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*Brief Report*

# Annexin A2 antibodies in post-treatment Lyme disease

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***Abstract***

**Background:** Anti-annexin A2 (AA2) antibodies have been described in Lyme arthritis and erythema migrans, although they have not been described in post-treatment Lyme disease (PTLD).

**Objectives:** Determine whether anti-AA2 antibodies are present among patients with PTLD and determine the clinical relevance of these antibodies.

**Design and methods:** Anti-AA2 levels were tested serially in a longitudinal cohort of 44 patients with acute Lyme disease, 22 with a return to health (EM RTH), and 22 with PTLD. Anti-AA2 antibodies were also assessed in a cross-sectional group of 281 patients with PTLD.

**Results:** Anti-AA2 antibodies were highest after antimicrobial therapy in both the EM RTH and PTLD cohorts. By 6 months, there was no difference between EM RTH and healthy controls. Anti-AA2 antibodies were higher in the cross-sectional PTLD group (79.69 *versus* 48.22 units, $p < 0.0001$), though with no difference in total symptom burden.

**Conclusion:** Anti-AA2 persists in PTLD, though did not identify a clinical phenotype.

***Keywords:*** anti-Annexin A2, autoantibodies, Lyme disease

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## Introduction

Infection with *Borrelia burgdorferi*, the causative agent of Lyme disease (LD), has the potential to lead to autoimmunity through excessive inflammation and dysregulation of the immune system.[1] Autoantibodies can provide unique insights into immune activation, in addition to characterizing disease phenotypes and prognosis. Through the use of human leukocyte antigen (HLA)-DR presented peptides, Steere *et al.* identified several antigens and autoantibodies associated with Lyme arthritis, including annexin A2 (AA2).[2] In that study, AA2 antigen and antibodies were associated with Lyme arthritis and post-infectious Lyme arthritis, though antibodies were also present in 15% ($n = 104$) of patients with acute LD at the time of the erythema migrans (EM) rash. This suggests autoantibodies develop early during the course of infection and may have other prognostic potential.

Steere *et al.* hypothesized that spirochetes bind to the AA2 antigen early during infection, which then predisposes the AA2 antigen to phagocytosis and subsequent antibody development.[2] In that study, T-cell reactivity to AA2 antigen, as measured by increased interferon secretion, was thought to be unique to Lyme arthritis. However, the proportion of patients with T-cell reactivity was also descriptively higher in patients with EM than in healthy controls (17% *versus* 0%). T-cell reactivity and antibody development after acute LD are of particular interest in the setting of post-treatment Lyme disease (PTLD), an infection-associated chronic illness of poorly understood etiology, and there is growing evidence that upregulation of interferons after early stages of LD may be associated with PTLD pathogenesis.[3,4]

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However, anti-AA2 antibodies have not been described in the setting of PTLD. In this study, we aimed to determine whether anti-AA2 antibodies are present among patients with PTLD, whether they persist after antimicrobial therapy, and whether these antibodies are associated with specific clinical characteristics.

## Methods

Anti-AA2 antibody levels were tested in two different samples of patients with LD. The first included adult patients with physician-diagnosed EM of >5 cm in diameter who were either antibiotic naïve or had received less than 72 h of antibiotics before enrollment. Patients were excluded if symptoms were present for more than 3 months or if there was a preceding history of LD or Lyme vaccination. This cohort included 44 patients with EM who were followed longitudinally at three time points: at the time of diagnosis with EM present (V1), 3 weeks later at the end of initial antibiotic treatment (V2), and 6 months after antibiotics (V3). Patients with EM were sampled such that half met an operationalized definition of PTLD at 6 months, based on the Infectious Diseases Society of America (IDSA) proposed definition requiring a documented episode of LD and post-treatment symptoms of fatigue, neurocognitive complaints, and/or musculoskeletal pain, while half met criteria for a return to health (RTH).[5,6] The median disease duration for this cohort was 6.5 days (range, 1–42 days), and patients were enrolled between 2008 and 2018.

Anti-AA2 antibodies were tested in a second, cross-sectional sample of 281 patients with well-characterized PTLD of heterogeneous illness duration [median 1.72 years (range, 7.1 months–4.0 years)] and more diverse clinical presentations at the time of LD onset.[7] Patients had medical-record confirmation of prior CDC-confirmed or probable LD and current fatigue, musculoskeletal pain, or cognitive complaints that significantly impacted function.[8] Patients from this cohort were enrolled between 2014 and 2021. In both cohorts, patients were excluded for comorbid, pre-Lyme conditions including fibromyalgia and chronic fatigue in addition to those with major immunosuppressive, psychiatric, or autoimmune illness, history of illicit drug or substance abuse, or current pregnancy.

Anti-AA2 was also tested in a cross-sectional sample of 94 healthy controls. Healthy control participants were excluded if there was a previous diagnosis of autoimmune disease (e.g. antiphospholipid syndrome, systemic lupus erythematosus). However, not all healthy controls were specifically screened for a history of LD or other conditions that can be associated with anti-AA2 antibodies.

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).[7] Musculoskeletal and neurologic symptom subdomains were generated by summing specific symptoms from the PLQS, and these were also evaluated.

Anti-AA2 ELISAs were developed, as previously described, using recombinant human AA2 (Novoprotein Scientific Inc., Short Hills, NJ, USA).[2] A positivity cutoff was determined as the 95th percentile of healthy control values (⩾293.8 units). Comparisons by group were performed using the $\chi^2$ test for categorical variables or the Wilcoxon rank sum test for continuous variables. Spearman's rank correlation tests were used to test for associations between two continuous variables.

These investigations adhered to the Declaration of Helsinki, and the Institutional Review Board of the Johns Hopkins University School of Medicine approved this study (NA_00011170, NA_00071455, IRB00035457, IRB00066509), and written informed consent was obtained from all participants prior to initiation of study-related activities. The reporting of this study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology statement.

## Results

Among all patients with EM followed longitudinally, median anti-AA2 antibody levels were the highest at V2 immediately following treatment [V1: 96.29 units interquartile range: (29.73–157.63), V2: 109.15 units (44.62–225.0), V3: 78.89 units (24.20–127.56)]. Similarly, the proportion of patients above the positivity cutoff was highest at V2 (V1: 15.9%, V2: 18.6%, V3: 6.8%). When compared to the median value from healthy

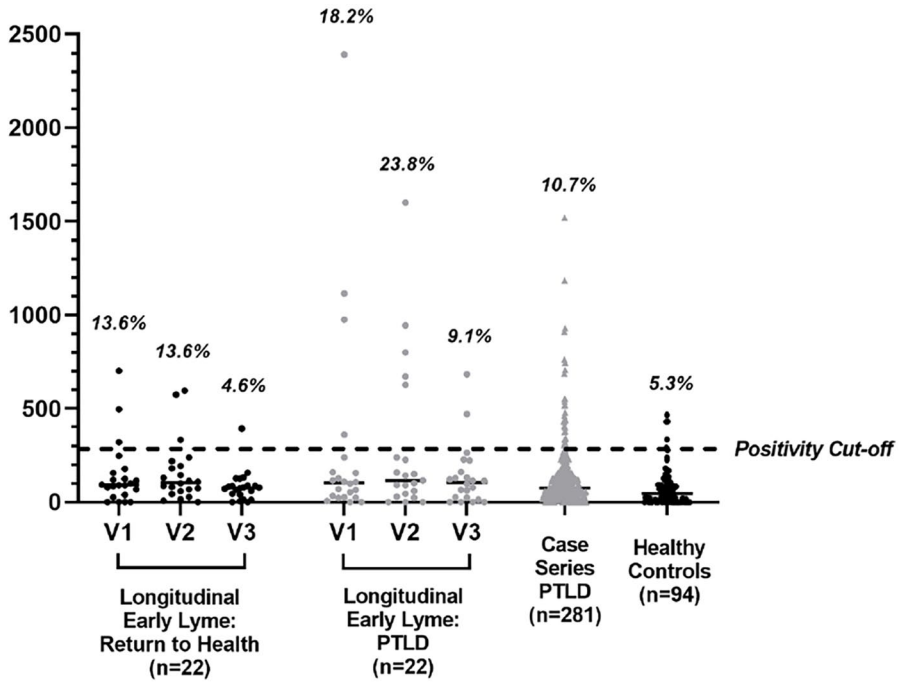


**Figure 1.** Anti-Annexin A2 antibody levels by cohort and visit over time. Also displayed is the percent above positivity cutoff determined by the healthy control sample.

controls [48.22 units (3.09–97.68)], anti-AA2 levels were significantly higher at V1 ($p = 0.009$) and V2 ($p = 0.004$) but not V3 ($p = 0.090$).

Participants in the longitudinal (median age 50 *versus* 34 years, $p = 0.001$) and cross-sectional (47 *versus* 34, $p < 0.0001$) cohorts were older than controls. There were no statistically significant differences by gender in the longitudinal cohort compared to controls; however, there were fewer females (41.96% *versus* 59.57%, $p = 0.003$) in the cross-sectional PTLD group compared to controls. There were no statistically significant differences in anti-AA2 levels by age or gender at any time point, except a weak negative association with age at V1 ($r = -0.334$, $p = 0.027$). There were no differences in anti-AA2 levels by age or gender among patients with PTLD. A secondary analysis controlling for both age and gender in all group comparisons outlined below resulted in similar conclusions and levels of statistical significance (results not shown).

Among patients with EM at V1, anti-AA2 antibodies were not significantly correlated with disease duration ($r = 0.288$, $p = 0.058$), or the number of acute, Lyme-specific symptoms ($r = 0.197$, $p = 0.200$). We also did not find a significant difference in anti-AA2 levels at V1 between those with single compared to disseminated EM [92.88 units (32.86–154.89) *versus* 100.24 units (26.60–246.79), $p = 0.720$]. There were no significant differences in anti-AA2 antibody levels between patients with EM who subsequently met RTH and those who met PTLD criteria at any of the three time points (Figure 1, $p > 0.358$ for each). Although median anti-AA2 levels were nearly twice as high for patients who met the criteria for PTLD at V3 compared to healthy controls, this was not statistically significant (105.89 *versus* 48.22, $p = 0.091$).

However, patients with PTLD in our cross-sectional sample did have higher anti-AA2 levels [79.69 units (41.79–155.89)] compared to controls ($p < 0.0001$). Likewise, the proportion of patients with PTLD above the positivity cut-off (10.7%) was similar to the proportion found among longitudinal cases at V3 (Figure 1, 9.1%) and approximately twice as high as the control proportion (5.3%). Anti-AA2 antibody levels were not significantly correlated with the total score on a measure of symptom burden ($r = -0.071$, $p = 0.251$), and there was no

difference in the neurologic or musculoskeletal symptom subdomains. Anti-AA2 antibody levels were very weakly negatively correlated with illness duration in this more heterogeneous cohort of patients ($r = -0.124$, $p = 0.038$).

## Discussion

Similar to previous studies, we found that anti-AA2 antibodies are present at the time of EM diagnosis. However, we are the first to show the trajectory of antibody levels after treatment of EM. In our longitudinal cohort, we found that anti-AA2 antibodies were highest immediately following antimicrobial therapy and then decreased afterward. By 6 months, there was no difference in anti-AA2 antibody levels between EM RTH and healthy controls.

Our study is also the first to show that anti-AA2 antibodies persist in patients with PTLD. In our cross-sectional group, we show that anti-AA2 antibodies can persist for years after antimicrobial therapy in patients with PTLD. While not statistically significant in the longitudinal cohort, anti-AA2 levels from patients with PTLD were higher than controls in the cross-sectional group. The lack of statistical significance may be due to the smaller numbers in the longitudinal cohort as the proportion of patients with anti-AA2 antibodies was numerically higher than healthy controls and similar to what was found in the cross-sectional group. Anti-AA2 levels were only very weakly correlated with illness duration, though not with total symptom burden nor with the musculoskeletal or neurologic symptom domains on our PLQS questionnaire.

While there was no difference in overall symptom burden or the presence of specific neurologic or musculoskeletal symptoms based on anti-AA2 levels, the persistence of anti-AA2 antibodies in patients with PTLD raises the question as to whether these antibodies provide insight into underlying disease pathophysiology. The AA2 antigen is a phospholipid-binding protein found in keratinocytes and endothelial cells, among other cell types.[9] AA2 facilitates binding of plasminogen, tissue plasminogen activator, and β-2-glycoprotein I, and anti-AA2 autoantibodies have been found to activate endothelial cells in the setting of antiphospholipid syndrome.[9,10] Anti-AA2 antibodies have also been shown to increase the expression of tissue factor on endothelial cells and are thought to contribute to a prothrombotic state, independent of other antiphospholipid antibodies (APLA).[10]

The relationship between anti-AA2 antibodies, APLA, and endothelial dysfunction is of particular interest. The AA2 antigen has been shown to mediate endothelial and subsequent toll-like receptor activation caused by APLA.[11] In previous studies of patients with persistent symptoms after LD, a high proportion of patients were found to have either anticardiolipin or anti-β-2-glycoprotein antibodies.[12] Hu *et al.* also described three novel lipid and phospholipid antibodies that were found in nearly all patients with untreated, acute LD.[13] Similar to the anti-AA2 antibodies in our study, these lipid/phospholipid antibodies often decreased after antimicrobial therapy.[13] However, the lipid/phospholipid antibodies were also assessed in a small group of patients with persistent symptoms after LD, and 33% [4 of 12] of these patients still had antibodies, up to 13 years after LD diagnosis and treatment.[13]

While there is much to learn about endothelial dysfunction in patients with PTLD, we find that post-acute sequelae of COVID-19 (PASC) is an interesting model for comparison. Both PTLD and PASC are post-infectious syndromes that appear to have some degree of overlapping symptom presentations. PTLD and PASC are also both associated with persistent APLA.[12,14] While anti-AA2 antibodies have not been described in PASC, these antibodies were studied in patients hospitalized with COVID-19 and were independently associated with an increased risk of death, with autopsies showing extensive thrombotic disease and endothelial disruption, and there has been interest in using these antibodies in efforts of characterizing patients with PASC.[15]

While several mechanisms have been proposed for PASC, endothelial dysfunction is considered a possible hypothesis to explain ongoing symptoms.[16,17] Ambrosino *et al.* found that endothelium-dependent flow-mediated dilatation was significantly lower in patients with PASC.[16] Lower diffusion capacity for carbon monoxide, in the absence of pulmonary fibrosis and anemia, has also been used to support the notion of

endothelial dysfunction in patients with PASC.[17] Dysautonomia and neurocognitive deficits have also been linked to endothelial dysfunction in patients with PASC.[18,19] Further characterization of endothelial function and dysautonomia is a major focus of ongoing PTLD research.

Our study has several limitations. Healthy controls in this study were excluded if there was a history of autoimmune disease, though other potential confounders (e.g. previous Lyme exposure) were not assessed among all control participants. While we found no associations between total symptom burden nor musculoskeletal or neurologic subdomains in patients with anti-AA2 antibodies, another limitation of this study is that it is possible that a larger sample size could potentially demonstrate clinical differences in these and other analyses.

## Conclusion

Our study is the first to show that anti-AA2 antibody levels are higher in patients with PTLD compared to healthy controls. That anti-AA2 levels were only very weakly correlated with illness duration, and not with symptom burden, suggests that more research is needed to determine the factors associated with the development and persistence of anti-AA2 antibodies among patients with PTLD.

## Declarations

### *Ethics approval and consent to participate*

These investigations adhered to the Declaration of Helsinki, and the Institutional Review Board of the Johns Hopkins University School of Medicine approved this study (NA_00011170, NA_00071455, IRB00035457, IRB00066509), and written informed consent was obtained from all participants prior to initiation of study-related activities.

### *Consent for publication*

Not applicable.

### *Author contributions*

**John B. Miller:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Writing – original draft; Writing – review & editing.

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**John N. Aucott:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

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### *Competing interests*

ED is a current employee of AstraZeneca. The remaining authors declare no conflicts of interest.

### *Availability of data and materials*

Datasets will be made available upon reasonable request.

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## Supplemental material

Supplemental material for this article is available online.

## References

1. Arvikar SL, Crowley JT, Sulka KB, *et al*. Autoimmune arthritides, rheumatoid arthritis, psoriatic arthritis, or peripheral spondyloarthritis following Lyme disease. *Arthritis Rheumatol* 2017; 69: 194–202.
2. Pianta A, Drouin EE, Crowley JT, *et al*. Annexin A2 is a target of autoimmune T and B cell responses associated with synovial fibroblast proliferation in patients with antibiotic-refractory Lyme arthritis. *Clin Immunol* 2015; 160: 336–341.
3. Sjöwall J, Fryland L, Nordberg M, *et al*. Decreased Th1-type inflammatory cytokine expression in the skin is associated with persisting symptoms after treatment of erythema migrans. *PLoS One* 2011; 6: e18220.
4. Hernández SA, Ogrinc K, Korva M, *et al*. Association of persistent symptoms after Lyme neuroborreliosis and increased levels of interferon-α in blood. *Emerg Infect Dis* 2023; 29: 1091–1101.
5. Aucott JN, Yang T, Yoon I, *et al*. Risk of post-treatment Lyme disease in patients with ideally-treated early Lyme disease: A prospective cohort study. *Int J Infect Dis* 2022; 116: 230–237.
6. Wormser GP, Dattwyler RJ, Shapiro ED, *et al*. The clinical assessments treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: Clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2006; 43: 1089–1134.
7. Rebman AW, Bechtold KT, Yang T, *et al*. The clinical, symptom, and quality-of-life characterization of a well-defined group of patients with posttreatment Lyme disease syndrome. *Front Med* 2017; 4: 224.
8. Centers for Disease Control and Prevention. Lyme disease (*Borrelia burgdorferi*) 2011 case definition, https://ndc.services.cdc.gov/case-definitions/lyme-disease-2011 (2011, accessed 25 September 2023).
9. Salle V, Mazière JC, Smail A, *et al*. Anti-annexin II antibodies in systemic autoimmune diseases and antiphospholipid syndrome. *J Clin Immunol* 2008; 28: 291–297.
10. Cesarman-Maus G, Ríos-Luna NP, Deora AB, *et al*. Autoantibodies against the fibrinolytic receptor, annexin 2, in antiphospholipid syndrome. *Blood* 2006; 107: 4375–4382.
11. Zhang J and McCrae KR. Annexin A2 mediates endothelial cell activation by antiphospholipid/anti-β2glycoprotein I antibodies. *Blood* 2005; 105: 1964–1969.
12. Greco TP, Conti-Kelly AM and Greco TP. Antiphospholipid antibodies in patients with purported 'chronic Lyme disease'. *Lupus* 2011; 20: 1372–1377.
13. Gwynne PJ, Clendenen LH, Turk S-P, *et al*. Antiphospholipid autoantibodies in Lyme disease arise after scavenging of host phospholipids by *Borrelia burgdorferi*. *J Clin Invest* 2022; 132: e152506.
14. Pisareva E, Badiou S, Mihalovičová L, *et al*. Persistence of neutrophil extracellular traps and anticardiolipin auto-antibodies in post-acute phase COVID-19 patients. *J Med Virol* 2023; 95: e28209.
15. Zuniga M, Gomes C, Carsons SE, *et al*. Autoimmunity to annexin A2 predicts mortality among hospitalised COVID-19 patients. *Eur Respir J* 2021; 58: 2100918.
16. Ambrosino P, Calcaterra I, Molino A, *et al*. Persistent endothelial dysfunction in post-acute covid-19 syndrome: A case–control study. *Biomedicines* 2021; 9: 957.
17. Kersten J, Wolf A, Hoyo L, *et al*. Symptom burden correlates to impairment of diffusion capacity and exercise intolerance in long COVID patients. *Sci Rep* 2022; 12: 8801.
18. Chopoorian AH, Wahba A, Celedonio J, *et al*. Impaired endothelial function in patients with postural tachycardia syndrome. *Hypertension* 2021; 77: 1001–1009.
19. Park KH, Han SJ, Kim HS, *et al*. Endothelial function and cardiovascular autonomic activity in neurally mediated syncope. *Cardiology* 2016; 134: 65–71.