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Les limites des tests diagnostiques des infections bactériennes

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Résumé

Le diagnostic de la maladie de Lyme est actuellement basé sur la sérologie, méthode de diagnostic indirect, du fait d'une culture en laboratoire fastidieuse. Le seul moyen de diagnostic direct qui peut être utile dans certains prélèvements (biopsies cutanées ou liquides de ponction) est la PCR. Nous détaillons dans cet article les principales limites de ces deux techniques pour le diagnostic des infections bactériennes, qui sont illustrées par des exemples tirés de l'histoire récente de la microbiologie. La principale limite de la sérologie bactérienne est la présence de nombreuses réactions croisées du fait de nombreux gènes communs entre espèces bactériennes. Certaines techniques sérologiques, pour le diagnostic des rickettsioses notamment, ont même été basées sur l'existence de réactions croisées. La principale limite de la PCR est la présence de contaminations de laboratoire possibles, ce qui nécessite une qualification des laboratoires qui pratiquent cette technique. De plus, la PCR ne renseigne pas sur la viabilité des bactéries détectées et doit donc être interprétée en fonction de la présentation clinique. Ceci illustre le fait que tout test de diagnostic microbiologique ne doit pas être interprété isolément mais doit toujours prendre en compte le contexte clinique et épidémiologique dans lequel il est effectué.

Abstract

Lyme disease diagnosis is currently based on serology – an indirect diagnostic method – as laboratory cultures are fastidious. The only direct diagnostic method that can be useful with some specimens (cutaneous biopsies or aspiration fluid) is PCR. We aimed to detail the main limitations of serology and PCR testing in the diagnosis of bacterial infections. Limitations are supported by examples from the recent history of microbiology. The main limitation of bacterial serology is the presence of numerous cross-reactions due to many genes that are common to various bacterial species. Some serological techniques, such as those used for the diagnosis of rickettsioses mainly, have even been based on the existence of cross-reactions. The main limitation of PCR testing is the potential presence of laboratory contaminations. PCR-performing laboratories must therefore be certified for the use of this technique. PCR testing also does not inform on the viability of the identified bacterium and should therefore be interpreted in light of the clinical presentation. These limitations highlight that all diagnostic test results should not be interpreted on their own; the clinical and epidemiological contexts should always be taken into consideration.

Current controversies on the diagnosis of Lyme disease contribute to putting into perspective the issue of the relevance of currently available diagnostic tools for bacterial infections. Species of the *Borrelia burgdorferi sensu lato* complex, i.e. the causative agents of Lyme disease, are associated with fastidious laboratory cultures and with a highly transitory bacteremia only occurring at the initial stage of the infection [1]. The main diagnostic method for this infection is serology, i.e. an indirect diagnostic method. Another method relies on DNA detection by PCR when tissue biopsies or synovial fluid or cerebrospinal fluid aspiration are performed. In the present article we detail the main limitations of both of these diagnostic tests, i.e. serology and PCR testing, drawing on examples from the recent history of microbiology.

Bacterial serology

Bacterial serology differs from viral serology. Viruses have great genetic variability and as a consequence, they have specific and rare proteins associated with an extremely low risk of cross-reactions. However, bacteria have a pool of common genes – especially genes encoding for the ribosome and genes encoding for heat-shock proteins – to which human serum is reactive. Bacterial peptidoglycan and lipopolysaccharide are also associated with numerous cross-reactions. Diagnostic interpretation difficulties thus remain for bacterial infections that can only be diagnosed by serology. The history of microbiology offers several examples of bacterial serologies based on cross-reactions. Serological testing may still contribute to the diagnosis when interpreted in light of the clinical context.

Spirochetes and the example of syphilis

Syphilis, caused by *Treponema pallidum* (a spirochete similar to *Borrelia*), is an example of infection that has been accused of causing a variety of unexplained syndromes because of the use of poorly reliable serological tests. Serological testing for syphilis, known

as “Wasserman test”, used to be based on the detection of human cardiolipin [2]. The serology was positive in many situations other than the syphilis infection, and was possibly temporarily positive, such as during pregnancy, viral infections, parasitic infections, or autoimmune diseases [3–5]. As a consequence, between the first and the second world war, physicians used to believe that Rh incompatibility between mother and child was due to seropositive or seronegative congenital syphilis. However, nontreponemal serological tests used in the diagnosis of syphilis have always lacked specificity.

As early as the 1980s, our team and other authors demonstrated the existence of cross-reactions between all spirochetes and mainly between the causative agents of syphilis, leptospirosis, and *Borrelia* infections [6]. It was demonstrated that the cross-reaction between *Borrelia* and *Treponema pallidum* could partly be inhibited by prior absorption by Reiter’s treponema (treponema strain that can be cultured in vitro, non-pathogenic species) [7]. We therefore used to systematically perform this prior absorption at the laboratory to avoid cross-reactions with other spirochetes before performing the Lyme serology [8]. The development of confirmatory Western Blot technology was associated with better specificity [9,10]. However, the Western Blot technique still does not distinguish *Borrelia* species responsible for recurrent fever from *Borrelia* species responsible for Lyme disease [11]. The serological diagnosis of borrelioses, including Lyme disease, is imperfect and will always be imperfect because of consubstantial limitations associated with bacterial serology.

Other historical examples of cross-reactions in bacterial serology

In 1916, as major typhus epidemics sparked during the first world war, Edmund Weil and Arthur Felix developed a serological test based on the finding that the serum of patients presenting with typhus cross-reacted with bacteria of the *Proteus* genus [12]. It is only in the 1940s that serological tests based on *Rickettsia* spp. antigens (true causative agents of typhus) were developed. For many years – and still today in countries such as India – Weil-Felix

serological test was used with OX19 (*Proteus vulgaris*) yielding cross-reactions with typhus-group *Rickettsia* spp. [13], with OX2 (*Proteus vulgaris*) yielding cross-reactions with spotted-group *Rickettsia* spp. [14], and with OXK (*Proteus mirabilis)* yielding cross-reactions with *Orientia tsutsugamushi* [15]. Lipopolysaccharides of *Proteus* are responsible for cross-reactions, not only with *Rickettsia* spp. but also with *Legionella* spp. [14–16].

Another example taken from the research field of *Rickettsia* spp. led to incorrect assumptions: the microagglutination test, developed by Giroud in the 1940s [17]. This method had very poor specificity and yielded many false positive results (25%-30%), as reported by Edlinger and Raoult [18,19]. This technique thus led several authors to believing that diseases without any proven etiology such as multiple sclerosis, vasculitis including Buerger's disease, schizophrenia or "tetany", were caused by *Rickettsia* spp. [20–24]. This technique was then no longer used in France because of its lack of specificity. It is, however, still being used in some regions of the world such as South Africa, leading some to say that *Rickettsia* spp. are responsible for syndromes such as chronic fatigue and fibromyalgia [25].

This type of cross-reactions may be observed with other bacteria. Musso *et al.* demonstrated that up to 35% of patients presenting with *Coxiella burnetii* infection had a cross-reaction with *Legionella micdadei*, that could also lead to pulmonary involvement [26]. These cross-reactions were once again mainly due to proteins and lipopolysaccharide antigens. Another study reported that the serum of patients presenting with a persisting *C. burnetii* infection was positive for *Bartonella henselae* in 50% of cases, as detected by serology [27].

PCR detection techniques

Bacterial DNA amplification by PCR contributed to major advances in human microbiology. However, just like any other diagnostic tests, it is associated with a risk of false

positive and false negative results. False positive results may be explained by an aspecific amplification of environmental contaminants or, within laboratories performing routine PCR tests, of previously amplified products. This is why laboratories performing these tests should meet specific certification requirements. An external validation, performed by the reference centers for infectious diseases, is required for all commercialized tests [28]. These reference centers have many samples available and perfectly documented, i.e. corresponding to various stages of the disease for positive specimens or collected from "healthy" patients or from patients not carrying the disease for negative specimens. Laboratories performing medical diagnoses should also acquire the COFRAC certification, as this agency has the monopoly on evaluating procedures in French medical laboratories. This certification is now mandatory in the French private and public sectors, and each step of the procedure, each reagent, and each technique has to be validated. Positive and negative controls should be performed.

Some patients practicing doctor-hopping to obtain a diagnosis have their samples tested – sometimes with a prescription delivered by a physician – in veterinary laboratories which do not have to comply with the above-mentioned obligations and required certifications for the diagnosis in humans. Results from such laboratories are therefore not reliable. These biological results usually do not inform on the technique used, the positive and negative controls, nor do they specify the method used. Such practices cannot be accepted in the diagnosis of human infectious diseases.

Besides the issue of false positive results yielded by PCR tests and of the quality of the technique used, the PCR diagnosis also raises the question of the viability of microorganisms, which DNA is detected inside tissues, and of their pathogenicity. Our team has, for instance, reported a case of *Streptococcus pneumoniae* endocarditis with a PCR result positive for *S. pneumoniae* on heart valve tissue seven years after the endocarditis episode, while the patient was clinically cured for the endocarditis [29]. A similar case was reported with *Streptococcus*

mutans endocarditis and a positive PCR on heart valve tissue 31 months following the episode [30]. Both these cases raise the question of the residual presence of bacterial DNA without any particular meaning. Several in vitro studies also demonstrated that the DNA of bacteria killed via exposure to ultraviolet rays, boiling water, acid, or autoclave could still be detected by PCR, and the same probably holds in the tissues of living organisms [29]. Studies trying to demonstrate the persistence of *Borrelia* infection on the sole basis of PCR detection, without any specific clinical sign, cannot prove the active infection.

Benefits and limitations of biological tests

How can the "reliability" of a test be assessed? We usually talk about the "performance" of a test, which can be characterized by its sensitivity and specificity. Lyme disease diagnosis does not solely rely on the result of a serological test or of a PCR test. Clinical and epidemiological characteristics should be taken into consideration in the test interpretation. This holds for Lyme disease, but also for any other disease. The predictive value of the serological test depends on the intrinsic characteristics of the test (sensitivity and specificity) and on the likelihood of having the disease (prevalence), which in turn depends on the tested sample. Let's take an example: a positive serology in a patient who do not present with any objective lesions suggestive of Lyme disease and who do not live nor has traveled to an endemic area, has a predictive value close to zero. Let's take another example: when performing seroprevalence studies in individuals excessively exposed to the disease (for instance forest rangers living in Alsace, France), up to 14% of them can have a positive serology without presenting any sign of the disease [31]. The proportion of people who contract Lyme disease following contact with *Borrelia burgdorferi* is variable, but the bacterium presence is not necessarily associated with disease onset in 100% of individuals following inoculation by a tick. This is why biological results cannot be interpreted on their

own. Physicians' knowledge is required to interpret biological results in light of the patient's clinical features.

Conclusion

Borrelia burgdoferi sensu lato early acute infections have well-defined clinical characteristics and should thus be treated without biological diagnosis. The issue of subjective manifestations in people with tick bites and/or in people who never had confirmed Lyme disease, will not be solved based on current medical and scientific knowledge. However, no literature data currently suggests that species of the *Borrelia burgdoferi sensu lato* complex play a role in the genesis of chronic subjective symptoms, as suggested in the history of microbiology for other intracellular bacteria for which the diagnosis relied on serological testing.

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