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Characteristics and clinical outcomes after treatment of a national cohort of PCR-positive Lyme arthritis

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ABSTRACT

Objectives: To describe the clinical and microbiological characteristics and outcomes after antibiotic treatment of a national cohort of patients with Lyme arthritis confirmed by PCR testing on synovial fluid and by serology, when available.
Methods: Using the French National Reference Center for *Borrelia* database, patients with a positive PCR on synovial fluid for *Borrelia* were identified. Patient clinical and biological characteristics were reviewed from patient records. Long-term outcomes after treatment were studied through a questionnaire and with follow-up data.
Results: Among 357 synovial fluid testing by PCR between 2010 and 2016, 37 (10.4%) were positive for *Borrelia*. Patients' median age was 36 years (range 6–78) with 61% of men and 28% patients under 18. The presentation was monoarticular in 92% and the knee was involved in 97%. Contrary to the *Borrelia* species repartition in European ticks, *B. burgdorferi* sensu stricto was the most prevalent species found in synovial fluid (54%) followed by *B. azfelii* (29%) and *B. garinii* (17%). Antibiotic treatments were mainly composed of doxycycline ($n = 24$), ceftriaxone ($n = 10$) and amoxicillin ($n = 6$), for a median duration of 4 weeks (range 3–12). Despite

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a properly conducted treatment, 34% of patients ($n = 12$) developed persistent synovitis for at least 2 months (median duration 3 months, range 2–16). Among those, 3 developed systemic inflammatory oligo- or polyarthritis in previously unaffected joints with no signs of persistent infection (repeated PCR testing negative), which mandated Disease-Modifying Antirheumatic Drugs (DMARD) introduction, leading to remission.

Conclusion: In France and contrary to ticks ecology, Lyme arthritis is mainly caused by *B. burgdorferi* sensu stricto. Despite proper antibiotic therapy, roughly one third of patients may present persistent inflammatory synovitis and a small proportion may develop systemic arthritis. In such cases, complete remission can be reached using DMARD.

Introduction

Lyme borreliosis (LB) is the most common vector-borne disease in the Northern Hemisphere [1]. The spirochete bacteria causing LB, *Borrelia burgdorferi* sensu lato complex, are transmitted by hard ticks belonging to the genus *Ixodes*[2]. In Europe, the most three frequent species responsible for human infections are *B. afzelii, B. garinii* and *B. burgdorferi* sensu stricto, whereas *B. burgdorferi* sensu stricto is almost the only species causing LB in North America[3]. In Europe, neuroborreliosis is the most frequent disseminated clinical manifestation of LB, followed by Lyme arthritis, acrodermatitis chronica atrophicans, and, more rarely, borrelial lymphocytoma, and Lyme carditis [4–6]. These different clinical pictures are linked to the great species diversity found in Europe. Conversely, in the US, Lyme arthritis (LA) is the most common feature of disseminated LB, and as *B. burgdorferi* sensu stricto is the main species found in the US it has been hypothesized that it is the main etiologic agent for LA in the US and in Europe.

The clinical manifestations of LA, include synovitis, usually in one or a few large joints, especially the knee[7]. For untreated cases, recurrent joint-swelling episodes may persist for months or even years. The diagnosis of LA can be challenging, especially in high endemic areas, and cannot be based on a single clinical or biological characteristic. High levels of IgG antibodies against *Borrelia* are found in serum from patients with LA, but although serological testing shows excellent sensitivity and specificity in LA, a positive result alone is not sufficient to make the diagnosis[8]. The bacterial culture in LA is ineffective, because of its weak sensitivity[8]. Molecular diagnosis on the synovial fluid using PCR detecting *Borrelia* DNA is an attractive add-on test in LA diagnostic algorithm, providing a 100% specificity and a 42–96% sensitivity[8–10].

The treatment of LA is based on antibiotics, generally for 3–4 weeks, and the vast majority of patients recover completely[5,11]. In the US, roughly 10% of patients develop persistent synovitis lasting ≥ 2 months, called slowly resolving or antibiotic-refractory LA[7]. Although slowly resolving LA pathophysiology remains obscure, there is strong evidence for an autoimmune or inflammatory mechanism[12–15], and little to none for persistent infection.

Since few data about clinical and microbiological features and outcome of patients with LA are available in Europe, we conducted a retrospective observational study to describe the clinical and biological characteristics and treatment outcomes of a national cohort of patients with LA confirmed with synovial fluid PCR.

Patients and methods

Patients

We conducted a retrospective observational study using the French National Reference Centre (NRC) for *Borrelia* database. Among its missions, the French NRC for *Borrelia* contributes to the epidemiological surveillance of LB. Between 2010 and 2016 all the patients referred to the NRC for *Borrelia* PCR testing and who had a positive *Borrelia* PCR ($n = 37$) in their synovial fluids were included. Patients' medical history and laboratory findings, when available, were reviewed from medical records (Fig. 1). Time to diagnosis was calculated as the time between the first reported articular symptoms (retrieved from the medical report) and the PCR testing. The duration and route of antibiotic therapy were retrieved from medical records. In order to study treatment outcome, patients were submitted a standardized questionnaire by their referent physician. This questionnaire included reported articular or other sequelae, asthenia or reported invalidity. Follow-up clinical data were also retrieved from their referent physician.

Borrelia ELISA and western-blot

Borrelia ELISA were realized using different commercial kits done by the considered hospital taking care of the patients (Euroimmun Lyme ELISA IgG IgM, Diasorin Liaison XL® *Borrelia* IgG IgM, Biomérieux Vidas® Lyme panel, Siemens Enzygnost® IgG IgM, Mikrogen recomwell® *Borrelia* IgG IgM).

Serum *Borrelia* western-blot using different commercial kits (Biosynex LYMECHECK® Optima IgG IgM, Mikrogen *recom*Line® *Borrelia* IgG IgM, Euroimmun Euroline® *Borrelia*-RN-AT-Adv) or an in-house test as previously described[17].

Borrelia *polymerase chain reaction testing*

Borrelia PCR in synovial fluid were conducted as previously described[18]. Briefly, presence or absence of *Borrelia* DNA was assessed by a specific real-time PCR assay targeting a 230bp DNA fragment from the conserved region of the flagellin (*fla*) gene of the *Borrelia burgdorferi sensu lato* complex and a Taqman® probe (11bp). Then *Borrelia* species identification was realized by a second real-time DNA amplification using hybridization probes targeting species-specific regions of the *fla* gene for *B. afzelii, B. garnii/B. bavariensis, B. burgdorferi* ss, *B. bissettii, B. valaisiana* and *B. lusitaniae*. All PCR tests were performed in the same laboratory (*Borrelia* NRC located in Strasbourg, France).

Statistical analyses

Data were analysed using GraphPad Prism Version 6.03. Non-parametric Kruskal–Wallis test followed by a Dunn's correction for multiple testing was used to compare the distribution of numerical variables. Mann-Whitney test was used when Kruskal–Wallis test was not applicable. Unadjusted associations between serum CRP levels and synovial fluid leucocytes count were estimated with Spearman correlation coefficient. Fisher's exact tests were used to compare categorical variables. P values (bilateral) < 0.05 were considered statistically significant.

Ethical considerations

This study was approved by the Ethic Committee of the Faculty of Medicine and Strasbourg University Hospital, with the reference number 2018-4.

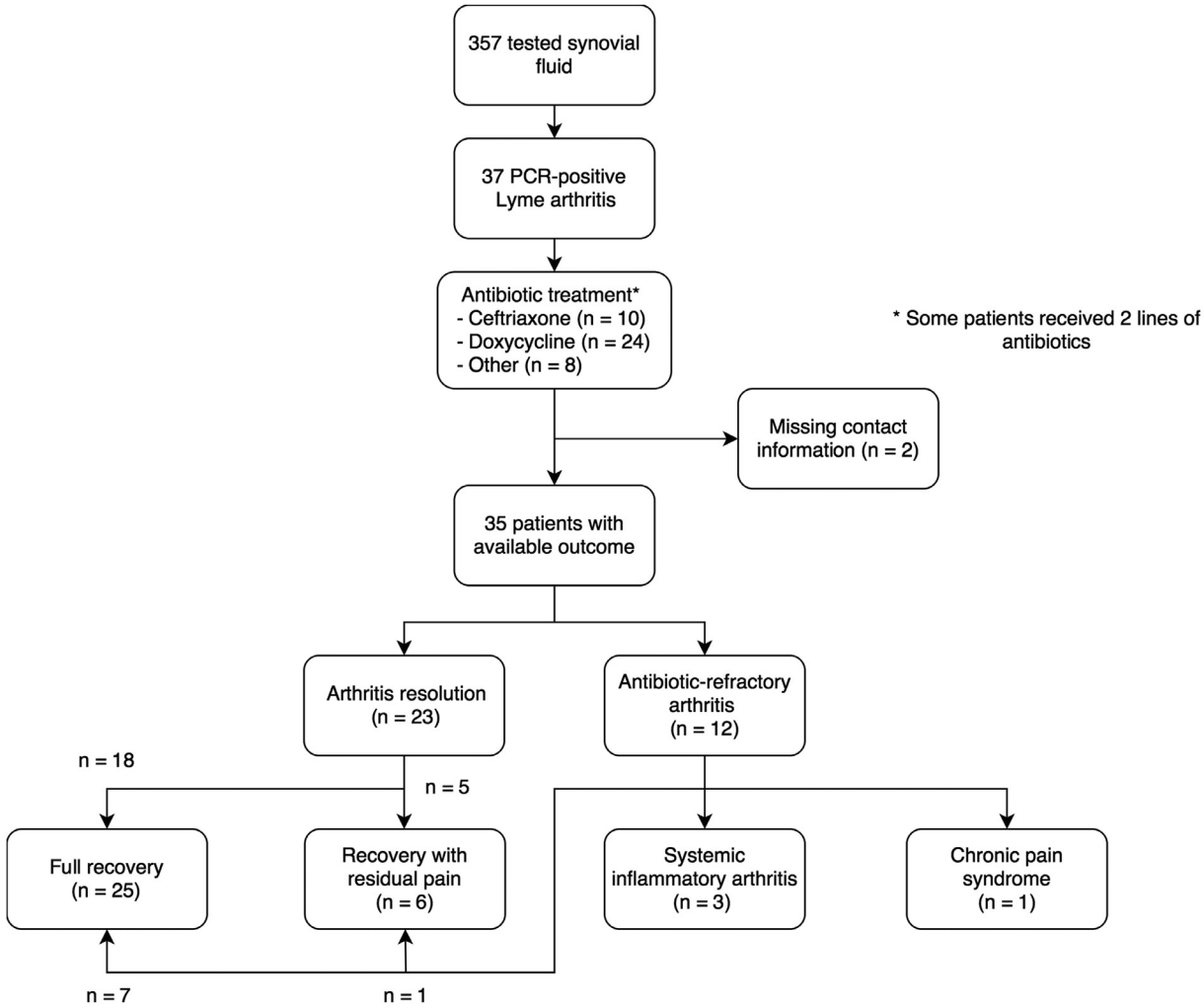

Fig. 1. Included patients' flowchart through the study. From our database of 357 tested synovial fluids from 2010 to 2016, 37 were positive for *Borrelia* DNA. Of these 37 patients, 35 had available follow-up data after treatment. Twenty-three patients had rapid resolution of the arthritis whereas 12 presented residual synovitis lasting ≥ 2 months. Among the 23 patients with rapid resolution of the arthritis, 18 presented a full recovery and 5 reported residual mechanical pain in the affected joint. Among the 12 patients with residual synovitis, 6 patients presented a full recovery, 2 reported residual mechanical pain in the affected joint, 3 developed systemic inflammatory arthritis in initially non-affected joints and 1 showed symptoms suggesting chronic pain syndrome.

Results

Patient characteristics

Between 2010 and 2016, 357 synovial fluids (SF) were tested by the French National reference centre. *Borrelia* DNA was detected in 37 (10.4%) of them (Fig. 1). Positive samples came from many regions of France where LB has been previously reported (Fig. 2) [19]. Patient characteristics are summarized in Table 1. Median time to diagnosis was 3 months (range 1–112 months) after clinical symptoms onset, and was significantly longer in adults than children (4 vs. 1.5 months, respectively, $p = 0.03$). On a seasonal point of view, date of the first clinical signs was observed during winter for 11 patients, spring for 6 patients, summer for 10 patients and fall for 8 patients (without significant difference). No significant differences were observed between diagnosis delay and *Borrelia* species, or between patients' age and *Borrelia* species.

Clinical presentation at diagnosis

The presentation was monoarticular in 92% (34/37) cases, and oligoarticular in the other cases. The knee was involved in 97% (36/37) patients, other involved joints were the ankle ($n = 1$) and the elbow ($n = 1$). Fever (> 38 °C) was reported in 22% (8/37) patients and was significantly more frequent in patients under 18 years old (45.5% vs. 11.5%, $p = 0.035$). Erythema migrans was reported by 14% (6/33) of patients. Other manifestations included headaches ($n = 2$), cervicobrachial nevralgia ($n = 1$), leukocytoclastic vasculitis ($n = 1$). None of the patients was diagnosed with concomitant cardiac Lyme disease manifestation. The characteristics are detailed in Table 1.

Laboratory findings

Lyme borreliosis serology was available for 33 patients, who all had IgG antibodies against *Borrelia* with the ELISA assay (Table 1). Western-blots were positive with the presence of at least 5 specific bands in 97% (32/33) of our cohort. One patient had a non-significant western-blot result with only 3 bands detected (41, 50 and 83 kDa bands). This patient had a 6-months diagnosis delay. Forty percent (12/30) of patients had detectable IgM antibodies against *Borrelia*. No association was observed between IgM positivity and *Borrelia* species, patients' age, or diagnosis delay or resolution of symptoms (data not shown).

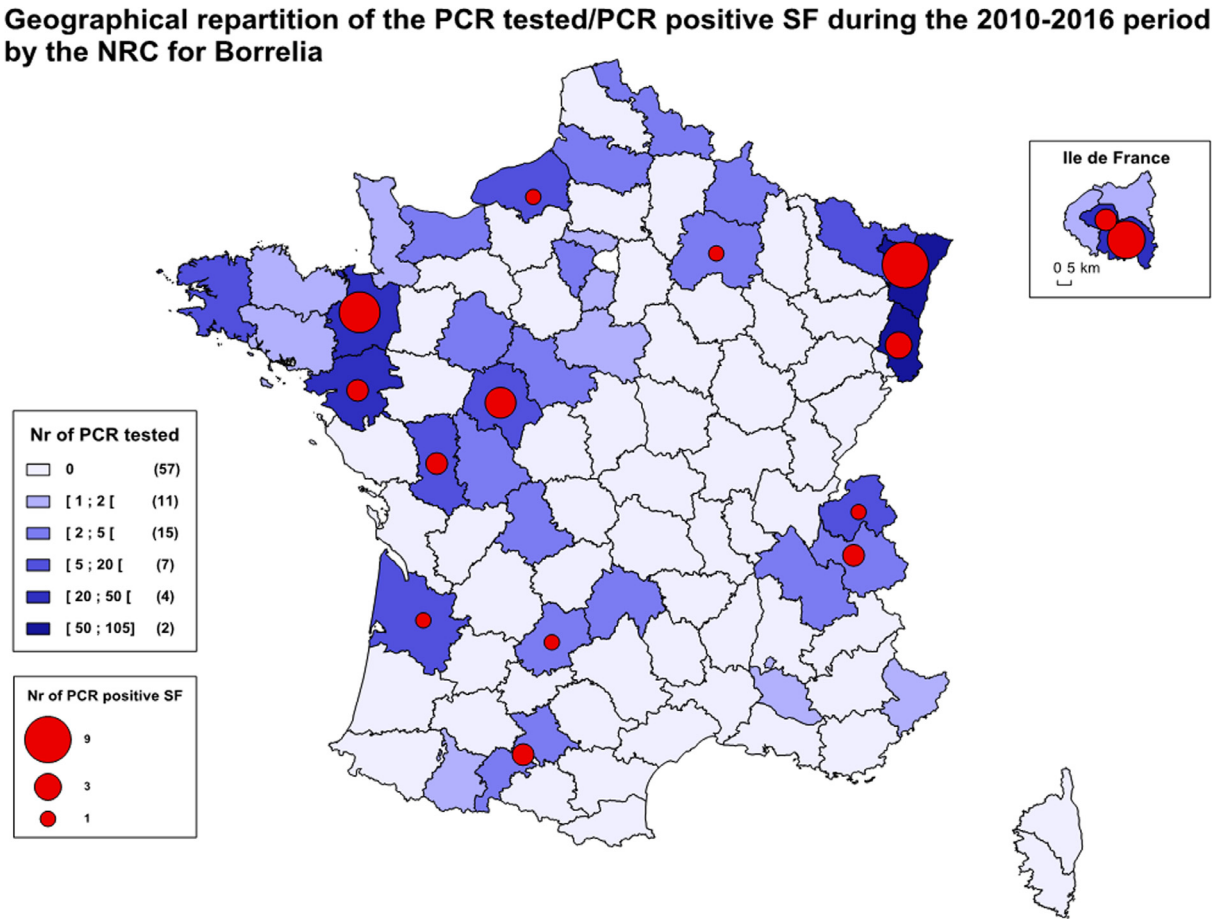

Fig. 2. Geographical distribution of synovial fluids tested by PCR and positive results for *Borrelia* DNA in the French National Reference Centre for *Borrelia* from 2010 to 2016.

Table 1
Patient, disease and treatment characteristics at diagnosis

Characteristics at diagnosis	PCR-positive Lyme Arthritis ($n = 37$)
Patient characteristics	
Age, median (range), years	36 (6 – 78)
Minor < 18 years	28% (11/37)
Male Sex	61% (23/37)
Tick exposure	91% (29/32)
History of tick bite	45% (15/33)
Diagnostic delay, median (range), months	3 (1 – 112)
Clinical presentation	
Previous erythema migrans	19% (6/32)
Monoarthritis	92% (34/37)
Oligoarthritis	8% (3/37)
Knee involvement	97% (36/37)
Fever (> 38 °C)	22% (8/37)
Laboratory findings	
C-reactive protein, median (range), mg/L	45 (3–182)
Positive *Borrelia* serology (ELISA)	100% (37/37)
Positive *Borrelia* western-blot	97% (36/37)
Negative *Borrelia* western-blot	3% (1/37)
Joint fluid aspiration	
leukocyte count > 2000/mm^3	95% (18/19)
Leukocyte count, median (range)	25,000 (1400 – 300 000)
% of neutrophils, mean (SD)	85% (± 10%)
Antobiotic regimen	
Treatment duration, median (range), weeks	4 (3 – 12)
Doxycycline	65% (24/37)
Ceftriaxone	27% (10/37)
Amoxicillin	16% (6/37)
Other	5% (2/37)

Among available synovial fluid counts ($n = 18$), the median SF count was 25,000 (range 1400–300,000) with a median neutrophil percentage of 90% (range 65–99). C-reactive protein (CRP) level was high (> 5 mg/L) in 91% of patients, with a median value of 45 mg/L (range 3–182). CRP level was associated with leucocytes count in synovial fluids (r^2: 0.423; $p = 0.003$).

Among the 37 patients with a positive *Borrelia* PCR in SF, PCR detected *B. burgdorferi* sensu stricto DNA in 19 patients (54%), *B. afzelii* DNA in 10 patients (29%), and *B. garinii* DNA in 6 patients (17%) (Fig. 3). No statistical difference was observed in the annual species repartition or in the annual ratio of positive samples on total samples tested. Most of the molecular analyses were performed on synovial fluids obtained from patients who hailed from the North of France.

Treatment

Antibiotic treatments used were oral doxycycline ($n = 24$), intravenous ceftriaxone ($n = 10$), oral amoxicillin ($n = 6$), oral tetracycline ($n = 1$) and oral cefaclor ($n = 1$). Five patients received a combination therapy (doxycycline and ceftriaxone). Three of them received ceftriaxone after doxycycline, one received doxycycline after 4 days of ceftriaxone and one received both at the same time. Ceftriaxone therapy was added when inadequate response to oral therapy was observed. Median antibiotic treatment duration was 4 weeks (range 3–12). After the diagnosis, 9 patients received intra-articular glucocorticoids including 8 patients who had antibiotic-resistant LA. Seven received triamcinolone hexacetonide synoviorthesis, as initial treatment for the arthritis ($n = 1$) or as adjunctive treatment for persistent synovitis ($n = 6$). One patient received isotopic synoviorthesis (detailed in next paragraph). None of them had to undergo surgical synovectomy.

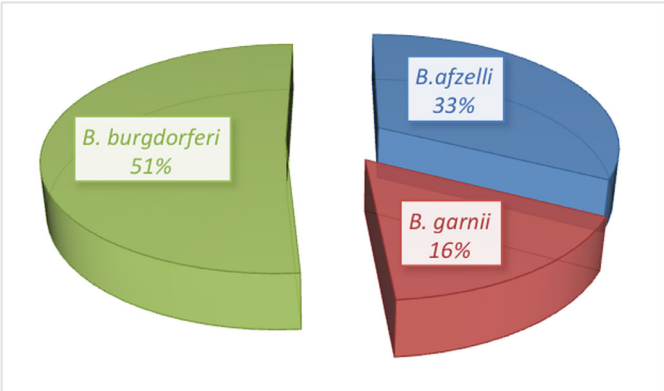

Fig. 3. Repartition of *Borrelia* species found in synovial fluids from 2010 to 2016.

Clinical outcome after treatment

Follow-up data after treatment were available for 35 (95%) patients with a median follow-up time of 17 (range 1–73) months (Fig. 1). Sixty-six percent of patients ($n = 23$) had a rapid resolution of the arthritis whereas 34% ($n = 12$) presented residual synovitis lasting ≥ 2 months after oral antibiotic or 1 month after IV antibiotic (also called antibiotic refractory LA).

Among the 23 patients with rapid resolution of the arthritis, 78% ($n = 18$) presented a full recovery and 22% ($n = 5$) reported long-lasting residual mechanical pain in the affected joint. The residual mechanical pain was moderate and did not significantly limit the patients' activities.

Among the 12 patients with residual clinical synovitis lasting ≥ 2 months (median duration 3 months, range 2–16) after one course of oral antibiotic treatment or for at least 1 month after IV treatment, *Borrelia* PCR testing in SF was repeated in 9 patients, and all tests were negative. Three of them received combination treatment with doxycycline and IV ceftriaxone. The initial treatment of the residual synovitis consisted of non-steroidal anti-inflammatory drugs and intra-articular glucocorticoid injections were used in 75% cases. Half ($n = 6$) of these patients with residual synovitis presented a full recovery, 17% ($n = 2$) reported long-lasting residual mechanical pain in the affected joint, 25% ($n = 3$) developed systemic inflammatory arthritis in initially non-affected joints (Fig. 1). One patient with persistent knee synovitis despite doxycycline (4 weeks) and IV ceftriaxone (3 weeks) underwent isotopic synoviorthesis (intra-articular injection of a radioactive isotope (i.e. ^{90}Ytrium) to decrease synovial inflammation). The isotopic synoviorthesis was performed after persistent *Borrelia* infection was ruled out by repeat synovial fluid PCR and after a relapse despite triamcinolone hexacetonide synoviorthesis and 3 months treatment with oral methotrexate (15 mg/week). He did not present subsequent articular flare-up. One patient with negative repeat synovial fluid PCR developed symptoms suggestive of chronic pain syndrome without inflammatory joint disease. Characteristics of patients with or without slowly resolving synovitis are presented in Table 2.

The characteristics of the 3 patients who developed diffuse inflammatory arthritis are detailed in Table 3. Briefly, the 3 patients presented oligo- or polyarthritis involving previously non-affected joints. One patient developed dactylitis, no patient developed axial of extra-articular involvement. *Borrelia* PCR testing on SF was repeated for all these 3 patients, with negative results. Laboratory testing for autoimmunity (including at least antinuclear antibody and ACPA) was negative for all the 3 patients. None of these 3 patients had HLA-DR locus typed. All these 3 patients were started on methotrexate at an oral dose of 15– 20 mg/week leading to sustained remission in 2 patients. Adalimumab was added to the third patient's treatment regimen, leading to sustained remission.

Discussion

To determine clinical and biological characteristics and treatment outcome of patients with proven Lyme arthritis, we examined data from 37 patients with a positive *Borrelia* PCR on synovial fluid.

The diagnosis of LA is currently based on the presence of specific symptoms, combined with laboratory evidence for infection. Serology is the cornerstone of Lyme disease laboratory diagnosis, and serological tests that are most often used are enzyme-linked immuno-sorbent assays (ELISAs) followed by immunoblots. In our cohort, all patients with available serological data (33/33) showed a positive ELISA IgG

Table 2
Characteristics of patients with or without persistent arthritis

Characteristics at diagnosis	Arthritis Resolution ($n = 23$)	Persistent Arthritis ($n = 12$)	P-value
Patient characteristics			
Age, median (range), years	33 (6–75)	41 (12–78)	0.48
Minor < 18 years	39% (9)	17% (2)	0.26
Male Sex	65% (15)	28% (7)	0.73
Diagnostic delay, median (range), months	3 (1–112)	5 (1–24)	0.27
Clinical presentation			
Monoarthritis	96% (22)	83% (10)	0.27
Oligoarthritis	4% (1)	17% (2)	0.27
Fever	26% (6)	17% (2)	0.69
Laboratory findings			
C-reactive protein, median (range), mg/L	37 (3–182)	61 (6–136)	0.46
Synovial fluid aspiration			
Leukocyte count, median (range)	17,000 (1400–300,000)	27,000 (2700–60,000)	0.31
Borrelia species			0.64
B. burgdorferi sensu stricto	48% (11)	67% (8)	
B. afzelii	30% (7)	25% (3)	
B. garinii	22% (5)	8% (1)	
Antibiotic regimen			
Treatment duration, median (range), weeks	4 (3–12)	6 (3–12)	0.09
Doxycycline	57% (13)	83% (10)	0.15
Ceftriaxone	22% (5)	33% (4)	0.69
Amoxicillin	22% (5)	8% (1)	0.64
Other	9% (2)	0%	0.53

Table 3
Characteristic of patients developing chronic systemic arthritis

Characteristics at diagnosis	Patient 1	Patient 2	Patient 3
Age	24 years	28 years	14 years
Sex	Female	Male	Male
			Acute left knee arthritis
		Acute right knee arthritis	Right knee arthritis
	Acute knee monoarthritis	Left knee arthritis 4 weeks earlier	6 weeks earlier
Initial clinical presentation	No fever no EM	No fever no EM	Leukocytoclastic purpura
			No fever no EM
CRP level	3 mg/L	8 mg/L	84 mg/L
SF leukocyte count	2400/mm^3	17,600/mm^3	25,000/mm^3
	75% neutrophils	neutrophil count NA	90% neutrophils
***Borrelia* species**	*B. garinii*	*B. burgdorferi* sensu stricto	*B. azfelii*
Initial treatments		**Before diagnosis:**	
	Tetracyclin	Oral MTX 15 mg/week	Doxycycline
	600 mg/day 4 weeks	Hexatrione acetonide	200 mg/day 8 weeks
	Naproxen	**At diagnosis:** Doxycycline 200 mg/day 12 weeks	Naproxen
		MTX discontinuation	
Inflammatory disease presentation	Asymmetric oligoarthritis: right knee and hip, left ankle	Knee bi-arthritis and dactylitis	Asymmetric polyarthritis: left wrist and elbow, right wrist and 2nd MCP
New SF PCR	Negative	Negative	Negative
Immune panel	ANA and ACPA negative	ANA and RF and ACPA negative	ANA and ACPA negative
B27 status	Negative	NA	Negative
Treatments		MTX 15 mg/week	
	MTX 15 mg/week	Hexatrione acetonide	MTX 15 mg/week
		Adalimumab 40 mg/2weeks	
Outcome	Rapid remission	Flare-up with MTX alone	Rapid remission
	MTX discontinuation after 24 months	Remission with MTX + ADA	MTX discontinuation after 18 months
	No recurrence at 3 months Lost of follow-up	Treatment still ongoing	No recurrence at 1 year of follow-up

Abbreviations: ACPA, anti-citrulinated protein antibody; ANA, anti-nuclear antibody; CRP, C-reactive protein; ECM, erythema migrans; MTX, methotrexate; RF, rheumatoid factor; SF, synovial fluid; NA, not available.

result. It is noteworthy that all *Borrelia* serology were sampled at the same time or after the synovial fluid and that PCR was performed independently of the serology. Therefore no bias of inclusion criteria related to the *Borrelia* serological result was possible in our cohort. In LA, serological tests such as ELISAs IgG are known to show a great sensitivity (93–100%) and specificity (91–97%) [20]. ELISAs IgM are known to be less sensitive than IgG in LA, and their presence was found in only 40% cases of our cohort. Thus, positive *Borrelia* IgG with negative IgM should not be considered as a "serological scar" and discard the diagnosis.

In our study, the diagnosis of LA was always confirmed by a positive SF PCR for *Borrelia*. Molecular diagnosis on the synovial fluid using PCR detecting *Borrelia* DNA is an interesting add-on diagnostic tool in LA diagnosis. In Europe, its sensibility is roughly 60–70% [8–10] and a PCR positivity in synovial fluid, in case of positive serology allows diagnosis certainty (100% specificity), which is not the case with serology alone. In a practical point of view, molecular diagnosis must be realized when serological tests are positive. Several points support this conception: On the one hand, some patients with LA will have a negative *Borrelia* PCR test in their synovial fluid, which can be due to low spirochaetal load, technical failure of the PCR or DNA degradation due to incorrect pre-analytic sample handling. On the second hand, since virtually all patients described in the literature as having LA have a positive serology, serology should be realized first considering its lower cost and that a false positive results due to external contamination is at least theoretically always possible. If PCR testing is negative (and serology is positive), differential diagnoses should be considered, but LA diagnosis remains still possible.

In comparison with serology, repeat PCR test turns negative after antibiotic treatment, sometime with a few months delay[16]. In one American study, spirochaetal DNA could be found in some patients after antibiotic therapy, however, amplification of mRNA (marker of spirochaetal viability) was negative in all patients, suggesting dead bacteria remnants rather than persistent infection [16]. In our study, all synovial fluid PCR repeated after antibiotic treatment among whom 9 had antibiotic refractory LA, were negative ($n = 14$).

In Europe, the most frequent species responsible for human LB are *B. afzelii, B. garinii* and *B. burgdorferi* sensu stricto, whereas the most frequent species that causes LB in North America is mainly *B. burgdorferi* sensu stricto[3]. In France and all over Europe, *B. burgdorferi* sensu stricto is the least frequent species found in the *Ixodes ricinus*, the main vector of LB in Europe. In a recent meta-analysis, the major *Borrelia* species found in European *I. ricinus* ticks was *B. afzelii* (46.6%), followed by *B. garinii* (23.8%), then *B. burgdorferi* sensu stricto, found in only 10.2% of ticks[21]. In European patients with LB, EM is the most frequent clinical manifestation, and is mainly associated with *B. afzelii*[22–24]; the second most frequent clinical picture is neuroborreliosis and is mainly associated with *B. garinii*[25]. Interestingly, the most frequent *Borrelia* species found in synovial fluids from our patients was *B. burgdorferi* sensu stricto (54%). A potential bias could be that PCR technique may have different sensibility, meaning that some organisms are more likely to be identified than others. But our PCR technique is routinely used and sensitivity is the same for all *Borrelia* strains. For example, in erythema migrans biopsies, *B. afzelii* is found in more than 90% of cases (personal communication). Our observations are concordant with some other previous studies, in which *B. burgdorferi* sensu stricto was found to be more frequent than the other species in European LA[26,27]. However, some studies did not observe this correlation[28,29]. In Europe, *Borrelia* strains causing Lyme arthritis are greatly heterogeneous, and our study, based on French data, might not be extrapolated to other European countries. Although our study provides the largest cohort of European LA patient with *Borrelia* species determination, a global survey involving other European countries may help to elucidate this question.

Initial clinical presentations in our LA cohort were classical, with joint swelling of a large articulation (especially the knee, in 97%), as

previously reported in a cohort of 65 US patients with LA[30]. Fever was rarely reported but significantly associated with children borreliosis which are known to experience a more acute presentation[31]. These observations occurred from weeks to months after a tick bite, although only 40% of patients remembered this bite. In a European case series, the period from tick bites or EM to the onset of LA ranged from 10 days to 16 months[32]. Because of this great variability of the latency period, there is no seasonal peak in the occurrence of LA[30]. We also observed this characteristic in our cohort, since the date of the first clinical signs was observed all over the year. History of a prior EM to LA is a rare phenomenon. Herein, only 6 (16.2%) of our 37 patients remembered an EM before LA presentation. Based on European studies, history of EM in case of LA is ranged between 10 and 32%[33–35].

Diagnosis delay was usually short, with 18/35 patients diagnosed between 1 and 3 months after clinical signs appearance; 4/35 patients were diagnosed after 12 months, with one patient after 112 months. This shows that LA is sometimes a difficult diagnosis, and should be systematically investigated for in the presence of a monoarticular or oligoarticular arthritis with negative culture and crystal observation, especially in case of knee involvement.

Antibiotic course mostly included doxycycline, ceftriaxone or amoxicillin for a median time of 4 weeks. All these treatment regimen have a proven efficacy in LA [35,36]. In our study, one third of patients presented persistent synovitis lasting at least 2 months after a well-conducted oral antibiotic treatment or 1 month after IV treatment. This condition is called slowly resolving or antibiotic-refractory Lyme arthritis in the literature and is usually reported in roughly 10% of patients in US cohorts and thought to be even rarer in Europe [37,38]. Although its pathophysiology remains unclear, the current preferred paradigm is an autoimmune reaction against *Borrelia* antigens or a loss of tolerance against an unidentified autoantigen rather than persistent *Borrelia* infection [37]. In line with this paradigm, all 9 repeated synovial fluid PCR testing in our patients with antibiotic-refractory LA were negative. Interestingly, matrix metalloprotease 10, an auto-antigen targeted by B and T lymphocytes, have been identified in some patients with antibiotic-refractory LA, reinforcing the autoimmune hypothesis[39]. Association between antibiotic-refractory LA and certain HLA-DR haplotype has been reported[12]. Unfortunately, HLA-phenotyping could not be performed in our study. Our data suggest that slowly resolving LA is not uncommon in Western Europe or at least in France. Clinical outcomes were favourable for 67% with symptomatic treatments (NSAID or intra-articular glucocorticoids). One patient with slowly resolving LA with negative follow-up PCR testing later developed symptoms suggestive of chronic pain syndrome. Finally, 3 patients (25% of patients with persistent synovitis and 9% from our follow-up cohort) developed systemic arthritis after antibiotic treatment. Systemic arthritis is a poorly identified complication of LA which incidence and pathogenesis are largely unknown. Infection-induced rheumatic disease (e.g., rheumatoid arthritis) or post-spirochaetal reactive polyarthritis are two viable hypothesis but further fundamental work is needed. A recent report described 30 rheumatoid arthritis-like and psoriatic arthritis-like cases following LA diagnosis in the US[40]. One critic voiced by Tuttle was that LA diagnosis was based on serology alone and that testing to investigate for persistent *Borrelia* infection in these patients was not reported [41]. However, in the 5 patients in whom synovial fluid was available, testing for *Borrelia* DNA was negative, which supports the conclusion that these patients did not have persistent *Borrelia* infection. In our study, the facts that all 3 patients developing systemic arthritis had negative repeated SF *Borrelia* PCR testing and an excellent response to DMARD treatment strongly argue against persistent infection.

One of the strengths of our study is the direct involvement of the French national reference centre for *Borrelia*. The French national reference centre for *Borrelia* based in Strasbourg since 2012 provides diagnosis testing for all regions of France thus allowing a representative capture of French PCR-positive LA cases. Besides, since patients' inclusion was based on synovial fluid PCR positivity, the LA diagnosis was proven in all cases. The main limit of our study is that due to the retrospective analysis, some data were not available for analysis. Two patients (5% of the cohort) could not be contacted in order to study the after treatment outcome. Another limitation is that due to the multicentre origin of our cohort, we were not able to reunite a cohort of LA with a negative PCR for use as a control group. However, the main aims of our study were to describe clinical characteristics and treatment outcome of PCR-proven LA, for which a control group was deemed unnecessary. Besides, as a positive serological test alone does not mean that a patient necessarily has active Lyme borreliosis, the additive value of this type of control group may be not relevant.

In conclusion we report 37 cases of synovial fluid PCR-positive Lyme arthritis in French patients. We identify *B. burgdorferi* sensu stricto as the main species involved in LA, in spite of its low prevalence in European ticks. After antibiotic treatment, follow-up identified 34% (12/35) patients with slowly resolving arthritis among whom 25% (3 patients, 9% of the follow-up cohort) developed systemic inflammatory arthritis without any sign of persistent infection (negative repeat PCR testing). Our study suggests that European patients with LA may present post-infectious inflammatory manifestation in a significant proportion of cases.

Conflict of interest

None to declare related to this work.

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