

Detection of *Borrelia burgdorferi* by species-specific and broad-range PCR of synovial fluid and synovial tissue of Lyme arthritis patients before and after antibiotic treatment

Sirs,

Lyme disease is caused by the tick-borne spirochete *Borrelia burgdorferi* (1). Due to the variation in clinical presentation and because of the limitations of currently available diagnostic assays it is often difficult to diagnose Lyme arthritis. *B. burgdorferi* could only be cultured from synovial fluid in a minority of patients (2). However, bacterial antigens and DNA of *B. burgdorferi* have been demonstrated in the synovial fluid (SF) and synovial tissue (ST) of patients with Lyme arthritis by immunological methods and the polymerase chain reaction (PCR). After antibiotic treatment PCR usually became negative (3). We therefore studied the sensitivity of species-specific and broad-range PCR in SF and ST samples of Lyme arthritis patients before and two month after treatment.

Samples from 11 Lyme arthritis patients and from 15 controls with the diagnosis of rheumatoid arthritis (n = 10) or calcium pyrophosphate dihydrate deposition disease (n = 5) were analyzed for bacterial DNA by broad-range PCR sequencing of a 16 s rRNA gene fragment and by a *B. burgdorferi* flagellin specific PCR. As summarized in Table I, patients were questioned for any history of tick bites, Erythema chronicum migrans or previous arthritic symptoms. Nine patients exhibited clinical symptoms of knee arthritis, and 2 had oligoarthritis involving the knee. Elevated CRP levels

and increased cell counts in the SF indicated an inflammatory process in all patients. All patients tested positive for *B. burgdorferi* by ELISA and the results were confirmed by Western blot. *B. burgdorferi* DNA was detected in 9 out of 11 patients (82%) with Lyme arthritis by species-specific PCR (SF 82%; ST 18%). Only 1 patient tested positive by broad-range PCR and the *B. burgdorferi* specific sequence was confirmed. PCR results became negative after treatment except for one SF specimen. SF obtained from this patient 6 months after antibiotic treatment demonstrated negative PCR without any additional therapy. ST and SF samples from control patients were negative at all time points. Synovitis resolved in all patients between 2 (n = 3) and 6 months after the diagnosis and initiation of treatment.

Our data support the conclusions of Nocton *et al.* (3) and Limbach *et al.* (4) with respect to the high sensitivity of the *B. burgdorferi* specific PCR in Lyme disease. Furthermore, we found a higher sensitivity with SF compared to ST. Conflicting findings by Jaulhac *et al.* (5) could be due to several reasons (6). Target imbalance as described by Persing *et al.* (7) seems an unlikely explanation for this phenomenon since the former study used the same PCR target as ours. In agreement with previous studies the sensitivity of broad-range PCR was low (8, 9). Unlike recently reported (10), persistence of *B. burgdorferi* DNA was not observed after antibiotic treatment in our patients. SF seems the preferable specimen to detect *B. burgdorferi* by PCR. Antibiotic therapy with ceftriaxon (2 weeks) or doxycycline (4 weeks) usually resulted in complete cure of Lyme arthritis and species-specific PCR proved a helpful tool in monitoring the treatment success.

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Table I. Clinical data, laboratory parameters and pre-and post-treatment results of species specific PCR for *Borrelia burgdorferi* and treatment modality of 11 patients with Lyme arthritis.

Patient with Lyme arthritis	Sex	Age	History of tick bite or ECM	Present. of arthritis	Duration of arthritis before [mos]	Synovitis knee				CRP [mg/l]	Cell count in SF [x10 ³ /ml]	ELISA		Western blot		Culture	Broad-range PCR		PCR results				AB
						Pre	2	6	12 months			IgM	IgG	IgM	IgG		SF	ST	Pre-treatment PCR		Post-treatment PCR		
																			SF	ST	SF	ST	
1	m	26	ECM	M	3	+	+	-	-	5	4.5	pos.	pos.	pos.	pos.	neg.	neg.	neg.	neg.	n.a.	n.a.	DX	
2	f	37	ECM	M	8	+	-	-	-	4	9.4	neg.	pos.	n.a.	pos.	neg.	neg.	neg.	pos.	neg.	pos.	neg.	DX
3	m	62	ECM	M	6	+	-	-	-	12	12.7	pos.	pos.	pos.	pos.	neg.	neg.	neg.	pos.	neg.	n.a.	n.a.	CF
4	m	31	-	M	2	+	+	-	-	61	9.0	neg.	pos.	n.a.	pos.	neg.	neg.	neg.	pos.	neg.	neg.	n.a.	CF
5	f	48	TB	M	36	+	+	-	-	18	13.3	pos.	pos.	pos.	pos.	neg.	neg.	neg.	pos.	neg.	neg.	neg.	DX
6	f	53	-	M	3	+	+	-	-	100	52.3	neg.	pos.	n.a.	pos.	neg.	neg.	neg.	pos.	neg.	neg.	neg.	CF
7	m	63	TB	M	0.1	+	+	-	-	129	42.0	pos.	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	CF
8	m	39	ECM	M	10	+	+	-	-	24	18.5	pos.	pos.	pos.	pos.	neg.	neg.	neg.	pos.	pos.	neg.	neg.	DX
9	m	24	TB	O	15	+	-	-	-	107	40.3	pos.	pos.	pos.	pos.	neg.	neg.	neg.	pos.	neg.	n.a.	neg.	CF
10	f	26	-	M	11	+	+	-	-	28	21.7	pos.	pos.	pos.	pos.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	CF
11	m	64	TB	O	14	+	+	-	-	100	24.0	pos.	pos.	pos.	pos.	neg.	neg.	neg.	pos.	neg.	neg.	neg.	CF

n.a. not available; AB: antibiotics; DX: Doxycycline (Vibramycin) 2 x 100 g/d po, 4 weeks; CF: Ceftriaxon (Rocephin®) 1 x 2 g/d iv, 2 weeks.

Presentation of arthritis: M = monoarthritis of the knee; O: oligoarthritis involving the knee; synovitis of the knee: (+) present, (-) absent.

Letters to the Editor

(abstract). *Rheumatology* 2000; 39 (Suppl. 1): 92.

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Efficacy of thalidomide in refractory adult Still's disease: A new case report

Sirs,

Adult Still's disease is a febrile disorder of unknown etiology which affects young adults during the second or third decade, presenting either as a primary disease or as a relapse of childhood onset Still's disease (1). Clinical features are characterized by an initial pharyngitis with prolonged fever, polyarthritis and maculopapular rash; liver and pericardium involvement are commonly observed (2). The biological data comprise a major increase in all inflammatory proteins and leukocytosis usually $>20,000$ cells/mm³. The poor prognosis of Still's disease is determined by the long time course of its evolution, the frequent relapses, and serious complications [thrombotic thrombocytopenic purpura (3), amyloidosis (4)]. Levels of tumor necrosis factor (TNF), like the levels of other inflammatory markers (C reactive protein, ferritin), increase dramatically in patients with Still's disease. TNF seems to play a role in the pathogenesis of Still's disease and therefore some anti-TNF drugs, such as infliximab (5) and etanercept (6), have been used with success in the treatment of adult Still's disease. Thalidomide is also known to inhibit the production of TNF and has consequently been tested in other inflammatory diseases (7-9). Taken together, these facts led us to test thalidomide in a case of refractory adult Still's disease.

A 23-year-old male presented in April 2000 with hyperthermia (39.5°C), polyarthritis, rash and pericarditis. He had a history of childhood onset Still's disease diagnosed at the age of 9 years and treated with corticosteroids for 12 years. The disease was considered to be in remission since 1998. The relapse of Still's disease was established according to the Yamaguchi criteria (10). Corticotherapy (prednisone) was started at 60 mg/day and remission occurred within 1 month; prednisone was progressively tapered to 10 mg/day and the patient remained

well for one year. In April 2001 fever, arthritis and rash relapsed. Prednisone was increased to 50 mg/day. One month later the disease was still evolving and treatment was complemented with i.v. immunoglobulin (400 mg/kg/day over 5 days each month), methotrexate (20 mg/week *per os*) and colchicine 1 mg/day.

Two months later, despite this quadritherapy, the disease was still clinically and biologically progressing. The C-reactive protein level was 112 mg/l. Therefore methotrexate and colchicine were stopped and thalidomide was started (150 mg/day). One month later the disease was in total remission, with a CRP level at 2 mg/l despite a reduction in the daily prednisone dosage to 30 mg/day. Nine months after the beginning of thalidomide treatment, remission was maintained with a CRP level at 9 mg/L, and a prednisone dosage of 15 mg/day. Throughout the follow-up the neurological examination was normal and electroneurograms performed after 3 and 9 months of treatment showed no sign of peripheral neuropathy.

Teratogenic effects led to the withdrawal of thalidomide from the world market in 1961. However, the recent discovery of the anti-inflammatory, immunomodulatory and anti-angiogenesis properties of thalidomide has increased interest in this molecule, especially in the field of inflammatory and immune-mediated diseases. Thalidomide is able to inhibit TNF production in human monocytes by stimulating mRNA degradation in a dose-dependent fashion and to reduce the half-life of TNF mRNA by 50% (11). Stambe *et al.* (12) reported the efficacy of thalidomide in a 44-year-old woman with adult onset Still's disease refractory to other treatments. The patient was therefore given thalidomide (200 mg/day, then 100 mg/day). Improvement was observed after 2 weeks of treatment (thalidomide + prednisone) and the disease was in remission during a 6-month follow-up period. During the study, the percentage of monocytes producing TNF decreased from 42.7% before thalidomide to 1.4% during the treatment.

Our results confirm the efficacy of thalidomide in adult Still's disease, even at a dosage of 150 mg/day. This efficacy is particularly remarkable when one considers the cost-effectiveness of this drug in comparison to other anti-TNF drugs. Moreover, no significant side effects were observed.

In conclusion, thalidomide seems to offer a new approach for the treatment inflammatory diseases linked to the overproduction of TNF. Refractory adult Still's disease might be a new indication for thalidomide as long as careful monitoring of peripheral

nerve conduction with electroneurograms every 6 months is conducted to detect asymptomatic neuropathy. In fertile women the use of thalidomide should be authorized only in women taking the birth control pill.

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