

Viewpoint

Whither smooth muscle antibodies in the third millennium?

Lupoid hepatitis (now known as autoimmune hepatitis type I) was defined as an autoimmune disease by Mackay and colleagues in 1965.¹ An immunological marker of the disease was identified by Johnson *et al* in 1965 in the form of an "antismooth muscle antibody".^{2,3} Over the following 30 years, smooth muscle antibody (SMA) has been used in the evaluation of patients with raised transaminases and has been given a defining role among diagnostic criteria for autoimmune hepatitis by the International Autoimmune Hepatitis Group.⁴ Testing of these criteria on defined patient cohorts showed the criteria to be robust in defining autoimmune hepatitis.⁵ Yet now, in the first year of the third millennium, SMA appears to have "fallen from grace". Hepatologists are dismissing the test as useless (numerous personal communications), whereas others assert in an influential publication that they "do not recommend its routine use for the diagnosis of autoimmune hepatitis".⁶ As immunopathologists, we may well ask "what has happened?"

The original description by Johnson *et al* of SMA in eight of 10 cases of "lupoid hepatitis" and in none of the 16 cases of systemic lupus erythematosus (SLE) provided a distinguishing marker between these two diseases, which shared the features of a positive lupus erythematosus (LE) test and a high concentration of serum γ globulin.² Whittingham *et al* then extended these observations to a larger number of individuals with lupoid hepatitis, of whom 26 of 32 were SMA positive, and demonstrated specificity by showing that this antibody was not detected in the serum of patients with SLE, other liver diseases, or healthy controls.⁷ However, further studies indicated that SMA, albeit in low titre, could be detected in sera from normal individuals⁸ and patients with a range of clinical problems including infections,^{9,10} systemic autoimmune disease,¹¹ and cancer,¹² thus raising the issue of the specificity of SMA for autoimmune hepatitis.

The SMA reaction is now known to detect a range of cellular autoantigens in the cytoskeleton of smooth muscle and other cells. The cellular cytoskeleton consists of a family of filaments that are broadly classified by size into microfilaments (including actin and vinculin), intermediate filaments (including vimentin and desmin), and microtubules (tubulin). Autoantibodies to many of these proteins have been described but the SMA specific for autoimmune hepatitis is antibody to F-actin.^{11,13,14} Bottazzo and colleagues¹⁵ classified the F-actin specific SMA detected by immunofluorescence as renal tubule and glomeruli staining in 1976. Although the broad family of SMA reactivity has low specificity for the diagnosis of autoimmune hepatitis,^{16,17} anti-F-actin antibody is highly predictive of autoimmune hepatitis, with a sensitivity of approximately 80%,^{7,18} and a specificity of about 90%.^{13,17} This specificity is further heightened in the clinical situation where the physician uses SMA as part of a diagnostic panel of tests—for example, hepatitis serology. Given that we have an autoantibody test with good sensitivity and specificity for the diagnosis of autoimmune hepatitis, why are the clinicians not using it?

Part of the problem lies in the fact that many laboratories that report on SMA reactivity do not give any information regarding F-actin specific antibody. This position is illustrated by the fact that the quality assessment programmes conducted by the Royal College of Pathologists of Australasia and the UK National External Quality Assessment Scheme do not seek information on the specificity of the reactivity for F-actin when they distribute sera for SMA immunofluorescence testing.

Most laboratories use rat/mouse stomach/kidney composite blocks as substrates to determine the presence of SMA in serum. If these are prepared in house, it is possible to use fresh frozen tissue, but commercially available preparations provide fixed tissue, which is said to destroy the native conformation of the F-actin antigen.¹⁹ Our own experience with detecting F-actin specific antibody is that it can be seen on a range of commercial blocks fixed with acetone or ethanol. A second recommended requirement for the sensitive detection of anti-F-actin antibodies is heat inactivation of the sera,²⁰ which is reported to remove the actin depolymerising activity of human sera. However, there is no evidence that this step increases the sensitivity or specificity of detection on a tissue composite block. Because many of the smooth muscle antibodies seen in diseases other than autoimmune hepatitis are of the IgM isotype,^{13,21} we recommend that the immunofluorescence staining procedure uses antihuman IgG rather than antihuman immunoglobulin.

There is a characteristic pattern of staining on composite block tissue that is given by F-actin specific antibody. This comprises the staining of mouse stomach muscularis mucosa, intraglandular muscle fibres coursing through the gastric mucosa and blood vessel wall, and submembranous actin of hepatocytes. However the staining specific for anti-F-actin antibody is the staining of the intracellular fibrils of the renal tubules and mesangial cells of the glomerulus (fig 1B,E).^{9,15} If the renal tubules and mesangial cells are stained, confirmatory testing using immunofluorescence on fibroblasts,⁹ or rat liver after phalloidin injection,²² enzyme linked immunosorbent assay,²³ or counterimmunoelectrophoresis for the detection of precipitating antibodies to the XR1 antigen²⁴ may be used. However, these additional steps are not necessary because immunofluorescent staining of the appropriate renal structures is highly specific.¹⁶

If an antinuclear antibody and/or an anti-F-actin antibody is used in the assessment of a patient with raised transaminases only 5% of patients with autoimmune hepatitis will be missed,²⁵ and all of these will still be defined by a positive SMA that is not F-actin specific. Importantly, autoimmune hepatitis/primary biliary cirrhosis overlap states will also be identified.

Accordingly, our message is that it is the presence or absence of F-actin specific antibody that the clinician needs to know. This has been disseminated over the past 10 years,^{9,22} but it would appear that neither immunopathologists nor hepatologists have been listening. Thus, if the SMA test is not to be consigned to immunological history

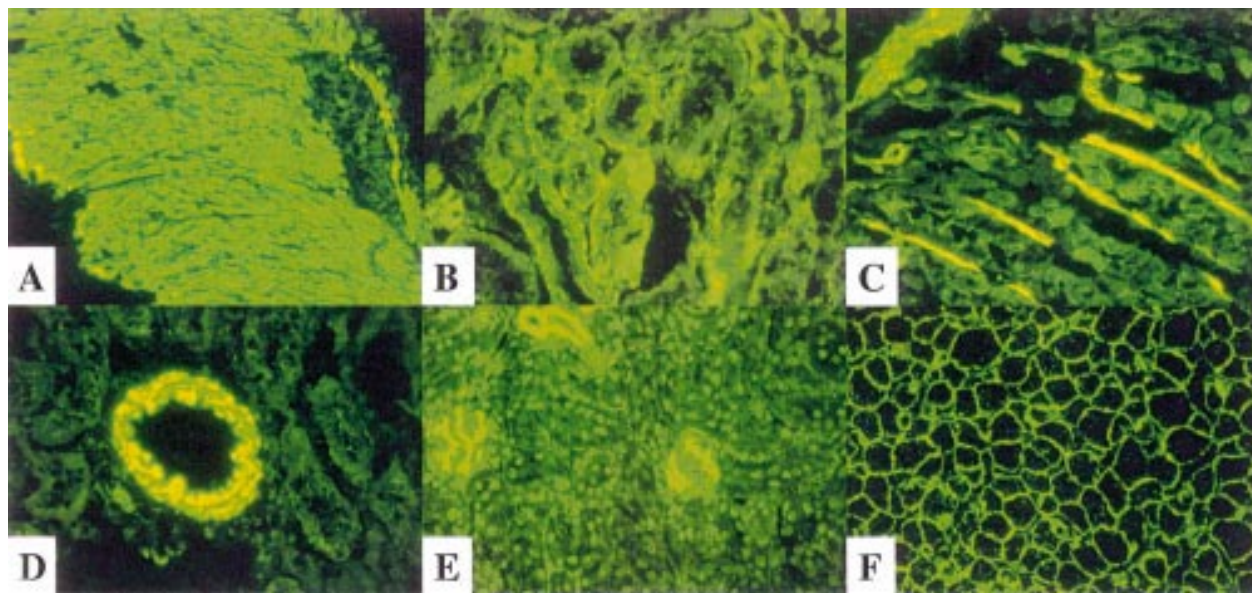


Figure 1 Patterns of F-actin specific smooth muscle antibodies. (A) Mouse muscularis mucosa; (B) mouse kidney tubules; (C) mouse gastric mucosa; (D) mouse kidney blood vessel; (E) mouse kidney glomerulus; (F) rat liver.

books, it is time that diagnostic laboratories took the lead and reported a test result that has both sensitivity and specificity for the diagnosis of autoimmune hepatitis. Such a report should define the presence or absence of SMA and the presence or absence of anti-F-actin antibody. Only then will the clinicians find the test useful in the assessment of patients with raised transaminases.

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