VERIFICATION OF A NEW ASSAY TO DETECT SYSTEMIC SCLEROSIS ANTIBODIES

Holly Walker and Catherine Sims
Immunology Laboratory, Queens Medical Centre, Nottingham University Hospitals, Derby Road, Nottingham, NG7 2UH

Nottingham University Hospitals NHS Trust

KEY WORDS • *SYSTEMIC SCLEROSIS (SSC)* • *ANTI-NUCLEAR ANTIBODY (ANA)* • *IMMUNOBLOT* • *NUCLEOLAR*

INTRODUCTION

Systemic sclerosis (SSc) is an immune-mediated rheumatic disease that is characterised by diffuse fibrosis of the skin and internal organs along with vasculopathy and production of autoantibodies. The clinical manifestations and prognosis of SSc vary, with the majority of patients having skin thickening and variable involvement of internal organs [(1)]. Diagnoses in patients are often concluded from these physical presentations alone, in the absence of serological testing being routinely available. In 2013 the American College of Rheumatologists/ European Alliance of Associations for Rheumatology updated their classification criteria to include SSc specific autoantibodies, namely Anti-centromere, Anti-Topoisomerase I and Anti-RNA polymerase III [(1)]. In addition to these, newer autoantibodies have since been described in literature that has associations with certain clinical subtypes of SSc. Therefore being able to provide a reputable laboratory assay ,within Immunology at NUH, that covers these antibodies is of ever growing importance to both our users and patients.
Our aim was to evaluate if introducing a novel immunoblot assay would provide both diagnostic and clinical benefit to the service users of NUH.

METHODOLOGY

It is widely accepted that Anti-Nuclear Antibodies (ANA) of a Nucleolar pattern are associated with Systemic Sclerosis specific auto-antibodies. For this reason a variety of patients with differing strengths of Nucleolar ANA were selected for testing.

Sera from Nucleolar ANA positive patients (n=15) and healthy controls (n=13) were evaluated using Euroimmun Systemic Sclerosis (Nucleoli) profile (IgG) kit on the fully automated euroBLOT one analyser to produce a qualitative result. See diagram below [(2)].

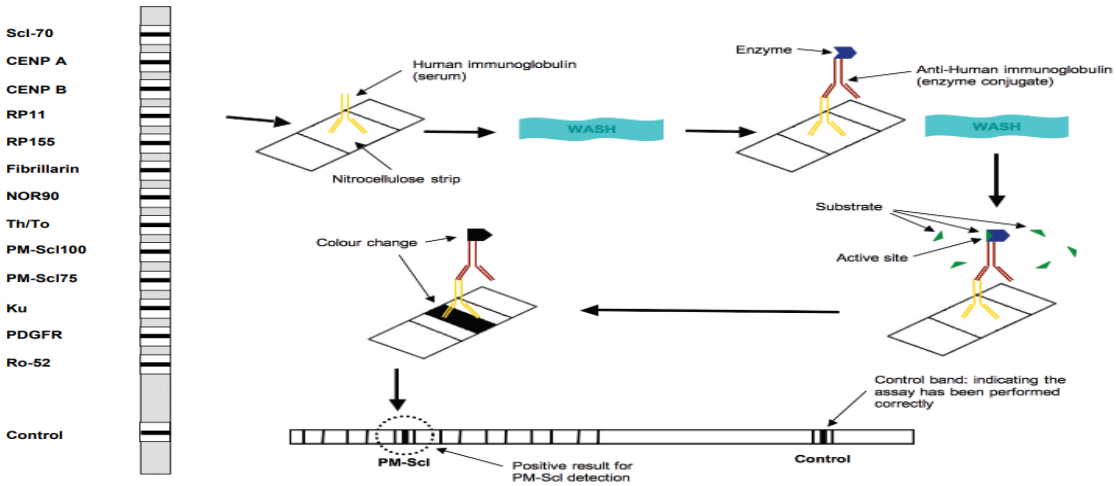

Figure 1. Methodology of novel SSc euroLINE immunoassay by euroIMMUN.

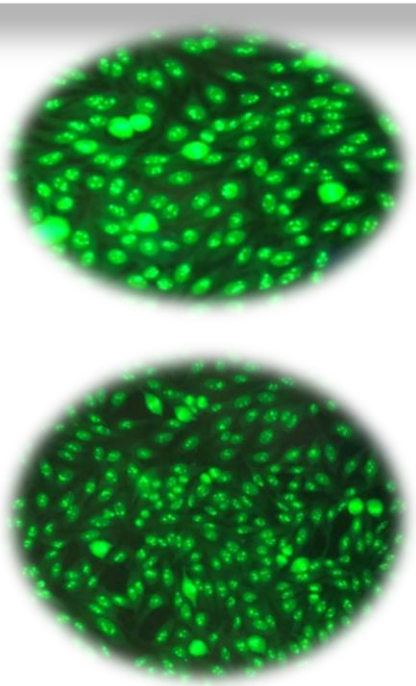

Figure 2. Nucleolar ANA Pattern at a 6400 titre

RESULTS

Of the patient sera, with a positive Nucleolar ANA, 53.3% showed positivity to one or more of the SSc related autoantibodies. Testing also included a NEQAS sample which showed good agreement with the consensus group. Disease negative samples did not yield any results of significance

Table 1. Antibodies detected using SSc (Nucleoli) Profile and the corresponding ANA titre strength.

SAMPLE	ANTIBODY POSITIVE	TITRE STRENGTH
1	RO52	400
2	PM-Scl75	400
3	PM-Scl75, PM-Scl100	400
4	N/A	<400
5	RO52, CENPB, CENPA	1600
6	N/A	400
7	RO52 & Fibrillarin	6400
8	N/A	400
9	N/A	400
10	RO52, Fibrillarin, CENP B	6400
11	NOR90	<400
12	N/A	<400
13	N/A	400
14	RO52, Fibrillarin, CENPB, CENPA	6400
15	PM-Scl75, Th/TO, NOR90, Fibrillarin	N/A

DISCUSSION

Serological diagnosis of systemic sclerosis associated antibodies can prove useful in the diagnosis of SSc particularly in those patients whom present with varying clinical manifestations. Our results displayed good consensus with nucleolar ANA, particularly those of high titre, showing associations with SSc autoantibodies. These antibodies would not have routinely been detected with our current Immunoblot assays that we offer to our users. Preliminary talks with service users about these results shows strong interest in this assay being offered to patients whom present with the vast range and types of SSc manifestations.

NEXT STEPS

With the promising preliminary verification of this assay we are beginning to have discussions surrounding how this assay is offered. Initial considerations are leading to a gating strategy being implemented which would only allow certain specialities, such as rheumatology; to request this test upfront for patients with a strong clinical suspicion of SSc. Going forward as a lab we will begin to implement all the necessary IT changes and communicate this to the suggested users with a plan to go live imminently. Following a successful go live of the assay we will look to audit the assay within 6 months to work out what is going well and to potentially look at how this test could be further utilised, i.e. reflexed from strong nucleolar ANA tests.

REFERENCES

1. Hoogen, F., et al., 2013., *2013 Classification Criteria for Systemic Sclerosis.* Vol 65, no.11.
2. Euroimmun., 2022., *Euroline – A line immunoassay for parallel detection of different autoantibodies* {online}., available via https://www.euroimmun.co.uk/eurolineUK