Ticks and Tick-borne Diseases 10 (2019) 398–406

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Borrelia burgdorferi sensu lato infection in patients with peripheral facial palsy

Tereza Rojko[a,*], Petra Bogovič[a], Stanka Lotrič-Furlan[a], Katarina Ogrinc[a], Tjaša Cerar-Kišek[b], Urška Glinšek Biškup[b], Miroslav Petrovec[b], Eva Ružić-Sabljić[b], Andrej Kastrin[c], Franc Strle[a]

[a] Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2, 1525 Ljubljana, Slovenia
[b] Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia
[c] Institute for Biostatistics and Medical Informatics, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1104 Ljubljana, Slovenia

ARTICLE INFO

Keywords:
Lyme borreliosis
Peripheral facial palsy
Bell's palsy
Symptoms
CSF analysis
Corticosteroid treatment

ABSTRACT

The aims of the study were to determine the frequency of borrelial infection in patients with peripheral facial palsy (PFP) and to compare clinical and laboratory characteristics of patients with borrelial PFP and patients with PFP of unknown etiology.

Adult patients with PFP who presented at our department between January 2006 and December 2013 qualified for the study if they had undergone lumbar puncture and also been tested for the presence of borrelial IgM and IgG antibodies in serum and cerebrospinal fluid (CSF) in indirect chemiluminescence immunoassay. Patients with PFP who had obvious signs/symptoms indicating a disease other than Lyme borreliosis (LB) were excluded. Patients who qualified for the study were classified into three groups according to the clinical and microbiological criteria: those having confirmed LB, those with possible LB, and those with PFP of unknown etiology.

Of 589 patients diagnosed with PFP during the eight-year period, 436 patients (240 males, 196 females) with median age 42.5 years (15–87 years) qualified for the study. Among these patients, 64 (14.7%) fulfilled criteria for confirmed LB, 120 (27.5%) had a diagnosis of possible LB, and in 252 (57.8%) the cause of their PFP remained unknown. When compared with patients with unknown cause of PFP, the patients with confirmed LB were older, more often presented in summer, more often reported tick bites, more frequently had LB in the past, more often complained of constitutional symptoms and radicular pain, and more often had bilateral palsy and CSF pleocytosis. Among the patients with possible LB and patients with unknown cause of PFP there were no differences in frequency of constitutional symptoms, radicular pain, bilateral palsy or CSF pleocytosis.

Presentation in summer, tick bites, constitutional symptoms and radicular pain, bilateral palsy, and CSF pleocytosis strongly suggest borrelial etiology of PFP.

1. Introduction

Peripheral facial palsy (PFP) is a relatively common disorder usually manifesting as Bell's palsy, which is defined as acute PFP of idiopathic origin. Various infectious and non-infectious conditions can produce isolated PFP identical to Bell's palsy (Gilden, 2004), with *Borrelia burgdorferi* sensu lato (s.l.) infection one of the leading causes of PFP in Lyme borreliosis (LB) endemic regions such as Slovenia (Lotrič-Furlan et al., 1999; Arnež and Ružić-Sabljić, 2010). Adults with Bell's palsy benefit from corticosteroid treatment given within 72 h after the onset of the palsy (Sullivan et al., 2007; Engström et al., 2008), but there is no reliable evidence that such treatment is helpful or detrimental for patients with LB who need antibiotic treatment, therefore it is of importance to distinguish between these two entities as early as possible.

The aim of our study was to assess the frequency of borrelial etiology of PFP in adult patients in Slovenia and to compare the clinical and laboratory characteristics and outcome in such patients with those in patients with PFP of unknown etiology.

2. Patients and methods

2.1. Patients and study design

Patients over 15 years of age who presented with PFP at the

Abbreviations: PFP, peripheral facial palsy; s.l., sensu lato; LB, Lyme borreliosis; CSF, cerebrospinal fluid; EM, erythema migrans; AI, antibody index

* Corresponding author.
E-mail address: tereza.rojko@kclj.si (T. Rojko).

https://doi.org/10.1016/j.ttbdis.2018.11.019
Received 2 August 2018; Received in revised form 7 November 2018; Accepted 26 November 2018
Available online 06 December 2018
1877-959X/

Department of Infectious Diseases, University Medical Center Ljubljana, Slovenia, between January 2006 and December 2013 qualified for the study if they had undergone lumbar puncture and been tested for the presence of borrelial IgM and IgG antibodies in serum and cerebrospinal fluid (CSF) in indirect chemiluminescence immunoassay (CLIA). Patients with PFP who had obvious signs/symptoms indicating a disease other than LB, such as Ramsay Hunt syndrome, were excluded. Patients with viral etiology indicated by the presence of viruses in CSF in molecular methods were also excluded.

Patients with PFP were classified into three groups according to clinical and microbiological criteria: those having confirmed LB, those with possible LB, and those with PFP of unknown cause.

A diagnosis of LB was considered confirmed by the presence of at least one of four criteria: 1) presence or reliable history of recent erythema migrans (EM) i.e. within 120 days before onset of PFP; 2) intrathecal production of borrelial IgM and/or IgG antibodies; 3) isolation of *Borrelia burgdorferi* s.l. from CSF; 4) seroconversion to borrelial antigens within two months after the first visit. EM was defined according to Stanek et al. (2011).

LB was considered possible in patients with PFP who tested positive for borrelial IgM and/or IgG antibody in serum but did not fulfill any of the criteria for confirmed LB. The group of patients with unknown cause of PFP comprised those who did not fulfill criteria for confirmed or possible LB and no other cause of PFP was found.

The large majority of patients with PFP and confirmed LB, fewer than half of those with PFP and possible LB, and some patients with PFP of unknown etiology were treated with ceftriaxone 2 g iv once daily or with oral doxycycline 100 mg twice daily for 14 days. The decision for treatment and choice of antibiotic were left to the discretion of the treating physician. The same was valid for treatment with corticosteroids.

Patients were followed clinically for six months. In a subset of patients who were seronegative at the initial visit, borrelial serum antibodies were determined two and six months after the first visit.

2.2. Laboratory testing

At the first visit, routine blood and CSF parameters (cell count, concentration of total proteins, glucose, albumin, IgG, IgA, IgM) were assessed in all patients. *Borrelial antibodies* Antibodies to *B. burgdorferi* s.l. were analyzed in CLIA (LIAISON®, Diasorin, Italy) with recombinant outer surface protein C (OspC) and recombinant VlsE antigens for IgM antibody, and recombinant VlsE as the antigen for IgG antibody. Results were interpreted according to the manufacturer's instructions. Intrathecal production of borrelial antibodies was determined as described by Reiber and Peter (2001). Antibody index (AI) values > 1.4 were considered positive. We decided for the single-tier approach due to the difficulties in interpretation of western blots in Europe and since the results of several studies suggest that the ELISA systems that use Vlse and OspC as antigens for detecting Borrelia-specific IgG and IgM antibodies show sufficient sensitivity and specificity to replace the two-tier test principle (Branda et al., 2013; Wormser et al., 2013; Moore et al., 2016).

During the initial part of our study, antibodies to *B. burgdorferi* s. l. were routinely determined in in-house indirect immunofluorescence assay (IFA). For the present study, we retested all available serum and CSF samples using CLIA; serologic findings were interpreted according to the results of CLIA. The samples had been stored at −80 °C for a median time of 1861.5 (range 731–3083) days prior to testing. *Culture and isolation of Borrelia* spp. CSF samples (1 mL) were cultivated in Modified Kelly-Pettenkofer medium for the presence of *Borrelia* spp. for up to nine weeks. For species identification, borrelial DNA was digested overnight at 37 °C with the restriction endonuclease *Mlu*I, and fragments were separated by pulsed-field gel electrophoresis as described elsewhere (Ruzic-Sabljic et al., 2002; Strle et al., 2006). *Molecular testing for HSV-1, HSV-2, and VZV DNA in CSF* Available CSF specimens that had been stored at −80 °C for a median time of 1508 (range 213–3083) days were analyzed. A description of the methods is given in Supplementary material.

2.3. Statistical methods

Statistical differences between groups were evaluated using Fisher's exact test for categorical variables and the Mann–Whitney U test for numeric variables. All p values below 0.05 were considered to indicate significance. We used a random forest classifier for prediction of a plausible target class from the set of clinical predictors. Random forest is a state-of-the-art machine learning classifier employing two types of randomness to construct a collection of decision trees, i.e. a bootstrap aggregation procedure (Breiman, 1996) and random feature selection (Amit and Geman, 1997). The random forest approach is a powerful tool for uncovering complex relationships among predictor variables (Breiman, 2001); it does not tend to over-fit, provides variable importance ranking, and is suitable in settings with relatively low sample sizes. For performance evaluation we used the area under a precision-recall curve (AUPR), which is a standard metric in settings with an unbalanced number of cases across classes. The theoretical range of AUPR is between 0 and 1; higher values indicate better classification.

The selected characteristics of patients with favorable and unfavorable outcomes were compared using multiple logistic regression. Selection of predictors was based on expert opinion and was independent of observed outcomes. Results were summarized with odds ratios (OR), their 95% confidence intervals (CI), and corresponding p values.

R programming language using the caret package (R Development Core Team, 2018) was used for all statistical computing, including prediction modeling.

3. Results

As shown in Fig. 1, among 589 patients diagnosed with PFP during the eight-year period, 17 were excluded from the study because they had disease other than LB (11 Ramsay Hunt syndrome, two tick-borne encephalitis, one pyramid apex tumor, one sarcoidosis, two others with neurologic disease). A further 136 patients did not satisfy the inclusion criteria because either CSF was not available (23 patients) or antibodies to *B. burgdorferi* s.l. were not determined with CLIA (112 patients), or the CSF tested positive for HSV-1, HSV-2, or VZV DNA (the latter in 1/364 patients). Thus, the data of 436 patients (240 males, 196 females) with median age 42.5 years (15–87 years) were further analysed. Among these patients, left-sided facial palsy occurred in 215 (49.3%), right sided palsy in 214 (49.1%), and bilateral palsy in seven (1.6%).

Among the 436 patients, 64 (14.7%) fulfilled criteria for confirmed LB, in 120 (27.5%) the diagnosis was possible LB, and in 252 (57.8%) the cause of PFP remained unknown.

3.1. Patients with PFP and confirmed LB

The presence or reliable history of recent EM, isolation of *B. burgdorferi* s.l. from CSF, and/or intrathecal synthesis of borrelial IgM and/or IgG antibodies were established in 21/64 (33%), 6/64 (9%) and 51/64 (80%) patients, respectively. Several patients fulfilled more than one criterion for confirmed LB (Table 1).

At presentation, serum IgG and/or IgM antibodies were established in the majority (58/64, 91%) of patients with confirmed LB; more often IgG (54/64 patients, 84%) than IgM (30/64 patients, 47%). Solely serum IgG antibodies were found in 28 patients and solely IgM antibodies in four patients. Serum IgG and/or IgM antibodies were present in all six patients in whom *B. burgdorferi* s.l. was cultured from CSF, in 17/21 (81%) patients with EM, and in 49/51 (96%) patients in whom borrelial infection was confirmed from intrathecal synthesis of borrelial IgM and/or IgG antibodies (Table 2). More detailed analysis of the

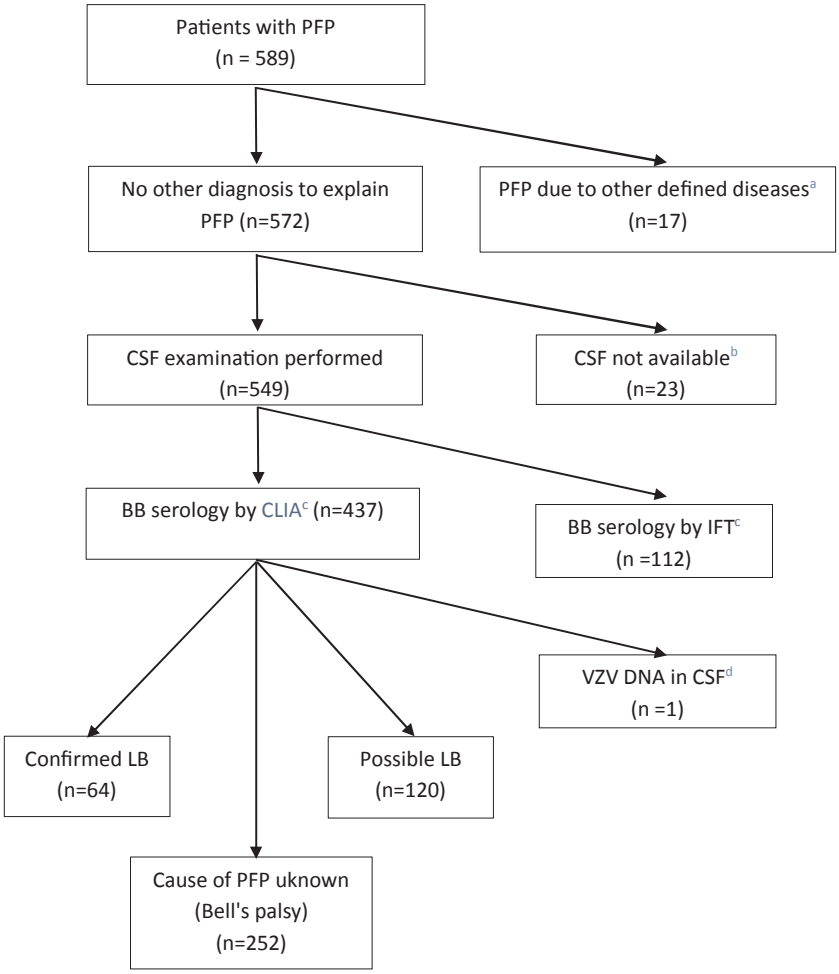

Fig. 1. Flowchart of the study. [a]Other defined diseases: 11 Ramsay Hunt syndrome, 2 tick-borne encephalitis, 1 pyramid apex tumor, 1 sarcoidosis, and 2 other neurological disease; [b]17 refusal of lumbar puncture, 2 coagulation disorders, 2 pregnancy, 2 technical difficulties with lumbar puncture; [c]the two groups did not differ significantly in age, sex, or number of symptoms; [d]364/437 CSF samples were tested for the presence of HSV-1, HSV-2 and VZV DNA.

latter subgroup showed that 15/37 (40%) patients with a positive AI for borrelial IgM had no borrelial IgM antibodies in serum, and that among 45 patients with intrathecal synthesis of IgG antibodies there was only one (2%) IgG seronegative patient.

Among six IgM and IgG seronegative patients, the diagnosis of confirmed LB was established from the history of EM in two patients, by the presence of EM in two patients, and by intrathecal synthesis of borrelial IgM and/or IgG antibodies in the remaining two (IgM and IgG intrathecal synthesis in one patient, solely IgM in one). No case of seroconversion was found in a subset of three seronegative patients (two with the presence of EM and one with the history of EM) who were tested two months after the initial visit.

3.2. Patients with PFP and possible LB

In 120 patients the presence of borrelial IgM and/or IgG antibodies in serum was the only finding indicating possible LB. In 17/120 (14.2%) patients both borrelial IgG and IgM antibodies were present, in 68/120 (56.7%) solely IgG, and in 35/120 (29.2%) solely IgM antibodies.

Table 1
Evidence of borrelial infection among 64 patients with confirmed Lyme borreliosis.

	Erythema migrans (N = 21)	Isolation of BB from CSF (N = 6)	IT synthesis of borrelial antibodies[a] (N = 51)
Erythema migrans (N = 21)	21	1	8
Isolation of BB from CSF (N = 6)[b]	1	6	6
IT synthesis of borrelial IgM and/or IgG antibodies (N = 51)	8	6	51
- IT synthesis of IgM	7	5	37
- IT synthesis of IgG	8	6	45
- IT synthesis of IgM and IgG	7	5	31

BB, *Borrelia burgdorferi* s.l.; CSF, cerebrospinal fluid; IT, intrathecal.

[a] In five of the patients, IgG antibodies to *B. burgdorferi* s. l. in serum only reached 240 IU/mL, therefore in these patients the intrathecal AI was calculated using a serum IgG level 702 IU/mL, which was the median value in 32 patients with IgG antibodies > 240 IU/mL, in whom complete dilutions were performed (range 299.4–3838 IU/mL in this group).

[b] *Borrelia garinii* in 5 cases and *Borrelia afzelii* in 1 case.

Table 2
Borrelia burgdorferi s.l. serum antibodies in 64 patients with confirmed Lyme borreliosis[a].

	Erythema migrans (N = 21)	Intrathecal production of borrelial antibodies (N = 51)				Isolation of borreliae from CSF (N = 6)[c]
		IgM[b] (N = 37)	IgG[b] (N = 45)	IgM and IgG (N = 31)	IgM and/or IgG (N = 51)	
Serum borrelial antibodies:						
IgM	12/21 (57%)	22/37 (59%)	21/45 (47%)	18/31 (58%)	25/51 (49%)	2/6 (33%)
IgG[a]	15/21 (71%)	33/37 (89%)	44/45 (98%)	30/31 (97%)	47/51 (92%)	6/6 (100%)
IgM and IgG	10/21 (48%)	20/37 (54%)	21/45 (47%)	18/31 (58%)	23/51 (45%)	2/6 (33%)
CSF borrelial antibodies:						
IgM	11/21 (52%)	37/37 (100%)	34/45 (76%)	31/31 (100%)	40/51 (78%)	5/6 (83%)
IgG	10/21 (48%)	32/37 (86%)	45/45	31/31 (100%)	46/51 (90%)	6/6 (100%)
IgM and IgG	8/21 (38%)	32/37 (86%)	34/45 (76%)	31/31 (100%)	35/51 (69%)	5/6 (83%)

[a] No cases of seroconversion among 72 patients who tested negative for IgG borrelial antibodies in CLIA and in whom borrelial serum antibodies were determined at follow-up examination with the same test.
[b] Median borrelial IgM AI value 8.3 (1.6–811.4) and median borrelial IgG AI value 10.1 (1.5–146.5).
[c] *Borrelia garinii* in 5 cases and *Borrelia afzelii* in 1 case.

3.3. Comparison of groups at the initial visit

The duration of PFP before CSF examination was longer in patients with possible LB than in those with confirmed LB or PFP of unknown cause (medians 7, 4, 4 days, respectively).

As shown in Table 3, in comparison with patients with unknown cause of PFP, patients with confirmed LB were older, more often reported tick bites and more frequently had LB in the past, more often complained of constitutional symptoms and radicular pain, and more often had bilateral palsy, but less regularly reported preceding symptoms of common cold. Furthermore, in patients with confirmed LB, PFP presented more often in summer (31/64; 48%), less often in fall (15/64; 23%) and spring (12/64; 19%), and rarely in winter (6/64; 9%), whereas patients in the other two groups presented more evenly throughout the year (Fig. 2).

CSF pleocytosis (leukocyte count $> 5 \times 10^6$/L) was significantly more frequent in patients with confirmed LB (40/64; 62% patients vs 24/252; 9.5% in patients with unknown cause of PFP), as were elevated CSF protein (> 0.45 g/L) levels and elevated albumin quotient (Table 3).

Comparison of patients with possible LB and patients with unknown cause of PFP found no significant differences, with the exception that patients in the possible LB group were older, had longer duration of PFP prior to examination, and were more often treated with antibiotics (Table 3).

To examine possible differences between the groups we also used the machine learning approach. The task was to build a statistical classifier that could separate among pairs of groups (e.g., between patients with confirmed LB and patients with possible LB). A random forest classifier showed several distinctions between patients with confirmed LB and those with possible LB (AUPR = 0.87) and also those with unknown cause of PFP (AUPR = 0.73). Differences between the possible LB group and the group with unknown cause of PFP were fewer and less marked (AUPR = 0.36) and were limited to patient age, CSF protein level, and albumin quotient. We also evaluated the relative importance of predictors during the classification process (Fig. 3). The differences were much more pronounced for CSF parameters than for other predictor variables such as season or age.

3.4. CSF pleocytosis in patients with unknown cause of PFP and in patients with possible LB

Elevated CSF cell counts were rare in both these groups. Among 252 patients with PFP of unknown cause, only 24 showed CSF pleocytosis (9.5%) and the CSF leukocyte count was > 10 cells/mL in only 11/252 (4.4%). The corresponding findings for the group with possible LB were 7/120 (5.8%) and 5/120 (4.2%), respectively. Patients with PFP of unknown cause who had CSF leukocyte counts > 10 cells/mL reported more frequent constitutional symptoms than patients without pleocytosis (7/11, 64% vs 70/228, 30.7%; $p = 0.029$). Bilateral palsy was rare in both groups (1/252 in patients with Bell's palsy, 1/120 in the group with possible LB) and was limited to patients with pleocytosis. In the group with PFP of unknown etiology and in the group with possible LB, the duration of symptoms before lumbar puncture was similar in patients with pleocytosis and in those with a normal CSF cell count: 4.5 (1–21) days vs 4 (0–270) days, $p = 0.297$ in patients with PFP of unknown etiology, and 9 (2–47) days vs 7 (0–90) days, $p = 0.188$ in patients with possible LB.

3.5. Antibiotic and corticosteroid treatment

Among the 436 patients in the study, 144 (33%) received antibiotics. Antibiotic treatment was given to 59/64 (92%) patients with borrelial PFP (48 ceftriaxone treatment, 11 doxycycline), 52/120 (43.3%) patients with possible LB (22 ceftriaxone, 30 doxycycline), and 33 (13.1%) patients with unknown cause of PFP (27 ceftriaxone, 6 doxycycline). In the latter two groups nearly all patients with pleocytosis but a much smaller proportion of patients with normal CSF cell counts received antibiotics. Corticosteroids (mostly methylprednisolone) were prescribed in 66/436 (15.1%) patients, including 7/64 (11%) with confirmed LB, 25/120 (20.8%) with possible LB and 34/252 (13.5%) with Bell's palsy.

3.6. Later course and outcome

Of 299 patients examined at a 2-month follow-up visit, 179 had tested seronegative for borrelial IgG at the initial examination. Of those 179 patients, 73 patients were retested 2 months later: three with confirmed LB, 13 with possible LB (the diagnosis was based on the presence of borrelial IgM antibodies; two patients had CSF pleocytosis) and 57 with PFP of unknown cause (including seven with CSF pleocytosis); there were no cases of seroconversion among these 73 patients. Of the retested patients, three patients with confirmed LB (2 with visible EM at the first examination and in one with the history of EM) and nine patients with CSF pleocytosis in the other two groups had been treated with antibiotics, the others not. Unfortunately, serological follow up with CLIA was not performed in the other 106 initially seronegative patients, including the patient with intrathecal borrelial IgM antibody synthesis.

Of the 436 patients in the study, 299 (68.6%) attended follow-up examination two and six months after the first visit: 59/64 (92%) with confirmed LB, 89/120 (74.2%) with possible LB, and 151/252 (59.9%) with PFP of unknown cause. During follow-up none of the patients developed any additional sign suggestive of LB. In 237/298 (79.5%) patients, regression of PFP was complete six months after its onset according to clinical assessment. The percentage of patients with

Table 3
Baseline data, clinical characteristics, and cerebrospinal fluid findings in patients with peripheral facial palsy of unknown cause in comparison with patients with confirmed and possible Lyme borreliosis.

	p_{FDR}	p[a]	Possible LB (N = 120)	PFP of unknown cause (N = 252)	Confirmed LB (N = 64)	p[b]	p_{FDR}
Age; median (range)	< **0.001**	< **0.001**	51.5 (16-83) (N = 120)	33 (15-87) (N = 252)	54 (15-79) (N = 64)	< **0.001**	< **0.001**
Sex, male	0.276	0.058	56/120 (46.7%)	145/252 (57.5%)	39/64 (61%)	0.672	0.751
Previous LB	> 0.999	> 0.999	6/120 (5%)	12/252 (4.8%)	12/64 (19%)	**0.001**	**0.002**
Tick bite (≤120 days before PFP)[c]							
1 tick bite	0.893	0.658	22/116 (19%)	42/249 (16.9%)	28/63 (44%)	< **0.001**	< **0.001**
≥2 tick bites	0.501	0.145	8/116 (6.9%)	30/249 (12 %)	13/63 (21%)	0.100	0.146
	0.122	0.016	14/116 (12.1%)	12/249 (4.8%)	15/63 (24%)	< **0.001**	< **0.001**
Common cold (within the past month)[c]	0.825	0.369	16/112 (14.3%)	47/252 (18.7%)	4/62 (6%)	0.020	0.033
Diabetes mellitus	0.255	0.044	18/120 (15%)	20/252 (7.9%)	6/64 (9%)	0.799	0.867
Exposure to draught/air condition[c]	> 0.999	0.816	8/52 (15.4%)	16/113 (14.2%)	2/39 (5%)	0.161	0.219
Duration of PFP before CSF examination (days)	< **0.001**	< **0.001**	7 (0-90) (N = 120)	4 (0-270) (N = 250)	4 (0-120) (N = 63)	0.302	0.383
Treatment with corticosteroids before CSF examination[d]	0.501	0.134	25/120 (20.8%)	36/252 (14.3%)	7/64 (11%)	0.548	0.663
Number of patients with bilateral palsy	0.887	0.542	1/120 (0.8%)	1/252 (0.4%)	5/64 (8%)	**0.002**	**0.003**
Local symptoms	0.861	0.439	61/120 (50.8%)	117/252 (46.4%)	27/64 (42%)	0.576	0.663
Local paresthesia	0.861	0.453	14/120 (11.7%)	22/252 (8.7%)	9/64 (14%)	0.238	0.312
Facial edema	0.830	0.393	6/120 (5.0%)	8/252 (3.2%)	2/64 (3%)	> 0.999	> 0.999
Local pain, affected side of face	0.893	0.649	17/120 (14.2%)	42/251 (16.7%)	8/64 (12%)	0.565	0.663
Disturbance of taste	0.887	0.568	24/120 (20.0%)	44/252 (17.5%)	11/64 (17%)	> 0.999	> 0.999
Constitutional symptoms	> 0.999	0.813	37/120 (30.8%)	81/252 (32.1%)	44/64 (69%)	< **0.001**	< **0.001**
Malaise	0.887	0.607	7/120 (5.8%)	11/252 (4.4%)	12/64 (19%)	< **0.001**	< **0.001**
Fever	> 0.999	> 0.999	3/119 (2.5%)	8/252 (3.2%)	5/64 (8%)	0.149	0.210
Headache	0.887	0.599	30/120 (25%)	56/252 (22.2%)	28/64 (44%)	**0.001**	**0.002**
Vertigo	0.870	0.481	5/120 (4.2%)	17/252 (6.7%)	4/64 (6%)	> 0.999	> 0.999
Myalgia	> 0.999	> 0.999	1/120 (0.8%)	2/252 (0.8%)	7/64 (11%)	< **0.001**	< **0.001**
Arthralgia	> 0.999	> 0.999	3/120 (2.5%)	7/252 (2.8%)	9/64 (14%)	**0.001**	**0.002**
Radicular pain	0.767	0.323	1/120 (0.8%)	0/252 (0%)	13/64 (20%)	< **0.001**	< **0.001**
CSF leukocyte number (x10^6/L)	0.608	0.210	2 (0-28) (N = 120)	2 (0-160) (N = 252)	59 (0-886) (N = 64)	< **0.001**	< **0.001**
> 5 × 10^6 leukocytes/L	0.767	0.316	7/120 (5.8%)	24/252 (9.5%)	40/64 (62%)	< **0.001**	< **0.001**
> 10 × 10^6 leukocytes/L	> 0.999	> 0.999	5/120 (4.2%)	11/252 (0.4%)	39/64 (60.9%)	< **0.001**	< **0.001**
Number of CSF leukocytes (x10^6/L; only patients with CSF leukocytes > 5 × 10^6/L)	0.945	0.721	15 (7-28) (N = 7)	10 (6-160) (N = 24)	138.5 (6-886) (N = 40)	< **0.001**	< **0.001**
Number of CSF leukocytes (x10^6/L; only patients with CSF leukocytes > 10 × 10^6/L)	0.255	0.047	16 (11-28) (N = 5)	40 (11-160) (N = 11)	144 (13-886) (N = 39)	**0.002**	**0.003**
CSF protein concentration	> 0.999	0.983	0.38 (0.15-0.99) (N = 120)	0.37 (0.17-1.35) (N = 252)	0.61 (0.20–4.06) (N = 64)	< **0.001**	< **0.001**
Elevated (> 0.45 g/L)	0.887	0.536	36/120 (30%)	67/252 (26.6%)	46/64 (72%)	< **0.001**	< **0.001**
Albumin quotient elevated (> 7.4)	0.532	0.168	30/118 (25.4%)	46/245 (18.8%)	40/63 (63%)	< **0.001**	< **0.001**
Number of patients with CSF/serum glucose ratio < 0.5[c]	0.608	0.224	18/115 (15.7%)	25/236 (10.6%)	27/63 (43%)[e]	< **0.001**	< **0.001**
Number of patients who came to follow-up examination 2 and 6 months after first visit	0.122	**0.008**	89/120 (74.2%)	151/252 (59.9%)	59/64 (92%)	< **0.001**	**0.002**
Number of patients with complete clinical regression of peripheral facial palsy at 6 months after the first examination	> 0.999	0.873	70/89 (78.7%)	116/151 (76.8%)	51/59 (86%)	0.084	0.128
Time (days) to complete clinical regression of peripheral facial palsy[c]	0.490	0.116	30 (7-450) (N = 64)	29 (3-120) (N = 94)	32.5 (5-356) (N = 40)	0.059	0.093
Antibiotic treatment[f]	< **0.001**	< **0.001**	52/120 (43.3%)	33/252 (13.1%)	59/64 (92%)	< **0.001**	< **0.001**
Ceftriaxone			22	27	48		
Doxycycline			30	6	11		

Data for categoric values are presented as number of patients (percentage of group total); data for numeric values are presented as median (range); LB, Lyme borreliosis; PFP, peripheral facial palsy; CSF, cerebrospinal fluid; *pFDR*, *p*-values presented as false discovery rate (FDR) corrected *p*-values.

[a] Comparison of findings in patients with PFP of unknown cause and possible Lyme borreliosis.

[b] Comparison of findings in patients with PFP of unknown cause and confirmed Lyme borreliosis.

[c] Data were available only for limited number of patients.

[d] Dosage of methylprednisolone varied from 16 mg/day to 64 mg/day, duration of treatment from 5 to 28 days.

[e] In 5/63 patients the CSFglu/Sglu was less than 1/3.

[f] Treatment approaches were left to the discretion of the treating physician. However according to recommendations, patients with PFP and confirmed Lyme borreliosis are treated with antibiotics, those with unknown cause of PFP are not, while for the group with possible Lyme borreliosis the recommendations are less precise; in addition, PFP associated with CSF pleocytosis is indication for antibiotic treatment. Five patients, allocated to the confirmed Lyme borreliosis group, had not received antibiotics because they were seronegative according to in-house indirect immunofluorescence assay (IFA) which was used during the initial years of the study, but were found to have intrathecal borrelial antibody synthesis after retesting serum and CSF samples for the presence of antibodies with CLIA. Patients without LB, who received antibiotics, had mostly pleocytosis (20/33); reported complaints such as headache and occipital pain; or had positive serum borrelial IgG antibodies determined by IFA, which were later negative with CLIA (4 patients).

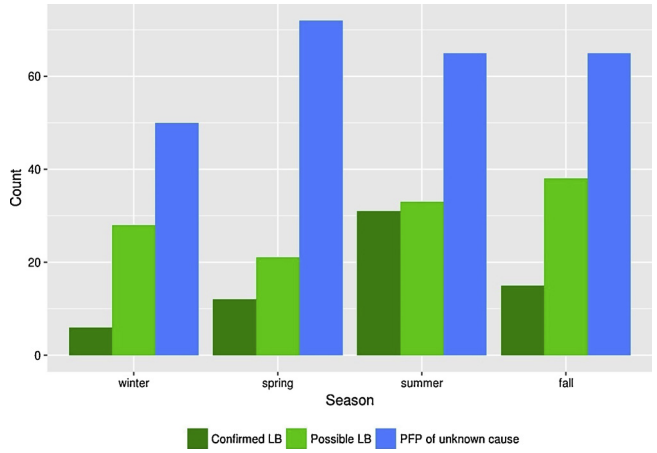

Fig. 2. Seasonal distribution of peripheral facial palsy (PFP) cases in the three different etiological groups (number of cases). The group with confirmed Lyme borreliosis (LB) more often presented in summer, less often in fall and spring and only rarely in winter, while patients in the other two groups presented more evenly throughout the year.

complete recovery of PFP was highest in the group with confirmed borrelial infection (51/59, 86%), followed by the group with possible LB (70/89, 79%), then the group with Bell's palsy (116/151, 76.8%) (Table 3). Patients with PFP of unknown cause who had pleocytosis had complete regression of PFP six months after the first examination more often than patients with normal CSF leukocyte counts (10/11, 91% vs 100/131, 76.3%; $p < 0.001$). In contrast, in the group with possible LB, complete regression at six months was less frequent in patients with pleocytosis than in those with normal CSF leukocyte count (2/7, 29% vs 68/82, 83%; $p = 0.004$).

There were no substantial differences between patients who received or did not receive methylprednisolone; this was valid for all patients as well as within individual "etiologic" groups (Table 4). In the group with confirmed LB, 6/7 (86%) patients who were treated with methylprednisolone and 45/52 (86%) patients who did not receive corticosteroids had favorable outcome of PFP ($p = 0.660$); the corresponding findings in the group with possible LB were 16/23 (70%) vs 54/66 (82%), $p = 0.172$.

The association of several patient characteristics (age, sex, duration of PFP, corticosteroid therapy, antibiotic treatment, patient group according to PFP etiology) with outcome (favorable or unfavorable) was assessed using multiple logistic regression. The statistical model exposed age ($p = 0.034$) and duration of PFP prior to diagnosis ($p = 0.017$) as significant predictors for unfavorable outcome, but not treatment with corticosteroids or antibiotics (Table 5).

4. Discussion

Abnormalities of the cranial nerves are probably the most common focal nervous system abnormality in Lyme neuroborreliosis, with facial nerve involvement in about three-fourths of cases (Halperin et al., 2013, 2008). Facial palsy in LB can present as part of a triad that includes radicular pain, cranial nerve involvement (most commonly facial palsy), and lymphocytic pleocytosis in the CSF, or the palsy can be the sole manifestation of the disease, in which case it is difficult to distinguish from Bell's palsy (Stanek and Strle, 2008; Lotrič-Furlan et al., 1999).

In Europe, the proportion of PFP resulting from infection with *Borrelia* spp. has been estimated as ranging between 2.2% and 33.3% (Arnež and Ružić-Sabljić, 2010; Bremell and Hagberg, 2011; Kindler et al., 2016; Ljøstad et al., 2005; Lotrič-Furlan et al., 1999; Peltomaa et al., 2002; Puhakka et al., 1992). Moreover, because of differences in the occurrence of LB in examined regions, the dissimilar criteria used for the definition of borrelial etiology, and different study populations

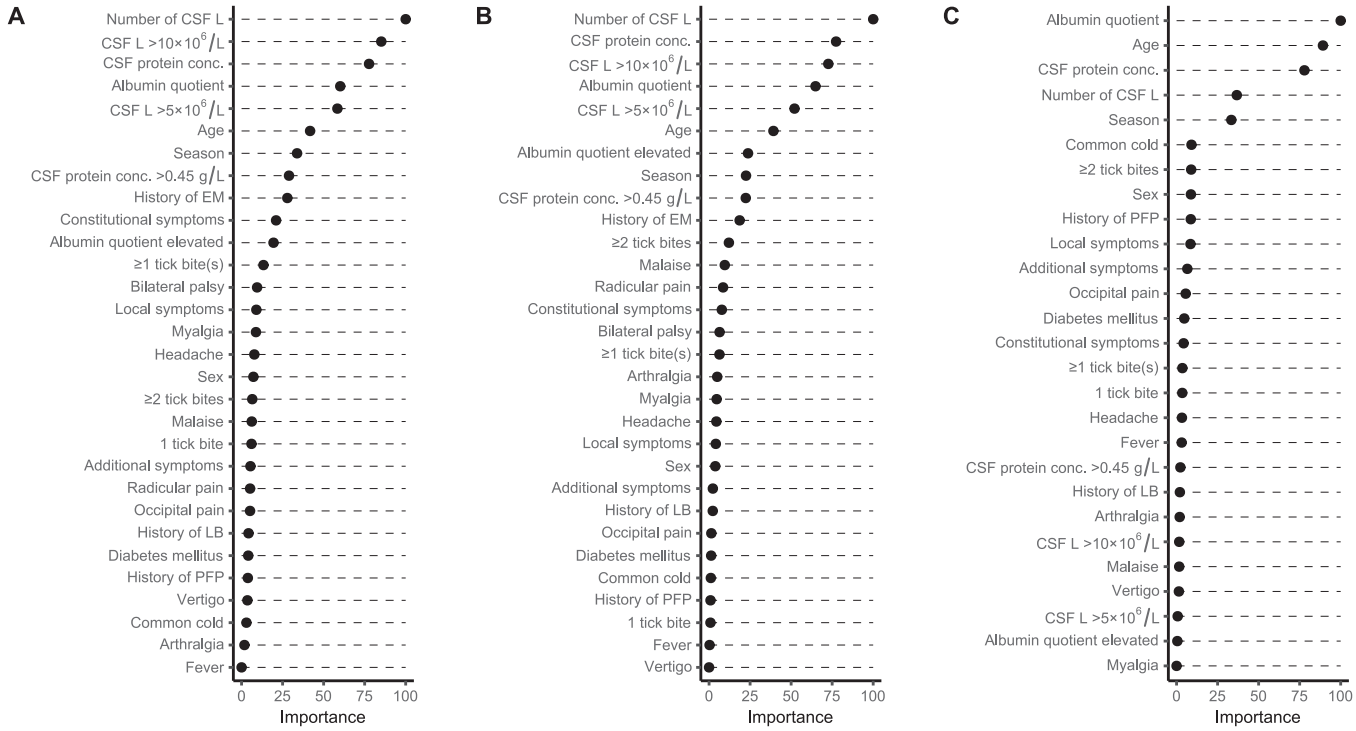

Fig. 3. Using a random forest classifier, we evaluated the relative importance of predictors during the classification process. Differences were much more pronounced for CSF parameters than for other predictor variables such as season and age. CSF, cerebrospinal fluid; L, leucocytes; EM, erythema migrans; LB, Lyme borreliosis; PFP, peripheral facial palsy. Panel A refers to the classification between confirmed LB and possible LB patients, panel B to the classification among confirmed LB and patients with PFP of unknown cause, while panel C depicts relative importance of predictors in classification between possible LB and patients with PFP of unknown cause.

Table 4
Association of clinical characteristics and outcome of peripheral facial palsy (PFP) at examination 6 months after onset of PFP for all patients and for patients within individual groups.

Group	Outcome	Age (years)	Sex (male)	Duration of PFP (days)	Medrol	Antibiotic	CSF Pleocytosis
All patients (N = 299)	Unfavorable	49.03	27/60	14.20	14/60	27/60	12/60
	Favorable	43.07	102/236	8.21	34/236	104/236	53/236
	p	**0.035**	0.884	0.242	0.116	> 0.999	0.731
Confirmed LB (N = 58)	Unfavorable	52.88	4/8	13.12	1/8	7/8	4/8
	Favorable	52.06	18/50	9.10	6/50	49/50	34/50
	p	0.946	0.462	0.821	> 0.999	0.259	0.428
Possible LB (N = 89)	Unfavorable	53.95	11/19	23.00	7/19	15/19	5/19
	Favorable	48.56	37/70	9.74	16/70	31/70	2/70
	p	0.384	0.798	**0.001**	0.244	**0.009**	**0.004**
Unknown etiology (N = 151)	Unfavorable	45.27	12/33	9.39	6/33	5/33	3/33
	Favorable	35.89	47/116	6.91	12/116	24/116	17/116
	p	**0.006**	0.693	0.353	0.234	0.621	0.566

PFP, peripheral facial palsy; CSF, cerebrospinal fluid; LB, Lyme borreliosis; Medrol, methylprednisolone.

Table 5
Factors associated with unfavorable outcome of peripheral facial palsy based on multiple regression analysis.

Variable	OR (95% CI)[a]	*p*-value[b]
Age	0.98 (0.96-1.00)	**0.032**
Sex (male vs female)	1.08 (0.59-1.96)	0.812
Duration of PFP prior to diagnosis (days)	0.97 (0.95-1.00)	**0.016**
Corticosteroids	0.66 (0.31-1.38)	0.266
Antibiotic	0.96 (0.42-2.24)	0.932
Pleocytosis	0.87 (0.34-2.24)	0.772
Possible LB vs confirmed LB	0.60 (0.21-1.69)	0.331
Unknown cause of PFP vs confirmed LB	0.36 (0.13-1.02)	0.054

OR, odds ratio; CI, confidence interval; PFP, peripheral facial palsy; LB, Lyme borreliosis.

[a] Estimated from multiple logistic model with outcome of PFP as the dependent variable.

[b] *p*-value < 0.05 statistically significant; significant *p*-values are shown in bold print.

(children vs adults), results are difficult to compare. The proportion of patients with confirmed borrelial etiology of PFP in the present study (64/436, 14.7%) is slightly lower than reported for adult patients in Slovenia more than 15 years ago (22/114, 19.3%) (Lotrič-Furlan et al., 1999), and lower than in children from the same region (12/52, 23%) (Arnež and Ružić-Sabljić, 2010); the higher percentage in children than in adults has been reported previously (Nigrovic et al., 2008; Paydar-Darian et al., 2017).

Our criteria for diagnosing confirmed LB (presence or reliable history of EM; intrathecal production of borrelial IgM and/or IgG antibodies; isolation of *B. burgdorferi* s.l. from CSF; seroconversion to borrelial antigens) and possible LB (presence of borrelia IgM and/or IgG antibody in serum as the only indicator of borrelia infection) are not completely congruent with those recommended by the EFNS (Mygland et al., 2010). Meeting just 1 of 4 criteria for confirmed LB could be problematic. For example, since patients with confirmed LB were more likely to have had prior LB, and since a positive AI can persist for years after successful LB treatment, it would be possible that some of these patients were labeled 'confirmed' based on a positive AI from a prior infection. Although we cannot exclude such possibility, the chances for misclassification are probably small, because: i) of 51 patients with intrathecal antibody synthesis of borrelial antibodies 13 fulfilled also other criteria for confirmed LB (the presence of EM and/or isolation of borreliae from CSF); and because ii) among 38 patients in whom the only fulfilled criterion of LB group was positive AI only five had LB in the past and none of them had Lyme neuroborreliosis, i.e. all of them had solitary EM without extracutaneous manifestations of LB.

Adult patients with Bell's palsy benefit from timely corticosteroid treatment (Sullivan et al., 2007; Engström et al., 2008) but such treatment may be harmful for patients with LB (Jowett et al., 2017; Wormser et al., 2018), therefore one of the main aims of the present study was to assess whether we could distinguish patients with borrelial PFP from patients with Bell's palsy early in the course of the disease using an approach as simple as possible. Our study has confirmed the findings of some previous reports in which presentation in summer (Bremell and Hagberg, 2011; Kindler et al., 2016), constitutional symptoms, and history of tick bite(s) in the four months prior to PFP (Lotrič-Furlan et al., 1999) indicate the borrelial etiology of PFP. As in other studies (Bremell and Hagberg, 2011; Kindler et al., 2016), the combination of PFP and radicular pain was highly specific for LB but was rare. LB is also considered one of the few causes of bilateral PFP (others being sarcoidosis and Guillain–Barre syndrome) (Halperin et al., 2013), which in the present study was found much more commonly in patients with confirmed LB than in the other two groups (5/64; 8% vs 2/372, 0.5%; $p < 0.001$).

However, our study also included 7/64 (11%) patients with confirmed LB without any suggestive history (no tick bites in the past four months, no history of EM) and with PFP as an isolated clinical manifestation (no constitutional symptoms and/or radicular pain or EM at presentation). Without serologic testing for *B. burgdorferi* s.l., corticosteroid treatment would most probably be given to these patients (if they presented within the first 72 h after PFP onset) instead of antibiotics, since Bell's palsy would be suspected. This confirms that in an LB endemic area a laboratory search for *B. burgdorferi* s.l. infection is needed in patients with PFP without other explanation.

Considerable debate has focused on the need for CSF examination in patients with suspected Lyme neuroborreliosis, particularly in those with PFP (Halperin, 2008). In Europe, positive CSF results are required for a diagnosis of Lyme neuroborreliosis (Mygland et al., 2010; Stanek et al., 2011), but in the USA diagnostic lumbar puncture is not always required (Halperin et al., 2013; Kowalski et al., 2011). As expected from the findings of earlier studies (Bremell and Hagberg, 2011; Lotrič-Furlan et al., 1999), CSF pleocytosis in the present study was significantly more frequent in patients with confirmed LB (62%) than in patients with PFP of unknown etiology (9.5%) or possible LB (5.8%); CSF protein levels and CSF/serum albumin quotients were also higher in confirmed LB. Moreover, CSF parameters were the most important variables for confirmed LB infection in the prediction model (random forest classifier), with much more weight than the non-CSF variables such as season, tick bites, constitutional symptoms, radicular pain, or bilateral facial palsy. Lymphocytic pleocytosis was confirmed in all patients with bilateral palsy (also in one patient with PFP of unknown origin and one with possible LB), indicating that in the case of bilateral facial palsy a more "generalized" nervous system involvement is present.

Although there were considerable differences between patients with borrelial PFP and those with PFP of unknown origin, the patients with

possible LB, in whom the only finding that suggested LB was the presence of borrelial antibodies in serum, did not differ significantly from those with PFP of unknown origin, apart from age and duration of PFP prior to examination.

A considerable proportion of the population living in LB endemic areas has positive serology for *B. burgdorferi* s.l., and the percentage of patients who tested positive for borrelial IgG antibodies in the groups of patients with possible LB and PFP of unknown cause combined (85/372, 22.8%) was only slightly higher than the percentage of IgG seropositive healthy Slovenian blood donors determined with the same test (9/49, 18%) (Cerar et al., 2006), therefore it is most likely that positive borrelial serology in patients with possible LB reflects prior (asymptomatic) infections, thus implying that the PFP is not due to LB, as was also suggested in a Swedish study (Bremell and Hagberg, 2011). Higher age in patients with possible LB is also in concordance with higher background seropositivity in an older population in LB endemic areas (Halperin et al., 2013).

In the present study, CSF pleocytosis was present in 9.5% (24/252) of the patients with PFP of unknown etiology, which corroborates findings from other studies reporting that 3–20% of patients with Bell's palsy have elevated CSF cell counts (Lotrič-Furlan et al. (1999) 15.9%, Bremell and Hagberg (2011) 20%, Kohler et al. (1999) 5.2%, Ljøstad et al. (2005) 3%). However, in our patients CSF pleocytosis was mild – fewer than half the patients with PFP of unknown etiology and pleocytosis had > 10 cells/mL. There were no cases of seroconversion in a subset of patients in whom borrelial antibodies were assessed using the same test 6–8 weeks after the first examination. Given that PFP may occur very early in the course of LB before any measurable antibody response, and since serologic response in such patients may not develop because of early antibiotic treatment (Halperin et al., 2013) and also because all our patients with pleocytosis were treated with antibiotics for LB, we could speculate that in some of these patients the PFP might be due to *B. burgdorferi* s.l. infection. However, in the group with PFP of unknown etiology the duration of symptoms before presentation and lumbar puncture was not shorter in patients with pleocytosis than in those with normal CSF cell counts and was similar to the duration in the group with confirmed borrelial infection.

Six months after the first examination, complete clinical remission of PFP was achieved in 86% of the patients with confirmed LB, which is in broad agreement with previous studies (Ljøstad et al., 2005; Lotrič-Furlan et al., 1999) and was higher than in the possible LB group and in the group with PFP of unknown origin. However, significantly fewer patients with PFP of unknown origin came to the follow-up examination compared with patients with confirmed LB. It is quite possible that patients who had a persistence of PFP were more likely to come to follow up than those whose condition improved, and that the proportion of patients with favorable outcome of PFP who attended follow up was lower in the group with PFP of unknown origin than in those with confirmed LB who were afraid of developing other manifestations of LB. Such a bias might explain the smaller proportion of improvements in the group of patients with PFP of unknown origin as found in our study.

The effect of corticosteroid treatment in PFP patients with borrelial infection remains uncertain. Corticosteroids decrease inflammation, but can also depress the immune system and increase the spirochete load (Hohman and Hadlock, 2014). In the present study, which was not designed for assessment of the influence of corticosteroids on the course and outcome of PFP, the outcome in the small subset of patients with confirmed LB who were treated with corticosteroids before antibiotics was similar to the outcome in other patients with confirmed LB, suggesting no detrimental effect of corticosteroids on the outcome, but the number of these patients was definitely too small for a firm conclusion. In the present study, the proportion of patients receiving corticosteroids was low not only in patients with confirmed borrelial etiology of PFP but also in the other two groups. There are several nonexclusive reasons for the low proportion. One is that our study began in 2006 while reliable reports on the efficacy of corticosteroids were published in 2007 and 2008 (Engström et al., 2008; Sullivan et al., 2007). The other is that duration of PFP before the initial examination at our outpatient clinic was rather long, i.e. median 7, 4, 4 days in patients with possible LB, in those with confirmed LB, and in PFP of unknown cause, respectively; thus, the majority of patients did not fulfill time criterium (72 h) for corticosteroid treatment. Furthermore, since LB is endemic in Slovenia and the results of corticosteroid treatment in patients with LB are not known, we were more careful in prescribing corticosteroid treatment.

An explanation for a curious observation that in the group of patients with possible LB the proportion of unfavorable outcome was higher in patients treated with antibiotics than in those who were not given antibiotics (15/19, 79% versus 31/70, 44%; $p = 0.009$; Table 4) could have been that those receiving antibiotics were less likely to get steroids, and therefore had worse outcomes. However, as shown in Table 4, there was no significant difference in the proportion of patients receiving steroids in these two subgroups (7/19 versus 16/70, $p = 0.244$.) as there were no significant difference in the proportion of patients receiving steroids in the antibiotic treated group in comparison to those without antibiotic treatment (13/46 versus 10/43; $p = 0.384$). Yet, patients with unfavorable outcome were older and had significantly longer duration of PFP prior the first examination at our outpatient clinic. The later covariates were found to be associated with the outcome of PFP (Table 5).

Our study has several limitations. In the majority of patients the etiology of PFP is unknown. However, the proportion of patients with idiopathic origin depends upon diagnostic approaches used to ascertain potential etiology. Routine clinical management usually do not include laboratory search for PFP causes, with the exception of testing for borrelia infection in LB endemic regions; and even studies usually do not include molecular diagnostics to determine possible viral etiologies. In the present study, stored CSF samples of the majority of patients (364 of 437) were tested for the presence of HSV-1, HSV-2, or VZV DNA. Very small number of diagnosed cases (only 1 out of 364 tested CSF samples was VZV positive by PCR) suggests that viral diagnostics had no real impact, and that extrapolation of the negative findings to the rest of our patients, in whom viral diagnostics had not been done, was rather safe. Additional limitation of the present study was that the computer search for initial case identification cannot ensure complete case capture. Moreover, although the diagnostic approach in patients with PFP has been partly standardized, the collection of data was based on the review of medical records and some relevant data could have been missed as they were gathered from the patient documentation only. Furthermore, the degree of PFP was not evaluated with an objective scale; consequently, we were not in a position to assess the severity of PFP at presentation with any precision. This drawback could influence the accuracy of the follow-up data. Our findings on the course and outcome of PFP are also limited by the relatively short time of follow up, as some adverse events, such as postparalysis synkinesis which were in our study also not specifically sought for, does not usually become apparent until several months following nerve insult (Kanaya et al., 2009).

Although our outpatient clinic for PFP has been active for more than 50 years and is well recognized, several patients with PFP are also seen by neurologists or ear, nose, and throat specialists. Thus, the findings of the present study cannot be simply extrapolated to all Slovenian populations of patients with PFP, as it is possible that patients referred to the clinic, which is staffed by infectious disease specialists, are already a selected group. Nevertheless, our findings are applicable to European regions with similar incidence rates and comparable ratios of *Borrelia* species causing LB but may not entirely pertain to other European regions or to North America, where LB is nearly exclusively caused by *B. burgdorferi* sensu stricto, a species that rarely causes LB in Europe.

From the laboratory perspective, demonstration of borrelial infection based on IgM antibodies might be of limited value (Stanek et al., 2014); the fact that 40.5% of patients with a positive borrelial IgM index had specific IgM antibody levels in serum below the cut-off value

strongly supports this limitation. In the present study this did not influence categorization of patients into the group with confirmed LB (the large majority of these patients also had an IgG antibody response and/or other reliable indication of borrelial infection), but it might have had an impact on the group with possible LB, since in some patients allocation to this group was based solely on the presence of borrelial IgM antibodies in serum.

In conclusion, in Slovenia, a highly endemic LB region, approximately 15% of adult patients with PFP had proven LB and an additional 28% had borrelial antibodies in serum as the sole indication of borrelial infection. Patients with proven LB differed in several parameters from patients with unknown cause of PFP, whereas those with borrelial antibodies in serum as the sole indication of borrelial infection were similar to the patients in whom the cause of PFP remained unknown. This similarity is in concordance with the absence of development of other manifestations of LB within six months after the onset of PFP in patients who were and who were not treated with antibiotics for LB, suggesting that in the majority of patients who fulfilled criteria for possible LB the borrelial antibodies were probably not associated with the current PFP. Consequently, in such patients treatment approach with corticosteroids as used in patients with Bell's palsy is probably safe.

CSF parameters were the most important variables for confirmed LB infection in the prediction model, with much more weight than non-CSF variables such as season, tick bites, constitutional symptoms, radicular pain, or bilateral facial palsy. The outcome, measured as complete clinical restitution of PFP, was most favorable in the group with proven LB (88%) followed by the possible LB group (79%) and patients with Bell's palsy (77%). The statistical model using multiple logistic regression exposed age and duration of PFP prior to diagnosis as significant predictors for unfavorable outcome; patient sex, corticosteroid therapy, antibiotic treatment, and patient group were not significant.

Funding

This research has not received any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical statement

The presented work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The study was approved by the Slovenian National Medical Ethics Committee.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2018.11.019.

References

Amit, Y., Geman, D., 1997. Shape quantization and recognition with randomized trees. Neural Comput. 9, 1545–1588.
Arnež, M., Ružić-Sabljić, E., 2010. Lyme borreliosis and acute peripheral facial palsy in Slovenian children. Pediatr. Infect. Dis. J. 29, 182–184.
Branda, J.A., Strle, F., Strle, K., Sikand, N., Ferraro, M.J., Steere, A.C., 2013. Performance of United States serologic assays in the diagnosis of Lyme borreliosis acquired in Europe. Clin. Infect. Dis. 57, 333–340.
Breiman, L., 1996. Bagging precistors. Mach. Learn. 24, 123–140.
Breiman, L., 2001. Random forests. Mach. Learn. 45, 5–32.
Bremell, D., Hagberg, L., 2011. Clinical characteristics and cerebrospinal fluid parameters in patients with peripheral facial palsy caused by Lyme neuroborreliosis compared with facial palsy of unknown origin (Bell's palsy). BMC Infect. Dis. 11, 215.
Cerar, T., Ružić-Sabljić, E., Cimperman, J., Strle, F., 2006. Comparison of immunofluorescence assay (IFA) and LIAISON in patients with different clinical manifestations of Lyme borreliosis. Wien. Klin. Wochenschr. 118, 686–690.
Engström, M., Berg, T., Stjernquist-Desatnik, A., Axelsson, S., Pitkäranta, A., Hultcrantz, M., Kanerva, M., Hanner, P., Jonsson, L., 2008. Prednisolone and valaciclovir in Bell's palsy: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet Neurol. 7, 993–1000.
Gilden, D.H., 2004. Clinical practice. Bell's Palsy. N. Engl. J. Med. 351, 1323–1331.
Halperin, J.J., 2008. Nervous system Lyme disease. Infect. Dis. Clin. N. Am. 22, 261–274.
Halperin, J.J., Baker, P., Wormser, G.P., 2013. Common misconceptions about Lyme diseases. Am. J. Med. 126 264.e1–264.e7.
Hohman, M.H., Hadlock, T.A., 2014. Etiology, diagnosis, and management of facial palsy: 2000 patients at a facial nerve center. Laryngoscope 124, E283–93.
Jowett, N., Gaudin, R.A., Banks, C.A., Hadlock, T.A., 2017. Steroid use in Lyme disease-associated facial palsy is associated with worse long-term outcomes. Laryngoscope 127, 1451–1458.
Kanaya, K., Ushio, M., Kondo, K., Hagisawa, M., Suzukawa, K., Yamaguchi, T., Tojima, H., Suzuki, M., Yamasoba, T., 2009. Recovery of facial movement and facial synkinesis in Bell's palsy patients. Otol. Neurotol. 30, 640–644.
Kindler, W., Wolf, H., Thier, K., Oberndorfer, S., 2016. Peripheral facial palsy as an initial symptom of Lyme neuroborreliosis in an Austrian endemic area. Wien. Klin. Wochenschr. 128, 837–840.
Kohler, A., Chofflon, M., Sztajzel, R., Magistris, M.R., 1999. Cerebrospinal fluid in acute peripheral facial palsy. J. Neurol. 246, 165–169.
Kowalski, T.J., Berth, W.L., Mathiason, M.A., Agger, W.A., 2011. Oral antibiotic treatment and long-term outcomes of Lyme facial nerve palsy. Infection 39, 239–245.
Ljøstad, U., Økstad, S., Topstad, T., Mygland, A., Monstad, P., 2005. Acute peripheral facial palsy in adults. J. Neurol. 252, 672–676.
Lotrič-Furlan, S., Cimperman, J., Maraspin, V., Ruzić-Sabljić, E., Logar, M., Jurca, T., Strle, F., 1999. Lyme borreliosis and peripheral facial palsy. Wien. Klin. Wochenschr. 111, 970–975.
Moore, A., Nelson, C., Molins, C., Mead, P., Schriefer, M., 2016. Current guidelines, common clinical pitfalls, and future directions for laboratory diagnosis of Lyme disease, United States. Emerg. Infect. Dis. https://doi.org/10.3201/eid2207.151694.
Mygland, A., Ljøstad, U., Fingerle, V., Rupprecht, T., Schmutzhard, E., Steiner, I., 2010. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur. J. Neurol. 17, 8–16.
Nigrovic, L.E., Thompson, A.D., Fine, A.M., Kimia, A., 2008. Clinical predictors of Lyme disease among children with a peripheral facial palsy at an emergency department in a Lyme diseases-endemic area. Pediatrics 122, e1080–e1085.
Paydar-Darian, N., Kimia, A.A., Lantos, P.M., Fine, A.M., Gordon, C.D., Gordon, C.R., Landschaft, A., Nigrovic, L.E., 2017. Diagnostic lumbar puncture among children with facial palsy in a Lyme disease endemic area. J. Pediatric Infect. Dis. Soc. 6, 205–208.
Peltomaa, M., Pyykkö, I., Seppälä, I., Viljanen, M., 2002. Lyme borreliosis and facial paralysis-a prospective analysis of risk factors and outcome. Am. J. Otolaryngol. 23, 125–132.
Puhakka, H.J., Laurikainen, E., Viljanen, M., Meurman, O., Valkama, H., 1992. Peripheral facial palsy caused by Borrelia burgdorferi and viruses in south-western Finland. Acta Otolaryngol. Suppl. 492, 103–106.
R Development Core Team, 2018. 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.
Reiber, H., Peter, J.B., 2001. Cerebrospinal fluid analysis: disease-related patterns and evaluation programs. J. Neurol. Sci. 184, 101–122.
Ruzic-Sabljic, E., Maraspin, V., Lotric-Furlan, S., Jurca, T., Logar, M., Pikelj-Pecnik, A., Strle, F., 2002. Characterization of Borrelia burgdorferi sensu lato strains isolated from human material in Slovenia. Wien. Klin. Wochenschr. 114, 544–550.
Stanek, G., Strle, F., 2008. Lyme disease–European perspective. Infect. Dis. Clin. N. Am. 22, 327–339.
Stanek, G., Fingerle, V., Hunfeld, K.P., Jaulhac, B., Kaiser, R., Krause, A., Kristoferitsch, W., O'Connell, S., Ornstein, K., Strle, F., Gray, J., 2011. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin. Microbiol. Infect. 17, 69–79.
Stanek, G., Lusa, L., Ogrinc, K., Markowicz, M., Strle, F., 2014. Intrathecally produced IgG and IgM antibodies to recombinant VlsE, VlsE peptide, recombinant OspC and whole cell extracts in the diagnosis of Lyme neuroborreliosis. Med. Microbiol. Immunol. 203, 125–132.
Strle, F., Ruzic-Sabljic, E., Cimperman, J., Lotric-Furlan, S., Maraspin, V., 2006. Comparison of findings for patients with Borrelia garinii and Borrelia afzelii isolated from cerebrospinal fluid. Clin. Infect. Dis. 43, 704–710.
Sullivan, F.M., Swan, I.R., Donnan, P.T., Morrison, J.M., Smith, B.H., McKinstry, B., Davenport, R.J., Vale, L.D., Clarkson, J.E., Hammersley, V., Hayavi, S., McAteer, A., Stewart, K., Daly, F., 2007. Early treatment with prednisolone or acyclovir in Bell's palsy. N. Engl. J. Med. 357, 1598–1607.
Wormser, G.P., Schriefer, M., Aguero-Rosenfeld, M.E., Levin, A., Steere, A.C., Nadelman, R.B., Nowakowski, J., Marques, A., Johnson, B.J., Dumler, J.S., 2013. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. Diagn. Microbiol. Infect. Dis. 75, 9–15.
Wormser, G.P., McKenna, D., Scavarda, C., Karmen, C., 2018. Outcome of facial palsy from Lyme disease in prospectively followed patients who had received corticosteroids. Diagn. Microbiol. Infect. Dis. 91, 336–338.