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Real-time PCR for early diagnosis of *Rickettsia conorii* and prompt management in patients with septic shock and multiple organ failure: two case reports

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ABSTRACT:

We herein describe two severe cases of Mediterranean spotted fever caused by Rickettsia conorii. The article presents polymerase chain reaction-restriction analysis as an early diagnostic tool for rickettsiosis caused by spotted fever group Rickettsiae. Timely microbiological diagnosis, the combined use of antibiotics, intensive care measures and a multidisciplinary team approach are fundamental to manage these serious diseases.

Key words: *Rickettsia conorii*

INTRODUCTION

Mediterranean spotted fever (MSF) is caused by Rickettsia conorii and transmitted by the brown dog tick Rhipicephalus sanguineus[1-2]. In Italy, more than 400 cases are reported every year and nearly half of them occur in Sicily. The disease usually has a benign course and is characterized by fever, myalgia and a papular rash with an inoculation eschar, called "tache noir", at the site of the tick bite. Nevertheless, severe forms have been sporadically reported. We report two cases of MSF, who developed septic shock and multiple organ failure (MOF), in which the diagnostic laboratory had a major role in their clinical management.

CASE REPORTS

We describe our experience with 2 patients (54-and 64-year-old males) who presented with fever and general maculopapular rash with rare petechial elements on the palms of the hands. Patient 1 also presented petechial elements on the soles of the feet. In both cases, laboratory tests revealed multi-organ involvement with acute renal failure, increased transaminases, lactate dehydrogenase and D-dimer, associated with high inflammatory markers. MSF was suspected and parenteral therapy with chloramphenicol 500 mg q6h and ciprofloxacin 500 mg q12h was initiated in both patients. Due to sepsis-induced MOF, both patients were transferred to the Intensive Care Unit (ICU), where they were intubated and resuscitated. After a week, the clinical condition of patient 1 improved with the stabilization of all vital parameters, and he was transferred to our Department. Patient 2's clinical condition worsened, and intubation and mechanical ventilation were required; however, he did not improve and he died because of respiratory failure ten days later.

In the fatal case, polymerase chain reaction (PCR) molecular investigation performed 6 hours (h) before

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Table 1. Characteristics of the two patients with Mediterranean spotted fever admitted to our Unit

Variables after hospital admission (d=days; h=hours)	Fatal case	Favorable case
Signs and symptoms		
Fever	3 d	2 d
Rash	24 h	18 h
Eschar	yes	no
Diagnostic assays		
Rickettsial DNA	Negative (28 h)	Positive (4 h)
Anti-rickettsial immunoglobulin M and IgG	Negative (2 d)	Positive (14 d)
Autopsy	yes	no
Delayed specific treatment after symptom onset (d)	4	2
Antimicrobial treatment after hospital admission		
Chloramphenicol e.v.	12 h	4 h
Ciprofloxacin e.v.	10 h	4 h
Oral tetracycline	4 h	None
ICU parameters		
Transferred to the ICU	24 h	6 h
Glasgow Coma Scale value on ICU admission	7	9
SOFA score on ICU admission	9	10
DIC score	5.5	7.5
Orotracheal intubation	On admission	After 8 h

death was negative as the serological assays, and the diagnosis was confirmed by autopsy, whereas in the case with a favorable evolution, PCR confirmed the diagnosis of MSF within 6 h of hospital admission and serological investigations were positive just two weeks after hospitalization. Table 1 shows the different clinical and therapeutic management in the two case reports.

METHODS

Members of the Rickettsia spotted fever and typhus groups were identified using a previously published highly sensitive and specific real-time TaqMan PCR assay specific for a 74-bp fragment of the gltA gene[3]. Rickettsial DNA was extracted from both whole blood and buffy coat samples with a DNA extraction kit (Qiagen, Hilden, Germany) as per manufacturer's specifications. Specific anti-rickettsial immunoglobulin M (IgM) and IgG determinations were performed by an enzyme-linked immunosorbent assay (ELISA) or an indirect immunofluorescence (IIF) assay. Antigens were freshly prepared in the hospital laboratory as described previously[4].

CONCLUSIONS

Mediterranean spotted fever (MSF) is a tick-borne disease caused by Rickettsia conorii. MSF is transmitted by the dog tick Rhipicephalus sanguineus[5]. The incubation period is around 7-8 days after the tick bite.

Spotted fever group rickettsiosis can be diagnosed by serological tests or molecular methods[6]. The detection of antibodies can rely on indirect immune fluorescence or immune enzyme assays. However, serological investigation is not useful to confirm the disease in its early stages, when diagnosis can be usually made considering clinical and epidemiological aspects.

Methods such as one-step, real time RT-PCR or nested RT-PCR are now widely used to detect Rickettsiae of spotted fever group in acute-phase serum samples. In the fatal case, delayed medical consultation and late initiation of antimicrobial therapy (4 days after the onset of symptoms), as well as older age, probably contributed to the unfavorable course.

Early and aggressive multidisciplinary management could influence the evolution of MSF severe complications, such as MOF. Our clinical cases confirm the limitations of serological assays in the acute phase of MSF, whereas early PCR assays to diagnose rickettsiosis may favorably affect therapeutic management. It is important to investigate if SOFA and DIC scores can influence DNA results. Finally, the role of chloramphenicol and combined antibiotic treatment in the acute phase of severe disease should be reconsidered.

Conflict of Interests:
The Authors declare that they have no conflict of interests.

REFERENCES

1. Oteo JA, Portillo A. Tick-borne rickettsioses in Europe. Ticks Tick Borne Dis 2012; 3: 271-278.
2. Cascio A, Iaria C. Epidemiology and clinical features of Mediterranean spotted fever in Italy. Parassitologia 2006; 48: 131-133.
3. Stenos J, Graves SR, Unsworth NB. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group Rickettsiae. Am J Trop Med Hyg 2005; 73: 1083.
4. Vitale G, Di Stefano R, Damiani G, Mansueto S. Characterization of Sicilian strains of spotted fever group rickettsiae by using monoclonal antibodies. J Clin Microbiol 1989; 27: 1081-1085.
5. Vitaliti G, Falsaperla R, Lubrano R, Rapisarda V, Cocuzza S, Nunnari G, Pavone P. Incidence of Mediterranean spotted fever in Sicilian children: a clinical-epidemiological observational retrospective study from 1987 to 2010. Int J Infect Dis 2015; 31: 35-40.
6. Gehrke FS, Angerami RN, Marrelli MT, de Souza ER, do Nascimento EM, Colombo S, da Silva LJ, Schumaker TT. Molecular characterization of mediterranean spotted fever rickettsia isolated from a European traveler in the state of São Paulo, Brazil. J Travel Med 2013; 20: 54-56.