Epidemiology and Infection

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Epidemiology and clinical characteristics of Mediterranean spotted fever suspects in a university hospital, Tunisia, 2000–2020

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Abstract

Mediterranean spotted fever (MSF) is a rickettsial disease caused by *Rickettsia conorii*, transmitted by brown dog ticks, and endemic in the Mediterranean region. Its incidence is increasing, with varied presentations and potential complications because of delayed diagnosis. This study retrospectively included 173 adult patients hospitalized for MSF at La Rabta University Hospital, Tunis, from 2000 to 2020. Patients, predominantly male (67.6%) and averaging 40 years of age, mostly resided in urban areas (82.7%). Animal exposure was reported in 74.6%, and cases peaked during the hot season (68.8%), with no cases in winter months. The classic triad of fever, rash, and eschar was observed in 69.9%, with maculopapular lesions affecting palms and soles in 83.8%. Headache (64.5%), myalgia (60.7%), and arthralgia (57.2%) were also common. Laboratory findings included elevated white blood cell count (36.4%), thrombocytopenia (48%), and increased aspartate aminotransferase (50.9%). Treatment with doxycycline (n = 161) resolved fever within 2.8±1.3 [1–5] days. Complications, including encephalitis, chorioretinitis, anterior uveitis and vasculitis, occurred in 2.3% of cases, but all patients recovered without relapse. No significant risk factors for severe forms were identified. Improved awareness of MSF's clinical features may be the key to an early diagnosis and successful treatment.

Introduction

Rickettsioses are infectious diseases caused by strict intracellular bacteria of the genus *Rickettsia*. The Mediterranean spotted fever (MSF) is an eruptive rickettsial disease caused by *Rickettsia conorii*. It is a zoonosis transmitted by the brown dog tick *Rhipicephalus sanguineus* sensu lato, which represents the vector of the disease and also the reservoir of bacteria [1]. Many authors theorize that the canine population especially domestic dogs, other mammals, and the ticks themselves could potentially contribute as reservoirs [2]. The warm climate is favourable to the survival and development of this tick [3]. MSF was first discovered in Tunisia in 1910 by Conor and Brüch [4]. The epidemiology of this rickettsial disease is directly related to the ecological characteristics of its vector. It is an urban and peri-urban zoonosis endemic in the Mediterranean area and in Africa [5]. In Tunisia, MSF is the most frequent rickettsial disease [1,6]. Its incidence in Tunisia would be in clear increase but remains underestimated because of lack of national epidemiological data.

The MSF is characterized by its several clinical presentations and should be diagnosed early by physicians in the presence of the triad of fever, cutaneous rash, and inoculation eschar. Serological testing is largely used for retrospective diagnosis. Molecular techniques such as real-time polymerase chain reaction (r-t-PCR) on skin biopsies or eschar swabs provide rapid and easy diagnosis with a good specificity and sensitivity [6].

Our primary objectives were to describe baseline and epidemiological characteristics in patients hospitalized for MSF, clinical features, biological findings, therapeutic management and outcomes. Our secondary objective was to evaluate the risk factors (RFs) for complicated forms or life-threatening complications in patients presenting infection caused by *Rickettsia. spp.*

Materials and methods

Study design and population

We conducted a retrospective descriptive study at the infectious diseases department of La Rabta University Hospital, Tunis (in the North of Tunisia), during 21 years (between 1 January2000 and 31 December 2020). We included all hospitalized adult patients (≥18 years old) with a diagnosis of MSF. We excluded patients' files with missing data, patients with rash fever related to another aetiology, adolescents and paediatric patients (< 18 years old), pregnant women, and outpatients.

All patients presenting with suspected MSF at the emergency department of our hospital were admitted.

Definitions

A suspected/probable patient with MSF was diagnosed on these arguments:

- Clinical criteria: fever of more than 5 days, presence of a skin rash, particularly involving palms and soles, and/or presence of inoculation eschar and systematic therapeutic response (defervescence after treatment with anti-rickettsial drugs) and/or
- Epidemiological criteria: Dog exposure and/or hot season (presentation on the month of June, July or August), and/or
- Biological criteria: thrombocytopenia (platelets count < 150 000/mm3, [normal range: 150000–450000/mm3]), increased liver enzymes, increased creatine kinase. and lactate dehydrogenase levels and/or
- A confirmed/definite patient with MSF was defined as patient with positive rickettsial disease serology (serum sample with IgG ≥ 1/64 of R. conorii) and/or r-t-PCR on skin biopsies or eschar swabs (microbiological criteria).

Complicated forms or life-threatening complications are defined as:

Neurological complications such as encephalitis or meningoencephalitis, cerebellitis, cerebral thrombophlebitis and ischemic or hemorrhagic

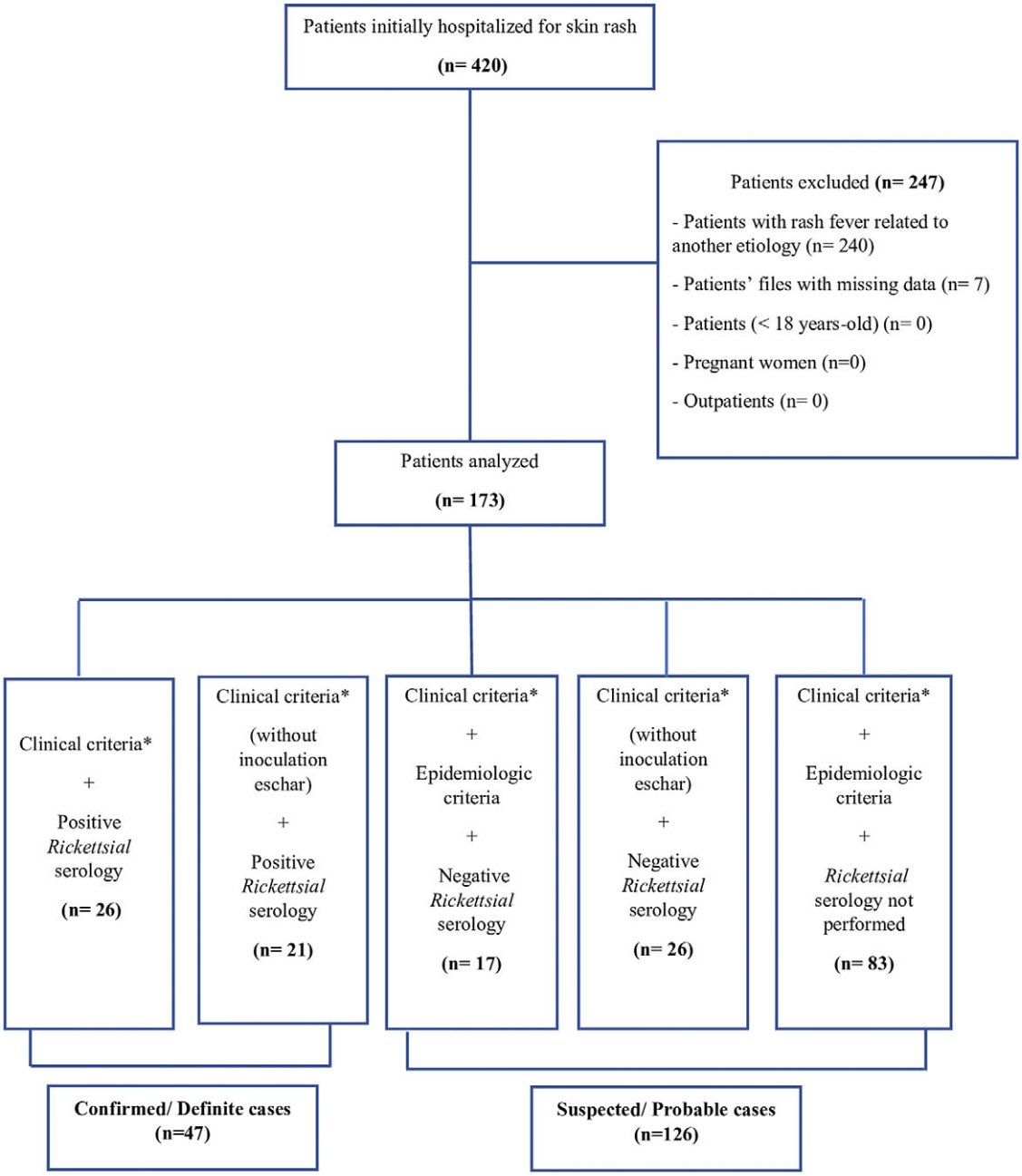

Figure 1. Flowchart detailing the overview of patients enrolled in the study.
*Clinical criteria were defined by the presence of the triad fever, skin rash and/or inoculation eschar with therapeutic response systematically.

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stroke. Ophthalmological complications such as uveitis, chorioretinitis or macular edema. Respiratory complications such as acute pulmonary edema, acute respiratory distress syndrome or lung embolism and other complications like pericarditis or myocarditis, pancreatitis or splenic infarction, septic shock and multi-organ failure.

Baseline characteristic, clinical and biological data

Clinical data regarding patients' history, demographic and baseline characteristics were collected through medical records. We also collected information regarding biological findings and outcomes.

Data analysis

The normality test of the quantitative data was performed. Continuous variables were expressed as mean and standard deviation (SD) and compared with Student's t-test. Categorical variables were expressed as a number (%). Relationships between two quantitative variables were studied by Pearson's correlation coefficient. A p-value < 0.05 was considered significant. We used SPSS v23.0 software (IBM, Armonk, NY, USA).

The time series of occurrences was aggregated by month to count the number of monthly occurrences. Missing data were reindexed to ensure temporal continuity. We then applied an additive seasonal decomposition to the time series to separate the trend, seasonality, and residual components. A Mann–Kendall test was performed to assess the significance of the trend in occurrences over time.

Results

During the study period, 420 patients were initially included. A total of 173 patients with a confirmed MSF were analysed. The flowchart detailed different criteria to diagnosed MSF (Figure 1).

Demographic and epidemiological data

The mean age of patients was 40±15 years, with predominance in the age group between 35 and 54 years (41.9%; Table 1). One hundred and seventeen (67.6%) of those patients were males. One hundred and twenty-eight patients (74%) were immunocompetent with no medical history. The underlying mainly comorbidities were hypertension (n = 10, 5.8%) and diabetes mellitus (n = 8, 4.6%). Glucose-6-phosphate dehydrogenase (G6PD) deficiency and chronic alcoholism were not presented in our patients. Patients with MSF were from a presumed urban area in 82.7% of the cases (n = 143). Animal exposure (commonly dogs) was reported in 129 patients (74.6%). Incubation period, defined as the time between the presumed contact and the symptoms onset, was 3.8±3 [1–11] days.

Our study observed a statistically significant decreasing trend in MSF cases over the 21-year period (Tau = −0.0707, Z = −2.042, p=0.04; Figure 2). The mean prevalence was 8.2 ± 5.7 cases per year, with notable outbreaks in 2005 (n = 22, 12.7%) and 2015 (n = 18, 10.4%). Seasonality was also evident, with more than two-thirds of cases (68.8%) occurring during the hot season, peaking in August (32.4%). In contrast, no cases were reported during the winter months of December, January, and February (Figure 3).

Table 1. Baseline characteristics and epidemiologic criteria of patients with Mediterranean spotted fever/comparison of confirmed/definite and suspected/probable patients with MSF

Characteristics			All patients (n = 173)	Confirmed/Definite cases (n = 47)	Suspected/Probable cases (n = 126)	p-value
Baseline characteristics						
Sex (n, %)	M		117/173 (67.6)	27/47 (57.4)	90/126 (71.4)	0.11
	F		56/173 (32.4)	20/47 (42.5)	36 (28.57)	-
Age (y, SD)			40 (±15)	41.3 (±14.9)	40.1 (±15.8)	0.67
Underlying comorbidities; (n, %)		No comorbidities	128 (74)	34 (72.3)	94 (74.6)	-
		Total	45 (26)	13 (27.6)	32 (25.4)	-
		Arterial hypertension	10/45 (22.2)	3/13 (23.1)	7/32 (21.9)	-
		Diabetes mellitus	8/45 (17.8)	3/13 (23.1)	5/32 (15.6)	-
		Asthma	5/45 (11.1)	0 (0)	5/32 (15.6)	-
		Coronary syndrome	4/45 (8.9)	1/13 (7.7)	3/32 (9.4)	-
		Others	18/45 (40)	6/13 (46.1)	12/32 (37.5)	-
Epidemiologic data						
Hot season* (n, %)	June		19/173 (11)	7/47 (14.9)	12/126 (9.5)	-
	July		44/173 (25.4)	14/47 (29.8)	31/126 (24.6)	-
	August		56/173 (32.4)	11/47 (23.4)	43/126 (34.1)	0.28
Dogs exposure** (n, %)			125/173 (72.3)	29/47 (61.7)	96/126 (76.2)	0.08
Patients' origins (n, %)		Urban area	143/173 (82.7)	38/47 (80.8)	105/126 (83.3)	0.87
		Rural area	30/173 (17.3)	9/47 (19.1)	21/126 (16.7)	-

Abbreviations: *n*: number; *SD*: standard deviation; *y*: years.
*The hot season was defined as the three months of June, July and August.
**"Dogs exposure" was to free-roaming dogs, street dogs, domestic dog pets

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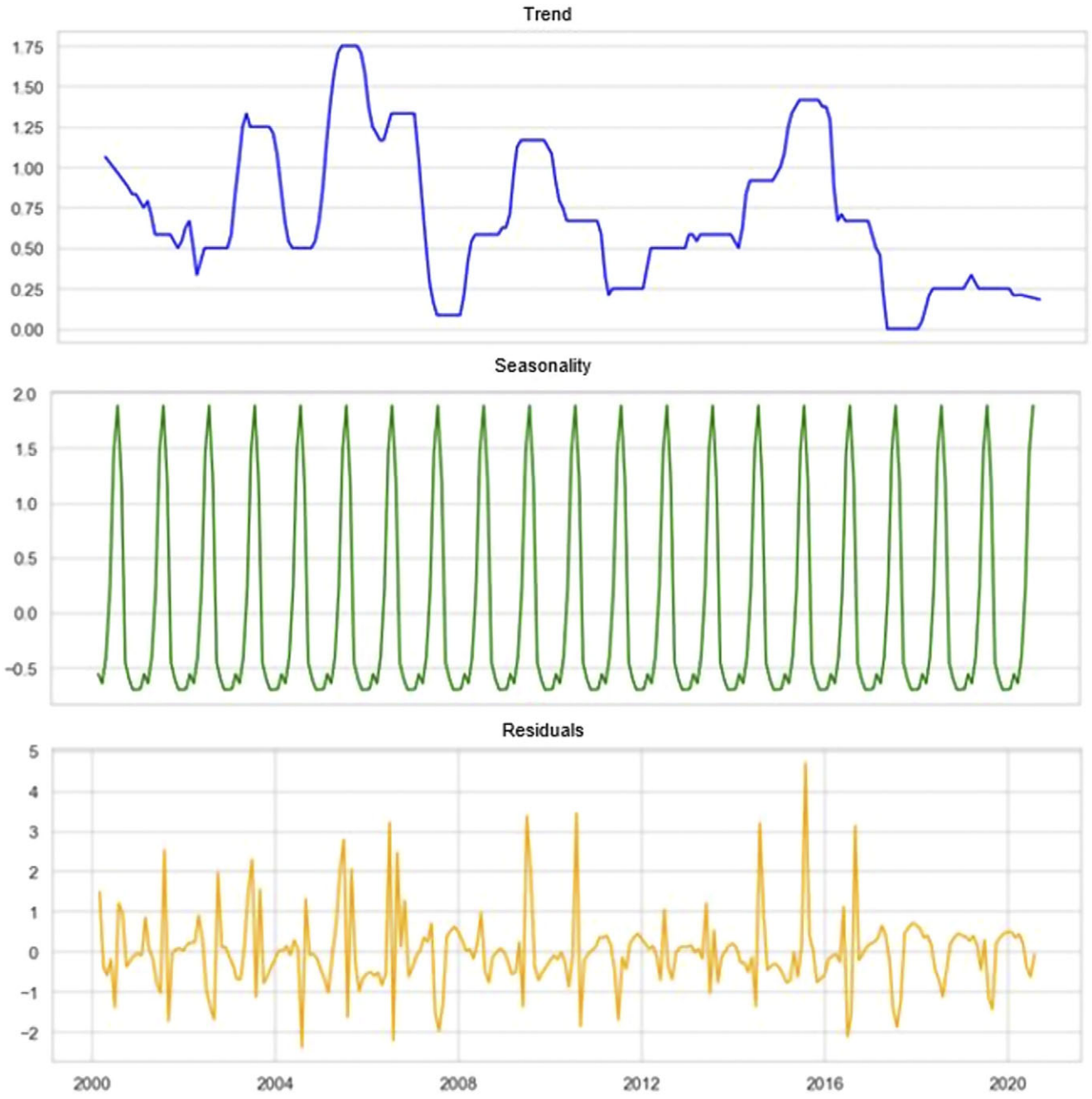

Figure 2. Testing and modeling trend and seasonality using Man-Kendall Test that revealed a statistically significant decreasing trend over time (Tau = -0.0707, Z = -2.042), with a p-value < 0.05 (p = 0.0411)*.

** Kendell's Tau statistic (-0.0707) indicates a week negative correlation, suggesting that symptoms tend to slightly decrease over time. The estimated slope being zero (slope = 0.0), which means that there is no marked net change in the frequency of occurrences from one month to another, although the overall trend is decreasing.*

Clinical features

Fever was common in all the patients. It was followed by influenza-like-illness consisting of headache (n = 115, 64.5%), myalgia (n = 105, 60.7%), arthralgia (n = 99, 57.2%), and asthenia (n = 73, 42.2%; Table 2). Other general symptoms such as chills and sweating were reported in 28.3% (n = 49) and 15.6% (n = 27) of patients with confirmed MSF, respectively. Respiratory signs such as cough and dyspnoea were observed in 10 patients (5.8%). Gastrointestinal (GI) symptoms such as vomiting and diarrhoea were reported in 15.6% and 4.6% of cases, respectively.

At admission, clinical examination revealed a regular triad of fever, skin rash and inoculation eschar in 121 patients (69.9%).

Skin examination showed mainly maculo-papular lesions (n = 145, 83.8%). Purpuric lesions were also noted in 13 patients (7.5%; Figure 4A). Involvement of palms and soles was common in 170 patients (98.3%) but not the face or scalp. The inoculation eschar was found in 69.9% (n = 121) of cases (Figure 4B). It was unique in the majority of the cases (n = 112, 92.6%) and was found in several locations (Supplementary Table S1).

Unilateral conjunctival hyperaemia was observed in 32.4% of cases (n = 56). In our cohort, no patients were with sepsis and

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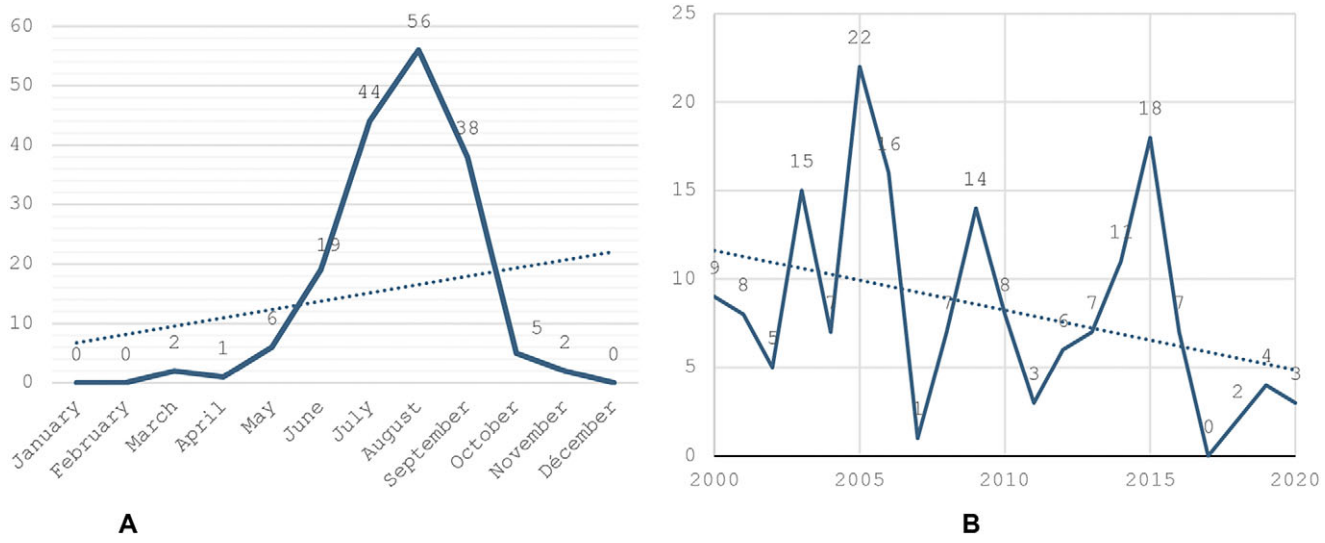

Figure 3. Demographics and epidemiologic data for patients with mediterranean spotted fever (n = 173) during 21 years in la Rabta Hospital (2000-2020). **(A)** Monthly distribution if cases **(B)** Annual distribution of cases and outbreaks.

Table 2. Clinical features, therapeutic management and outcomes of patients with Mediterranean spotted fever/comparison of confirmed/definite and suspected/probable patients with MSF

Clinical features		All patients (*n* = 173)	Confirmed/Definite cases (*n* = 47)	Suspected/Probable cases (*n* = 126)	*p*-value
Fever (*n*, %)		173/173 (100)	47/47 (100)	126/126 (100)	-
Skin rash (*n*, %)		173/173 (100)	47/47 (100)	126/126 (100)	-
Inoculation eschar (*n*, %)		121 (69.9)	29/47 (61.7)	92/126 (73)	0.21
Headache (*n*, %)		121/173 (64.5)	35/47 (74.5)	86/126 (68.2)	0.24
Myalgia (*n*, %)		105/173 (60.7)	31/47 (66)	74/126 (58.7)	0.43
Arthralgia (*n*, %)		99/173 (57.2)	30/47 (63.8)	69/126 (54.8)	0.25
Asthenia (*n*, %)		73/173 (42.2)	18/47 (38.3)	55/126 (43.6)	0.64
Chills (*n*, %)		49/173 (28.3)	14/47 (29.8)	35/126 (27.8)	0.94
Sweating (*n*, %)		27/173 (15.6)	8/47 (17)	19/126 (15.1)	0.94
Cough and dyspnoea (*n*, %)		10/173 (5.8)	0/47 (0)	10/126 (8)	0.10
Vomiting (*n*, %)		27/173 (15.6)	4/47 (8.5)	23/126 (18.25)	0.18
Neurological symptoms (*n* = 12); (*n*, %)	Meningeal syndrome	9/12 (75)	4/47 (8.5)	5/126 (4)	0.41
	Slow walking	1/12 (8.3)	1/47 (2.1)	0/126 (0)	-
	Shaky gait	1/12 (8.3)	0/47 (0)	1/126 (0.8)	-
	Cutaneous hyperesthesia	1/12 (8.3)	0/47 (0)	1/126 (0.8)	-
Therapeutic management					
Prior antimicrobial therapy (*n*= 100) (*n*, %)					
Beta-lactams	Total	54/100 (54)	7/100 (7)	47/100 (47)	**0.008**
	Amoxicillin clavulanic-acid	25/100 (25)	9/100 (9)	16/100 (16)	0.74
	Amoxicillin	24/100 (24)	7/100 (7)	7/100 (7)	-
	Third Generation Cephalosporins	5/100 (5)	1/100 (1)	4/100 (4)	-
Aminoglycosides	Total	12/100 (12)	3/100 (3)	9/100 (9)	0.87
	Gentamicin	10/100 (10)	3/100 (3)	7/100 (7)	-
Tetracyclines	Total	10/100 (10)	2/100 (2)	8/100 (8)	0.87
	Doxycycline	10/100 (10)			

(*Continued*)

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Table 2. (*Continued*)

Clinical features		All patients (*n* = 173)	Confirmed/Definite cases (*n* = 47)	Suspected/Probable cases (*n* = 126)	*p*-value
Fluoroquinolones (FQ)	Total	7/100 (7)	1/100 (1)	6/100 (6)	0.73
	Ciprofloxacin	5/100 (5)	1/100 (1)	4/100 (4)	1
	Ofloxacin	2/100 (2)	1/100 (1)	1/100 (1)	-
Macrolides	Total	6/100 (6)	1/100 (1)	5/100 (5)	0.90
	Azithromycin	5/100 (5)	1/100 (1)	4/100 (4)	1
	Clindamycin	1/100 (5)	1/100 (1)	0/100 (0)	-
Other antibiotics	Total	4/100 (4)	1/100 (1)	3/100 (3)	1
Antibiotic therapy during hospitalization (*n*, %)	Doxycycline	147/173 (85)	43/47 (91.5)	104/126 (82.5)	-
	Doxycycline + FQ	15/173 (8.7)	7/47 (14.9)	8/126 (6.3)	-
	Ofloxacin	7/173 (4)	3/47 (6.4)	4/126 (3/2)	-
	Ciprofloxacin	4/173 (2.3)	2/47 (4.2)	2/126 (3.2)	-
Treatment duration (d, SD)		6,4 (±3)	8.28 ± 3.40	7.69 ± 2.99	0.29
Side/adverse effects (n, %)		0/173 (0)	0/47 (0)	0/126 (0)	-
Outcomes and mortality					
Complications (*n*, %)	Total	4/173 (2.3)	2/47 (4.2)	2/126 (1.6)	0.64
Neurologic complications (*n*, %)	Cerebral vasculitis	1/4 (25)	0/47 (0)	1/126 (0.8)	-
	Encephalitis	1/4 (25)	0/47 (0)	1/126 (0.8)	-
	Meningitis	0/4 (0)	0/47 (0)	0/126 (0)	-
	Cerebellitis	0/4 (0)	0/47 (0)	0/126 (0)	-
	Cerebral thrombophlebitis	0/4 (0)	0/47 (0)	0/126 (0)	-
	Ischemic or haemorrhagic stroke	0/4 (0)	0/47 (0)	0/126 (0)	-
Ophthalmic complications (*n*, %)	Chorioretinitis	1/4 (25)	1/47 (2.1%)	0/126 (0)	-
	Anterior uveitis	1/4 (25)	1/47 (2.1%)	0/126 (0)	-
	Macular oedema	0/4 (0)	0/47 (0)	0/126 (0)	-
Cardiac complications (*n*, %)	Myocarditis	0/4 (0)	0/47 (0)	0/126 (0)	-
	Pericarditis	0/4 (0)	0/47 (0)	0/126 (0)	-
Digestive complications (*n*, %)	Pancreatitis Splenic infraction	0/4 (0) 0/4 (0)	0/47 (0) 0/47 (0)	0/126 (0)	-
Respiratory complications (*n*, %)	Acute respiratory distress syndrome	0/4 (0)	0/47 (0)	0/126 (0)	-
	Acute pulmonary oedema	0/4 (0)	0/47 (0)	0/126 (0)	-
	Lung embolism	0/4 (0)	0/47 (0)	0/126 (0)	-
Transfer to ICU (*n*, %)		0/173 (0)	0/47 (0)	0/126 (0)	-
Septic shock (*n*, %)		0/173 (0)	0/47 (0)	0/126 (0)	-
Mortality (*n*, %)		0/173 (0)	0/47 (0)	0/126 (0)	-

Abbreviations: d: days; *n*: number; ICU: intensive care unit; *SD*: standard deviation.

the quick Sequential Organ Failure Assessment (SOFA) score was <2 in all cases. Pulmonary auscultation revealed rhonchis and crackling sounds in 7 (4.1%) and 4 (2.3%) patients, respectively. Neurological examination was normal in 161 patients (93.1%). However, a physical meningeal syndrome was reported in 9 patients (5.2%). Lumbar puncture (LP), performed in these nine patients, was abnormal in four patients (2.3%). It showed pleocytosis with a mean rate of 37.8±9,9 cells/mm^3, with lymphocytes predominant and normal glucose and protein concentration in all patients.

Infrequent signs such as rhonchus, crackling sounds on pulmonary auscultation, splenomegaly, and hepatomegaly were noted in 4.1%, 2.3%, 1.7%, and 0.6% of cases, respectively.

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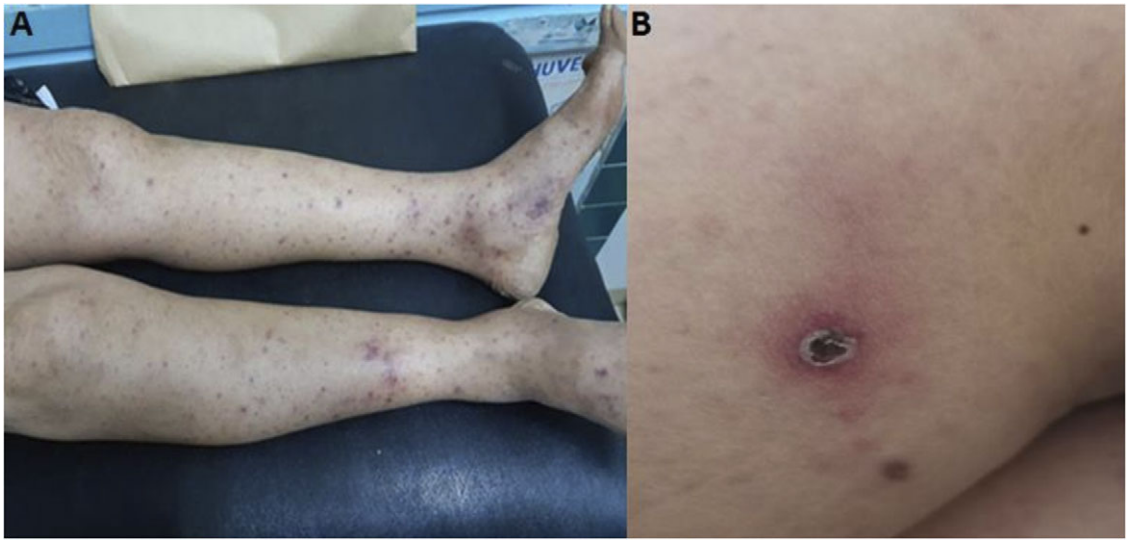

Figure 4. (A) Maculo-papular and purpuric lesions and **(B)** inoculation eschar in a 56-year-old Tunisian immunocompetent patient.

Table 3. Microbiological findings of patients with Mediterranean spotted fever/comparison of confirmed/definite and suspected/probable patients with MSF

Microbiological findings		All patients (*n* = 173)	Confirmed/Definite cases (*n* = 47)	Suspected/Probable cases (*n* = 126)
Serological results (immunofluorescence) (*n* = 90) ; (*n*, %)	Single serum sample with IgG ≥ 1/64 of *R. conorii* (n, %)	47/90 (52.2)	47/47 (100)	0/126 (0)
	Single serum sample with IgG ≥ 1/64 of *R. typhi* (n, %)	1/90 (1.1)	0/47 (0)	1/126 (0.8)
	Single serum sample with IgG ≥ 1/64 of *R. conorii* +*R .typhi* (n, %)	2/90 (2.2)	2/47 (4.2)	0/126 (0)
	Two serum samples with fourfold titre elevation within 2 weeks (n, %)	0/90 (0)	0/47 (0)	0/126 (0)
	Negative rickettsial disease serology (n, %)	43/90 (47.8)	0/47 (0)	43/126 (34.1)

Abbreviation: *n*: number

Biological and microbiological data

We tested serum samples for antibodies against *R. conorii* by immunofluorescence assay (cutoff for IgG ≥ 1/64) in 90 patients (52%; Table 3). All patients had only a single serum sample with no follow-up sample. Serology was positive to *R. conorii* in more than half of samples; 47 patients (27.2% of all patients).

Concerning laboratory data revealed a high white blood cell count (WBC; normal range: 4000–10,000/mm^3) in 63 patients (36.4%) with a mean value of 8310/mm^3. However, decreased WBC level was observed in 5.8% of the cases (*n* = 10). Thrombocytopenia was present in 48% of cases (*n* = 83) with a mean value of 169.331/mm^3. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (normal range: 10–40 U/L) were increased in 50.9% (*n* = 88) and 40.5% (*n* = 70) of cases, respectively. C-reaction protein (CRP) and lactate dehydrogenase (LDH) levels were also increased in 91.9% (*n* = 159) and 65.9% (*n* = 114) of cases, respectively. Hyponatremia (normal range: 135–145mmol/L) was noted in 50.9% (*n* = 88) of cases (Table 4).

We also decided to make comparative analysis of confirmed/definite patients defined as patients with positive serology (serum sample with IgG ≥ 1/64 of *R. conorii*; n=47) and suspected/probable patients.

Therapeutic management and outcomes

Antimicrobial drugs consisted of an oral single-day dose of 200 mg of doxycycline regimen in 161 patients (93.1%; Table 2). Fluoroquinolones such as ofloxacin and ciprofloxacin were administrated intravenously in 4% and 2.3% of cases, respectively, with favourable outcome. Antibiotics were administrated with a mean of 6.4±3 [3-15] days. The different treatments were equally well-tolerated and no severe side-effects were observed. Fever disappeared after 2.8±1.3 [1-5] days of treatment initiation. Four of the 173 patients (2.3%) had complications such as chorioretinitis, encephalitis, bilateral anterior uveitis, and cerebral vasculitis. Chorioretinitis and anterior uveitis occurred respectively on Days 5 and 7 of hospitalization. In the case of encephalitis, the patient had an altered level of consciousness with a Glasgow coma scale of 14/15, with a normal LP. Cerebral vasculitis was objectified on magnetic resonance imaging and required the use of steroids. In our cohort, no patients were transferred to intensive care unit or mechanically ventilated. All patients recovered

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Table 4. Laboratory findings in patients with Mediterranean spotted fever/comparison of confirmed/definite and suspected/probable patients with MSF

Biological abnormalities		All patients (n = 173)	Confirmed/ Definite cases (n = 47)	Suspected/Probable cases (n = 126)	p-value
White-cell count/mm^3 (4,000–10,000/mm^3)	Mean rate	8310±6730 /mm^3	9633 ± 4977 /mm^3	9351 ± 4808 / mm^3	0.77
	Leucocytosis (>10.000/mm^3) (n, %)	63 (36.4)	14 (29.8)	49 (38.9)	0.65
	Leukopenia (<4000/mm^3) (n, %)	10 (5.8)	2 (4.3)	8 (6.3)	0.87
Platelets count/mm^3 (150,000–450,000/mm^3)	Mean rate	169 331±125 036 /mm^3	160318 ± 90209 /mm^3	175631 ± 100607 /mm3	0.35
	Thrombocytopenia (<150.000/ mm^3) (n, %)	83 (48)	21 (44.7)	62 (49.2)	0.94
Aspartate aminotransferase, U/L (10–40 U/L)	Mean rate	114±70 U/L	115± 125 U/L	81 ± 75 U/L	0.20
	Increased level (>40U/L) (n, %)	88 (50.9)	30 (63.8)	58 (40)	0.86
Alanine aminotransferase (10–40 U/L)	Mean rate	65±43 U/L	79 ± 69 U/L	63± 61 U/L	0.46
	Increased level (>40U/L) (n, %)	70 (40.5)	26 (55.3)	44 (34.9)	0.96
C-Reactive Protein (<5 mg/L)	Mean rate	160±65 mg/L	125± 119 mg/L	148± 132 mg/L	0.28
	Increased level (> 6mg/L) (n, %)	159 (91.9)	36 (76.6)	123 (97.6)	0.06
Lactate dehydrogenase (190–445 U/L)	Mean rate	550±123 U/L	782 ± 436 U/L	646 ± 330 U/L	0.06
	Increased level (>445U/L) (n, %)	114 (65.9)	27 (57.4)	87 (69)	-
Creatine kinase, U/L (15–200 U/L)	Mean rate	380±200 U/L	174 ± 189 U/L	211 ± 238 U/L	0.39
	Increased level (>200U/L) (n, %)	110 (63.6)	32 (68.1)	78 (61.9)	-
Natremia (135–142mmol/L)	Mean rate	136±1.4 mmol/L	136 ± 0.98 mmol/L	135. ± 1.11 mmol/L	0.11
	Hyponatremia (<135mmol/L)	88 (50.9)	26 (55.3)	62 (49.2)	0.586
Kalemia (3.5–5 mmol/L)	Mean rate	3,8±0.3 mmol/L	3.6 ± 0.4 mmol/L	3.72 ± 0.5 mmol/L	0.08
	Hypokalaemia (<3.5mmol/L)	23 (13.3)	7 (14.9)	16 (12.7)	0.89

with no relapse and were discharged from hospital after a mean of 6 ±4.8 [1-19] days of hospitalization.

Focus on RFs for life-threatening complications

Regarding clinical and paraclinical variables, no significant differences were found in patients with complicated forms (n = 4) compared to patients with no complicated forms (n = 169); p>0.05. The mean WBC was more important in patients with complicated forms with a significant trend (12332±4855 /mm^3 [2840-31800] vs 9949±3371 /mm^3 [2390-14320]; p = 0.08).

Discussion

MSF is a vector-borne zoonosis endemic in the Mediterranean region, particularly in southern Europe and northern Africa. A clear increase in the incidence of this zoonosis in different European countries was observed in the last decades, especially in Portugal, Romania, and Italy [3,5–7]. This was also observed in several Maghreb countries such as Algeria, Morocco, and Tunisia [8]. In Tunisia, the most frequent eruptive rickettsial disease is due to *R. conorii* (80,8%), followed by *R. typhi* (10,6%) and *R. massiliae* [1]. The observed incidence of MSF probably reflects better diagnostic methods, improvements in the Tunisian epidemiological surveillance systems (ESS) and a true increase in the disease burden.

According to epidemiological findings in medical literature, there appears to be a seasonal pattern in the incidence of MSF, with a significant clustering of cases occurring during the summer months. For instance, in our study, we observed that 68.8% of cases were documented during the summer season, with the highest rate of hospitalizations recorded between June and September [4,9]. Indeed, environmental factors such as high temperature strongly affected *Rh. sanguineus* behaviours by altering its development and thus leading to a higher affinity for humans. Ticks, particularly *R. sanguineus*, have a life cycle that is closely linked to climatic conditions. In summer, higher temperatures and relative humidity favour their activity and reproduction. This season is ideal for tick eggs to hatch and for larvae, nymphs, and adults to develop. As ticks become more active, they seek hosts to feed on, increasing the risk of human bites [2,3,10]. In our patients, animal exposure with presence of a dog, main host of this tick [4,5,11], was reported in 72.3% of the cases.

Our clinical findings were consistent with previous data. The clinical polymorphism of this rickettsial disease is sometimes misleading [3]. However, the classical triad symptoms: fever,

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maculopapular rash, and an inoculation eschar at the tick bite site is characteristic of MSF [1]. The majority of patients initially sought medical care due to fever. This predominant symptom was commonly accompanied by a maculopapular or purpuric rash in 86.4% to 100% of cases [8,9,11,12]. Atypical forms of MSF without skin rash were described by Crespo and al. in 13% of cases in a large 24-year retrospective study[13]. Inoculation eschar is a pathognomonic symptom of MSF, with an estimated frequency of 60% in different locations [3]. Conjunctival hyperaemia was frequently observed during MSF. This conjunctival involvement could be explained by accidental contamination from tick-infected hands, and it can be considered as the inoculation eschar [14]. *Rickettsia* tends to target the endothelial cells found in small and medium blood vessels all over the body, leading to the development of systemic symptoms [15]. Extra-cutaneous manifestations of MSF were commonly reported in the literature [1]. Neurological signs such as meningeal syndrome, and other extra-cutaneous symptoms such as respiratory, GI symptoms, and hepato-splenomegaly were reported, not only in our cohort but also in several studies [1,5,12,13].

Concerning biological findings, WBC count is variable during *R. conorii* infection in a biphasic pattern. In fact, leukopenia is usually observed during the first days of the disease. Then, from the 15th day, it is often followed by a late hyperleukocytosis [16]. As demonstrated in our results, thrombocytopenia is common during this zoonosis and can be considered as major biological diagnostic criteria. In fact, *R. conorii* targets and damages vascular endothelial cells, which could explain the thrombocytopenia [16]. As we have shown in our study and because of endothelial damage of liver cells, MSF is usually associated to high levels of liver enzymes (AST/ALT), reported in 79% of the cases [9].

Several laboratory diagnostic tools exist for the diagnosis of *R. conorii* infection. Serological tests are widely used for the diagnosis of MSF, with immunofluorescence antibody (IFA) assay as test of choice Diagnostic confirmation requires two serum samples taken 2 weeks apart to detect seroconversion or a fourfold increase in IFA titres against *R. conorii.* One of major limitations of our study was the lack of second serum sample, this factor explains why seroconversion or a substantial increase in the antibody titre was not noted in our patients [17]. The presence of cross-reactions between the different species of *Rickettsiae* is considered another problem with this laboratory technique [4]. On the other hand, PCR on skin biopsies or eschar swabs can perform a rapid direct diagnosis by specifying the species [18,19]. However, negative PCR results should not exclude the diagnosis [6]. Unfortunately, this technique was not a common practice in our facility during our study period. The presence of PCR would have led to the diagnosis being confirmed, particularly in the acute phase before seroconversion, even after the beginning of antibiotics. In our cohort, this would probably have increased the number of patients in the confirmed/definite group compared with the suspected/probable group.

Therapeutically, it is recommended to use a single dose of 200 mg of doxycycline per day as a first-line treatment. This third-generation tetracycline, with a well-known intracellular diffusion, was prescribed in 94.2% of cases, in our study, with a mean duration of 6.4 days. Some authors recommended to continue this treatment for at least 3 days after fever disappearance. Others suggested administering a single dose of doxycycline for a single day, for mild-to-moderate manifestations [20], an approach that may merit consideration for our patients with mild symptoms. The use of fluoroquinolones in patients with *R. conorii* infection is controversial. Some authors had noted a correlated severity with the use of these molecules [21]. However, in our series, ofloxacin and ciprofloxacin were administered in 4.1% and 2.3% of cases respectively with a favourable clinical and biological outcome. This deleterious effect of fluoroquinolones was explained by toxin liberation [22].

Although it is a non-life-threatening infection, complications are reported during MSF in 1% to 20% of patients. In contrast with Rocky Mountain Spotted Fever, the most lethal of the spotted group rickettsiae, MSF has a case fatality rate of 0–3% [3,15]. In our cohort, there were no instances of mortality observed, consistent with findings from the Israeli multicentric study [19]. Related RFs according to the literature were: encephalitis with cerebral oedema, acute respiratory distress syndrome, acute non-cardiogenic pulmonary oedema, gastrointestinal haemorrhage, necrotizing haemorrhagic pancreatitis, and acute renal failure [23,24]. However, neurological manifestations such as encephalitis and cerebral vasculitis were observed in two of our patients and both recovered with no relapse afterwards. Concerning our study secondary objective, we thought that no work analysing RFs has been concluded due to the lack of more life-threatening cases.

Finally, in this current paper, we approach an important medical and public health topic. Although tick-borne diseases are widely reported to be increasing globally, possibly due to improved diagnostics, ESS, or changes in host-vector dynamics, our study showed a downward trend in reported cases over the years. The study period is enough large to provide a better understanding on the clinical and epidemiological profile of MSF. We believe that our data are significant, representing one of the largest monocentric studies conducted in North Africa that describes clinical and laboratory features, therapeutic management and outcomes in adult patients with MSF. However, there are a few limitations of this study: (i) our study is monocentric and retrospective with 52 cases which were confirmed by serology and only 27% patients confirmed with *R. conorii*, none of the patients were documented to have seroconversion or a fourfold increase in IFA titres against *R. conorii.* Furthermore, 83 of the suspected patients did not undergo rickettsial serology and could therefore have been confirmed cases if they had had the opportunity to do so, which could also reconsider the results of our comparison. We thought that this content reflects considerable expertise of the Tunisian practitioners in the clinical diagnosis and treatment of MSF, contrasting with poor microbiological data, in a study dates back to the early 2000s where the diagnostic techniques may be missing in low-to-middle-income countries; (ii) this hospital-based cohort did not include patients with molecularly diagnosis; PCR on skin biopsies or eschar swabs has not been carried out and *Rickettsia* species were unidentified; (iii) the disease is described as mild and non-life-threatening, yet all the patients were admitted to the hospital and other series of patients with MSF have reported well documented fatalities; the hospitalization bias of mild-to-moderate forms made our cohort non-representative to evaluate mortality or complicated forms.

Conclusion

MSF deserves a special attention with a better knowledge of this disease, its clinical polymorphism and collection of exposures to possible zoonoses. With a long series of diseases with low prevalence in a clinical epidemiology study, our results showed that MSF is an infection that can affect all age groups, occurs during the summer season, especially in urban aera. Clinical diagnosis in suspected/probable cases is based on the triad of fever, skin rash, and inoculation eschar, which was almost constant in our patients. Although our study highlighted that it remains a mild disease, a

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delayed diagnosis can lead to life-threating complications. The biggest clinical challenge to achieve successful treatment is making an early and prompt diagnosis. Biological tests have significantly enhanced the diagnosis of rickettsioses. The PCR amplification, on skin biopsies or eschar swabs, is a rapid test for detecting rickettsioses. It should be increasingly available in Tunisian hospitals. Further research should attempt to identify the different species and involve other areas of the country.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S095026882400178X.

Data availability statement. Data available on request due privacy to restrictions. The data presented in this case study are available on request from the corresponding author.

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Informed consent statement. Due to the retrospective nature of the study, the Ethics Committee Rabta University Hospital, Tunisia determined that patients' consent was not required. We have made sure to keep patients' data confidential and compliance with the Declaration of Helsinki.

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Ethics statement. Due to the non-prospective nature of the study, the Ethics Committee of La Rabta University Hospital, Tunis, in Tunisia determined that participant consent was not required. The confidentiality of participant data has been respected in accordance with the Declaration of Helsinki.

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