Autoantibodies in post-treatment Lyme disease and association with clinical symptoms

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ABSTRACT

Objective. *Autoantibodies have been described in the post-infectious state, specifically after Lyme disease and COVID-19. We aimed to describe the prevalence and potential clinical utility of several commercially available autoantibodies after these infections.*

Methods. *Euroimmun panels (myositis, scleroderma and ANA5) were assayed using sera from patients with Lyme disease with return to health (RTH) (n=70), post-treatment Lyme disease (n=58), COVID-19 RTH (n=47) and post-acute symptoms of COVID-19 (n=22). The post-Lyme questionnaire of symptoms (PLQS) was used to determine symptom burden after Lyme disease.*

Results. *There was no statistically significant difference in autoantibody prevalence across the four groups (p=0.746). A total of 21 different antibodies were found in the Lyme cohorts and 8 different antibodies in the COVID-19 cohorts. The prevalence of scleroderma-associated antibodies was higher after Lyme disease than COVID-19 (12.5% vs. 2.9%, p=0.026). There was no statistically significant difference in symptom burden based on antibody status.*

Conclusion. *Several autoantibodies were found after Borrelia burgdorferi and SARS-CoV2 infection, although the prevalence was similar in those with persistent symptoms and those who returned to health. While our data show no difference in autoantibody prevalence across the four post-infectious states, we do not imply that autoantibodies are irrelevant in this setting. Rather, this study highlights the need for novel antibody discovery in larger cohorts of well-defined patient populations.*

Introduction

Autoantibodies have diagnostic and prognostic potential in rheumatic disease, provide insight into immune activation, and can characterise clinical phenotypes. However, clinical context is important as autoantibodies are present in the general population and can develop after many infections. There is often limited data about their clinical value in the post-infectious setting.

Following infection, autoantibodies are usually transient and often do not lead to rheumatic disease. However, immune dysregulation may persist in some individuals, and this has the potential to lead to autoimmunity (1). Interpretation of these antibodies is further complicated by the presence of pre-clinical rheumatic disease, in which autoantibodies may be present years before symptoms develop (2). Nilsson *et al.* reported that myositis-specific and myositis-associated antibodies are found in 20% of patients with persistent symptoms after Lyme disease (3). Our study expands on this by describing the prevalence of myositis-specific and -associated antibodies, and several other commonly tested autoantibodies, following *Borrelia burgdorferi* infection, in patients with post-treatment Lyme disease (PTLD) and in those who returned to health (RTH) after appropriate antimicrobial therapy. SARS-CoV-2 infection also associates with autoantibody development and prolonged symptoms after infection. Patients with SARS-CoV-2 infection were therefore included to evaluate whether these findings were unique to Lyme disease.

Materials and methods

We utilised two Lyme disease cohorts to understand the clinical utility of autoantibodies in the post-infectious state. The first consisted of participants with prior acute Lyme disease, defined by physician-documented erythema migrans, who met criteria for returning to health (RTH, n=70) or PTLD (n=15) after antibiotic treatment (4, 5). Blood was collected two months after completion of antibiotics, and clinical status determined six months after antibiotics. The second (n=43) included participants with PTLD, with prior medical-record confirmed Lyme disease and persistent symptoms severe enough to impact function (6). In this sample, subjects with shorter illness durations were selected, with blood collection at a median of 4.4 months (IQR: 3–5.6 months) after Lyme disease onset. Participants with PTLD from the longitudinal and cross-sectional cohorts were combined in the analyses.

Table I. Autoantibody positivity among four cohorts of patients following acute infection. Displayed are the percent positive for each of the antibody specificities. For the positive sera, mean antibody levels (arbitrary units, as obtained per manufacturer's protocol) are shown.

Euroimmun line blot antibody panel	Post-treatment Lyme disease (PTLD) n=58	Erythema migrans w/return to health (RTH) n=70	Post-acute symptoms of COVID n=22	COVID-19 w/return to health n=47
ANA5	4 (6.9%)	4 (5.7%)	1 (4.5%)	1 (2.1%)
	Scl70 [a], Ro52 (2), Jo1, SSA	PM-Scl100, dsDNA, RNP70-A/C, Jo1	RNP/Sm	Histone
	118.8	46.5	49.0	68.0
Scleroderma	7 [a] (12.1%)	9 [b] (12.9%)	1 (4.5%)	1 (2.1%)
	PM-Scl75 (2), Scl70, Ro52 (2), RP11, fibrillarin, RP155	NOR90 (4), CENP-A, PM-Scl100, Th/To (4), RP11, PM75, fibrillarin, Ku	RP155	NOR90
	78.0	62.3	37.0	40.0
Myositis	7 (12.1%)	7 (10.0%)	1 (4.5%)	4 (8.5%)
	PM-Scl75(2), Ro52 (2), Mi2β, Jo1, MDA5	PM-Scl100, PM-Scl75 TIF1γ, SAE, OJ, Ku, NXP2, Jo1, PL12	NXP2	TIF1γ, PL7, NXP2, Mi2α,
	72.6	78.2	52.0	51.0
Any positive antibody	10 (17.2%)	14 (20.0%)	3 (13.6%)	6 (12.8%)
	86.3	66.8	46.0	52.0

The following antibodies are assayed in these Euroimmun lineblot panels:
ANA5: M2 mitochondrial, ribosomal P protein, histone, nucleosome, dsDNA, PCNA, CENP-B, Jo1, PMSCL100, Scl-70, SSB, Ro52, SSA, RNP70-A/-C, Sm, RNP/Sm;
Scleroderma: Scl-70, CENP-A, CENP-B, RP11, RP155, fibrillarin, NOR90, Th/To, PMSCL100, PMSCL75, Ku, Ro52, PDGFR;
Myositis: Ro52, OJ, EJ, PL12, PL7, SRP, Jo1, PMSCL75, PMSCL100, Ku, SAE, NXP2, MDA5, TIF1γ, Mi2β, Mi2α. Antibody levels considered negative (0-5 units), low (6-25 units), moderate (26-50 units) and strong (>50 units).
[a] In the PTLD cohort, one patient tested positive for two antibodies (PMScl75 and Scl70).
[b] In the Lyme RTH cohort, three patients tested positive for more than one antibody (Th/To and RP11; PMSCL75 and NOR90; RNP70-A/C, Ku, Th/To, NOR90, fibrillarin, OJ, and NXP2)

In both Lyme disease cohorts, participants were excluded at enrollment for a prior diagnosis of autoimmune disease. Symptom burden was evaluated among Lyme patients by generating a total score representing the sum of the 36-items included in the post-Lyme questionnaire of symptoms (PLQS) (6). Blood was also obtained from participants with mild pre-Alpha strain SARS-CoV-2 infection in 2020, a median of 1.5 months from COVID-19 onset (IQR: 1–2 months). Participants completed surveys at the time of blood draw indicating the presence (n=22) or absence (n=47) of prolonged symptoms after COVID-19.

The following antibody panels were assayed in sera by line blot using the Euroimmun platform: ANA5 (16 autoantibodies), scleroderma (13 autoantibodies), and myositis (16 autoantibodies). Cut-off values defined by the manufacturer for low, moderate, and strong positivity were used. In the current study, only moderate and strong positivity was interpreted as a positive test (7). Several antibodies (*e.g.* Ro52, Ku, PMSCL100) were included in more than one panel and considered positive only if there was moderate-to-strong positivity in all.

Results

Table I describes the proportion of PTLD, Lyme RTH, post-COVID-19, and COVID-19 RTH participants with autoantibodies (see Supplementary Table S1 for complete data). There was no statistically significant difference in autoantibody prevalence across the four groups (*p*=0.787). Twenty-one different antibodies were found in the Lyme cohorts and 8 different antibodies in the COVID-19 cohort. Seven antibodies (anti-PMSCL75, anti-NOR90, anti-Th/To, anti-fibrillarin, anti-Jo-1, anti-Ro52, and anti-RP11) were found in more than one Lyme disease patient. Only anti-NXP2 was found in more than one patient after COVID-19. Four participants had more than one autoantibody. When comparing across cohorts, there was no statistically significant difference in positivity by age (*p*=0.980) or sex (*p*=0.357).

Compared to COVID-19, a significantly higher proportion of patients with prior Lyme disease had scleroderma-associated antibodies (12.5% *vs.* 2.9%, *p*=0.026). There was no statistically significant difference in the ANA5 or myositis panels. No participants had both anti-RP11 and anti-RP155, or both anti-PMSCL75 and anti-PMSCL100 antibodies.

We then sought to determine if there was any difference in symptom burden in individuals with PTLD. When com-

Table II. Post-Lyme questionnaire of symptoms (PLQS) total score stratified by autoantibody positivity status among patients following treatment for Lyme disease (median, inter-quartile range).

	Post-treatment Lyme disease (PTLD) n=56			Return to Health (RTH) n=70			All Lyme n=126		
	Positive n=9[a]	Negative n=47[a]	p-value	Positive n=14	Negative n=56	p-value	Positive n=23	Negative n=103	p-value
Total Score	18.0 (14.0-25.0)	20.0 (12.0-31.0)	0.579	2.0 (0.0-5.0)	2.5 (1.0-4.5)	0.673	5.0 (0.0-18.0)	8.0 (2.0-19.0)	0.297
Neurologic sub-score	9.0 (6.0-11.0)	9.0 (6.0-12.0)	0.885	0.5 (0.0-2.0)	1.0 (0.0-2.0)	0.616	2.0 (0.0-8.0)	3.0 (1.0-8.0)	0.370
Musculoskeletal sub-score	2.0 (2.0-3.0)	3.0 (1.0-5.0)	0.400	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.609	1.0 (0.0-2.0)	1.0 (0.0-3.0)	0.615

[a] Two PTLD participants (one autoantibody positive and one autoantibody negative) with missing PLQS total score.

bining across the Lyme PTLD and RTH cohorts, there was no statistically significant difference in PLQS total score by autoantibody positivity status. Similar results were found among those with RTH and those with PTLD (Table II). There was no difference in individual symptom domains (*e.g.* musculoskeletal, neurologic) between these groups. With similar proportions of autoantibody positivity in these cohorts, we assessed the expected false positivity rate. Autoantibody specificity was determined for each autoantibody compared to healthy controls, with 13 tests having a specificity <100% (range: 97–99.5%). Since each autoantibody is considered independent, the probability of having at least one positive test in a healthy control is 17%. This expected false positivity rate is similar to the prevalence of autoantibody positivity in the cohorts presented here.

Discussion

Studies performed in Sweden have found similar rates of myositis-specific and myositis-associated antibodies in patients with PTLD using the same Euroimmun assays (3). In that study, myositis-specific and myositis-associated antibodies were detected in 22% (19 of 85) of patients with PTLD and in 20% (45 of 224) of all patients with persistent symptoms after Lyme disease. In the current study, we find a lower prevalence of myositis-specific and myositis-associated antibodies in PTLD (12%, 7 of 58). This is likely explained by the more stringent antibody positivity cut-off criteria we used. If patients with low positive assays and inconsistent positive tests across panels were instead included as positive, 29%

(17 of 58) patients with PTLD would be considered to have positive myositis-specific and myositis-associated antibodies, slightly higher than the Swedish study. The proportion of positive tests similarly increased in the scleroderma and ANA5 panels using less stringent criteria: 29% (17 of 58) and 19% (11 of 58) in the PTLD cohort, respectively, with a total of 47% (27 of 58) of patients with PTLD making at least one antibody. This was similar in the Lyme RTH cohort, with a total of 54% (38 of 70) of patients making at least one antibody.

While the small sample size is a limitation of our study, we were able to (i) show that autoantibodies are not uniquely detected in patients with persistent symptoms, (ii) extend findings to additional Euroimmun panels (ANA5 and scleroderma), and (iii) show that these findings are not unique to Lyme disease as a similar proportion of patients have autoantibodies following SARS-CoV-2 infection, though scleroderma-associated antibodies were more prevalent after Lyme disease. We also found that there was no difference in total symptom score based on the presence of antibodies, nor were there differences within musculoskeletal or neurologic sub-scores generated from examining specific symptom items (*p*>0.29 for all, Table II).

A limitation is the cross-sectional nature of this study, which does not allow us to explore antibody persistence nor whether there was later evolution into a rheumatic disease. As autoantibodies have traditionally been thought to be transient after infection, our study was enriched with participants with shorter disease duration. However, in the study

of patients with PTLD in Sweden, the median disease duration was four years at the time of sample acquisition, with >80% of patients having disease duration more than one year (3). Given similarities with our study, this suggests that these autoantibodies may persist.

This study highlights an important issue when assessing multiple autoantibodies in post-infectious states: the probability of a type I error (false positivity) increases when multiple, independent variables are tested. While the proportion of participants with at least one autoantibody is high (17%) in this study, this was identical to the expected false positive rate (17%) and was similar to the proportion of healthy individuals (9–14%, n=197) with these autoantibodies in a different study.(8) This is further supported by the low prevalence of any single antibody, which was no higher than 3% (5 of 197).

While our data show no difference in autoantibody prevalence across the post-infectious states, we do not imply that antibodies are irrelevant in this setting. Instead, this highlights a need for discovery of new antibody specificities in these patient cohorts. For example, Steere *et al.* identified MMP-10, apoB-100, ECGF, and annexin A2 antibodies in patients with Lyme arthritis(9). These antibodies associated with distinct synovial tissue pathology and increased risk of persistent inflammation after antibiotics (post-infectious Lyme arthritis).(10)

In this study, we found that several autoantibodies are present following *Borrelia burgdorferi* and SARS-CoV-2 infection. Our study is the first to show that these antibodies are not associated with increased risk of persistent symp-

toms after the acute infection. However, these findings do not exclude autoimmunity as a potential cause of persistent symptoms. This study highlights the need for novel autoantibody discovery in addition to validation and phenotyping in larger cohorts of well-defined patient populations. Such findings will likely provide important novel insights into the relationship between autoimmunity and post-infectious states.

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