"*Borrelia*-associated early-onset morphea": A particular type of scleroderma in childhood and adolescence with high titer antinuclear antibodies?

Results of a cohort analysis and presentation of three cases

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Background: Morphea is an inflammatory autoimmune skin sclerosis of unknown etiology. A causative role of *Borrelia burgdorferi* infection has been controversially discussed, but no conclusive solution has yet been achieved.

Objective: Intrigued by 3 young patients with severe *Borrelia*-associated morphea and high-titer antinuclear antibodies, we retrospectively examined the relationship between *Borrelia* exposure, serologic autoimmune phenomena and age at disease onset in morphea patients.

Methods: In 90 morphea patients the presence of *Borrelia*-specific serum antibodies was correlated to the age at disease onset and the presence and titers of antinuclear antibodies. Patients with active *Borrelia* infection or high-titer antinuclear antibodies due to systemic sclerosis or lupus erythematosus served as controls.

Results: We observed a statistically highly significant association between morphea, serologic evidence of *Borrelia* infection, and high-titer antinuclear antibodies when disease onset was in childhood or adolescence.

Limitations: Because pathogenic *Borrelia* species may vary in different geographic regions the relevance of *Borrelia* infection in morphea induction may show regional variations.

Conclusion: *B burgdorferi* infection may be relevant for the induction of a distinct autoimmune type of scleroderma; it may be called "*Borrelia*-associated early onset morphea" and is characterized by the combination of disease onset at younger age, infection with *B burgdorferi*, and evident autoimmune phenomena as reflected by high-titer antinuclear antibodies. As exemplified by the case reports, it may take a particularly severe course and require treatment of both infection and skin inflammation (J Am Acad Dermatol 2009;60:248-55.)

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BACKGROUND

Morphea, also named localized scleroderma, is an inflammatory connective tissue disease that affects the skin and leads to a pronounced sclerosis with increased collagen deposition and atrophy of skin appendages. Morphea displays a wide clinical spectrum ranging from single localized patches to disseminated devastating forms, such as disabling pansclerotic morphea of childhood or progressive facial hemiatrophy.[1] Lesions usually present as ivory sclerotic patches surrounded by an inflamed lilac ring. Involvement of subcutaneous fat and underlying muscles may result in profound atrophy or, in

Abbreviations used:

ANA: antinuclear antibody
ELISA: enzyme-linked immunosorbent assay
ROC: receiver operating characteristic

children, growth retardation of the affected limb or anatomic region.[1,2] Histologically, the different types are similar but differ in regard to severity and extent of the inflammatory infiltrate and the degree and level of location of the newly formed collagen in the skin.[3]

Etiology and pathogenesis of morphea are still unknown. It is generally viewed as an autoimmune disease that may involve the formation of autoantibodies.[4,5] A suspected trigger of morphea is tick-borne infection with the spirochete *Borrelia burgdorferi* sensu lato.[6] *B burgdorferi* sensu lato is the cause of Lyme disease and considered a serious public health challenge for the population in the northern hemisphere.[7-10] *Borrelia* infection preferentially affects the skin and joints, but also involves the muscular, nervous, or cardiovascular system.[8,9] Furthermore, it has been associated with potentially autoimmune sequelae such as post-Lyme arthritis.[11]

After the first report by Aberer, Neumann, and Stanek[6] in 1985 on an association between morphea and *B burgdorferi* infection, the pathogenic role of *Borrelia* in triggering morphea onset has been an open matter of intense debate.[12-17]

Intrigued by 3 young patients suffering from severe or disseminated morphea in association with *Borrelia* infection, we reevaluated the enigmatic relationship between these two conditions and examined retrospectively a cohort of 90 morphea patients, identifying a unique autoimmune type of scleroderma in which *B burgdorferi* seems to have an important role.

PATIENTS AND METHODS

Patient cohort

The data of 90 patients with histologically verified morphea seen at the Department of Dermatology of the University of Munich between 1999 and 2007 that had been tested for *Borrelia* infection and antinuclear antibodies were examined retrospectively.

Serologic testing

Antibodies against *B burgdorferi* had been determined by enzyme-linked immunosorbent assay (ELISA, Enzygnost Borreliosis Dade Behring Marburg GmbH, Germany). All positive results were confirmed by Western blot analysis as described (Borrelia ViraStripe Test Kit, Viramed Biotech AG, Planegg, Germany).[18] Execution of tests and interpretation of results was performed according to current guidelines and recommendations.[19,20] Each case of positive *Borrelia*-specific antibodies was screened for syphilis by the *Treponema pallidum*-particle agglutination test. Antinuclear antibodies in serum (ANAs) had been determined by indirect immunofluorescence on HEp-2 cells. According to current guidelines a titer of $\geq$ 1:160 was considered positive.[21]

Statistical analysis

The age at disease onset was correlated with the presence of *Borrelia*-specific antibodies and ANAs in serum. The cut-off age between early and later onset patients was defined by the receiver operating characteristic (ROC) curve. ROC curves illustrate the relationship between statistical sensitivity and specificity and discriminate between alternative states of health over the complete spectrum of operating conditions. They summarize the inherent capacity of a biomarker for discriminating a diseased from a non-diseased subject across all possible levels of positivity.[22,23] The Mann-Whitney *U* test was used for comparing the ANA titers, and the chi-square test and Fisher's exact test were used for comparing the frequency data in the different groups. Data are described by the mean $\pm$ standard deviation and by median and the first and third quartiles for the ANA titer values that were not normally distributed. Statistical analysis was performed using the SPSS 15.0 software (SPSS Inc, Chicago, Ill).

RESULTS

Typical cases with *Borrelia*-associated morphea and high-titer ANAs

The 3 patients lived in endemic areas for Lyme disease in Bavaria, Germany, showed typical clinical and histopathological findings of morphea without symptoms of systemic sclerosis, were seropositive for *B burgdorferi* antibodies and displayed high-titer serum ANAs. One patient remembered a tick bite.

Patient 1 (Fig 1, *A*) was a 14-year-old girl with a 6-year history of widespread progressive skin lesions on the trunk, left arm, and left leg. She displayed multiple extensive erythematous hypopigmented and hyperpigmented indurated and atrophic patches as well as sharply demarcated white sclerotic plaques. The affected left breast was hypoplastic, and the affected left leg was 1.5 cm too short, resulting in an oblique pelvis. The clinical diagnosis was disabling, partly linear morphea of childhood.[1]

Serum contained *Borrelia*-specific IgM antibodies against the *Borrelia* antigens p41, p17 and OspC, and ANAs at a titer of 1:5,120. Intravenous therapy with ceftriaxone 2 g daily for 21 days was initiated. Ten months later, *Borrelia*-specific serum antibodies had

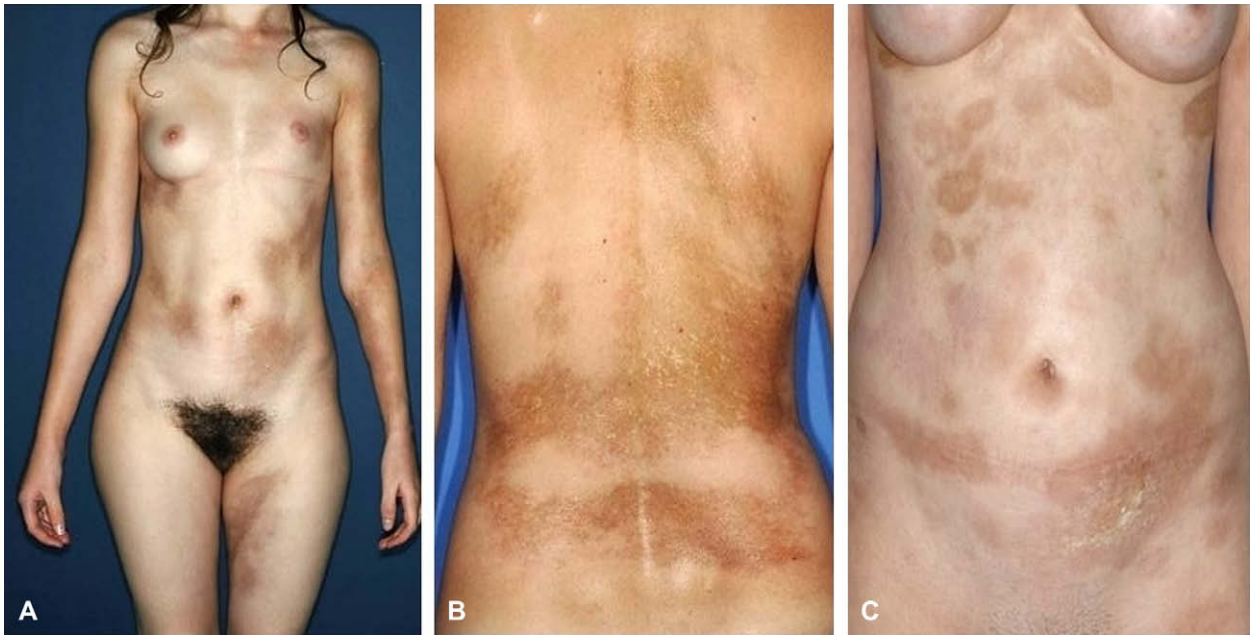

Fig 1. Patients with *Borrelia*-associated early-onset morphea and high-titer antinuclear antibodies. **A**, Patient 1: Disabling, partly linear morphea of childhood with extensive skin involvement has resulted in undergrowth of the left breast and left leg with an oblique pelvis. **B**, Patient 2: Hyperpigmented areas of sclerosis and white sclerotic plaques cover the back of this patient with generalized morphea. **C**, Patient 3: Erythematous or hyperpigmented atrophic lesions or white skin sclerosis affect large areas of the trunk.

largely disappeared, with only a weak residual OspC reactivity. Bath PUVA (psoralen bath plus UVA irradiation) treatment halted inflammation and reduced the sclerotic induration. Within the following year, no morphea relapse was observed.

Patient 2 (Fig 1, *B*) was a 20-year-old woman with a 4-month history of widespread patchy erythematous hypopigmented and hyperpigmented, indurated and atrophic skin lesions with white sclerotic plaques on her trunk, which were diagnosed as generalized morphea. A tick bite had occurred 1 year before disease onset.

High-titer *Borrelia*-specific serum-IgG antibodies reactive against the antigens VlsE, p14, Osp17, p21, p39, p43/45, p58, and p83/100, and high-titer ANAs (>1:10,240) were present. U1RNP and Sm antibodies were positive. The patient received twice-daily 10 mega units penicillin G intravenously for 21 days. Skin lesions were treated with bath PUVA, which induced a reduction of sclerosis and skin thickening as measured by 20-MHz sonography. Sixteen months after antibiotic therapy, titer and spectrum of *Borrelia*-specific serum antibodies had strongly declined, with only one distinct reactivity left in Western blotting against VlsE, while the inflammatory activity of morphea slowly started to increase again.

Patient 3 (Fig 1, *C*) was an 18-year-old woman who had developed slowly progressive areas with hyperpigmented or white sclerotic plaques at the age of 12 years. Skin lesions finally covered large areas of the trunk. First serologic testing 3 months after morphea onset demonstrated the presence of *Borrelia*-specific serum IgM antibodies against the antigen p41, p21 and OspC, and high-titer ANAs ($\geq$1:10,240). *Borrelia*-specific antibodies had disappeared 6 months after treatment with amoxicillin (500 mg 3 times daily for 14 days). Over the following 6 years inflammatory morphea relapses were controlled by repetitive courses of high-dose ultraviolet A1 (UVA1) phototherapy.

Borrelia-exposed early-onset morphea patients show high-titer ANAs

Of the 90 patients with morphea, 16 were male and 74 female. The age at disease onset ranged from 6 to 80 years (41.54 $\pm$ 19.81 years). Antibodies against *B burgdorferi* were present in 20 of the 90 cases (22.2%). Forty-two of the morphea patients (46.7%) tested positive for ANAs in serum (ANA titer $\geq$1:160). ANAs were more often present in female (39/74; 52.7%) than in male morphea patients (3/16; 18.8%). In all patients with positive *Borrelia*-specific antibodies active or former syphilis had been excluded by a treponemal screening test, *Treponema pallidum*-particle agglutination.

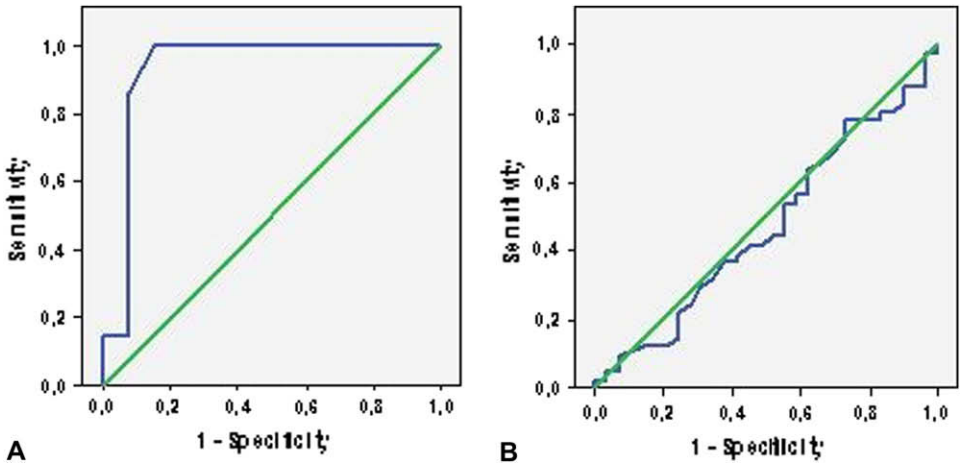

Fig 2. ROC curves with regard to the cut off age for high ANA titers in patients seropositive (**A**) or seronegative (**B**) for antibodies against *B burgdorferi*. **A**, ROC curve showed 1.0 sensitivity and 0.846 specificity at the age of 26 years in the seropositive group. Area under the curve: 0.929 (± 0.066 standard error [SE]); difference is significant (P = .002). **B**, ROC curve showed no cut-off age in the seronegative group. Area under the curve: 0.472 (± 0.071 SE); difference from the 0.5 area reference line is not significant (P = .69).

Both *Borrelia*-specific antibodies and high-titer ANAs appeared to be more prevalent in younger patients. Therefore, by the ROC curve we analyzed the presence and titers of ANAs regarding the age at morphea onset (Fig 2). For the occurrence of high ANA titers in *Borrelia*-exposed patients, it defined a cut-off age at disease onset of 26 years or less that distinguished "early-onset" from "later-onset" morphea patients. In seronegative patients, no such link was observed.

According to this definition, the total morphea cohort consisted of 28 early-onset ($\leq$ 26 years at disease onset) and 62 later-onset patients ($\geq$ 27 years). *Borrelia*-specific antibodies were present in 11 of 28 early-onset patients (39.3%) and in 9 of 62 later-onset morphea patients (14.5%) (P = .0089, chi-square test).

ANAs were present in 19 of the early-onset patients (67.9%) and in 23 of the later-onset patients (37.1%) (Table I). When the presence of *Borrelia*-specific antibodies was associated with the presence of ANAs, positive ANA titers were seen in 8 of 17 (47.1 %) early-onset patients seronegative for *Borrelia* infection, in 21 of 53 (39.6%) *Borrelia*-negative later-onset patients, and in 2 of 9 (22.2 %) *Borrelia*-positive later-onset patients. ANA titers in these patients were usually low, as reflected by a median of 1:80 in all 3 groups (see Table I).

All 11 early-onset morphea patients (100%) with serologic evidence for *B burgdorferi* infection tested positive for ANAs at high titers (median ANA titer, 1:5120) (see Table I). When this group was compared to the seronegative early-onset patients or to the seronegative or seropositive later-onset patients, the differences in ANA frequency (Fisher exact test) and ANA titers (Mann-Whitney U test) were statistically highly significant (comparison with the seronegative early-onset patients: P = .004, Fisher exact test; $P < .001$, Mann Whitney U test; comparison with the later-onset patient groups: $P < .001$ regarding both Fisher exact and Mann-Whitney U tests). There were no statistically significant differences when the ANA frequencies or titers of the *Borrelia*-negative early onset patients were compared with the *Borrelia*-negative (P = .59, χ^2 test; P = .27, Mann-Whitney U test) or *Borrelia*-positive later-onset morphea patients (P = .399, Fisher exact test; P = .241, Mann Whitney U test). *Borrelia*-associated early-onset morphea with high-titer ANAs had the lowest age at onset and started on average at 12.5 ± 6.95 years of age, as compared to 17.76 ± 6.32 years for *Borrelia*-negative early-onset morphea and 50.21 ± 14.64 or 51.53 ± 13.39 years for *Borrelia*-positive or *Borrelia*-negative later-onset morphea (see Table I).

The fine specificity of the humoral *Borrelia*-specific immune response in early-onset morphea patients with high-titer antinuclear antibodies

Ten of the 11 patients with *Borrelia*-associated early-onset morphea were female. In 4 cases a tick bite before onset of morphea had been recognized. The timing of serologic analysis and the fine specificity of the humoral immune response in the patients with *Borrelia*-associated early-onset morphea and high-titer ANAs are given in Table II.

Table I. Patient characteristics and prevalence and titers of ANAs in patients with early- and later-onset morphea grouped according to serologic evidence to *B burgdorferi* infection

	All morphea patients	Seropositive early-onset morphea patients	Seronegative early-onset morphea patients	Seropositive tater-onset patients	Seronegative later-onset morphea patients
No. of patients	90	11	17	9	53
Female/Male	74/16	10/1	14/3	8/1	42/11
Mean age at disease onset ± SD	41.54 ± 19.81	12.5 ± 6.95	17.76 ± 6.32	50.21 ± 14.64	51.53 ± 13.39
ANA titer ≥ 1:160	42/90 (46.6%)	11/11 (100%)	8/17 (47.05%)	2/9 (22.2%)	21/53 (39.6%)
ANA 1:160	5/90	1/11	—	—	4/53
ANA 1:320	18/90	—	4/17	2/9	12/53
ANA 1:640	2/90	1/11	—	—	1/53
ANA 1:1280	8/90	2/11	3/17	—	3/53
ANA 1:2560	1/90	1/11	—	—	—
ANA 1:5120	3/90	2/11	—	—	1/53
ANA 1:10,240	5/90	4/11	1/17	—	—
ANA percentiles					
25th		1280	40	.00	.00
50th (median)		5120	80	80	80
75th		10,240	800	200	320

—, No patient with this ANA titer in this group; *ANA*, antinuclear antibody; *SD*, standard deviation.

Morphea duration at the time of testing ranged from 2 months to 6 years. In general, the antibody pattern correlated with the duration of morphea. Patients with a short morphea history of several months (patients 3, 6, 9, 11) had antibody patterns compatible with early *Borrelia* infection, as exemplified by the presence of IgM antibodies to proteins such as p41 or OspC. With increasing morphea duration, *Borrelia*-specific IgG antibodies became more frequent, showing a broad IgG reactivity against different *Borrelia* antigens (patients 2, 4-8, 10). Only in patient 1 the humoral immune response did not reflect the overall morphea duration of 6 years. Here, only *Borrelia*-specific IgM-antibodies were detected that declined after intravenous therapy with ceftriaxone 2 g daily for 21 days. As reported by her parents the patient's history included two courses of penicillin treatment during childhood due to streptococcal angina. Although insufficient to cure an infection with *B burgdorferi*, the antibiotic exposure may have affected the humoral immune response. As reported by Wilske, Fingerle, and Schulte-Spechtel et al,[20] inadequate antibiotic therapy in early *Borrelia* infection may abrogate the IgM to IgG class switch of *Borrelia*-specific antibodies without curing the infection itself. Thus, the lack of *Borrelia*-specific IgG antibodies in this case does not contradict a relationship between *Borrelia* infection and morphea onset.

Thus, the age-related evaluation of our patient sample proposes a distinct entity of scleroderma. It is characterized by early onset, exposure to *Borrelia* infection, and autoimmune phenomena as represented by high-titer serum ANAs.

Borrelia-specific antibodies and ANA titers in other conditions

To exclude a direct relationship between ANAs and *Borrelia*-specific antibodies, 20 patients with high-titer ANAs of at least 1:5120 or greater suffering either from systemic lupus erythematosus (n = 9) or systemic scleroderma (n = 11) were analyzed regarding the presence of *Borrelia*-specific antibodies. Only one of the 20 sera contained *Borrelia*-specific IgG antibodies as detected by ELISA and confirmed by Western blotting, while in the remaining 19 sera no *Borrelia*-specific antibodies were detected. To determine whether *Borrelia* infection by itself may induce ANAs, sera of 14 patients with late-stage borreliosis and an intense serologic *Borrelia*-specific reactivity in ELISA and Western blotting were tested regarding the presence of ANAs. Two of the 14 sera contained low-titer ANAs not exceeding a titer of 1:320. These data demonstrate that high-titer ANAs by themselves are not associated with antibodies against *B burgdorferi* and vice versa, that exposure to *B burgdorferi* by itself is not sufficient to induce high-titer ANAs.

DISCUSSION

We present data on 90 morphea patients; this is one of the largest cohorts from a single institution or a defined geographic environment for which

Table II. Fine specificity of the humoral *Borrelia*-specific immune response in early-onset morphea patients with high-titer antinuclear antibodies

Patient No.	Sex	Age at disease onset (y)	Inverse ANA titer	IgM antibodies to *Borrelia* antigens in Western blotting	IgG antibodies to *Borrelia* antigens in Western blotting	Duration of morphea at testing	Tick bite remembered
1	F	8	5120	p17, OspC, p41	n.d.	6 y	No
2	F	21	10,240	n.d.	VlsE, p14, Osp17, p21, p39, p43/45, p58, p83/100	1 y	Yes
3	F	12	10,240	p21, p41, OspC	n.d.	3 mo	No
4	F	6	5120	p21, OspC, p30, p39	OspC, p30, p39, p41, p58	4 y	No
5	F	6	640	p41	VlsE, p14, p17, p41, p83	2 y	Yes
6	F	7	10,240	p41, OspC	p17, p21, OspC	3 mo	No
7	F	7	1280	OspC, OspB, p41	OspC, p41, p83	2 y	Yes
8	F	12	10,280	n.d.	p21, p30, OspB, p39, p41, p58, p83/100	1 y	Yes
9	M	16	1280	OspC, p41		2 mo	No
10	F	17	160		p21, p30, p41,	1 y	No
11	F	26	2560	OspC, p30, OspA, p39, p41, p83/100	n.d.	3 mo	No

ANA, Antinuclear antibody; *F*, female; *M*, male; *mo*, months; *n.d.*, not detected; *y*, years.

evidence of *Borrelia* infection has been determined.[13] Our patient cohort seems to constitute a representative sample of morphea patients. Similar to a recent meta-analysis,[13] in which 144 of 641 (22.5%) pooled morphea patients showed serologic evidence of *Borrelia* infection, 22.2% of our patients had been exposed to infection with *B burgdorferi*. This is much greater than the seroprevalence observed for the general population of the geographic origin of our patients in Bavaria, Germany.[24] Without the 11 cases of *Borrelia*-associated early-onset morphea, however, the seroprevalence for the remaining morphea patients of 11.4% (9/79) would not differ much from the endemic *Borrelia* exposure.

The ANA prevalence of 46.7% in our patient cohort also corresponds to former reports.[2,4] The age distribution of both the *Borrelia*-specific antibodies and ANAs was opposite to what would have been expected in healthy individuals, showing the highest prevalence and titers in the younger morphea patients.[25,26] In these early-onset morphea patients, the presence of *Borrelia*-specific antibodies was inevitably associated with high ANA titers. Together with the epidemiological considerations, this statistically highly significant association proposes a distinct entity of skin sclerosis resulting from *Borrelia* exposure preferentially at a young age. It translates our clinical experience of *Borrelia* infection in severe childhood morphea into a select disease entity.

The decline in *B burgdorferi*–specific serum antibody titers following antibiotic treatment in our 3 case presentations suggested that the patients had an active *Borrelia* infection before therapy. We could not detect *B burgdorferi* DNA by polymerase chain reaction within the lesional biopsy specimens from these patients (data not shown). Thus the presence of *B burgdorferi* within the skin lesions occasionally observed in other patients[13] may not be necessary to sustain inflammation and sclerosis once morphea has started. This concept is supported by the observation that in two of the cases reported herein in more detail, morphea remained active despite a decline in *Borrelia*-specific antibodies following antibiotic treatment. Furthermore, an individual predisposition is likely to be required for *B burgdorferi* to induce morphea, since in general *Borrelia* skin infections such as erythema migrans heal without sequelae when treated appropriately.[27] These results suggest that *Borrelia*-associated early-onset morphea may reflect a select type of infection-induced autoimmunity that persists or progresses independent from the initiating event. Whether this process involves activation of potentially autoaggressive bystander T cells or molecular mimicry, as proposed for post-Lyme arthritis[11] needs to be determined.

B burgdorferi sensu lato is a heterogeneous complex comprising different species. Because pathogenic *Borrelia* species may vary in different geographic regions, the relevance of *Borrelia* infection in morphea induction may show regional variations. *B burgdorferi* sensu stricto is the only human

pathogenic species in the United States. In Europe, Lyme borreliosis is largely caused by *B afzelii* or *B garinii*, while *B burgdorferi* sensu stricto is rare. Another recently identified European species is *B spielmanii*.[7,8,20]

Major immunogenic proteins of *B burgdorferi* such as the outer surface proteins A or C (OspA, OspC), but also other *Borrelia* proteins, are highly polymorphic and display substantial levels of sequence variability between and within the different *Borrelia* species or local *Borrelia* populations.[28-30] This diversity is relevant for virulence and *Borrelia*-directed immune responses and may also influence the induction of autoimmunity in infected hosts, as has been demonstrated for post-Lyme arthritis. Treatment-resistant Lyme arthritis has been associated with a T-cell response to a particular OspA peptide (OspA161-175) of *B burgdorferi* sensu stricto. While this particular sensu stricto-peptide differs in 6 or 3 amino acids from the same OspA peptide region of *B afzelii* or *B garinii*, it shares sequence identity with 8 of 9 amino acid residues of a human peptide, MAWD-BP276-288, from the MAWD-binding protein, a phenazine biosynthesis-like protein expressed by synoviocytes and other tissues. The immune response to these homologous peptides provides a species-dependent and region-specific explanation for *Borrelia*-induced autoimmunity in post-Lyme arthritis, which is more prevalent in the United States than in Europe.[31-33] In a similar mode, infection-induced autoimmunity in *Borrelia*-associated early-onset morphea might be influenced by the genetic heterogeneity of *B burgdorferi* or depend on certain immunogenic factors that may vary between different *Borrelia* species or geographic *Borrelia* populations and thus explain regional differences in the geographical prevalence of this novel disease entity.

Finally, the geographical distribution of Lyme borreliosis worldwide largely correlates with the known distribution of the ixodid vectors in northern temperate climate zones around the world.[34] Within the clinical spectrum of localized scleroderma, the occurrence of *Borrelia*-associated early-onset morphea would largely be expected in these tick-exposed populations.

Together, our data propose a distinct type of scleroderma that provides an explanation for the controversially discussed association of morphea with *Borrelia* infection. *Borrelia*-associated early-onset morphea may be considered a particular type of infection-induced autoimmunity in predisposed individuals. On average, within the cohort of morphea patients, it shows the earliest age at onset and is characterized by infection with *B burgdorferi* and evident autoimmune phenomena as reflected by high-titer ANAs.

The severity of the disease and its mutilating consequences in the cases reported here indicate that *Borrelia*-associated early-onset morphea may be a particularly aggressive type of localized scleroderma in childhood or adolescence. Therefore, based on our findings, testing for both *Borrelia* infection and ANAs appears especially relevant in patients with early age at morphea onset to identify patients suffering from this variant. Further studies will have to determine whether antibiotic treatment of *Borrelia* infection early in the course of *Borrelia*-associated early-onset morphea may improve the prognosis of this often devastating skin disease.

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