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Supporting Patients through Education & Research

Scientific Summary & Progress Report - Myositis Autoantibodies- Dr Zoe Betteridge

The University of Bath research group is well regarded as one of the leading centres for myositis serology research, and in the last 12 months has established a clinical service for autoantibody testing. Through this new service and our continued collaboration with the UKMyoNet, EuMyonet and Juvenile Dermatomyositis Cohort Biomarker Study networks we have expanded our myositis serum biobank to nearly 3000 myositis samples. All of these samples have been screened for myositis specific and associated autoantibodies by immunoprecipitation and work has been finalised verifying the results (as appropriate) by either repeat immunoprecipitations, in house ELISAs or using commercial assays. Where applicable, the serological and clinical data for these patients has been fully analysed with the assistance of the Department of Mathematics (University of Bath) and a manuscript describing the clinical associations of each myositis autoantibody, their mutual exclusivity and myositis specificity is in preparation. Additionally, an abstract regarding the mutual exclusivity of myositis specific autoantibodies (MSAs) has been accepted as a poster presentation at the British Society of Rheumatology annual conference (April 2016) and work from this project has been presented as an oral presentation at the 1st International Myositis meeting (Stockholm May 2015). This work has also led to two invited reviews / book chapters on the clinical and diagnostic utility of myositis autoantibodies;

- Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. Betteridge Z, McHugh N. J Intern Med. 2016 Jul;280(1):8-23. Epub 2015 Nov 25.
- Other Myositis-associated and Myositis-Specific Autoantibodies. Z Betteridge. The Myositis Handbook: An Inclusive Guide to the Inflammatory Myopathies. Epub 2016

Data from this study has also been included in a number of associated studies by collaborating groups across Europe and has formed part of a number of publications;

- Increasing incidence of immune-mediated necrotizing myopathy: single-centre experience. Klein M, Mann H, Pleštilová L, Zámečník J, Betteridge Z, McHugh N, Vencovský J.Rheumatology (Oxford). 2015 Nov;54(11):2010-4. doi: 10.1093/rheumatology/kev229. Epub 2015 Jun 24.
- Rare myositis-specific autoantibody associations among Hungarian patients with idiopathic inflammatory myopathy. Bodoki L, Nagy-Vincze M, Griger Z, Betteridge Z, Szöllősi L, Jobanputra R, Dankó K. Acta Reumatol Port. 2015 Oct-Dec;40(4):337-347.
- [Comparison of clinical characteristics and laboratory parameters of patients with dermatomyositis-specific autoantibodies and autoantibody-negative

patients]. Bodoki L, Budai D, Nagy-Vincze M, Griger Z, Betteridge Z, Dankó K. Orv Hetil. 2015 Sep 6;156(36):1451-9. Hungarian.

Furthermore, this myositis serology study has led to the development of a number of sub-studies as detailed below;

Anti-EIF3

The discovery of a novel autoantibody (anti-EIF3) in patients with mild polymyositis has been previously presented as oral and poster abstracts at a number of meetings. A manuscript describing the findings was submitted to Arthritis and Rheumatology but was rejected after review. In the past 12 months, the reviewers' comments have been addressed and a revised manuscript has been prepared for submission to Rheumatology. The project is now awaiting additional serum from the anti-EIF3 positive patients in order to complete the final figures for the paper.

Validation of Commercial Assays

In the past year we have initiated a collaboration with a US diagnostic's company 'Genalyte'. This company has developed a rapid autoanalyser (the Maverick System) capable of multiplex screening of samples in less than 20 minutes. Having been provided one of these autoanalysers our group has been testing the myositis autoantibody panels using samples from our vast serum bank. In addition, we have also been screening the same samples on the commercially available EuroImmun blotting panel and comparing the data. The preliminary results have been presented by Prof Neil McHugh at the American College of Rheumatology annual meeting and will form the basis of a grant application to 'The Myositis Association' investigating the validation and standardisation of commercial myositis autoantibody tests. Additionally, the lineblot versus immunoprecipitation data is currently being analysed and prepared for submission both as a meeting abstract and a full manuscript.

ELISA Development (TIF1, NXP2, MDA5)

We previously developed in-house ELISAs for the detection of NXP2, MDA5 and TIF1-gamma. These ELISAs have been used to screen for, and confirm the presence of, anti-NXP2, anti-MDA5 and anti-TIF1 gamma in the myositis and control cohorts. Additionally, the ELISAs have been enhanced to be a quantitative assay, and serial samples from autoantibody positive patients have been screened to determine whether the autoantibody levels vary over time and if this has any correlation with clinical symptoms.

In addition, with the assistance of two University of Bath final year pharmacology undergraduates preliminary work in establishing ELISAs and western blots for the detection of TIF1 alpha and beta has been initiated. These initial findings have proven the feasibility of

establishing these assays within the lab and a BIRD summer studentship has been appointed to undertake this work in August and September 2016.

Scleroderma Autoantibody Studies

After completion of the additional work requested by reviewers, our publication on the novel scleroderma specific anti-EIF2B autoantibody has been accepted by Arthritis and Rheumatology as a concise report. Additional work on this topic is now underway by the group (Miss Hui Lu, Dr Zoe Betteridge, Dr John Pauling and Prof Neil McHugh) investigating the presence of this autoantibody in a US Systemic Sclerosis cohort

- Anti-Eukaryotic Initiation Factor 2B Autoantibodies are Associated with Interstitial Lung Disease in Patients with Systemic Sclerosis. Betteridge ZE, Woodhead F, Lu H, Shaddick G, Bunn CC, Denton CP, Abraham DJ, du Bois RM, Lewis M, Wells AU, McHugh NJ. Arthritis Rheumatol. 2016

Autoantibodies in Lung Disease

As part of a collaboration with the University of Liverpool 250 idiopathic pulmonary fibrosis (IPF) patients have been screened for autoantibodies by immunoprecipitation with approximately 2% of patients resulting in a known connective tissue disease (CTD) autoantibody and a further 40% of patients demonstrating strong bands of unknown specificity. Since patients with IPF have a poor prognosis with limited treatment options, the detection of CTD autoantibodies, demonstrating a potential misdiagnosis, in a subset of these patients has clinical relevance. These preliminary findings are now been used as part of an MRC Partnership Grant application to investigate the use of autoantibody screening as part of IPF / CTD diagnosis in a larger cohort of lung disease patients.

Additionally, since 4% of patients in the cohort resulted in the same novel pattern on immunoprecipitation, work is now being completed investigating this potential novel lung disease specific autoantibody.