) small nuclear ribonucleoprotein polypeptides as antigens to investigate the clinical significance of autoantibodies to these polypeptides. Clin Immunol Immunopathol 50:213-230, 1989.
16. Fatenejad S, Brooks W, Schwartz A, Craft J: Pattern of anti-small nuclear ribonucleoprotein antibodies in MRL/Mp-lpr mice suggests that the intact U1 snRNP particle is their antigenic target. J Immunol 152:5523-5531, 1994.

This paper characterizes the antigen in the Sm/RNP antigen antibody system.

Anti SS-A and SS-B Antibodies

17. Clark G, Reichlin M, Tomasi TB Jr: Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythematosus. J Immunol 102:117-122, 1969.

This is the paper describing the characterization of the Ro autoantibody system.

18. Mattioli M, Reichlin M: Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. Description of a cytoplasmic nonribosomal antigen. Arthritis Rheum 17:421-429, 1974.

This is the original description of the La autoantibody system.

19. Asplough MA, Tan EM: Antibodies to cellular antigens in Sjögren's syndrome. J Clin Invest 55:1067-1073, 1975.

This is the description of anti SS-A in patients with Sjögren's syndrome.

20. Alspaugh M, Maddison P: Resolution of the identity of certain antigen-antibody systems in systemic lupus erythematosus and Sjögren's syndrome: and interlaboratory comparison. *Arthritis Rheum* 22:796-798, 1979.

In this brief communication two laboratories employed the Ouchterlony double diffusion technique and exchanged serum and tissue samples. They report that Ro is identical to SS-A and La is identical to SS-B. The current correct terminology employs both names: Ro/SS-A and La/SS-B.

21. Lerner MR, Boyle JA: Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science* 23:400-402, 1981.

This paper describes the antigenic specificity of anti-Ro/SS-A as a ribonucleoprotein-containing small uridine-rich nucleic acids designated as hY¹, hY³, hY⁴, and hY⁵ (H for human and Y for cYtoplasmic).

22. Yamagata H, Harley JB, Reichlin M: Molecular properties of the Ro/SS-A antigen and enzyme-linked immunosorbent assay for quantitation of antibody. *J Clin Invest* 74:625-633, 1984.

This paper describes an ELISA method for detecting anti Ro/SS-A. It also reports that Ro/SS-A's major protein is a 60kD molecule and the SS-A/Ro particle contains one molecule of protein and one molecule of hYRNA.

23. Reichlin M, Brucato A, Frank MB, Maddison PJ, McCubbin VR, Wolfson-Reichlin M, Lee LA: Concentration of autoantibodies to native 60-kd Ro/SS-A and 52-kd Ro/SS-A in eluates from the heart of a child who died with congenital complete heart block. *Arth Rheum* 37:1698-1703, 1994.

24. Reichlin M: Antibodies to nuclear proteins in systemic lupus erythematosus. In: McCune WJ, ed. *Rheumatic Disease Clinics of North America*. Philadelphia, WB Saunders Co., 1994;20:29-43.

This paper discusses the pathogenic role of anti Ro/SS-A antibodies in human diseases.

25. Pourmand N, Blomberg S, et al: Ro 52 KD Autoantibodies are Detected in a Subset of ANA- negative Sera, *Scand J Rheumatol* 29:116-23, 2000.

Testing for Ro 52kD antibodies may be helpful in evaluating ANA negative patients with a suspected connective tissue disease.

Anticentromere Antibodies:

26. Wade JP, Sack B, Schur PH: Anticentromere antibodies - clinical correlates. *J Rheumatol* 15:1759-1763, 1988.

This is a retrospective study that evaluates the clinical characteristics of patients found to be positive for anticentromere antibody. The authors conclude that ACA may not be as specific a marker for CREST as originally thought when used as a screening test in a preselected group of patients with rheumatic disease complaints.

27. Earnshaw W, Bordwell B, Marino C, Rothfield N: Three human chromosomal autoantigens are recognized by sera from patients with anti-centromere antibodies. *J Clin Invest* 77:426-430, 1986.

This paper describes the ground work for the characterization of anti-centromere antibody. Three distinct centromeric autoantigens are reported.

28. Sun D, Martinez A, Sullivan KF, Sharp GC, Hoch SO: Detection of anticentromere antibodies using recombinant human CENP-A protein. *Arthritis Rheum.* 39:863-867, 1996.

This paper examines the reactivity of anticentromere antibodies in an ELISA system using a recombinant CENP-A antigen. They found 95% of ACA positive sera by indirect immunofluorescence (n=38) to be positive in this ELISA system. There was a false positive rate of 2% among the normals (n=100). The authors suggest this assay could be an adjunct to the previously described ELISA using recombinant CENP-B antigen.

29. Parveen S, Morshed SA, Nishioka M: High prevalence of antibodies to recombinant CENP-B in primary biliary cirrhosis: nuclear immunofluorescence patterns and ELISA reactivities. *J Gastroenterol Hepatol* 10:438-445, 1995.

The authors of this paper suggest that using an ELISA method with CENP-B antigen is more sensitive than using indirect immunofluorescence (IFA) to identify anticentromere antibodies in patients with primary biliary cirrhosis. No false positives were observed among the normal controls using either ELISA or IFA. The ability to identify anticentromere antibody reactivity in the patient group improved from 44% using IFA, to 60 % using the recombinant CENP-B ELISA method.

30. Bernstein RM, Callender ME, Neuberger JM, Hughes GR, Williams R: Anticentromere antibody in primary biliary cirrhosis. *Ann Rheum Dis* 4:612-614, 1982.

This paper identifies the anticentromere antibody in a subset of patients with scleroderma and primary biliary cirrhosis. A 50% prevalence rate for the antibody was observed in the patient group.

Antineutrophil Cytoplasmic Antibodies

31. Franssen CF, Huitema MG, Kobold AC, Oost-Kort WW, Limburg PC, Tiebosch A, Stegeman CA, Kallenberg CG, Tervaert JW. In vitro neutrophil activation by antibodies to proteinase 3 and myeloperoxidase from patients with crescentic glomerulonephritis. 1999, *J Am Soc Nephrol* 10(7):1506-1515.

This study examines the pathogenic role that antineutrophil cytoplasmic antibodies play in disease. They compare the potency of anti-MPO to anti Pr-3 from patients with necrotizing crescentic glomerulonephritis. They conclude that anti Pr-3 antibodies are more potent activators of the neutrophil respiratory burst and degranulation in vitro than anti-MPO antibodies.

32. Goeken JA: Antineutrophil cytoplasmic antibody - A useful serologic marker for vasculitis. 1991, J Clin Immunol 11:161-174.

This paper reviews the classification of vasculitis and each of the disease entities. It also includes a review of the history of ANCA, predictive value, use in disease monitoring and disease mechanisms.

33. De'Olivera J, Gaskin G, Dash A, Rees AJ, Pusey CD: Relationship between disease activity and antineutrophil cytoplasmic antibody concentration in long term management of systemic vasculitis. Am J Kidney Dis 1995, 25:380-389.

The authors state that ANCA is useful in diagnosing vasculitis and that changes in ANCA reflect changes in disease activity.

34. Segelmark M, Baslund B, Wieslander J: Some patients with anti-myeloperoxidase autoantibodies have a C-ANCA pattern. Clin Exp Immunol 1994, 96:458-465.

This paper points out that P-ANCA does not always mean antibody to myeloperoxidase (MPO) and C-ANCA does not always mean antibody to proteinase-3 (Pr-3). The Perinuclear staining usually associated with anti-MPO is an artifact of ethanol fixation. Ethanol promotes redistribution of the neutrophil's primary granules (which contain MPO) from the cell's cytoplasm to the edge of the nucleus, which accounts for the perinuclear pattern. Other fixatives such as formalin do not induce granule migration and therefore, all staining occurs in the cytoplasm. This paper indicates that not all of the myeloperoxidase relocates with ethanol fixation.

35. Hardarson S, LaBrecque, Mitros FA, Neil GA, Goeken JA: Antineutrophil cytoplasmic antibody in inflammatory bowel and hepatobiliary diseases. High prevalence in ulcerative colitis, primary sclerosing cholangitis and autoimmune hepatitis. Am J Clin Pathol 1993, 99:277-281.

This paper discusses the prevalence of ANCA in several hepatobiliary disease states. The sensitivity and specificity of ANCA was determined to be 72% and 90% respectively in patients with either ulcerative colitis or primary sclerosing cholangitis. The staining pattern identified in these patients was perinuclear. We now know that the antibody producing this perinuclear pattern is distinct from MPO producing P-ANCA. The antigen has not been characterized, but the staining is termed very perinuclear or atypical ANCA.

36. Choi HK, Merkel PA, Walker AM, et al. Drug-Associated Antineutrophil Cytoplasmic Antibody-Positive Vasculitis: Prevalence Among Patients with High Titers of Antimyeloperoxidase Antibodies. *Arthritis Rheum* 43:405-413,2000.

In patients with ANCA-positive vasculitis, high titer anti-MPO antibodies are associated with drug (most commonly hydralazine and PTU) exposure.

37. Kyndt X, Reumaux d, Bridous F, et al: Serial Measurements of Antineutrophil Cytoplasmic Autoantibodies in Patients with Systemic Vasculitis. *Am J Med* 106:527-533, 1999.

High-titer ANCA is a predictor for relapse in patients with systemic vasculitis; lung involvement is also a poor prognostic sign.

Antinuclear Antibody Testing:

38. Moncé NM, Cappel VL, Saqueton CB: A comparison of two fixatives on IFA HEp-2 slides for the detection of antinuclear antibodies. *J Immunoassay* 15:55-68, 1994.

The authors demonstrate the variable preservation of autoantigens SS-A and SS-B with different fixation processes. They conclude that acetone fixation maintains the antigenic structure of the SS-A molecule in HEp-2 cell nuclei better than ethanol fixation. Employing the acetone fixation process routinely in the substrate preparation will assist in the identification of a positive ANA in the presence of anti SS-A antibody.

39. Lipscomb MF, Cape LD, Stephens GL, Deng JG, Gilliam JN: Comparison of substrates for the detection of antinuclear antibodies in normals and in patients with connective tissue and other diseases. *Diagnostic Immunol* 2:181-187, 1984.

This paper discusses the differences in ANA tests performed using tissue culture cell lines or frozen tissue sections. They found an increased false positive rate using the cell line substrates as compared to using tissue section substrates. They also found better sensitivity among patients with connective tissue diseases. Other advantages of using the cell lines include the identification of anticentromere antibodies and better identification of anti SS-A antibodies.

40. Harmon CE, Deng JS, Peebles CL, Tan EM: The importance of tissue substrate in the SS-A/Ro antigen-antibody system. *Arthritis Rheum* 27:166-173, 1984.

This paper addresses the differences in antigenic expression of the SS-A/Ro antigen in among various species and in tissue locations. ANA substrate cells must be chosen carefully with respect to SS-A/Ro antigen expression and distribution.

41. Smolen JS, Butcher B, Fritzler MJ, Gordon T, Hardin J, Kalden JR, Lahita R, Maini RN, Reeves W, Reichlin M, Rothfield N, Takasaki Y, van Venrooij WJ, Tan EM: Reference sera for antinuclear antibodies II. Further definition of antibody specificities in international antinuclear antibody reference sera by immunofluorescence and western

blotting. *Arthritis Rheum* 40:413-418, 1997.

This paper reevaluates the reactivities of antinuclear antibody reference sera available from the Arthritis Foundation and the CDC. These sera are available to laboratories to assist in standardizing ANA testing by indirect immunofluorescence and other methods. The data confirms most of the previously defined antigen specificities. The sera were also tested via western blot to establish the range of antigenic specificity contained in each of the serum samples.

42. Jaskowski TD, Schroder C, Martins TB, Mouritsen CL, Litwin CM, Hill HR: Screening for antinuclear antibodies by enzyme immunoassay. *Immunopathol* 105:468-473,1996.

This is a comparison of the traditional way to screen for antinuclear antibodies using indirect immunofluorescence and a the newer ELISA method. Comparable sensitivities and specificities were observed. The authors propose ELISA as a more objective measure for ANA testing in light of the inherent subjective nature of the traditional IFA ANA test.

Antithyroid Antibodies (anti thyroid peroxidase, TPO or antimicrosomal & anti thyroglobulin, Tg)

43. Dayan, CM, Daniels GH: Chronic autoimmune thyroiditis. *N Engl J Med* 35:99-107, 1996.

This is a comprehensive review of the autoimmune thyroid diseases.

44. Endres DB: Antithyroid peroxidase antibodies (antimicrosomal antibodies) in thyroid disease. *Clin Chem* 10:3-5, 1991.

This paper discusses the antibody tests used to evaluate thyroid disorders with particular attention to the clinical significance of anti microsomal antibodies. The antigenic specificities of both major thyroid antibodies are detailed. In addition methods, disease associations, trends with treatment and clinical utility of the tests are covered. This paper presents data supporting the use of anti TPO (antibody directed against thyroid peroxidase) in diagnosis and management of autoimmune thyroid disease.

45. Kasagi K, Kousaka T, Higuchi K, Iida Y, Misaki T, Alam MS, Miyamoto S, Yamabe H, Konishi J: Clinical significance of measurements of antithyroid antibodies in the diagnosis of Hashimoto's thyroiditis: Comparison with histological findings. *Thyroid* 6:445-450, 1996.

This paper presents a conflicting view of the importance of anti thyroid antibodies in management and diagnosis of Hashimoto's thyroiditis. In contrast to other authors, Dr Kasagi states that anti thyroglobulin (Tg) antibodies have the greatest positive predictive value of all antibody tests, and highest correlation with histological

changes found in Hashimoto's. Tg antibody levels are stated to reflect the destructive changes of thyroid follicular cells and are therefore correlated with thyroid status.

46. Beever K, Bradbury J, Phillips D, McLachlin SM, Pegg C, Goral A, Overbeck W, Feifel G, Rees Smith B: Highly sensitive assays of autoantibodies to thyroglobulin and to thyroid peroxidase. Clin Chem 35:1949-1954, 1989.

The authors describe a modification of the current ELISA method for detecting antithyroid antibodies. A comparison of the ELISA system with hemagglutination is presented. The paper also includes clinical correlations.

C-Reactive Protein & Erythrocyte Sedimentation Rate

47. Linares LF, Gomez-Reino JJ, Carreira PE, Morillas L, Ibero I: C-reactive protein (CRP) levels in systemic lupus erythematosus. Clin Rheumatol 5:66-9-69, 1986.

The authors evaluate the use of CRP in differentiating lupus flare from systemic infection.

48. Hart WR: C-reactive protein: the best laboratory indicator available for monitoring disease activity. Cleveland Clinic J Med 56:126-130, 1989.

This paper evaluates the clinical utility of CRP in various conditions and disease states. The author reviews methods of CRP testing and compares its use to that of measuring other acute phase reactants.

Rheumatoid Factor

49. Waaler E: The occurrence of a factor in human serum activating the specific agglutination of sheep corpuscles. Acta Pathol Microbiol Scand 1:172-188, 1940.

This is the original description of rheumatoid factor.

50. Del Puente A, Knowler WC, Pettitt DJ, Bennett PH: The incidence of rheumatoid arthritis is predicted by rheumatoid factor titer in a longitudinal population study. Arthritis Rheum 31:1239-1244, 1988.

The authors studied the Pima Indians of Arizona for 19 years. In that study period, 70 new cases of RA developed. They conclude that the presence of rheumatoid factor is a risk factor for developing RA and the risk is related to the titer. RF may represent the earliest manifestation of the disease process.

51. Painter PC, Lyon JM, Evans JH, Powers WW, Whitaker RL, Decker MJ: Performance of a new rate-nephelometric assay for rheumatoid factor, and its correlation with tube-titer results for human sera and synovial fluid. Clin Chem 28:2214-2218, 1982.

This is a comparison of two methods for detecting rheumatoid factor. The authors provide an interpretive correlation between the two methods. A clinical correlation to rheumatoid factor level is also provided.

52. Kalsi J, Isenberg D: Rheumatoid factor: primary or secondary event in the pathogenesis of RA? *Int Arch Allergy Immunol* 102:209-15, 1993.

Review of rheumatoid factor.

53. Thomas MJ, Adebajo A, Chapel HM, Webley M: The use of rheumatoid factors in clinical practice. *Postgrad Med J* 71:674-677, 1995.

The authors discuss the use of rheumatoid factor as a screening test in the hospital setting. They point out the need for awareness about the limitations of the test.

Anti Topoisomerase I (Scl-70) Antibodies:

54. Douvas AS, Achten M, Tan EM: Identification of a nuclear protein (Scl-70) as a unique target of human antinuclear antibodies in scleroderma. *J Biol Chem* 254:10514-10522, 1979.

This is the original description of the Scl-70 antibody system.

55. Steen VD, Powell DL, Medsger TA: Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. *Arthritis Rheum* 31:196-203, 1988.

The authors make the association of anti-topo I antibody with kidney involvement, pulmonary fibrosis, and ischemic ulcers of the finger tips.

56. Weiner ES, Earnshaw WC, Senecal JL, Bordwell B, Johnson P, Rothfield NF: Clinical associations of anticentromere and antibodies to topoisomerase I. A study of 355 patients. *Arthritis Rheum* 31:378-385, 1988.

This paper concludes that patients with anti-topo I antibodies are more likely to have heart and facial skin involvement than antibody negative patients.

57. Hildebrandt S, Weiner ES, Senecal JL, Noell S, Earnshaw WC, Rothfield NF: Autoantibodies to topoisomerase I (Scl-70): analysis by gel diffusion, immunoblot, and enzyme-linked immunosorbent assay. *Clin Immunol Immunopathol* 57:399-410, 1990b.

This paper compares several methods of detecting anti topo-I antibodies. The ELISA method was more specific than the Western immunoblot.

58. Shoenfeld Y, Gruenbaum E, Laufer M, Zurgil N, Bakimer R, Lunderschmidt A, Valentini G, Tirri G, Blank M: Anti-topoisomerase I and clinical findings in systemic sclerosis. *Israel J Med Sci* 32:537-542, 1996.

This paper evaluates 191 patients with systemic sclerosis. The autoantibody profile of the group is correlated to the clinical findings. The prevalence of antibody to DNA topoisomerase I (Scl-70) is found to be 37% in systemic sclerosis.

59. Sato S, Ihn H, Soma Y, Shimozuma M, Shishiba T, Takenhara K: A case of systemic sclerosis with anticentromere, anti-topoisomerase I and anti-U1RNP antibodies. *J Rheumatol* 20:1961-1963, 1993.

Case report of an unusual antibody presentation in systemic sclerosis.

Anitendothelial cell Antibodies

60. D'Cruz D, Keser G, Khamashta MA, et al: Anitendothelial cell Antibodies in Inflammatory Myopathies: Distribution Among Clinical and Serologic Groups and Association With Interstitial Lung Disease. *J Rheumatol* 27:161-164,2000.

In patients with idiopathic inflammatory myopathies, AECA are a marker for interstitial lung disease.

