

ISOLATION OF BORRELIA BURGDORFERI FROM SKIN IN PATIENTS WITH ERYTHEMA MIGRANS, UNSPECIFIC SKIN LESIONS AND GRANULOMA ANNULARE

F. Strle, E. Ružić, V. Preac-Mursic, J. Cimperman, B. Wilske

ABSTRACT

The report includes 5 patients in whom *Borrelia burgdorferi* was isolated from the skin. One patient had typical erythema migrans, another one had skin changes, which corresponded to the early, homogeneous erythema migrans, and two patients had non-specific skin lesions at the site of an insect bite, lasting for several months. *Borrelia* antibodies were not present in the blood of any of our patients, nor did any of them report a tick-bite. Also described is a case of *B. burgdorferi* isolated from a granuloma annulare. The isolated *Borreliae* belong to serotypes 1, 4, and 6.

KEY WORDS:

Lyme borreliosis, Borrelia burgdorferi, cultivation from skin biopsies, non-typical skin lesions, granuloma annulare

INTRODUCTION

In Europe and in the USA, Lyme borreliosis is the most widespread disease being transmitted by ticks (1,2). The same disease is also to be found in Slovenia (3). The term Lyme was taken from the county of Lyme in the USA, where a considerable number of patients developed this hitherto unknown disease over 15 years ago (4). Borreliosis was named after the bacterium causing the disease (5). Lyme borreliosis is usually transmitted by ticks.

Like syphilis, Lyme borreliosis progresses through two phases, early and late infection (6,7). The early infection is divided into the first stage, in which a localized infection appears, and the second stage, characterized by a disseminated infection. The second stage follows within a few days or a few weeks from the initial stage, and is marked by intermittent

symptoms which may last for some weeks to some months. The late phase of the disease (persistent infection) corresponds to the third stage and usually begins after one year or more following inoculation (6, 7). The skin can be affected both in the early as well as in the late phase.

In the majority of cases the disease develops about 10 days (i.e. some days to some weeks) after a tick bite, when a redness of the skin appears and spreads outward. Initially the skin lesion is homogeneous, but later it grows paler in the central region. A typical ringlike erythema gradually enlarges, i.e. "migrates" outward, and is therefore named erythema migrans. As a general rule, *Borreliae* enter the skin via a bite of an infected tick. Changes in the skin (erythema migrans), following a tick bite, reflect a local inflammation probably caused by spreading of *B. burgdorferi* over the skin. In some

patients *Borreliae* enter into the blood and disseminate into different tissues. After a period of latency, which may last for some days, or, in most cases, several weeks or months, and occasionally even several years, the clinical evidence of involvement of the nervous system, heart, joints, skin and/or other tissues or organs may appear (7).

It is characteristic of Lyme borreliosis that its clinical picture is rarely complete, and sometimes any of its clinical stages may be completely absent (7). The pathognomonic clinical sign of the disease (i.e. erythema migrans, which is the main manifestation of the first stage of Lyme borreliosis) may also be either absent, or the skin lesions are atypical. In such cases, a borrelial infection is usually confirmed by determining *Borrelia* antibodies. Extreme caution, however, must be taken when interpreting serological tests, as negative results do not necessarily exclude *Borrelia* infection, while on the other hand, positive results do not necessarily mean that (all) symptoms and signs, which the patients experience, are a consequence of borrelial infection. In clinically unclear cases, much greater significance is therefore attached to the isolation of *B. burgdorferi* than to the determination of antibodies.

This report includes 5 cases of patients with different skin lesions, in whom *B. burgdorferi* was isolated from the skin. Among 848 patients registered in Slovenia between 1985 and 1988, 73 % reported a tick bite, 10 % an insect bite, and 17 % did not recall being bitten either by a tick or by an insect at the site of later skin lesion (11). About 10 % of the lesions remained homogeneous (12).

MATERIAL AND METHODS

All 5 patients underwent physical examination at the Outpatient Clinic of the Department of Infectious Diseases in Ljubljana. For the present report, some patients were purposely selected whose clinical picture itself enabled recognition of the disease, while the isolation of the causing agent from the skin additionally confirmed an infection with *Borrelia*. In other patients presented, the isolation of *B. burgdorferi* was essential in diagnosis of the cause of skin lesions, thereby enabling appropriate treatment.

A biopsy of the skin was carried out for culture and a blood sample was taken for the presence of antibodies to *B. burgdorferi* in all patients on the same day.

The skin biopsy was performed at the periphery of the skin lesion after disinfection with 70 % alcohol and local anaesthesia with 2 % xylocaine. A piece of tissue of about 10 x 5 x 3 mm was removed. The obtained material was cultivated in MKP medium, as previously described (8). The protein profile of the isolated *Borrelia* was determined with SDS-PAGE. According to the protein profile and the reactivity with monoclonal antibodies, the isolated *Borreliae* were categorized into serotypes, as has been reported by Wilske et

al. (9).

The IgM and IgG antibody titers were determined with the IFA without absorption (10). As an antigen, *Borrelia burgdorferi* PKo strain serotype 2, was used. Antibody titers of $\geq 1 : 256$ were considered as positive.

CASE REPORTS

Case 1 : MJ, male, 49 yrs

A 49-year-old patient was referred to our Department after the development of dizziness, headache, nausea and generally feeling unwell. All these symptoms lasted for one day. His previous medical history was uneventful. Over the last few years he experienced occasional pain in his joints, particularly in his shoulders and elbows. In the last 6 months before admission he did not receive any antibiotics. He did not recall being bitten by a tick. Upon physical examination, the patient was afebrile. Meningeal signs were not present. His physical state did not show any abnormality except that a ringlike, oval-shaped erythema, measuring 30 cm in length was observed on the left hemithorax on his back. He was not aware of his skin changes. When he was asked a target question, he replied in the affirmative, stating that around July 7 he was most probably bitten on the left side of his back by an insect. Some days later, the site of the insect bite began to itch. A small redness appeared which after July 20 began to expand, but afterward he was not so aware of it anymore. The basic laboratory tests were within normal limits. Lyme serology was negative, however, *B. burgdorferi*, serotype 2, was isolated from the patient's skin. A lumbar puncture was not carried out. The patient received 100 mg doxycycline twice daily over 14 consecutive days. The skin lesion completely disappeared 7 days after initiation of therapy. However, headaches, occasional dizziness and general feeling of being unwell persisted until the middle of December.

Case 2: TB, male, 36 yrs

This patient was cutting grass at the end of May and at the beginning of June. He was not aware of being bitten by a tick or an insect. Around June 9th, i.e. a week prior to undergoing an examination at our clinic, a redness had appeared on the inner side of this right calf, which enlarged over the next few days. The last two days before the examination he experienced a burning pain in the affected area. He also had minor headaches, but he denied having fever or chills, or any other problems.

Upon examination on June 16th, a homogeneous redness of 25 cm in length and around 20 cm in width was observed on the inner part of the patient's right calf. In the center of it a small crust was seen. The area of redness was slightly warmer and its border was well-demarcated from the surrounding skin. There were no other significant abnormalities in the patient's physical state. Serological tests

failed to confirm the presence of *B. burgdorferi* antibodies, however, *B. burgdorferi*, serotype 4, was isolated from the material obtained at biopsy.

Case 3: MK, female, 38 yrs

The patient had been well until the onset of the present illness. Toward the end of May she was bitten by an insect (most probably a horse - fly) in her right calf. Already some days later she noticed a redness, which gradually spread. She occasionally experienced minor itching and burning sensations in the affected area. She did not report any other symptoms and she denied having been bitten by a tick. She did not receive any antibiotics. The examination on November 23rd was uneventful apart from a roundish redness on the distal part of her right calf, measuring 4 cm in diameter, the border of which was slightly pronounced. The skin in the region of the erythema was harder, dry, and slightly flaking. The results of serological tests were negative, but *B. burgdorferi*, serotype 6, was isolated from the skin. Treatment with 100 mg doxycycline twice a day over 14 consecutive days led to partial improvement, but complete disappearance of the erythema of the skin only occurred after treatment with ceftriaxone.

Case 4: PD, female, 40 yrs

The 40-year-old patient, a mother of three children, was in good health. She was able to carry out her normal duties at work and at home without a difficulties. In July 1988 she was bitten by an insect in her right calf. A redness appeared at the site of the bite, but disappeared within some days. In the autumn of that year she developed pain in her rib cage, which was associated with fatigue and gradually progressing myalgia, arthralgia, pain, and headaches. She grew forgetful, had difficulties concentrating, was also psychically changed. In October 1988, a small redness appeared at the site of an insect bite, which did not enlarge significantly in the following few months. The patient underwent several tests, which all failed to show any abnormality. She was also referred to a neurologist, a psychiatrist, dermatologist and orthopaedic surgeon, who did not discover any definite cause of her problems. In April 1989, when the patient came to our Outpatient Clinic, on the anterior part of her right calf, in its distal third, there was a round reddish-purple erythema, slightly above the level of the skin, measuring 1 cm in diameter.

Serological tests for *B. burgdorferi* were negative. The same results were obtained by repeated tests, carried out 3 weeks later. Due to perseverance on the part of the patient's physician, who treated her because she was experiencing severe pain, a biopsy of the skin was performed. From the obtained specimen, *B. burgdorferi* serotype 2 was isolated. Although the skin lesion disappeared after antibiotic treatment, only slight and temporary alleviation of the remaining

symptoms occurred. The patient remained serologically negative, however, a positive result was obtained by a test of transformation of lymphocytes with *B. burgdorferi* in blood.

Case 5: MW, female, 36 yrs

This patient's course of disease has already been described elsewhere (12), she resides in an area where Lyme borreliosis is endemic (13). In brief, 36-year-old patient had been in good health until the summer of 1989, when she received a tick bite and several insect bites. At the end of September 1989, a reddish-purple papule appeared on the dorsum of her right foot. The papule gradually increased in size and acquired the form of an irregularly shaped horseshoe with a diameter of about 4 cm. In December, a similar skin change appeared in the area of the right foot. Later on, pain in both knees, extreme fatigue and almost constant headache appeared. Examination of the patient in the early April 1990 established no physical abnormalities other than skin changes typical of granuloma annulare. Serological examinations showed borderline titers of antibodies to *B. burgdorferi* (IgM 1 : 128, IgG 1 : 256). Histological findings in a biopsy taken from the right foot were typical of granuloma annulare. *Borrelia burgdorferi* was isolated from the skin lesion. The isolated strain was identified as *Borrelia burgdorferi* with monoclonal antibodies (L32 IF11 L22 IF8) by Western blot. According to the reactivity pattern with eight monoclonal antibodies against Osp A, the isolate is Osp A serotype 2 (9).

The patient was administered ceftriaxone 2 g daily i.v. for 14 days. In the weeks following the therapy the headache ceased and arthralgia decreased. In late September, 5 months after antibiotic therapy was completed, the patient was free of subjective problems. The skin changes cleared and serological values for *B. burgdorferi* were negative. Tissue removed from immediate vicinity of the former biopsy revealed no signs of granuloma annulare, and culture for *Borrelia burgdorferi* was negative (Table 1).

DISCUSSION

Following the description of Lyme disease in the USA, the discovery of its causing agent, and the introduction of serological tests, it has become clear that the newly discovered borrelial infection occurs in many different manifestations, which were for many years known in Europe as separate nosologic entities, such as acrodermatitis chronica atrophicans (14), erythema migrans (15), and Garin - Bujadoux - Bannwarth's syndrome (16). In addition to this, serological tests for *Borrelia* were also carried out and attempts for the isolation of the etiological agent were done in certain other, relatively well-defined skin lesions. The results have shown borrelial etiology in the majority of patients with a solitary lymphocytoma (17, 18, 19) and probably also in some patients with sclerotic and atrophic skin lesions (19).

Table 1. Basic data on patients in whom *B. burgdorferi* was isolated from affected skin.

Patient	tick bite	insect bite	type of skin lesion	serological tests	serotype of isolated B.b.
1	N.A.	yes	erythema migrans	negative	serotype 2
2	N.A.	N.A.	erythema	negative	serotype 4
3	N.A.	yes	erythema	negative	serotype 6
4	N.A.	yes	erythema	negative	serotype 2
5	yes	yes	granuloma annulare	IgM 1: 128 IgG 1: 256	serotype 2

B.b. = *Borrelia burgdorferi*

N.A. = not aware

Serological tests and the visualization of spirochetes in tissue slides by staining suggest that some other skin entities might be *Borrelia*-induced as well. There have been reports that in granuloma annulare IgG antibodies to *B. burgdorferi* are positive in 26 to 65 % of cases and that skin lesions show a marked improvement with the use of antibiotics effective against *B. burgdorferi* (20, 21). So far we have been aware of only one report (12) of successful isolation of *B. burgdorferi* from granuloma annulare skin lesion (our patient number 5). This case supports the thesis that *Borrelia burgdorferi* may be the causative agent in some patients with granuloma annulare.

In a typical erythema migrans, borrelial etiology is undisputable. There are many reports on the isolation of *B. burgdorferi* from the skin in the area of erythema migrans (22 - 27), and a few reports on the isolation of *B. burgdorferi* from the surrounding skin, which appeared healthy (27). *B. burgdorferi* was also isolated from skin lesions which were not typical of erythema migrans nor did they belong to any well-known skin entity (24, 28). In such cases the data about a tick

bite at the site of subsequent skin changes and the presence of *Borrelia* antibodies are diagnostically helpful. Among our patients with non-specific skin changes, none reported a tick bite nor did anyone have specific *Borrelia* antibodies in serum, despite the fact that the skin changes had lasted for 16 weeks, 25 weeks, and 7 months respectively (cases 2, 3, and 4) before a blood sample and a skin biopsy were taken.

Our results confirm that in addition to typical erythema migrans also atypical borrelial skin changes are possible (24, 28), and that antibodies against *B. burgdorferi* in blood can be absent even when several months have already elapsed from the start of the infection (29). Atypical borrelial skin lesions represent a diagnostic challenge and - particularly in the absence of antibodies - they can also give rise to severe diagnostic problems. Their recognition is critical as it enables appropriate treatment.

The isolation of three different serotypes of *B. burgdorferi* from skin is indicative of a diversity of *Borreliae* in Slovenia, and confirms the findings (30) about the heterogeneity of *B. burgdorferi* in Europe.

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AUTHORS' ADDRESSES

Franc Sterle M.D., Ph. D, Dpt of Infectious Diseases, University Medical Center, Japljeva 2, 61000 Ljubljana, Slovenia
Eva Ružič M.D., mr sc, Institute for Microbiology, Zaloška 4, Medical faculty, Ljubljana University, Zaloška 4, 61000 Ljubljana, Slovenia

Vera Preac-Mursic Ph.D., Max von Pettenkofer Institute, Medical Faculty, München University, Pettenkoferstrasse 9a, 8000 München, Germany

Bettina Wilske M.D., Max von Pettenkofer Institute, Medical faculty, München University, Pettenkoferstrasse 9a, 8000 München, Germany.

Jože Cimperman M. D., University Medical Center, Japljeva 2, 61000 Ljubljana, Slovenia