Infect Dis Ther (2019) 8:5–22
https://doi.org/10.1007/s40121-018-0223-0

REVIEW

Biologic Markers of Antibiotic-Refractory Lyme Arthritis in Human: A Systematic Review

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Received: October 16, 2018 / Published online: November 30, 2018

ABSTRACT

Introduction: Lyme disease—also known as Lyme borreliosis (LB)—is the most common vector-borne disease in North America and Europe. It may result in substantial morbidity, primarily from persistent Lyme arthritis (LA) that—although treatable—can develop into antibiotic-refractory LA (A-RLA). The aim of this study is to systematically review and evaluate a range of biomarkers for their potential predictive value in the development of A-RLA.

Methods: We conducted a systematic review of studies examining biomarkers among patients with A-RLA from MEDLINE via OVID, EMBASE and Web of Science databases and identified a total of 26 studies for qualitative analysis.

Results: All studies were of patient populations from the USA, with the exception of one from Europe. We identified an array of biomarkers that are commonly modulated in the A-RLA compared with subjects with antibiotic-responsive LA. These included a range of inflammatory markers (IL-6, IL-8, IL-10, IL-1β, IL-23, IL-17F, TNFα, IFNγ, CXCL9, CXCL10, CCL2, CCL3 and CCL4, CRP), factors along the innate and adaptive immune response pathways (e.g., $CD4^+$ T cells, GITR receptors, OX40 receptors, $IL\text{-}4^+CD4^+$Th2 cells, $IL\text{-}17^+CD4^+$ T cells) and an array of miRNA species (e.g., miR-142, miR-17, miR-20a, let-7c and miR-30fam).

Conclusion: The evidence base of biologic markers for A-RLA is limited. However, a range of promising biomarkers have been identified. Cytokines and chemokines related to Th17 pathway together with a number of miRNAs species (miR-146a, miR-155 and let-7a) may be promising candidates in the prediction of A-RLA. A panel of multiple biomarkers may yield clinically relevant prediction of the possible resistance at the time of LA first diagnosis.

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s40121-018-0223-0) contains supplementary material, which is available to authorized users.

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Funding: Public Health Agency of Canada.

Keywords: Antibiotic-refractory Lyme arthritis; Biomarkers; Human; Inflammation; Systematic review

INTRODUCTION

Lyme disease or Lyme borreliosis (LB) is caused in humans by at least three genospecies of the *Borrelia burgdorferi* sensu lato complex: *B. burgdorferi*, *B. garinii* and *B. afzelii*. A bite from an infected *Ixodes scapularis* and *Ixodes pacificus* blacklegged ticks initiates this bacterial infection that leads to LB. The early stage of the disease can develop later to a number of long-term complications such as Lyme arthritis (LA) and Lyme carditis [1]. In North America and Europe, LB presently is the most common vector-borne disease [1] with > 30,000 cases reported annually in the USA [2]. However, the actual prevalence estimates are thought to be up to ten times as high because of the underreporting [3]. Moreover, approximately sixfold increased incidence in LB was noted in Canada between 2009 (128 cases) and 2015 (707 cases) [4].

Early symptoms of LB usually start 1–2 weeks following the tick bite with a proportion of the infected subjects developing the characteristic erythema migrans (EM) rash. EM can last for a period of 4 weeks or more. Symptoms such as headache, myalgia, fever, malaise, fatigue and chills may also accompany this stage. If untreated, systemic dissemination of the bacteria may occur via the lymphatic system or blood to the cardiovascular system, nervous system and joints. Weeks to months after the tick bite, early disseminated LB may emerge with symptoms such as Lyme-associated facial nerve palsy and an array of cardiac conditions such as palpitations, shortness of breath or chest pain [5]. A range of inflammatory processes may occur about 6 months after infection as suggested by the development of joint pain and swelling and synovial fluid findings. Months to years after the initial tick bite, the disease can progress to the late disseminated stage. This stage may lead to substantial morbidity, primarily from persistent arthritis (LA) that may occur in ~ 60% of untreated patients [5], rendering it as one of the most common long-term consequences of the late disseminated LB stage. In this case, LA clinically manifests as intermittent or persistent arthritis in the joints for several years.

Current recommendations for treatment of LA patients include initial oral doxycycline (100 mg twice daily) or amoxicillin (500 mg thrice daily) for 30 days. In patients who are unable to take either of these agents, cefuroxime axetil (500 mg twice daily) may be used as an alternative [6]. In patients with antibiotic-refractory LA (A-RLA) treated with antibiotics, PCR testing for *B. burgdorferi* DNA in the affected joint fluid is usually negative, which suggests that the A-RLA persists despite near or total eradication of the pathogen from the joint [7]. One of the major clinical and public health challenges related to A-RLA is the lack of ability to determine which LB patients may develop A-RLA [8]. This is particularly true given the possible post-treatment eradication of the pathogen [9]. Biologic markers evaluated prior to or at the time of treatment may be useful in the prediction of those individuals who may be at risk of developing A-RLA [9].

We conducted a systematic review to summarize the literature documenting the changes in immunologic (e.g., immune response or cytokine and chemokine expression) and genetic biomarkers (e.g., expression and polymorphisms in genes regulating the immune system) in response to LB and their role in the development of A-RLA. The objective of this study was to evaluate the potential predictive value of these biomarkers in the progression of LA to A-RLA.

METHODS

Literature Search

We conducted a systematic review of studies examining biomarkers among patients with A-RLA from MEDLINE via OVID, EMBASE and Web of Science. We examined studies published from 1 January 1982 to 15 December 2017. A

broad search using the following MeSH terms was conducted: (Lyme) AND (arthritis) AND (antibiotic-refractory OR antibiotic OR refractory). We limited our search to studies conducted in humans and published in English with the inclusion of biologic markers associated with or predictive of disease risk and/or prognosis. The systematic review was conducted in line with the Preferred Reporting for Systematic Review and Meta-Analyses (PRISMA; see Fig. 1 and Supplementary Table 1) [10]. All abstracts and titles were screened independently in duplicate with any conflicts determined by a third reviewer.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were defined using the Population, Exposure, Comparator, Outcome, Study Design (PECOS) table (Supplementary Table 2). We included studies examining biologic markers of any type among adults or children with no age or sex restrictions. The exposure of interest was LA. A broad array of outcomes of interest was included, such as associations of biomarkers with A-RLA in a population sample, association of biomarkers with A-RLA compared with responsive LA and prediction of A-RLA at the time of diagnosis of

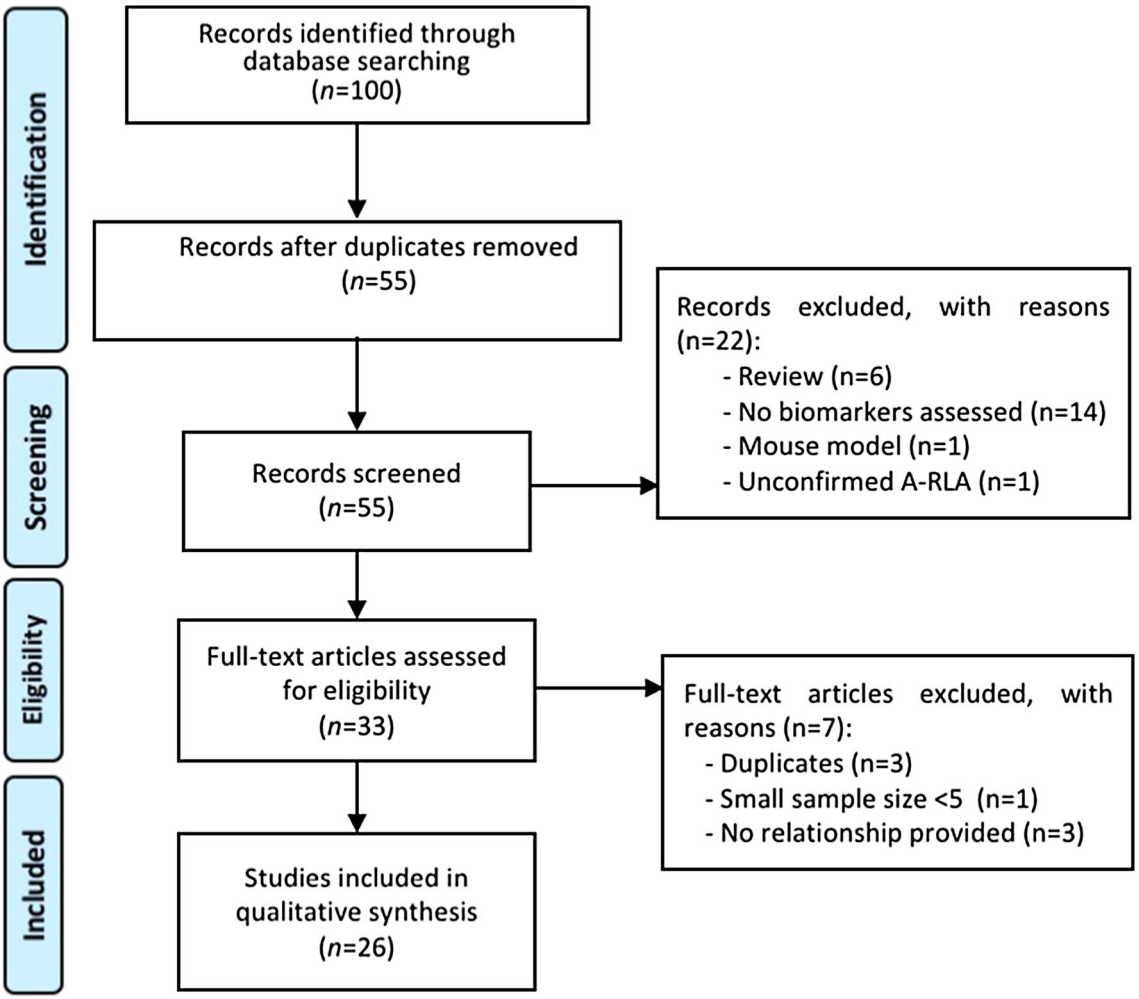

Fig. 1 Flowchart of the study selection and systematic literature review process. The flow diagram describes the systematic review of literature on the biologic markers of antibiotic-refractory LA in humans. A total of 26 unique studies were identified for qualitative analysis

LA. Any potentially relevant study design, either intervention or observational, was included. Case reports and review articles were excluded. Studies prior to 1982 were not considered as it is the discovery date of *B. burgdorferi*. Only publications in English were included in this study.

Data Extraction

We developed and tested a data extraction template using two blinded reviewers. All relevant study and population characteristics were extracted in addition to specific methods around biologic sample collection and biomarker quantification (Supplementary Table 3). All data extracted were performed in duplicate. Upon completion of data extraction, we grouped studies into those reporting associations with immune response and genetic biomarkers separately for synthesis and comparative discussion.

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

RESULTS

In total, 26 studies were identified that met the specified search criteria and contained relevant results of interest, which are summarized in Table 1. All studies were of patient populations from the USA, with the exception of one from Germany [11]. These studies represented a relatively homogeneous study population, with most cases being ascertained from Tufts Medical Center or Massachusetts General Hospital [12–26]. However, there were a few reports that did not state where cases were ascertained [17], many of which were conference abstracts [28–35]. One study was conducted across the USA [36]. Of the studies that reported sex distributions within the study populations, all included both males and females [11, 12, 17, 18, 20–25, 36]. In addition, of the studies that reported study population ages, most were from adult populations but also included children above the age of 12 years [17, 18, 20–25]. The study from Germany [11] was exclusively from a pediatric population with two reports that were only in adults [12, 36]. All biologic samples used for analysis were derived from peripheral blood, serum or synovial fluid and/or tissue.

The immune responses to *B. burgdorferi*, its antigens, or other proteins in A-RLA were evaluated in ten studies [14–16, 19, 24, 28, 30–33], whereas six studies examined other biomarkers (predominantly immune markers) of A-RLA [11, 13, 17, 21, 27, 36]. Of the selected studies, five examined various types of immune cells in A-RLA [18, 20, 22, 23, 34], while five other studies evaluated an array of genetic markers [12, 26, 28, 29, 35]. As shown in Table 2, the studies that investigated immune responses demonstrated significantly higher reactivity to matrix metalloproteinase (MMP)-10 [14, 30], annexin A2 [15, 33], apolipoprotein B-100 [16, 32] and endothelial cell growth factor (ECGF) [19] in A-RLA patients compared with healthy controls. Additionally, compared with antibiotic-responsive LA, A-RLA patients had significantly higher reactivity to the variable major protein-like sequence-expressed (VlsE) lipoprotein [24] as well as to human ECGF (hECGF) [31]. When stimulated with *B. burgdorferi* or interferon (IFN)-γ, A-RLA patients also exhibited particularly high levels of MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor (TNF), C–C motif chemokine ligand (CCL)-2, C–X–C motif chemokine ligand (CXCL)-9 and CXCL-10 in fibroblast-like synoviocytes [28].

Compared with healthy controls, A-RLA patients exhibited significantly higher levels of C-reactive protein (CRP) in their blood or synovial fluid/tissue [36]. Compared with antibiotic-responsive LA patients, studies found a significantly higher level of immune mediators associated with T-helper 1 and T-helper 17 cell immune responses, such as CCL2, CCL3, CCL4, TNF-α, CXCL9, CXCL-10, IL-6, IL-8, IL-10, IL-1β, IL-17F, IL-23 and IFN-γ [13, 21, 27, 31]. Furthermore, A-RLA patients demonstrated

Table 1 Characteristics of the selected studies

Objective	Study[a]	Number of subjects		Males (%)	Age (years)		Specimens
		A-RLA	Comparative group(s)		Range	Average	
Etiology of A-RLA vs. responsive LA and/or other arthritis	Strle et al. 2017 [13]	81	60 Antibiotic-responsive LA				Serum and SF
	Crowley et al. 2016 [14]	114	58 Healthy controls 91 Antibiotic-responsive LA				Serum, PBMCs, SF
	Strle et al. 2014 [31]	159					SF
	Drouin et al. 2013 [19]	109	74 Healthy controls 21 Rheumatoid arthritis 77 Antibiotic-responsive LA				SF and serum
	Strle et al. 2012 [21]	101	76 Antibiotic-responsive LA		12–79		PBMCs
	Strle et al. 2011 [35]	12	5 Antibiotic-responsive LA				SF
	Kannian et al. 2007 [24]	41	23 Antibiotic-responsive LA	66	13–64	41	Serum
	Shin et al. 2007 [25]	35	17 Antibiotic-responsive LA	75	12–79	38	SF and tissue
	Nimmrich et al. 2014 [11]*	8	23 Antibiotic-responsive LA	37.5		11 ± 2	Serum
	Londono et al. 2014 [17]	14	6 Other arthritis	50	11–43	16	SF
	Vudattu et al. 2011 [34]	16	15 Antibiotic-responsive LA				PBMCs and SF mononuclear cells
	Shen et al. 2010 [22]	12	6 Antibiotic-responsive LA	67	11–54	29	PBMCs and SF mononuclear cells
	Kannian et al. 2007 [23]	7	6 Antibiotic-responsive LA	77	12–64	25	PBMCs and SF mononuclear cells

Table 1 continued

Objective	Study[a]	Number of subjects		Males (%)	Age (years)		Specimens
		A-RLA	Comparative group(s)		Range	Average	
Lochhead et al. 2017 [12]	27	5 Pre-treatment controls 8 Other arthritis		69	17–76	45	SF
Lochhead et al. 2015 [29]	10	6 Antibiotic-responsive LA 5 Other arthritis					SF
Strle et al. 2011 [27]	101	76 Antibiotic-responsive LA					SF
Steere et al. 2006 [26]	71	50 Antibiotic-responsive LA					Not specified
Mechanisms of A-RLA vs. healthy controls	Pianta et al. 2015 [15]	89	52 Healthy controls				Serum, SF
	Crowley et al. 2015 [16]	94	57 Healthy controls				Serum, SF
	Vudattu et al. 2013 [18]	16	15 Antibiotic-responsive LA 13 healthy controls	69	12–62	29	Peripheral blood and SF
	Crowley et al. 2015 [30]	114					SF
	Crowley et al. 2014 [32]						Serum
	Pianta et al. 2014 [33]						SF and serum
	Katchar et al. 2013 [20]	15	8 Antibiotic-responsive LA 4 Healthy controls	53	12–78	29	Peripheral blood and SF
Genetics of A-RLA	Strle et al. 2015 [28]						SF

Table 1 continued

Objective	Study[a]	Number of subjects		Males (%)	Age (years)		Specimens
		A-RLA	Comparative group(s)		Range	Average	
Early vs. late manifestations of LD	Uhde et al. 2016 [36]	11	67 Healthy controls	63.6		53 ± 21	Serum

All studies were of patient populations from the USA, except as noted (*) from Germany
A-RLA antibiotic-refractory Lyme arthritis, *EM* erythema migrans, *RA* rheumatoid arthritis, *PBMCs* peripheral blood mononuclear cells, *FS* synovial fluid, *LD* Lyme disease
[a] Empty cells denote information not available from the original study

significantly higher expression of the *B. burgdorferi* proteins p58 and outer surface protein (Osp) C compared with their antibiotic-responsive LA counterparts [11]. One study investigated histologic findings in the synovial tissue of A-RLA patients compared with other arthritis patients and found differences in lining layer thickness, global cellular infiltration, lymphoid aggregates and obliterative macrovascular lesions, which were more common in A-RLA patients [17].

When examining immune cell types, compared with healthy controls, A-RLA patients had higher levels of memory $CD4^+$ T cells [18] in peripheral blood, while the $CD3^+$ T cells were significantly lower [20]. Compared with antibiotic-responsive LA patients, A-RLA patients had lower levels of $CD4^+$ T cells [18] (although higher levels were observed in one study [34]), CD56bright natural killer (NK) cells [20] and $V\alpha24^+$ NKT cells [20] in synovial fluid. Higher numbers of $IL\text{-}4^+CD4^+$ Th2 cells, $IL\text{-}17^+CD4^+$ T cells and $FoxP3^+$ Treg cells were all found in A-RLA patients compared with antibiotic-responsive subjects [22]. One study, however, did not find a significant difference in OspA161-175-specific T-cell frequencies or proliferation responses between A-RLA and responsive patients [23].

Five studies evaluated the genetic markers of A-RLA (Table 3). Of those, two studies examined the microRNA (miRNA) expression and reported that miR-146a, miR-142, miR-17, miR-155, miR-223 and miR-20a were significantly elevated in post- vs. pre-antibiotic treated A-RLA [12]. These miRNA species, in addition to let-7a, let-7c and miR-30fam, were also significantly higher in A-RLA patients compared with patients with osteoarthritis [12, 29]. Patients with A-RLA also exhibited higher levels of miR-146a, miR-155, miR-223 and miR-142 than the antibiotic-responsive LA patients [29]. Another study reported the frequency of the 1805GG single-nucleotide polymorphism (SNP) in the Toll-like receptor-1 gene (*TLR-1*) to be significantly lower in A-RLA patients than in the antibiotic-responsive LA patients [35]. Cells with this 1805GG SNP were shown to have an altered mRNA expression of the suppressor of cytokine signaling (SOCS)-3, suggesting that greater inflammatory responses in A-RLA patients with this polymorphism may be due to the loss of a cytokine regulatory pathway [28]. Lastly, allele haplotype frequencies of human leukocyte antigen (HLA) were investigated in one study and found that HLA-DRB1-DQA1-DQB1 haplotype frequencies were similar between A-RLA patients and subjects with antibiotic-responsive LA [26]. However, the frequency of DRB1 alleles differed markedly, and a significantly larger number of A-RLA patients showed binding to the outer surface protein A (OspA) than their antibiotic-responsive LA counterparts [26]. Our systematic assessment of the literature allowed us to identify an array of biomarkers that are commonly upregulated in the A-RLA compared with subjects with antibiotic-responsive LA (Table 4). These included a range of inflammatory markers (IL-6, IL-8, IL-10, IL-1β, IL-23, IL-17F, TNFα, IFNγ, CXCL9, CXCL10, CCL2, CCL3 and CCL4, CRP), factors related to innate and adaptive

Table 2 Summary of studies reporting immune biomarkers in antibiotic-refractory LA in humans

Study	Biomarker class	Biomarker(s)	Assay	Summary of results
Strle et al. 2017 [13]	Innate immune cytokine response	CCL3, TNF-α, CXCL9, IL-17F	Luminex assay	A total of 21 mediators associated with innate, T-helper 1 cell and T-helper 17 cell immune responses were assessed in serum and SF IL-17F in serum and CCL2, CCL3, TNF-α and CXCL9 in SF were significantly higher in A-RLA compared with antibiotic-responsive LA
Crowley et al. 2016 [14]	Immune response in A-RLA	MMP-10 stimulated PBMCs, serum MMP-10, synovial fluid MMP-10, synovial fluid MMP-3	Bead-based multiplex coupled with Luminex assay	The response was specific to MMP-10-stimulated PBMCs that had a significantly higher T-cell and B-cell reactivity in A-RLA compared with healthy controls and antibiotic-responsive LA
Pianta et al. 2015 [15]	Cytokine response and expression	T-cell reactivity (IFN-γ response) and IgG response to annexin A2/annexin A2 protein levels in SF and serum	ELISA	Significantly higher response (IFN-γ and IgG) to annexin A2 and elevated annexin A2 protein levels (in SF and serum) among A-RLA compared with healthy controls
Crowley et al. 2015 [16]	Cytokine response	T-cell reactivity (IFN-γ response) and IgG response to apolipoprotein B-100 and apolipoprotein B-100 in serum	ELISA	Apolipoprotein B-100 protein levels were also significantly higher in serum of A-RLA compared with healthy controls Significantly higher IgG response to apolipoprotein B-100 among A-RLA compared with healthy controls T-cell reactivity (IFN-γ) was borderline significant ($p = 0.06$) in A-RLA compared with healthy controls

Table 2 continued

Study	Biomarker class	Biomarker(s)	Assay	Summary of results
Strle et al. 2015 [28]	Cytokine response	MMP1, MMP2, MMP3, MMP9, MMP13, IL-6, IL-8, IL-10, TNF, CCL2, CXCL9, CXCL10 from FLS simulated with *B. burgdorferi*	Luminex assay	A total of 8 MMPs and 21 cytokines and chemokines were assayed in FLS A-RLA exhibited significantly higher levels of IL-6, IL-8, IL-10, TNFα, CCL2, CXCL9, CXCL10
Crowley et al. 2014 [32]	Cytokine response	Response of T cell (autoantibody) to MMP-10	Not specified	Higher numbers of A-RLA patients had robust or autoantibody T-cell responses to MMP-10—compared with antibiotic-responsive LA, healthy controls or rheumatoid arthritis patients
Strle et al. 2014 [31]	Levels of inflammatory cytokines and chemokines and response to cytokines	Th17-associated mediators and frequency of autoantibody responses to hECGF	Luminex	Higher levels of Th17 associated mediators (e.g., IL-23) and a greater frequency of autoantibody responses to hECGF among A-RLA compared with antibiotic-responsive LA
Crowley et al. 2014 [32]	Cytokine response	T-cell and B-cell reactivity (IgG anti-ApoB antibodies) to apolipoprotein B-100	ELISA	Significantly higher frequency of A-RLA had T-cell and B-cell responses to anti-ApoB IgG antibodies compared with healthy controls and patients with EM
Pianta et al. 2014 [33]	Cytokine response	Anti-annexin A2 IgG autoantibody response in serum	ELISA	Significantly higher autoantibody response in A-RLA compared with healthy controls, but similar to that in antibiotic-responsive LA

Table 2 continued

Study	Biomarker class	Biomarker(s)	Assay	Summary of results
Drouin et al. 2013 [19]	Autoantibody and autoantigen responses	Anti-ECGF IgG autoantibody response in serum and ECGF in serum	ELISA	Significantly higher number of A-RLA had positive autoantibody responses to ECGF compared with healthy controls A-RLA exhibited ECGF autoantibodies more frequently than in antibiotic-responsive LA A-RLA showed significantly higher levels of ECGF in SF compared with antibiotic-responsive LA
Strle et al. 2012 [21] Strle et al. 2011 [35]	Chemokine and cytokine levels	CXCL9, CXCL10, IL-6, IL-8, IL-10, IL-1β, CCL2, CCL3, CCL4, TNF, IFNγ	Bead-based multiplex assay	CXCL9, CXCL10, IL-6, IL-8, IL-10, IL-1β, CCL2, CCL3, CCL4, TNF, IFNγ were more common in (and in significantly higher levels in the SF of) A-RLA compared with antibiotic-responsive LA
Kannian et al. 2007 [23]	Antibody response	IgG antibody titers in response to *B. burgdorferi* antigens in serum	ELISA	In A-RLA, during the first 1–3 months after treatment, antibody response to the VlsE peptide declined while the titers to *B. burgdorferi* DbpA, OspA and Arp increased Synovial inflammation persisted in A-RLA after infection compared with antibiotic-responsive LA
Shin et al. 2007 [25]	Chemokines and cytokines levels in response to antibiotic treatment	CXCL8, CXCL9, CXCL10, IL-1β, IL-5, IL-6, CCL2, CCL3, CCL4, TNF, IFNγ	Cytometric bead array	Compared with antibiotic-responsive LA, A-RLA exhibited significantly higher CXCL8, CXCL9, CXCL10, CCL4, IL-6, IL-1β, TNF and IFNγ during the antibiotic treatment period and higher CXCL9, CXCL10, IL-5, IL-1β, CCL2, CCL3 and CCL4 following the treatment

Table 2 continued

Study	Biomarker class	Biomarker(s)	Assay	Summary of results
Uhde et al. 2016 [36]	Acute phase reactants	CRP and amyloid A	ELISA	Significantly higher CRP but not amyloid A in A-RLA compared with healthy controls
Nimmrich et al. 2014 [11]	Protein expression	p58, OspC, P100, VlsE, P39, Ospa and p18	Western blot	Significantly higher IgG p58 and OspC expression—but not P100, VlsE, P39, Ospa and p18—in A-RLA compared with antibiotic-responsive LA
Vudattu et al. 2013 [18]	Leukocytes	Monocytes, $CD4^+$ T cells, in peripheral blood or SF	Flow cytometry	Compared with healthy controls, A-RLA exhibited higher levels of monocytes and $CD4^+$ T cells in peripheral blood Compared with antibiotic-responsive LA, A-RLA had lower $CD4^+$ T cells in SF
Katchar et al. 2013 [20]	Lymphocytes	$CD3^+$ T cells in peripheral blood, CD56 bright NK cells and $V\alpha24^+$ iNKT cells in SF	Flow cytometry	A-RLA had lower $CD3^+$ T cells in peripheral blood compared with healthy controls and lower CD56 bright NK cells and $V\alpha24^+$ iNKT cells in SF compared with antibiotic-responsive LA
Vudattu et al. 2011 [34]	Lymphocytes and phenotypes of lymphocytes	$CD4^+$ T cells and expression of GITR and OX40 receptors	Flow cytometry	Increased $CD4^+$ T cells and GITR and OX40 receptors expression in A-RLA compared with antibiotic-responsive LA
Shen et al. 2010 [22]	Phenotypes of lymphocytes	IL-4^+CD4^+Th2 cells, IL-17^+CD4^+ T cells, FoxP3^+Treg cells	Flow cytometry	Significantly higher numbers of IL-4^+CD4^+Th2 cells, IL-17^+CD4^+T cells and FoxP3^+Treg cells were found in A-RLA compared with antibiotic-responsive LA
Kannian et al. 2007 [23]	Lymphocytes	OspA161–175-specific T cells	Flow cytometry	No significant differences in OspA161–175-specific T-cell frequencies or proliferation responses between A-RLA and antibiotic-responsive LA

Table 2 continued

Study	Biomarker class	Biomarker(s)	Assay	Summary of results
Londono et al. 2014 [17]	Histologic findings	Lining layer thickness, global cellular infiltration, lymphoid aggregates, obliterative macrovascular lesions	Histologic analysis (tissue staining)	Lining layer thickness, global cellular infiltration, lymphoid aggregates, obliterative macrovascular lesions were all more common in A-RLA compared with other arthritis cases

MMPs matrix metalloproteinases, *PBMCs* peripheral blood mononuclear cells, *SF* synovial fluids, *FLS* fibroblast-like synoviocytes, *hECGF* human endothelial cell growth factor, *EM* erythema migrans, *DbpA* decorin binding protein A, *VlsE* variable major protein-like sequence expressed lipoprotein, *Arp* arthritis-related protein, *OspA* outer surface protein A, *OspC* outer surface protein C, *CRP* C-reactive protein, *GITR* glucocorticoid-induced TNFR-related protein, *A-RLA* antibiotic-refractory Lyme arthritis

immune responses (e.g., $CD4^+$ T cells, GITR receptors, OX40 receptors, IL-4^+CD4^+ Th2 cells, IL-17^+CD4^+ T-cells) and biomarkers such as annexin A2, hECGF.

DISCUSSION

To date, there is a relatively limited evidence base of large, adequately powered studies to assess the predictive ability of biomarkers for A-RLA. Most studies presented here did not include more than 120 A-RLA cases. However, there is emerging information to suggest an array of both immunologic and genetic biomarkers that can be utilized in characterizing A-RLA. While most studies focused on immune response biomarkers, including cytokines, chemokines and immune cell typing, there were also a number of studies evaluating a range of genetic biomarkers, such as miRNAs, SNPs and haplotype frequencies that can be used to identify or predict a status of A-RLA in LB patients.

Of the genetic biomarkers that have been studied, there are several miRNAs that appear to be the highly promising. The miRNAs are small non-coding RNA molecules that function in post-transcriptional gene regulation [37, 38]. Altered miRNA expression has been implicated in the pathogenesis of several inflammatory and autoimmune diseases, including rheumatoid arthritis [39, 40]. In addition to inflammatory responses, miRNAs can also play a role in bone destruction and remodeling [41]. Only two studies examined differential miRNA expression in A-RLA patients in which some of the miRNAs identified here are consistent with those characterized in rheumatoid arthritis, i.e., miR-146a, miR-155, miR-223 and let-7a [39]. Other miRNAs, however, appear to be unique to A-RLA such as miR-142, miR-17, miR-20a, let-7c and miR-30fam [12, 29]. The miR-146 and miR-155 species are known to play a role in immune functioning as shown in mouse models where both were upregulated during *B. burgdorferi* infection [42, 43]. These studies demonstrated that miR-146a may act as a negative regulator of immune activation whereas miR-155 as a positive regulator [42, 43]. Important roles in cellular proliferation and regulation of inflammatory processes and responses were also suggested for miR-223 and miR-17 [44–46]. Higher levels of these miRNA species were reported in A-RLA patients [12, 29], indicating a status of dysregulated repair of the damaged tissue. With new insights into the roles of miRNAs in human diseases, it is likely that future research will reveal specific mechanisms through which these miRNAs contribute to A-RLA and how they may be used not only as predictive biomarkers of A-RLA patients but also as potential therapeutic targets.

There has been considerably more research on immune biomarkers as potential predictive

Table 3 Summary of studies reporting genetic biomarkers in antibiotic-refractory LA in humans

Study	Biomarker class	Biomarker(s)	Assay	Summary of results
Lochhead et al. 2017 [12]	miRNA	miR-146a, miR142, miR17, miR-155, miR-223, miR20a, let-7a, let-7c	PCR	miR-146a, miR142, miR17, miR-155, miR-223 and miR20a were higher in post- vs. pre-antibiotic treated A-RLA miR-146a, miR142, miR17, miR-155, miR-223, miR20a, let-7a and let-7c were higher in A-RLA compared with osteoarthritis
Lochhead et al. 2015 [29]	miRNA (extracellular)	miR-146a, miR-155 (inflammatory signature), miR-30fam (vascularization signature), miR223, miR142	qPCR	miR-146a, miR-155, miR-223 and miR-142 were higher in A-RLA compared with antibiotic-responsive LA miR-146a, miR-155, miR-30fam, miR223 and miR-142 were upregulated in A-RLA compared with osteoarthritis patients
Strle et al. 2015 [28]	mRNA expression	*SOCS3* mRNA expression in cells with 1805GG polymorphism in *TLR1*	QuantiGene and whole-genome RNASeq	Altered *SOCS3* mRNA expression in A-RLA compared with antibiotic-responsive LA, i.e., greater inflammatory responses
Strle et al. 2011 [35]	SNPs	Frequency of 1805GG polymorphism in *TLR1* gene	PCR	Frequency of the 1805GG polymorphism was lower in A-RLA compared with antibiotic-responsive LA
Steere et al. 2006 [26]	HLA typing and binding to OspA	HLA-DRB1-DQA1-DQB1/DRB1 allele frequency	High-resolution molecular HLA typing	HLA-DRB1-DQA1-DQB1 haplotype frequencies were similar between A-RLA and responsive LA Larger number of A-RLA patients showed binding to OspA compared with antibiotic-responsive LA

SNPs single-nucleotide polymorphisms, *TLR* Toll-like receptors, *OspA* outer surface protein A, *SOCS-3* suppressor of cytokine signaling-3, *HLA* human leukocyte antigen

factors of A-RLA. Of the most promising biomarkers examined, Th17-related cytokines appear to be of significant value. While several autoantigens have been identified that may be more prominent in A-RLA patients, such as MMP-10, annexin A2 [15, 33], apolipoprotein B-100 and ECGF [14–16, 19, 30, 32, 33], T-cell responses tend to occur later in LB manifestations and appear to be particularly associated with the manifestation of A-RLA [47]. Th17 cells are a subgroup of T-helper cells that have been shown to play a key role in autoimmune diseases, such as rheumatoid arthritis [48]. These cells are characterized by their ability to

Table 4 Summary of commonly upregulated biomarkers in antibiotic-refractory vs. -responsive LA in humans

Biomarker class	Biomarker	Specimens
Inflammatory markers	IL-6, IL-8, IL-10, IL-1β, IL-23, IL-17F, TNFα, IFNγ, CXCL9, CXCL10, CCL2, CCL3, CCL4, CRP	Serum and synovial fluids
Immunity-related markers	miR-146a, miR-155, miR-223 and miR-142, $CD4^+$ T-cells, GITR receptors, OX40 receptors, IL-4^+ $CD4^+$ Th2 cells, IL-17^+CD4^+ T-cells and $FoxP3^+$Treg cells	Peripheral blood and synovial fluids
Other markers	Annexin A2, hECGF	Serum and synovial fluids

synthesize IL-17, a proinflammatory cytokine [49] previously implicated in the development of LA [8, 50]. Furthermore, inhibition of IL-17 was shown to inhibit the development of LA in mouse models infected with *B. burgdorferi* [51]. Findings from the present study indicate that IL-17F and IL-23 from Th17 cells are significantly elevated in A-RLA patients compared with antibiotic-responsive LA patients [13, 31]. The Th17 cells were also shown to play a role at the different LB stages in humans with a pronounced effect particularly at the early stages to help in combating the infection [13]. A continued Th17 response appears, however, to evolve at the later infection stages to trigger autoimmunity and lead to inflammatory arthritis [52]. Although their role in A-RLA in humans has yet to be fully elucidated, observations suggesting a role of the Th17 responses in the early as well as late stages of LB provide promise for their use in predicting disease complications, e.g., LA and/or A-RLA. In addition to Th17 responses, Th1 cells were also shown to play an important part in LA by providing a dominant immune response in the synovial fluid of LB patients [53]. Other Th1 response or innate immune response biomarkers that can be employed to differentiate A-RLA from the responsive LA phenotype include IL-6, IL-8, IL-10, IL-1β, IL-17F, IL-23, IFN-γ, TNF-α, CCL2, CCL3, CCL4, CXCL9 and CXCL-10 [13, 21, 27, 31].

Despite targeting an emerging field in LB research, the present study has a number of limitations. As previously mentioned, most of the selected studies recruited a small number of A-RLA cases (i.e., $n < 120$). These studies were likely, therefore, to be underpowered in detecting smaller changes in biomarker levels. In addition, a variety of controls were used between studies, which were typically healthy controls, other arthritis patients (i.e., rheumatoid arthritis, osteoarthritis) or antibiotic-responsive LA patients. This inconsistent selection on controls caused difficulty in generating a decisive evaluation for the predictive ability of the biomarkers across the studies and in the conversion of LA to A-RLA. Lastly, the reports included here were all case-control studies in which stored biospecimens were assessed retrospectively and may have not been standardized for all patients, e.g., in terms of timing for sample collection or if studies were multi-site in nature, leading to a lack of harmonized protocols across the selected studies. Furthermore, of the evaluated reports only one included examination of synovial tissue and synovial fluid [25]. It has been well documented that in LB patients with persistent arthritis, the spirochetes are preferentially detected in synovial tissue rather than synovial fluid [54]. This limitation may lead to an unsubstantiated conclusion that persistent spirochetal infection may be absent in A-RLA patients based on lack of synovial fluid testing.

As a few of the studies identified here were designed to examine the prediction of progression from LB to LA and subsequently to A-RLA, future studies need to be developed prospectively to adequately evaluate the predictive power of a selected set of factors from the biomarkers characterized here. Moreover, a comparison of the biomarkers proposed here—between patients with LA-related true joint

swelling and those with joint pain—would be illuminating and can be proposed for future studies. Findings from these and other studies should also be validated against other similar study populations to evaluate the true predictive ability of the biomarkers of interest. Furthermore, future studies should investigate multiple biomarkers and combine both immune and genetic biomarkers to better compare—and strengthen—the prediction of A-RLA. It is likely that a combined biomarker approach in predicting resistance may yield the most clinical relevance as it examines multiple dysregulated pathways that play a concerted role in the etiology of A-RLA. Indeed, A-RLA is a joint disease that persists despite two-months of oral or one-month of intravenous antibiotics treatment [6] and may simply require prolonged antibiotic therapy beyond these limited courses [55]. Thus, the definition of "antibiotic refractory" appears to be an evolving concept, and the biomarker profiles described here could change with the different disease stages and therapy regimen.

CONCLUSION

In conclusion, the evidence base of biologic markers for A-RLA is limited to date. However, in the small set of studies conducted to date, a range of promising biomarkers have been identified. It appears that cytokines and chemokines related to the Th17 pathway together with a number of miRNAs species (miR-146a, miR-155 and let-7a) may be promising candidates in the prediction of A-RLA. Within well-powered and properly designed studies, a panel of multiple biomarkers may yield clinically relevant prediction of the possible antibiotic resistance at the time LA is first diagnosis.

ACKNOWLEDGEMENTS

Funding. Public Health Agency of Canada funded the work presented in this article and the article processing charges (AB).

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole and have given their approval for this version to be published.

Authorship Contribution. Alaa Badawi conceived the overall concept of the study and assisted in drafting the manuscript. Darren Brenner conducted the literature search and analysis and provided the first draft of the manuscript. Paul Arora advised on the analysis and assisted in interpretation of the results. All of the authors critically reviewed the manuscript, contributed substantive intellectual content and approved the final version submitted for publication.

Disclosures. Alaa Badawi, Darren Brenner and Paul Arora have nothing to disclose.

Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Data Availability. All data generated or analyzed during this study are included in this published article and supplementary information files.

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