Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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DOI: <https://doi.org/10.1016/j.pathol.2020.10.018>

A distinctive staining pattern with anti-3-hydroxy-3-methyl-glutaryl-coenzyme a reductase (HMGCR) antibodies on mouse kidney

Sir,

We report a distinctive indirect immunofluorescence staining pattern on mouse kidney with autoantibodies to anti-3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR). The specificity of this staining pattern was confirmed using immunoabsorption experiments. The staining pattern was an incidental finding observed when testing a sample for tissue autoantibodies by immunofluorescence. The pattern may not have utility for routine immunofluorescence screening; however, it should prompt a HMGCR confirmatory test when observed.

In 2010, a link between necrotising myopathy and HMGCR autoantibodies was established.¹ The gold standard test for necrotising myositis confirmation is biopsy. However, the presence of HMGCR autoantibodies may be confirmed using radio-immunoprecipitation or counter-current immunoelectrophoresis (CIEP)^{2,3} to precipitate HMGCR recombinant protein. Automation of laboratory techniques has led to a reduction in labour intensive methods such as radio immunoprecipitation and/or CIEP. The importance of the immunoprecipitation assays is that they precipitate higher affinity antibodies that are considered more clinically relevant than those detected on screening tests such as enzyme-linked immunosorbent assay (ELISA). Thus, it is recommended that ELISA are used to screen samples when the pretest probability of anti-HMGCR myopathy is high in those with

significant creatinine kinase (CK) elevations and muscle weakness.⁴ A unique immunofluorescence pattern has also been described as a confirmatory/screening test. On rat liver, a scattered cytoplasmic staining of hepatocytes around the liver lobules, namely anti-HMGCR antibody associated liver immunofluorescence pattern (HALIP) is described.⁵

Our experience has found that of all samples sent for myositis testing, anti-HMGCR autoantibodies make up a third of those positive for a known myositis antibody, making it the most common form of myositis.³ The availability of anti-HMGCR positive samples by both ELISA and CIEP within our laboratory led to the identification of a distinctive immunofluorescence staining pattern on mouse kidney. The anti-HMGCR antibodies stain the distal kidney tubules which appear as distinct bands in the kidney. Mouse HMGCR is moderately expressed in kidney tissue (<http://www.informatics.jax.org/marker/MGI:96159>) and experimental nephropathy increases mouse renal HMGCR expression.⁶ We confirmed the specificity of the mouse kidney staining pattern to HMGCR using immunoabsorption experiments.

A 74-year-old female presented to her general practitioner with non-specific malaise, profound muscle weakness and decreased energy. Despite being a keen golfer, she was struggling to walk the golf course. Muscle pain, shortness of breath and intermittent palpitations were also recorded. She was known to have thyroid disease (Hashimoto's thyroiditis) and low B12 concentration. She had been taking the statin, atorvastatin, for 8 years. Tests for parietal cell and intrinsic factor autoantibodies were requested. Gastric parietal cell (GPC) antibodies were detected using indirect immunofluorescence (IIF) on ready made slides with mouse kidney and stomach (MKS) sections (Inova Diagnostics, USA) and patient serum at 1 in 20 dilution in phosphate buffered saline. In addition to the strong classical GPC pattern, an unusual pattern was observed in the distal kidney (Fig. 1A). This pattern was previously observed on IIF using murine tissue substrate from a patient with 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) antibody positive necrotising myositis (Fig. 1B,C). When the patient's sample was tested for anti-HMGCR antibodies on our in-house ELISA it was strongly positive, 38 AU (arbitrary units; reference range 0–3) which was confirmed with immunoprecipitation to HMGCR on CIEP. CK was subsequently found to be elevated at 16,432 U/L (female reference range 30–180 U/L). The myositis line blot (Euroimmun, Germany) was negative for other myositis-associated autoantibodies (Mi-2 α , Mi-2 β , TIF1 γ , MDA5, NXP2, SAE1, Ku, PM-Scl 75, PM-Scl 100, Jo-1, SRP, PL-7, PL-12, EJ, OJ and Ro-52). The patient was commenced on prednisone and methotrexate for presumed autoimmune necrotising myositis. Regular atorvastatin was stopped, and clinical improvement was observed following initiation of immunosuppressive therapy. No muscle biopsy was undertaken.

Immunoabsorption experiments confirmed the pattern specificity. Using a strong positive anti-HMGCR sample at 1 in 20 dilution it was pre-incubated with 16, 4 and 1 μ g/mL of HMGCR antigen (Sigma, USA) prior to loading onto the mouse tissue slides. The strong distal kidney staining observed progressively diminished with increasing amounts of HMGCR added; staining at 1 μ g/mL was moderate-strong, reducing to weak at 4 μ g/mL and marginal at 16 μ g/mL. As further evidence, eight confirmed HMGCR positive samples

Fig. 1 Immunofluorescence staining on mouse kidney and stomach sections. (A) Strong staining of gastric parietal cells with faint HMGCR staining of distal kidney using subject's serum at 1 in 20 dilution. Gastric parietal tissue (far right), and thin muscularis layer, exterior to the kidney, where there is faint staining. (B) A CIEP confirmed anti-HMGCR positive serum at 1 in 20 dilution staining of mouse distal kidney. (C) A CIEP confirmed anti-HMGCR positive serum at 1 in 20 dilution staining of mouse distal kidney. (Nikon Image Navigator.)

all displayed to varying intensities the same distinctive immunofluorescence staining pattern on mouse kidney.

While this is an interesting observation it may not be suitable as a screening test for HMGCR antibodies. It is possible the presence of anti-mitochondrial or nuclear antibodies staining the entire tissue may mask this distinctive staining pattern. We also noted variability with the intensity of staining on different lots of tissue slides used, and slides from a different manufacturer did not give equivalent staining. Manufacturers' slide preparation process may contribute to the variability in staining. Further investigation is required to determine the suitability of mouse kidney tissue for routine anti-HMGCR immunofluorescence. However, as an incidental finding this unique staining pattern should prompt anti-HMGCR testing when observed during tissue autoantibody screening.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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DOI: <https://doi.org/10.1016/j.pathol.2020.10.020>

Mecillinam susceptibility in Victorian third generation cephalosporin-nonsusceptible *Escherichia coli* urinary isolates

Sir,

Antimicrobial resistance has become an increasing challenge for clinicians managing urinary tract infections (UTIs). The worldwide emergence of the highly successful *Escherichia coli* ST131 clone is particularly problematic because it harbours multiple genes that confer resistance to commonly used antibiotics. Data from the Australian Group on Antimicrobial Resistance (AGAR) reported third generation cephalosporin resistance in 14.5% of *E. coli* causing bloodstream infections, for which the urinary tract was the most frequent source.¹ Consecutive AGAR reports have also reported increasing rates of fluoroquinolone and trimethoprim-sulfamethoxazole resistance. Comprehensive Australian data on third generation cephalosporin-nonsusceptible *E. coli* (3GCNS-EC) from urinary specimens are lacking. One Melbourne laboratory has reported 3GCNS-EC rates of 5.7% from urinary isolates.² Increasing rates of resistance to cephalosporins, ciprofloxacin and trimethoprim-sulfamethoxazole has resulted in limited treatment options for some infections. Fosfomycin, nitrofurantoin and pivmecillinam are frequently the only oral options available for 3GCNS-EC UTIs.³ Of these, nitrofurantoin is the only antibiotic listed on the Pharmaceutical Benefits Scheme, fosfomycin has been approved by the Therapeutic Goods Administration for use in females greater than 12 years of age, and pivmecillinam is available in Australia via the pharmaceutical Special Access Scheme. Fosfomycin is an attractive option for clinicians wishing to avoid the infrequently encountered adverse effects attributed to nitrofurantoin, but may be less effective.⁴

Pivmecillinam, an amidinopenicillin, is the orally active prodrug of mecillinam. Mecillinam is available in an intravenous formulation in a limited number of Scandinavian countries. In the laboratory pivmecillinam susceptibility is extrapolated from the results of antimicrobial susceptibility disk testing using mecillinam disks. Pivmecillinam demonstrates higher *in vitro* stability to β -lactamase hydrolysis compared to other penicillins and maintains good *in vitro* susceptibility against 3GCNS-EC.^{3,5} Furthermore, despite the use of pivmecillinam in Scandinavian countries for many years, widespread pivmecillinam resistance has not emerged,