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Review Article

Epidemiology, Clinical Aspects, Laboratory Diagnosis and Treatment of Rickettsial Diseases in the Mediterranean Area During COVID-19 Pandemic: A Review of the Literature

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Abstract. The purpose of the present review is to give an update regarding the classification, epidemiology, clinical manifestation, diagnoses, and treatment of the *Rickettsial* diseases present in the Mediterranean area.
We performed a comprehensive search, through electronic databases (Pubmed – MEDLINE) and search engines (Google Scholar), of peer-reviewed publications (articles, reviews, and books). The availability of new diagnostic tools, including Polymerase Chain Reaction and nucleotide sequencing has significantly modified the classification of intracellular bacteria, including the order Rickettsiales with more and more new *Rickettsia* species recognized as human pathogens. Furthermore, emerging *Rickettsia* species have been found in several countries and are often associated with unique clinical pictures that may challenge the physician in the early detection of the diseases.
Rickettsial infections include a wide spectrum of clinical presentations ranging from a benign to a potentially life treating disease that requires prompt recognition and proper management. Recently, due to the spread of SARS-CoV-2 infection, the differential diagnosis with COVID-19 is of crucial importance. The correct understanding of the clinical features, diagnostic tools, and proper treatment can assist clinicians in the management of Rickettsioses in the Mediterranean area.

Keywords: Rickettsiosis; Review; Epidemiology; Microbiology; Zoonosis; COVID-19.

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Introduction. Human rickettsial diseases are a variety of different clinical zoonoses caused by the genus Rickettsia and Orientia (order Rickettsiales, family Rickettsiaceae) that comprises a small (0.3–0.5 by 0.8–2.0 mm), obligately, intracellular and gram-negative bacilli, within α-proteobacteria.[1–3]

The development of Polymerase Chain Reaction (PCR) and nucleotide sequencing for the study of 16S rRNA has significantly modified the taxonomic classification of bacteria, in particular, intracellular bacteria. Some species sequencing for the study of 16S rRNA has demonstrated the phenotypic and genotypic differences between some microorganisms and the genus Rickettsia. For example, this is the case of *Orientia tsutsugamushi* that was reclassified from the genus Rickettsia into a new genus Orientia. The order Rickettsiales currently comprised the genera Anaplasma, Ehrlichia, Neorickettsia, Orientia, Rickettsia, and Wolbachia.[4,5]

Rickettsial infections are transmitted to human hosts mostly through arthropod bites or arthropod faeces that infect scratching lesions. The most frequent vectors responsible for the transmission are ticks, which also act as reservoirs, but some infections are associated with lice, fleas, or mites.[2]

Rickettsia species are split into four pathological groups or clades, based on their phenotypic characteristics, vector hosts and phylogenetic organization, that include the ancestral group, the spotted fever group (SFG), the typhus group, and the transitional group. The SFG is the largest group, and it is composed of the most common rickettsiae, such as *Rickettsia aeschlimannii, Rickettsia africae*, *Rickettsia conorii* subsp. *caspia*, *Rickettsia conorii* subsp. *conorii, Rickettsia conorii* subsp. *indica*, *Rickettsia conorii* subsp. *israelensis, Rickettsia massiliae, Rickettsia monacensis, Rickettsia raoultii*, *Rickettsia rickettsii, Rickettsia rioja*, *Rickettsia sibirica* subsp. *mongolitimonae*, *Rickettsia sibirica* subsp. *sibirica,* and the *Rickettsia slovaca.* The typhus group is composed of *Rickettsia prowazekii* and *Rickettsia typhi*.[6] The ancestral group includes *Rickettsia bellii* and *Rickettsia canadensi*.[3] The transitional group includes species of clinical importance, as *Rickettsia akari Rickettsia australis* and the *Rickettsia helvetica,* phylogenetically similar to SFG species such as *Rickettsia felis*.[7] Several authors discuss the validity of this group; in fact, these species have no relevant differences with other SFG species, except for their phylogenetic position.[8]

Due to their adaptation from a free-living to an obligate intracellular life in eukaryotic cells, Rickettsia species modified and reduced their genome size progressively.[9,10] Unexpected property of the rickettsial genome is the presence of plasmids, the first described in obligate intracellular bacteria.[11] This discovery suggests possible exchanges of genetic material by conjugation, a mechanism that was previously considered to be absent in obligate intracellular bacteria.[9,11]

The transmission of the infection depends on the group. SFG is transmitted by the bite of an infected tick; whereas, organisms of typhus group are transmitted through inoculation via infected louse or flea faeces (*Rickettsiae prowazekii* and *Rickettsia typhi*, respectively) through a bite, wound or mucous membranes. Once inoculated into the skin, organisms are phagocytized by dendritic cells and transported via lymphatics to local lymph nodes where they replicate. Subsequently, the bacteria spread in the bloodstream and disseminate to infect the endothelium of the microcirculation, where the Rickettsiae can infect vascular endothelial cells of the small and medium-sized blood vessels. The damage of the endothelium and the subsequent endothelial dysfunction is followed by alteration in coagulation and the cytokine network. The endpoint of this pathogenetic results in a reduction in circulating peripheral CD4 T lymphocytes and perivascular infiltration by CD4 and CD8 T lymphocytes, B cells, and macrophages, causing a vasculitis.[12–14]

Epidemiology. There are several pathological Rickettsia species in Europe, and in the last years, new species and subspecies have been implicated as human pathogens, and new rickettsial syndromes have been described.[15]

Mediterranean spotted fever (MSF) caused by *Rickettsia conorii* subsp. *conorii* is the most frequent rickettsiosis in Europe. It is endemic in southern Europe, but sporadic cases have been reported in all the continents.[15,16] The first cases were first described in Tunisia in 1909 by Conor and Buch. The brown dog tick, *Rhipicephalus sanguineus,* is the vector and the potential reservoir of *Rickettsia conorii* subsp. *conorii* in the Mediterranean area.[15,17] Most MSF cases occur in summer when climatic conditions seem to be an essential factor in increasing the aggressiveness of *Rhipicephalus sanguineus* ticks to bite humans.[15–18]

Rickettsia conorii subsp. *israelensis* is the agent of Israeli spotted fever (ISF), which was first reported in 1946 in the Haifa Bay area, Israel.[17–20] In Europe and the Mediterranean region, the brown dog tick, *Rhipicephalus sanguineus*, is recognized to be the vector of *Rickettsia conorii* subsp. *israelensis*.[21] The geographic distribution of the disease appears to be spread more widely in the Mediterranean countries than previously thought. Cases have been reported in Italy, Portugal, Tunisia, and Libya.[22–25]

Other *Rickettsia conorii* subspecies reported in the Mediterranean area are *Rickettsia conorii* subsp. *caspia* and *Rickettsia conorii* subsp. *indica*. The first one is the agent of Astrakhan fever, endemic in the Astrakhan region, adjacent regions of the Caspian Sea, and described in *Rhipicephalus sanguineus* ticks in Kosovo

and southern France.[16]

Rickettsia sibirica subsp. *mongolitimonae,* the microorganism that cause of lymphangitis-associated rickettsiosis (LAR), was isolated for the first time in China, from *Hyalomma asiaticum* ticks collected in Mongolia in 1991.[16] *Rickettsia sibirica* subsp. *mongolitimonae* was detected in *Hyalomma anatolicum excavatumt* ticks in Greece and Cyprus; in *Rhipicephalus pusillus* ticks in France, Portugal, and Spain; and in *Rhipicephalus bursa* ticks in Spain.[26–30] The first human infection with *Rickettsia sibirica* subsp. *mongolitimonae* was reported in France in 1996.[31] *Rickettsia sibirica* subsp. *mongolitimonae* is implicated in human pathogen in different countries, as France, Spain, Turkey, and Egypt.[32–35]

Rickettsia slovaca and *Rickettsia raoultii* are associated with a syndrome characterized by scalp eschars and neck lymphadenopathy following tick bites (SENLAT). These microorganisms have been found in *Dermacentor marginatus* and *Dermacentor reticulatus* ticks in a vast majority of European countries.[36–42] After MSF, SENLAT is the most prevalent tick-borne rickettsiosis in Europe. It has been reported in different countries, including Hungary, Spain, France, Germany, Italy, Bulgaria, and Portugal.[43–48] SENLAT occurs most frequently from March to May and from September to November, which corresponds to the periods of most considerable activity of Dermacentor adult ticks in Europe.[47–49]

Rickettsia helvetica is transmitted by *Ixodes ricinus*, which is the primary vector and the natural reservoir. However, human infection is rare, and it has been documented only in Austria, Denmark, France, Italy, Sweden, Slovakia, and Switzerland.[16,50,51]

Other rare rickettsial pathological species in Europe and the Mediterranean area are *Rickettsia massiliae, Rickettsia monacensis, Rickettsia aeschlimannii,* and *Rickettsia sibirica* subsp. *sibirica*.

Clinical Manifestation. Rickettsiosis is a rare disease: the incidence is around 1 case per 100.000 people by year, but it has been increasing during the last years, probably due to better diagnostic techniques.[15]

In Europe, the most important diseases are three: Mediterranean Spotted Fever (MSF), Lymphangitis-associated rickettsioses (LAR), and scalp eschar and neck lymphadenopathy (SENLAT).[19] The other significant disease caused by *Rickettsia rickettsii* is the Rocky Mountain Spotted Fever (RMSF), but no cases have been reported in Europe to date.[19]

Apart from these three pathologies, there are other minor forms caused by different pathogens.

Mediterranean spotted fever. MSF, caused by *Rickettsia conorii,* is the most common rickettsial-disease in Europe, where the highest incidence is during summer.[52] Not all people who come into contact with this bacterium develop the disease. A Spanish study, indeed, shows that 4-8% of the population carry antibodies against Rickettsia but without a previous clinical history of MSF.[53]

The most common symptoms are fever (93-98%), myalgia (64-75%), headache (48-65%), and asthenia (27%). The maculopapular rash is present in 85-94% of the patients; the tache noir has been noticed in 58-64% of the patients. The classic triad, fever, maculopapular rash, and inoculation eschar, is present in 40-50% of the patients.[54–56]

In most cases, MSF is a self-limiting disease but sometimes could be life-threatening. It was estimated that about 5-10% of MSF cases could be severe. Cases of severe respiratory distress syndrome,[57] cardiovascular symptoms (coronary ectasia,[58] myocarditis,[59,60] vasculitis[61]) ocular symptoms,[62–65] neurological symptoms[66,67] (sensorineural hearing lost,[68,69] polyneuropathy,[70,71] encephalitis,[72,73] meningitis[74,75]), acute pancreatitis,[76] splenic rupture,[77] acute renal failure,[73] hemophagocytic syndrome[78–80] and arthritis[81,82] have been reported.

The most frequent hematological and biochemical modifications are thrombocytopenia, leukocyte count abnormalities, elevated hepatic enzyme levels and an increase of c-reactive protein.[54,83]

Mortality was around 1-3%[84] before the antimicrobial drug era. Thus, it has been considered a benign illness. In some recent studies, MSF appears to be more severe than it has been thought. Mortality rates were 5.4% in France, 3.6% in Portugal, 3.2% in Algeria, 0.8% in Spain and 0.36% in Italy.[52,54,84–86]

Risk factors for severe MSF include advanced age, immunodeficiency, chronic alcoholism, G6PDH deficiency, diabetes, prior prescription of an inappropriate antimicrobial drug, or delay in treatment.[84,85]

Scalp eschar and neck lymphadenopathy after a tick bite. SENLAT[87] syndrome is also known as TIBOLA[88] (tick-borne lymphadenopathy) or DEBONEL[44] (*Dermacentor*-borne necrotic erythema and lymphadenopathy), and it is caused by *Rickettsia slovaca* and *Rickettsia raoultii*[19] but also by other bacteria such as *Bartonella henselae*.[87] This disease is developed mostly during spring and autumn.[49]

The clinical description of SENLAT includes asthenia, headache, painful adenopathies (especially to the neck's lymph nodes), and a painful scalp eschar surrounded by a perilesional erythematous halo. Low fever, rash, and face edema have also been reported less frequently.[45,87,89] No malignant or fatal cases have been described in the literature. After the therapy, alopecia could potentially last for several months, with persistent asthenia.[89]

Lymphangitis-associated rickettsioses. LAR is caused

by *Rickettsia sibirica* subsp. *mongolitimonae*. Just a few cases have been reported in Europe. In particular, until 2013, only 24 cases have been reported in the Mediterranean area.[19]

The typical period of this disease is spring. Commons symptoms include fever, headache, an eschar (frequently more than one) on the site of inoculation, and lymphangitis, which starts from the eschar and reaches an enlarged lymph node. The difference between LAR and the other two diseases are the period of occurrence (spring), and the presence of lymphangitis and multiple eschars. [90]

Until now, no deaths have been reported in patients that have been infected by Rickettsia. However, some severe cases have been reported, in particular: a retinal vasculitis,[91] sepsis with disseminated intravascular coagulation,[92] myopericarditis[93] and a septic shock.[94,95]

Mediterranean spotted fever-like. Other Rickettsiae in Europe could infect humans; most of them cause a disease very similar to MSF. For example, *Rickettsia conorii* subsp. *caspia* causes an illness called "Astrakhan fever." This disease is typical of the Caspian Sea area, but some cases have also been reported in France.[96] Astrakhan fever diverges from MSF in the percentage of patients who present with an eschar (only 20%) and because it could cause thrombocytopenia and bleed.[97]

Another similar disease is the Israeli spotted Fever (ISF), caused by *Rickettsia conorii* subsp. *israelensis*. In Europe, this bacterium has been found only in Portugal and in Italy. The symptoms are quite similar to MSF except for the presence of gastrointestinal symptoms in half of the patients. The main difference is the malignity; indeed, the mortality is higher (more than 25%).[24,40,56,98,99]

Other Rickettsiae who could cause a MSF-like illness are *Rickettsia monacensis*,[100,101] *Rickettsia massiliae*,[102,103] *Rickettsia aeschlimannii,*[104,105] and *Rickettsia helvetica* which could be malignant.[51,106,107]

Differential Diagnosis with other infectious diseases including COVID-19. Clinically, the patients with MSF present the classic triad, fever, tache noir, and maculopapular rash in 40-50% of cases. In the absence of this typical clinical picture, the diagnosis could be challenging.

A small percentage of patients could present only the tache noir, which is generally pathognomonic of rickettsial diseases. However, clinical cases in which the tache noir was present in other zoonoses have been reported in the literature.[108–110]

The presence of fever without other signs is, probably, the most difficult challenge for clinicians because it is the expression of many diseases, both infective (bacterial, viral, fungal, and parasitic) and not infective. In these patients, a proper anamnesis, laboratory findings, and radiological features are mandatory to permit the correct diagnosis. Blood cultures should be collected at the fever peak to exclude a bacterial or fungal infection. Furthermore, in the area where SARS-CoV-2 is circulating in the population, the nasopharyngeal swab, together with acute phase serology, is recommended to rule it out. Indeed, the common symptom in patients with COronaVIrus Disease (COVID-19) is the fever.[111,112] The other symptoms that these two diseases have in common are headache, asthenia, and myalgia. The associations of dysgeusia, anosmia, and gastrointestinal symptoms could suggest the diagnosis of COVID-19.[113–115]

The maculopapular rash is an expression of several diseases.[116] In these cases, clinicians should pay attention to the distribution, the pattern, and the relationship between the localization at the start of it and other clinical signs, especially the fever. Although respiratory symptoms are the most frequent in COVID-19, skin involvement should always be considered. Galván Casas C et al.[117] described the most common cutaneous pattern, and Magro et al.[118] demonstrated how SARS-CoV-2 is associated with microvascular damage and thrombosis.

Moreover, different cutaneous vasculitis-like patterns correlated with COVID-19 or SARS-CoV-2 therapy have been described.[119,120]

Diagnosis. Nowadays, the majority of reference laboratories in developed countries can provide quick identification of rickettsial pathogens thanks to molecular and serological assays. In many cases, the diagnosis could be made by the clinical manifestation, but the laboratory tests are necessary at the support of it.

The choice of the most appropriate diagnostic technique requires consideration of the suspected pathogen, the timing of symptoms onset, and the type of sample available for testing.[121]

Serological tests remain essential diagnostic tools,[122] but Rickettsiae can be isolated from or detected in clinical specimens. The diagnostic tools available include serologic assays, molecular testing, cultures, immunochemistry, and Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF).[123]

The diagnostic technique could be divided into two groups:

1) **Diagnostic techniques used as routine.**
2) **Less common diagnostic techniques.**

Diagnostic techniques used as routine (Table 1)

Serologic tests:

Indirect immunofluorescence antibody assay (IFA) is a widely accepted serologic test for the detection of rickettsial infection.[124,125] It is considered the most

Table 1. Tests used as a routine.

Type of test	Methods	Vantages	Disadvantages
Indirect immunofluorescence antibody assay (IFA)	Serologic	High sensitivity and specificity for IgG[124,125].	Low sensibility for IgM; Operator-dependent [129,130]
The enzyme-linked immunosorbent assay (ELISA)	Serologic	More sensitive than IFA for the detection of low antibodies level; Absorbance of the enzyme reaction is measured with a spectrophotometer[131,132].	Could be negative during the early phase[131,132].
Nucleic acid amplification tests (NAATs) (molecular methods)	Molecular	Quick response; could be used during acute disease[123]; provides the differentiation between different species[136].	High costs; low sensitivity if used peripheral blood and serum[139]; antibiotics reduce the sensitivity[116].

sensitive and specific method among serological assays.[126] IFA consists of rickettsial antigens fixed on a slide and detected by specific antibodies present in the patient's serum, which can be identified by a fluorescein-labeled conjugate. Serum of patients with clinical manifestation of disease must be collected on the day of the admission and 2-4 weeks after illness onset.[127] IFA assays are highly sensitive at detecting antibodies after 2-3 weeks after illness onset, and their results are best interpreted if serum samples collected in acute and convalescent phases are tested at the same time.[128] Most laboratories test for IgG antibodies because IgM antibodies reactive with *Rickettsia rickettsii* are frequently detected in patients with no other supportive evidence of a recent rickettsial infection. Therefore, the detection of IgM during the acute phase should not be considered diagnostic for an ongoing illness as there could be cross-reactivity with other species and persistence of IgM beyond acute status.[129,130]

The enzyme-linked immunosorbent assay (ELISA) detects the binding of specific antibodies to antigens in a serum sample. When secondary anti-human antibodies conjugated with an enzyme are bound to antibodies from a serum sample and subjected to a substrate, an enzymatic reaction will be measurable in a positive specimen.[131] ELISA is sensitive, reproducible, and allows the differentiation of IgG and IgM antibodies. The results are more sensitive than IFA for the detection of low antibodies level, such as during late convalescence.[121] ELISA has the advantage, compared to IFA, of eliminating the subjective evaluation since the absorbance of the enzyme reaction is measured with a spectrophotometer. The inhibition ELISA has been used only for the diagnosis of scrub typhus and seems to be more sensitive than IFA in the early phase of the disease.[132]

Molecular diagnostic methods:

These assays are more appropriate than serology in the diagnosis of acute infection; a sample collected early at disease onset, before the development of antibodies, is more likely to produce a positive result in PCR assays. When antibody production has increased to detectable levels, bacteria are rarely found in the bloodstream or at the inoculation site. Furthermore, if antibiotic treatment has been initiated, the sensitivity of PCR assays decreases for the same reason.[133,134]

The most used method is *nucleic acid amplification tests (NAATs)*, such as PCR, which has acquired increasing importance over the past few years. The quick response allows a prompt diagnosis without the need to wait for seroconversion or cell culture's growth time, which can take from 10 to 30 days.[123] Amplification of species-specific DNA by PCR provides a useful method for the differentiation between the several Rickettsia spp. and to gain knowledge about the genomic differences within the genus.[124]

The conventional PCR format, due to a large number of PCR products, is more prone to contamination. For this reason, a single-use primer PCR has been introduced.[135] Another molecular method is real-time PCR that offers the advantage of speed, reproducibility, quantitative capability, and reduced risk of contamination compared with conventional PCR assays.[136]

Several clinical samples are suitable for PCR amplification: skin biopsy, eschar, swab, or CSF. Peripheral blood and serum could also be used, but PCR on these samples has a lower sensitivity compared to skin samples or eschar collected on the bite site.[137,138]

PCR detection of *Rickettsia rickettsii* in the blood is possible. Still, its sensitivity is lower because of the small numbers of rickettsiae in the blood in the first stages of the disease.[139] For this reason, during the acute phase, it is better to use the SFG tissue specimen.[116,140] Doxycycline treatment decreases the sensitivity of PCR; therefore, obtaining blood before starting antibiotic therapy is recommended to minimize false-negative results.[116]

Less common diagnostic technique (Table 2)

Shell Vial:

This method requires a large number of bacteria and specific cell lines to proliferate, such as Vero E6 cells, human embryogenic lung fibroblast, and the promyelocytic HL-60 leukemia cell line (the most widely used cell line for growing *A.*

Table 2. Less common diagnostic technique.

Type of test	Methods	Vantages	Disadvantages
Weil-Felix test	Serologic	Easy to use; low cost. It is still used in developed countries[125].	Cross-reaction with other antigens. Low sensitivity, low sensibility[122,168]
Western Blot	Serologic	Highest sensitivity to early antibody, high specificity[144].	Expensive, technically difficult to perform, longer procedure[144].
Line Blot	Serologic	High specificity and sensitivity; a large number of antigens tested[121].	No quantitative titers available; expensive[121].
Indirect hemagglutination test	Serologic	More sensitive than either the complement fixation or Weil-Felix[126]	Rarely used, low sensitivity, long preparation[143].
Latex agglutination	Serologic	High sensitivity[145]	Rarely used for the high cost[145].
Micro immunofluorescence (MIF)	Serologic	Hight sensitivity and specificity; tests multiple rickettsial antigens simultaneously[123]	Cross-reactivity[123]; high costs.
Complement Fixation (CF)	Serologic	Very specific; used for sero-epidemiologic studies[143]	Poor sensitivity, especially during the early stage of the disease[126]
Indirect immunoperoxidase assay (IPA)	Serologic	Similar to IFA; very sensitive and specific[143]	Needs specific instrument and trained personal[143]
Shell Vial	Culture	Highest specific; could be used during acute disease[121,141].	Long times[141].; low success rate; needing specific cell lines[141]; low sensitivity[142]
Circulating endothelial cells (CECs)	Other	Not influenced by previous antibiotic treatment; CECs level detected could be correlated with the severity of the disease[127]	Low sensitivity; not easy to perform[146].
Immunohistochemistry (IHC)	Other	High sensitivity[123,143]	Need bioptic sample, not easy to perform[123,143].
MALDI-TOF	Spectrometry	Early diagnoses, differentiation between species[123]	High costs; not always available[123]. Only used to identify infections inside the arthropods[123,147,148]

phagocytophilum).[141] Specimens for cell cultures should be collected before starting antibiotic treatment and should not be frozen.[121] To identify the cultivated small intracellular Rickettsiae, the laboratories should label bacteria by fluorescent antibodies or staining with the Gimenez method.

The low success rate and the complexity of this method do not permit the routinely use of this methodic.[142]

Serologic methods:

The Weil-Felix test, based on the detection of immune-response to different Proteus antigens that cross respond with Rickettsia[125] should not be considered a first-line testing method anymore, even if it remains an option developing countries. It allows the detection of IgM antibodies 5-10 days after clinical manifestations.

Western blot assay (WBA) was demonstrated to be more sensitive than IFA for the detection of early antibodies in Rickettsia spp. Nevertheless, it is generally more expensive and technically challenging to perform than other serological methods.[143] Furthermore, Rickettsia cultures are required. For these reasons, its use is limited to only a few reference laboratories.[144]

The *line, or dot, blot immunoassay,* may be particularly useful for screening the many antigens that might be considered for patients with nonspecific or atypical clinical presentation. This test can be regarded as valuable only as a first-line test for the rapid diagnosis of acute cases in areas with high prevalence.[121]

The microagglutination test could be divided into two different methods, which included the indirect hemagglutination test and the Latex agglutination method. The first one is specific for the detection of IgG and IgM for all Rickettsiae.[143]

The Latex agglutination permitted the directed detection of the *R. conorii*, *R. prowazekii*, *R. rickettsia, R. typhi*, and infections. This method has a high sensitivity, but it is not routinely used for the high cost.[126,145]

Micro immunofluorescence (MIF) assay is similar to IFA except that wells are spotted with multiple rickettsial antigens for simultaneous detection. The negative aspect of this method is cross-reactivity, and its costs.[123]

Complement Fixation (CF) test permitted the identification detection of antibodies specific for rickettsiae. It is peculiar, but it has shown a reduced sensitivity, especially during the early stage of the

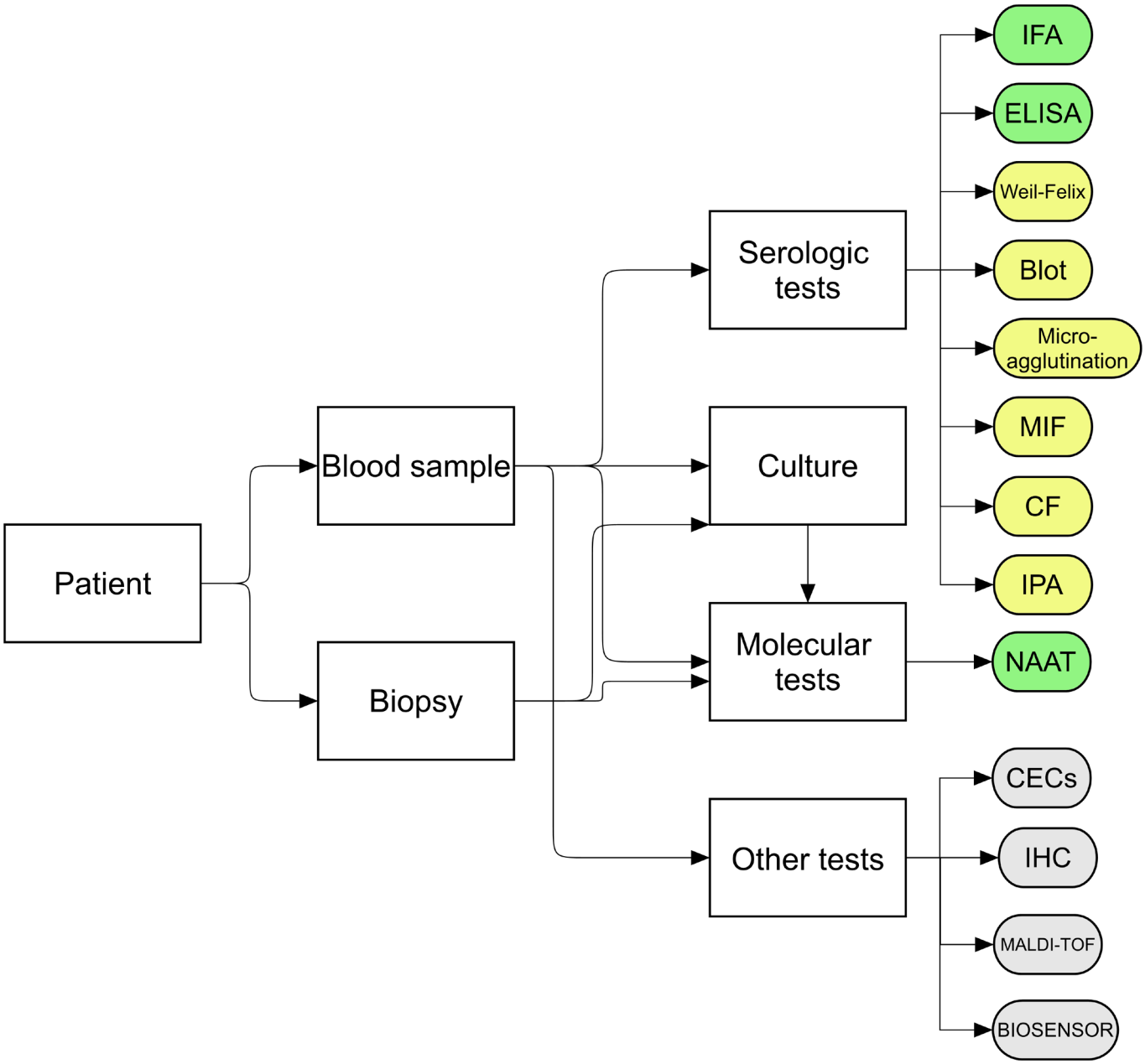

Figure 1. Samples that can be obtained from patients and the diagnostic techniques that could be performed on them. Green: tests commonly used; Yellow: tests used less frequently or used in the past; Grey: tests used only for research studies.

disease. For this reason, it is only used for seroepidemiological studies.[126]

Indirect immunoperoxidase assay (IPA). The procedure is the same as IFA, but it used the peroxidase instead of fluorescein. It needs a specific instrument and trained personal. For this reason, it is not commonly used.[143]

Other tests:

Circulating endothelial cells (CECs) method allows the detection of *R. conorii* in circulating endothelial cells isolated from whole blood by using immunomagnetic beads coated with an endothelial cell-specific monoclonal antibody.[127] The sensitivity is about 50%, and it is not influenced by previous antibiotic treatment. Furthermore, the CECs level detected correlates with the severity of the infection, so it can be considered a prognostic indicator.[146]

Immunohistochemistry (IHC) permits the Rickettsia's detection directly from biopsy specimens, but it could only be used during the acute phase and only if there is a rash or tache noir.[123,143]

The most recent diagnostic tool is the *matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF)*. This technique has been using with promise application for the Tick-borne infections inside the arthropods.[123] The future role of this new method could be applied to help the clinical decision. The identification of Rickettsiae inside the vector[147] or in the hemolymph[148] is showing great potential but remained a niche method.[123]

Biosensors emerging technology allows the fast detection of Rickettsia-induced immune response. For example, the OmpA antigen, an outer membrane protein present in the R. rickettsia, the agent

responsible for the spotted fever, allows the detection of anti-OmpA human IgG. This is possible through an amperometric immune-sensor by using a synthetic peptide, obtained from the H6PGA4 R. rickettsia protein, homologous to OmpA.[149]

Treatment. Rickettsiae spp. are obligate intracellular bacteria; therefore, the standard treatment is based on tetracyclines or chloramphenicol. The gold standard therapy is indeed represented by doxycycline 100 mg per os twice daily x 7 days in adults and 2.2 mg/kg of body weight per dose twice daily, orally or intravenously.[140,150] It has been demonstrated, in several studies, that doxycycline shortens the course of MSF and induces a rapid remission of symptoms. The problem is that tetracycline should be avoided in childhood, during pregnancy,[151,152] in patients who are allergic to it, and in those who have a G6PDH deficiency. An alternative to doxycycline is chloramphenicol. It should be administered at a dosage of 50 mg/Kg/day in four doses for seven days.[56] Since 2000, chloramphenicol was used only for patients suffering from allergy, those having adverse effects to doxycycline or if fever persisted for more than five days or in those that relapsed after the therapy with tetracycline. However, chloramphenicol should also be avoided during pregnancy (grey baby syndrome), and because of the various adverse effects (aplastic anemia, bone marrow suppression), it is not recommended in children. Furthermore, in a randomized study on 415 children, the chloramphenicol group had a longer hospitalization.[56]

For this reason, in pregnant women and children, the first choice is a macrolide. Different randomized studies have shown the macrolides' non-inferiority. In particular, Meloni et al., Bella et all and Cascio et al., in their randomized studies, have demonstrated the non-inferiority, respectively, of azithromycin, josamycin, and clarithromycin vs. doxycycline.[153–155] On the contrary, Munoz-Espin et al. have shown that erythromycin is less effective than doxycycline.[156]

Studies in vitro have tested the efficacy of fluoroquinolones against Rickettsiae spp., showing encouraging results.[150,157–159] Furthermore, randomized studies have shown that there is no difference between tetracycline and fluoroquinolones.[160–162] However, other studies found that fluoroquinolones are associated with increased MSF severity and the worst outcome.[163] Ciprofloxacin has been shown to have a deleterious effect on *Rickettsia conorii*-infected cells.[164]

Rickettsiae spp. showed to be susceptible also to rifampicin,[165] but in 1991 a small trial showed its inferiority in comparison with doxycycline.[166]

Even trimethoprim-sulfamethoxazole has been considered as a possible therapeutic option, but in vitro and in vivo studies have demonstrated that it is not active against Rickettsia spp.[150,167]

In the presence of unspecific symptoms during the spring to summer months, starting azithromycin seems reasonable given the ongoing COVID-19 epidemic in Mediterranean countries.[164]

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