Cellulitis of the face associated with SENLAT caused by Rickettsia slovaca detected by qPCR on scalp eschar swab sample: An unusual case report and review of literature

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Cellulitis of the face associated with SENLAT caused by *Rickettsia slovaca* detected by qPCR on scalp eschar swab sample: an unusual case report and review of literature

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Abstract

Background: Tick-borne rickettsioses are infectious diseases caused by obligate intracellular Gram-negative bacteria belonging to the spotted fever group *of Rickettsia.*

Methods: We describe an unusual case of SENLAT (Scalp eschar and neck lymphadenopathy after tick bite), caused by *Rickettsia slovaca*, associated with a cellulitis of the face in a 70-year-old woman, and diagnosed using qPCR on a scalp eschar swab. We review the literature regarding cases of SENLAT-associated-cellulitis and case of SENLAT diagnosed by qPCR on scalp eschar swabs.

Results: We found only one previous report of SENLAT associated with a cellulitis of the face. It was a nine-year-old French girl diagnosed by seroconversion for *Rickettsia* sp. Our review of the literature showed that qPCR on eschar swab samples is a less invasive method than performing cutaneous biopsy of the eschar and has good sensitivity and specificity (90% and 100% respectively).

Conclusions: We report the second case of cellulitis of the face associated with the SENLAT syndrome. Detection of *Rickettsia* by qPCR on swab sample of the scalp eschar is a simple, noninvasive technique allowing rapid diagnosis and treatment when SENLAT is suspected.

Introduction

Tick-borne rickettsioses are infectious diseases caused by obligate intracellular Gram-negative bacteria of the spotted fever group (SFG), belonging to the genus *Rickettsia* in the order Rickettsiales. Hard ticks may act as vectors, reservoirs, and/or amplifiers of SFG rickettsia*e* (Delord et al., 2014). The pathogenic role of *Rickettsia slovaca* was first demonstrated in 1997 in a patient who presented with a single inoculation lesion of the scalp and enlarged cervical lymph nodes after being bitten by a *Dermacentor* tick (Raoult et al., 2002). The acronym TIBOLA (tick-borne lymphadenopathy) was proposed because of painful lymphadenopathy next to the region of the tick bite. Later, a similar clinical entity was described and named DEBONEL (*Dermacentor*-borne necrosis erythema lymphadenopathy) to emphasize the role of the *Dermacentor* ticks. Finally, the name SENLAT (scalp eschar and neck lymphadenopathy after tick bite) was suggested to collectively describe this entity because, although *R. slovaca* is frequently involved, there have been reports describing other bacteria, such as *R. raoultii, R. sibirica mongolitimonae, R. massiliae, Bartonella henselae, Francisella tularensis.* (Angelakis et al., 2010; Dubourg et al., 2014). "*Candidatus Rickettsia rioja*", *Coxiella burnetii* and *Borrelia burgdorferi* sensu lato are possible etiological agents due to indirect molecular evidence in ticks involved in SENLAT cases (Dubourg et al., 2014*).* However, in some cases (20-25%), the causative agent remains undetermined suggesting that other microorganisms might be involved (Dubourg, G. et al. 2014).

Rickettsia slovaca, the most frequent reported agent of SENLAT, is transmitted by ticks of the genus *Dermacentor* and especially by *D. marginatus* (**Supplementary Figure 1**) or *D. reticulatus*. The geographical distribution of *R. slovaca* most likely corresponds to the geographical distribution of these ticks in Europe and North Africa (Dubourg et al., 2014). *Rickettsia slovaca* has also been found in *D. marginatus* ticks in the Kurgan region (Ural) of Russia and in Georgia, and in 6.5% of *D. silvarum* ticks in China. Human cases have not been

reported in Asia (Parola et al., 2013). Infection is most frequent among women (67 to 100%) and children under 12 years old (41 to 43%). The median incubation period is of 5 to 10 days (range, 1 to 15 days). In Europe, infections occur most frequently from March to May and from September to November, which corresponds to the periods of greatest activity of *Dermacentor* adult ticks (Parola et al., 2013). *Rickettsia slovaca* has also been reported as an agent of fever of unknown origin (Botelho-Nevers and Raoult, 2007) among travelers returning from Mongolia, Italy and Corsica (Delord et al., 2014).

Clinical manifestations of SENLAT include inoculation lesion at the upper half of the body, mainly on scalp in humans, probably because *Dermacentor* ticks are used to parasitize long-haired animals like horses and wild boar(Parola et al., 2009). The inoculation lesion is often centered by the tick bite with an erythema of at least 5mm that encircles the lesion and second cervical or occipital lymphadenopathies, satellite of the eschar, which may be large, inflammatory and painful (Parola et al., 2009). These two elements may be associated with fever, rash, localized alopecia, headache, asthenia, sweats, arthralgia and myalgia (Dubourg et al., 2013).

We describe an unusual case of SENLAT associated with a cellulitis of the face diagnosed by qPCR on a scalp eschar swab and review the literature about this entity and the diagnosis by qPCR on eschar swabs.

The Case

A 70-year-old woman with medical history of hypothyroidism was admitted in our department in Marseille on April 2017 with a periorbital cellulitis associated with a scalp eschar and cervical lymphadenopathies. The patient reported a walk in the forest about ten days ago with the apparition, a few days later, of odynophagia, bilateral cervical lymphadenopathies and lesion of the scalp, without fever. Her family doctor thought she had

tonsillitis and prescribed amoxicillin. Subsequently, a large erythema and edema of the face appeared, so that she consulted to our unit. At physical examination we recorded a scalp eschar on the left side of the head, an erythema and edema at the forehead with rapid extension to periorbital, retro auricular and cervical areas with left-sided lymphadenopathies **(Figure 1**). Laboratory tests were normal except a slight increase in the C- reactive- protein (15mg/l). Cervical and face tomodensitometry showed a cellulitis without fascia involvement or abscess. Empirical antibiotherapy with amoxicillin- clavulanic acid and clindamycin had been started before at the emergency unit and we added oral doxycycline (200mg/daily) for ten days because of the presence of the inoculation eschar on the scalp. A dry sterile swab sample was collected from the inoculation eschar. The swab, while being rotated vigorously, was directed to the base of the eschar at a 50°–60° angle for 5–6 times (Mouffok et al., 2011). qPCR on scalp eschar swab targeting a fragment of the *gltA* gene, as previously described (Aubry et al., 2016), was positive for *Rickettsia* sp. and sequencing of a fragment of the *ompA* gene (Aubry et al., 2016) identified *Rickettsia slovaca*. Culture of the scalp swab sample on human embryonic lung cells using shell vial methods as previously reported (Raoult et al., 2002) were negative. Serum sample were analysed using indirect Immunofluorescence Assay (IFA) against two spotted fever group rickettsial antigens (*R.conorii conorii*, *Rickettsia felis*) and a typhus group antigen (*R.typhi*). The IFA test during the acute phase was considered positive if antibody titres were > 1:128 for immunoglobulin G (IgG) and > 1:64 for immunoglobulin M (IgM) for the spotted fever group, and > 1:64 for IgG and > 1:32 for typhus group, as previously described (Hayet et al., 2018). These serological testings were negative in the acute phase serum sample and in a convalescent serum sample one month after the resolution of symptoms. The patient completely recovered after ten days of doxycycline except for a small cicatricial alopecia at the site of the eschar **(Figure 1**).

Discussion

In 2009, Parola et al. described facial edema without cellulitis as a new clinical feature of SENLAT because it was found in 19% of cases of *R. slovaca* and 40% of *R. raoultii* infections (Parola et al., 2009). A literature search revealed only one previous reference reporting a case of SENLAT associated with a similar cellulitis of the face. It was a nine-year-old French girl with an acute hemifacial edema and erythema requalified as TIBOLA in 2011 (Gaston et al., 2011). The removal of two ticks in the scalp had occurred 10 days earlier. The edema appeared suddenly in a context of fever at 39.5 °C, headache and vertigo. The clinical examination revealed an erysipeloid placard of the left hemiface, associated with a homolateral jugulo-carotid inflammatory adenitis. At the same time, the examination of the scalp revealed two necrotic eschars surrounded by an erythematous halo. The diagnosis was done by seroconversion for *Rickettsia* fifteen days later (Gaston et al., 2011).

The microbiological methods available for the diagnosis of tick-borne rickettsioses are serology, culture and PCR. Serology is the most widespread method for the diagnosis of tick-borne rickettsioses, but seroconversion occurs only 15 to 21 days after the beginning of the symptoms (Foissac et al., 2013). Moreover, even when using IFA, which is the reference method, cross reactions are frequent and the specific serological sensitivity for *R. slovaca* is low (about 12%), probably due to a limited locoregional dissemination of the infection (Foissac et al., 2013), as illustrated by our case, where even convalescent serum sample was negative. Culture is fastidious and requires cell cultures that are only performed in specialized BSL 3 laboratories. On the other hand, molecular techniques (qPCR targeting the gltA gene for *Rickettsia* group and species-specific qPCR) are useful given their good sensitivity at the beginning of the infection, especially on eschar samples (Foissac et al., 2013). qPCR on a swab sample of the eschar requires the use of a sterile swab to obtain a sample from inside the eschar, simply by inserting the swab into the eschar and rotating it to the basis of the eschar. The material obtained via swabbing can be then processed for nucleic acid extraction and

136 subsequent PCR analysis (Luce-Fedrow et al., 2015). It has a sensitivity of 85 to 90% and a
137 specificity close of 100% (Bechah et al., 2011; Mouffok et al., 2011; Renvoise et al., 2012;
138 Wang et al., 2009). These performances are almost comparable to the ones of qPCR on eschar
139 cutaneous biopsy (sensitivity >95% and specificity >98%) (Foissac et al., 2013). qPCR on
140 eschar swab samples has been used with success around the world for detection of *R. parkeri,*
141 *R. conorii, R. sibirica mongolitimonae, R. africae, R. australis, R. felis* and *O. tsutsugamushi*
142 (**Table 1)**. To date, only 2 references reported the detection of *R. slovaca* by qPCR on a swab
143 eschar sample (Bechah et al., 2011; Dubourg et al., 2014). Swabbing an eschar has a lot of
144 advantages because it is a noninvasive, simple, rapid and painless technique that can be easily
145 performed without risk, especially in children. This test can be used at the bedside or in an
146 outpatient clinic and could be useful for epidemiologic, surveillance and clinical studies even
147 although health care workers are not able to perform biopsies (Le Viet et al., 2017; Luce-
148 Fedrow et al., 2015; Renvoise et al., 2012). In a recent study, opinions of health care
149 providers and patients were evaluated and they both preferred collecting swab samples over
150 biopsy samples (46 vs. 5 and 57 vs. 2, respectively; $p = 0.0001$) (Mouffok et al., 2011).
151 In a clinical setting, the treatment of choice for SFG rickettsia is doxycycline
152 (Botelho-Nevers et al., 2012), including in children for whom its safety has been
153 demonstrated, notably on the absence of dental damage (Todd et al., 2015; Volovitz et al.,
154 2007). There is no data in the literature to determine the exact duration of treatment, which is
155 related to the clinical response and is classically of 7 to 14 days (Foissac et al., 2013), or at
156 least 3 days after apyrexia (Renvoise et al., 2009). The complete resolution of symptoms is
157 long but antibiotic treatment can reduce the duration of symptoms from 62 to 50 days
158 (Foissac et al., 2013). Sequelar alopecia at the tick bite site, as observed in our patient, is
159 reported in 20 to 51% of cases (Foissac et al., 2013).

Rickettsia are strict intracellular bacteria and conventional microbiological techniques cannot be used to evaluate the efficacy of antibiotics. Cell culture techniques are used to test in vitro susceptibility of *Rickettsi*a to antibiotics by determining the minimum inhibitory concentrations but can be performed only in specialized laboratories (Renvoise et al., 2009). However, beta-lactams, aminoglycosides and cotrimoxazole are ineffective on rickettsia (Renvoise et al., 2009). As a consequence, in a patient with a cellulitis, the presence of an eschar and lymphadenopathies should prompt clinicians to add doxycycline, because beta lactam are inefficient on SENLAT-associated cellulitis.

Conflict of interest

All the authors have no conflict of interest to disclose

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250
251

Table 1: Agents of Rickettsia spp. detected by qPCR on eschar swab

Agents	Authors	Positive SWAB	Clinical presentation/ Localization of eschar
R. parkeri	Myers et al., 2013	2	Fever, chills, night sweats, a diffuse maculopapular rash, headache, myalgia, neck stiffness, arthralgia, and malaise.
R. conorii	Bechah et al., 2011	25	Fever, eschar and generalized maculopapular rash.
	Khrouf et al., 2016	12	Acute fever and cutaneous rashes and/or eschars.
	Mouffok et al., 2011	2	NS
R. sibirica mongolitimonae	Bechah et al., 2011	2	NS
	Solary et al., 2014	1	Fever, 2 eschars on the lower right eyelid, periorbital edema, cervical lymphadenopathies.
R. africae	Bechah et al., 2011	2	NS
	Socolovschi et al., 2012	4	Fever, eschar, rash, lymph-adenopathy
R. australis	Bechah et al., 2011	1	NS

	Wang et al., 2009	4	Case 1: inguinal eschar, tender local lymphadenopathy, fever to 39.8°C, severe headache, myalgia, arthralgia, and generalized sparse rash Case 2: Fever, torso eschar, severe headache, myalgia, arthralgia, and rash Case 3: Fever, eschar on the scalp, tender local lymphadenopathy, and generalized rash with maculopapular and vesicular components. Case 4: Fever, eschar on the torso, tender local lymphadenopathy, and generalized sparse rash characterized by papular and vesiculopustular lesions.
R. felis	Botelho-Nevers et al., 2007	5	Fever and eschar.
O. tsutsugamushi	Kim et al., 2006	1	Fever with eschar or a maculopapular skin rash and also had > 2 symptoms (such as headache, malaise, myalgia, coughing, nausea, and abdominal discomfort)
	Le Viet et al., 2017	17	>15 years, axillary temperature >37.5˚C and at least one of the following four secondary findings: eschar, skin rash, lymphadenopathy, hepatomegaly and/or splenomegaly
R. slovaca	Bechah et al., 2011	4	Fever, inoculation lesion, enlarged nodes, localized alopecia
	Dubourg et al., 2014	1	NS

Figures legends

Figure 1

Scalp eschar (Panel a) and peri-orbital cellulitis of the face before (Panel b) and after treatment (Panel c) in a 70-year-old women, revealing a SENLAT syndrome caused by *R. slovaca* detected by qPCR on scalp eschar swab.

Supplementary Figure

Supplementary Figure 1

Dermacentor marginatus ticks: female (Panel a) and male (Panel b).


a)
b)
c)