Morphoea and *Borrelia burgdorferi*: results from the Scottish Highlands in the context of the world literature

J R Goodlad, M M Davidson, P Gordon, R Billington, D O Ho-Yen

**Aims:** Previous studies investigating the link between infection with *Borrelia burgdorferi* and morphoea have produced conflicting results. Often, these studies have been undertaken in patients from different regions or countries, and using methods of varying sensitivity for detecting *Borrelia burgdorferi* infection. This study aimed to establish whether a relation could be demonstrated in the Highlands of Scotland, an area with endemic Lyme disease, with the use of a sensitive method for detecting the organism.

**Methods:** The study was performed on biopsies of lesional skin taken from 16 patients from the Highlands of Scotland with typical clinical features of morphoea. After histological confirmation of the diagnosis, a nested polymerase chain reaction (PCR) using primers to a unique conserved region of the *Borrelia burgdorferi* flagellin gene was performed on DNA extracts from each biopsy. A literature search was also performed for comparable studies.

**Results:** None of the 16 patients had documented clinical evidence of previous infection with *B. burgdorferi*. DNA was successfully extracted from 14 of the 16 cases but all of these were negative using PCR for *B. burgdorferi* specific DNA, despite successful amplification of appropriate positive controls in every test. The results were compared with those of other documented studies.

**Conclusions:** Examination of the literature suggests that there is a strong geographical relation between *B. burgdorferi* and morphoea. These results, in which no such association was found, indicate that morphoea may not be associated with the subspecies of *B. burgdorferi* found in the Highlands of Scotland.

**Erythema chronicum migrans, acrodermatitis chronica atrophicans (ACA), and lymphocytoma cutis are all cutaneous manifestations of Lyme disease, a multisystem disorder that follows infection with *Borrelia burgdorferi*.** An aetiological role for *B. burgdorferi* has also been proposed for other skin disorders, including primary cutaneous B cell lymphoma (PCBCL) and morphoea. Although there is increasing evidence to support this hypothesis in the context of PCBCL, studies investigating the link between *B. burgdorferi* and morphoea have produced conflicting results, some reports suggesting a positive association, but others not. This has led to the proposal that regional variations in *B. burgdorferi* may be important in dictating the spectrum of clinical disease following infection, with morphoea only being caused by certain subspecies endemic to specific geographical areas.

"Studies investigating the link between *Borrelia burgdorferi* and morphoea have produced conflicting results, some reports suggesting a positive association, but others not"

Although Lyme disease is endemic in many areas of the UK, only two previous studies have investigated the possible link with morphoea specifically in the UK. Both gave a negative result, but this was based primarily on patients with morphoea lacking serological evidence of previous infection with *B. burgdorferi*. However, because *B. burgdorferi* infection may occur without the production of specific antibodies these results are inconclusive. Therefore, we used a polymerase chain reaction (PCR) technique to evaluate skin biopsies taken from patients with morphoea in the Highlands of Scotland, an area with endemic Lyme disease, for the presence or absence of *B. burgdorferi* specific DNA. Recently, this approach has been used successfully to demonstrate a significant association between *B. burgdorferi* and PCBCL in patients from the same region. A literature review was also performed to interpret our results in the context of the possible geographical variations that may exist in the relation between *B. burgdorferi* infection and the subsequent development of morphoea.

**MATERIALS AND METHODS**

**Case selection**

The pathology department, Raigmore Hospital, Inverness, UK, processes all surgical pathology specimens for the Highland and Western Isles regions of Scotland, an area with a population of approximately 250,000 in which Lyme disease is endemic. By searching the surgical pathology files, Raigmore Hospital, Inverness (from 1976 to date), and reviewing case records of all patients with histology compatible with morphoea we were able to identify 16 patients for study, all with histological and clinical features typical of morphoea.

**Pathological studies**

Sections (5 µm thick) were cut from formalin fixed, routinely processed, paraffin wax embedded tissue blocks of each biopsy and stained with haematoxylin and eosin. Each case was assessed to confirm a diagnosis of morphoea and to approximate the stage of the disease using standard criteria.

**Demonstration of *B. burgdorferi* DNA**

Sections (6 × 5 µm) cut from formalin fixed, paraffin wax embedded tissue blocks were dewaxed and DNA extracted using the Intergen EX-WAX DNA extraction kit (Intergen,......
Manhattanville, New Jersey, USA). All DNA extracts were tested by PCR with primers for β-globin to ensure that DNA of sufficient quality and quantity was obtained for subsequent analysis.

To detect *B burgdorferi*, a nested PCR assay was used to amplify a sequence of the highly conserved flagellin encoding region, as described previously. The first stage PCR was performed with 20 μl of sample in a reaction volume of 50 μl, containing final concentrations of 10 mM Tris/HCl (pH 8.5), 50 mM KCl, 3.5 mM MgCl₂ (Applied Biotechnologies), 0.2 mM dNTP (Pharmacia Biosystems, Milton Keynes, UK), 0.5 U Taq polymerase (Applied Biotechnologies), and 0.2 μM each primer F1 (5′-ATT AAC GCT GCT AAT CTT AGT -3′) and F3 (5′-GTA CTA TTC TTT ATA GAT TC-3′) (Severn Biotech, Kidderminster, UK). The thermal cycling conditions were 35 cycles of one minute at 94°C, two minutes at 41°C, and three minutes at 66°C, followed by a further extension period of five minutes at 72°C. For the nested PCR, 20 μl of a 1/5 dilution of the first stage product was amplified in a 50 μl reaction mix with a reduced concentration of MgCl₂ (2.5 mM) and primers F6 (5′-TTC AGG GTC TCA AGC GTC TTC TTG GAC T-3′) and F8 (5′-GCA TTT TCA ATT TTA GCA AGT GAT G-3′) (Severn Biotech). The thermal cycling conditions were 35 cycles of one minute at 94°C, one minute at 50°C, and one minute at 72°C, with a final extension of five minutes at 72°C. A positive control (~10 cultured *B burgdorferi* organisms) and negative control were included in all cases. The PCR products were visualised under ultraviolet transmission of ethidium bromide stained agarose gels.

**Literature review**

A textword Medline search was conducted for the years 1966 to date using the terms “morphea”, “morphoea”, “Lyme disease”, and “*Borrelia*” in various combinations. Abstracts of all articles were read and papers selected for inclusion if they were published in English and used laboratory techniques to investigate the relation between *B burgdorferi* infection and morphoea.

**RESULTS**

**Clinical features**

There were five male and 11 female patients ranging in age from 3 to 69 years at diagnosis. According to the classification of Peterson et al, there were 13 cases of plaque morphoea (12 morphoea en plaque, one atrophoderma of Pasini and Pierini), two cases of linear morphoea (one linear morphoea, one en coup de sabre), and one case of generalised morphoea. None of the patients had a history of antibiotic treatment in their hospital notes and none had a documented history of manifestations of Lyme disease.

**Pathological findings**

All 16 cases showed histological features consistent with a diagnosis of morphoea, the principle finding being the presence of thickened collagen bundles in the reticular dermis. The histological stage of the lesion was approximated by additional changes. Three cases were interpreted as early stage lesions. All displayed a moderate lymphocytic infiltrate with fewer numbers of plasma cells arranged around blood vessels lined by swollen endothelial cells (fig 1). Six biopsies were regarded as showing features of late stage lesions. In these, there was increased eosinophilia of the thickened collagen bundles, together with homogeneous collagen in the papillary dermis, atrophy of eccrine glands with marked loss of periglandular fat, some fibrous thickening of blood vessel walls, and a minimal or absent chronic inflammatory cell infiltrate (fig 2). The remaining seven cases displayed features in keeping with lesions of an intermediate duration in which the “late” changes were either less pronounced, or only some were present.

**PCR analysis**

Amplifiable DNA was obtained from 14 of the 16 biopsies, as determined by PCR with primers for β-globin. These 14 cases were tested using primers for the *B burgdorferi* flagellin gene. In each test, there was successful amplification of control DNA extracted from cultured borrelia organisms, giving a product of 275 bp after the nested run. However, all 14 samples from patients with morphoea were negative, as were the negative controls (fig 3).
UK. patients with morphoea tested have been seronegative, clinical manifestations of morphoea. In several series, all evidence of previous infection and sought to correlate this with patients with morphoea may display specific cellular humoral immune response to the organism, large numbers of been demonstrated.

cess, and in only a small number of cases have spirochaetes staining or immunohistochemistry have met with mixed suc-

strate the organism in biopsies of lesional skin taken from more definite evidence of a causal link by seeking to demon-

merely represent coincidental infection, other studies sought

manifestation of chronic borreliosis.

DISCUSSION

Table 1 summarises the results of the literature review.14–37

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Direct visualisation in tissue sections of morphoea biopsies</th>
<th>Serology</th>
<th>Culture from biopsies of morphoeic lesions</th>
<th>PCR analysis of DNA extracts of morphoea biopsies</th>
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*Eleven of 39 cases in this study also showed an increased B burgdorferi induced proliferation of peripheral blood lymphocytes. †This represents the only positive culture where spirochaetes have been subtyped; in this instance PCR analysis confirmed B afzelii. §All patients in this study were selected on the basis of positive serology for borrelia infection; 7 of 7 tested also showed an increased B burgdorferi induced proliferation of peripheral blood lymphocytes.

**Summary of previous studies investigating the relation between Borrelia burgdorferi infection and morphoea in different geographical locations**

Literature review

Table 1 summarises the results of the literature review.14–37

The suspicion that morphoea may occur as a consequence of Borrelia burgdorferi infection and morphoea in different geographical locations. In view of problems in

Once more the results have been contradictory. Studies reporting a positive association between Borrelia burgdorferi infection and morphoea have shown evidence of the organism in between 26% and 100% of cases,2,3,14–37 whereas in a further 10 reports, including our current one, no positive cases have been identified in a total of 190 cases tested.22,23,25,26,28,34–37 Lastly, the culture of borrelia from biopsies of morphoeic lesions has also been attempted. Although several studies have produced completely negative results, 9,19,25,26,28 success has been achieved in a small number of cases,14,16,17,20,24 and the negative results may be partly or wholly attributable to the fastidiousness of the organism in culture. Two possible conclusions can be drawn from this diasp]
the particular strains of the subsequent development of morpoea. This phenomenon is already well documented for other accepted facets of borrelia infection. For example, ACA rarely occurs during the course of Lyme disease in North America where Borrelia burgdorferi sensu stricto is the dominant species, but is commonly seen in Europe where B. afzelii and B. garinii are more prevalent.35

“These results indicate that in the Scottish Highlands there is no association between infection with indigenous strains of Borrelia burgdorferi and the subsequent development of morpoea”

If this hypothesis is correct, then the literature suggests that Borrelia burgdorferi is implicated in the pathogenesis of at least some cases of morpoea in Austria (especially in the vicinity of Vienna).34–36 But almost certainly not in the USA or some, but possibly not all, parts of Germany.21–24 33–37 Isolated studies reporting a positive association in countries such as Italy, Switzerland, Puerto Rico, Turkey, and Japan, and a negative association in Spain, Finland, Holland, and France25–32 require corroboration before definite conclusions can be drawn about these geographical locations.

The results of our study can be assessed in a similar light using the Austrian studies as reference for an example of a population in which B. burgdorferi plays a role in the pathogenesis of morpoea. Given the cohort of patients studied, a similar positive result should have been forthcoming if the strains of borrelia endemic in the Scottish Highlands also possessed the potential to initiate the development of morpoea, because this region has the highest incidence of Lyme disease in Scotland, and probably the UK.44 The breakdown of morpoea subtypes studied was similar to that described in one of the Austrian papers in which a positive association was also demonstrated, meaning that case selection was unlikely to have biased our results.36 In addition, we are confident that although PCR is prone to false negative results, our technique is sufficiently sensitive to have detected the organism were it present. Positive controls were amplified in each reaction and we have also used this technique to identify B. burgdorferi in archival material from patients with PCBCL.1 In addition, our cases were not biased towards late stage morpoea and, coupled with the fact that B. burgdorferi has been identified in biopsies of patients with morpoea up to 20 years after the onset of cutaneous lesions, this means that the age of the lesions sampled was unlikely to have produced a false negative result.31 Moreover, although not directly questioned, there was no evidence that our patients were receiving antibiotic treatment before biopsy and none had a history documenting evidence of previous infection with Borrelia burgdorferi.54

“Take home messages”

• None of the 16 patients with morpoea had clinical evidence of previous infection with Borrelia burgdorferi
• All of the 14 cases tested were negative for B. burgdorferi specific DNA (using the polymerase chain reaction), despite successful amplification of appropriate positive controls in every test
• Because the literature suggests that there is a strong geographical relation between B. burgdorferi and morpoea, these results suggest that morpoea is probably not associated with the subspecies of B. burgdorferi found in the Highlands of Scotland.

ACKNOWLEDGEMENTS

This work was part funded by a grant from Raigmore Hospital, Research and Endowments Fund and part funded by grant K/MRS/50/C2774 awarded by the Chief Scientist Office, Edinburgh. The authors would also like to thank Dr J McPhee, Head of Pathology, Raigmore Hospital, Inverness, for his support and I Christie for technical assistance.

References


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*Mol Path* 2002 55: 374-378
doi: 10.1136/mp.55.6.374

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