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Spinal Infections

Laboratory diagnostics for primary spinal infections in pediatric and adult populations: a narrative review

Romain Dayer, MD, Giacomo De Marco, MD, Oscar Vazquez, MD, Anne Tabard-Fougère, PhD, Blaise Cochard, MD, Nathaly Gavira, MD, Giorgio Di Laura Frattura, MD, Nastassia Guanziroli Pralong, MD, Christina Steiger, MD, PhD, Dimitri Ceroni, MD*

Pediatric Orthopedics Unit, Pediatric Surgery Service, Geneva University Hospitals and University of Geneva Rue Willy-Donzé 6, Geneva 1205, Switzerland

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ABSTRACT

Primary spinal infection (PSI) is a generic term covering a heterogeneous group of infections that can affect the vertebral body, intervertebral disks, the content of the medullary cavity, and adjacent paraspinal tissues. Patients' characteristics can vary significantly, notably according to their age, and some of these characteristics undoubtedly play a primordial role in the occurrence of a PSI and in the type of offending pathogen. Before approaching the subject of laboratory diagnostics, it is essential to define the characteristics of the patient and their infection, which can then guide the physician toward specific diagnostic approaches. This review critically examined the roles and usefulness of traditional and modern laboratory diagnostics in supporting clinicians' decision-making in cases of pediatric and adult primary spinal infection (PSI). It appears impossible to compare PSIs in children and adults, whether from an epidemiological, clinical, bacteriological, or biological perspective. The recipients are really too different, and the responsible germs are closely correlated to their age. Secondly, the interpretation of traditional laboratory blood tests appears to contribute little guidance for clinicians attempting to diagnose a PSI. Biopsy or needle aspiration for bacterial identification remains a controversial subject, as the success rates of these procedures for identifying causative organisms are relatively uncertain in pediatric populations.Using nucleic acid amplification assays (NAAAs) on biopsy samples has been demonstrated to be more sensitive than conventional cultures for diagnosing PSI. Recent advances in next-generation sequencing (NGS) are particularly interesting for establishing a microbiological diagnosis of a PSI when standard cultures and NAAAs have failed to detect the culprit. We can even imagine that plasma metagenomic NGS using plasma (known as "liquid biopsy") is a diagnostic approach that can detect not only pathogens circulating in the bloodstream but also those causing focal infections, and thus eliminate the need for source sample collection using costly invasive surgical procedures.

Introduction

Primary spinal infection (PSI) is a generic term covering a heterogeneous group of infections that can affect the vertebral body, intervertebral disks, the content of the medullary cavity, and adjacent paraspinal tissues [1,2]. Infections are rarely contained in one compartment and usually diffuse to others, infecting multiple structures in the vertebral column [3]. There are thus many potentially confusing terms referring to different spinal infections as if they were separate entities. The current trend is to consider PSIs as a continuum of infections that can affect several anatomical structures [3]. The term PSI is thus recognized to encompass a host of infections such as discitis, spondylodiscitis, vertebral osteomyelitis, epidural abscess, subdural abscess, facet joint pyogenic arthritis, paravertebral abscess, and even meningitis [1,3].

By definition, a PSI originates from a distant site, and the microorganism reaches spinal structures by hematogenous spread [4]. Thus,

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* Corresponding author. Service of Pediatric Orthopedics, The Children's Hospital, Geneva University Hospitals, Rue Willy-Donzé 6, Geneva 1205, Switzerland, Tel: +41 22 3724791, Fax: +41 22 3724783.

E-mail address: dimitri.ceroni@hcuge.ch (D. Ceroni).

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in this specific scenario, a PSI occurs without any previous history of injury, diagnostic rocedures, surgery, or dissemination of the infection from contiguous tissue. Among the pediatric population, virtually all spinal infections are primary, whereas, among adults, the rate of PSIs drops to 65% [5,6]. This distinction between the 2 forms is crucial because it is recognized that PSIs progress more severely and result in higher morbidity rates than secondary or postoperative infections [5].

Indeed, PSIs are uncommon in children, with an incidence estimated at 1 to 2 cases per year per 32,500 pediatric hospital consultations or 1 in 250,000 of the pediatric population [7]. In pediatric patients, children from 6 to 48 months old represented 60% to 80% of all cases of PSI [8]. The overall incidence among adults varies from 1 to 5 per year per 100,000 of the adult population [2], and there is a bimodal mode of onset, with the first peak in young adults and the second in patients from 50 to 70 years old [9,10]. The incidence of PSIs increases drastically in mature and advanced patients, and, for reasons not fully elucidated, a PSI is twice as common in men [11]. PSI most commonly affects drug abusers [12–14], HIV-positive patients [15], and adults >50 years old [16]. The most frequently affected spinal segment is the lumbar spine (58%), followed by the thoracic segment (30%) and the cervical spine (11%) [17,18]. A PSI of the sacral spine is exceptional, and most cases of sacral osteomyelitis are secondary, resulting from pressure ulcers and previous surgery, or are consecutive to the contiguous spread from a pelvic infection [19,20].

A good understanding of PSIs invariably requires knowledge of the medical conditions that result in micro-organisms circulating in the bloodstream (bacteremia). Indeed, bacteremia depends on very specific medical conditions that are different at each age, that is, the patient's characteristics and especially the medical conditions that predispose them to those bacteria. In children, oropharyngeal infections are probably the most common sources of transient bacteremia that are responsible for subsequent PSIs [8,21,22]. Among adults, the situation differs somewhat as urinary tract infections are primarily responsible for bacteremia, often following genitourinary procedures [23,24]. Other common potential sources for a PSI include dental infections, oral cavity infections, oropharyngeal infections, otitis media, respiratory tract infections, skin and soft tissue infections, gastrointestinal infections, infective endocarditis, infected intravenous catheter sites, and orthopedic nonspinal device-related infections [24]. However, in almost 50% of cases, the primary source of infection remains unidentifiable [24], so it is worth remembering that such innocuous events as tooth brushing or venipuncture can potentially lead to a hematogenous PSI.

The present article will expressly ignore infections resulting from trauma, prior surgery, or associated with osteosynthesis. It will therefore aim to comprehensively review, summarize and critically examine the current evidence for PSI and focus specifically on high-quality modern laboratory diagnostics.

Patient characteristics according to age

Patients' characteristics can vary significantly, notably according to their age, and some of these characteristics undoubtedly play a primordial role in the occurrence of a PSI and in the type of offending pathogen. Before approaching the subject of laboratory diagnostics, it is essential to define the characteristics of the patient and their infection, which can then guide the physician toward specific diagnostic approaches.

Clinical forms of PSI in pediatric populations

Three main clinical forms of childhood PSI have been described according to patients' ages [8,21,22,25–28]. Neonate forms affect infants under 6 months old and represent the most severe manifestations of the disease; fortunately, they are also the rarest forms [8,22]. These infections occur mainly in premature children, who are then moved to intensive care units and undergo invasive procedures such as intubation, endotracheal suction, and peripheral venous cannula, venous long-line, peripheral arterial line, and umbilical catheter insertions [29]. These children often present with septicemia and multiple infectious foci, with *Staphylococcus aureus* being the most prevalent cause, responsible for approximately 80% to 90% of cases. Other less frequently identified infectious agents are *coagulase-negative Staphylococcus, α-hemolytic Streptococcus, Streptococcus pneumoniae, Escherichia coli,* and *Salmonella* spp [30]. Due to their atypical presentation, diagnosis is usually delayed, leading to extensive vertebral destruction, neurological complications, and subsequent permanent deformations [25,31]. Infantile forms of PSI involve children from 6 months (corresponding to the end of maternally derived immunity) to 48 months old, an age group representing 60% to 80% of childhood cases of spondylodiscitis [8]. Children in this age group are characterized by a developing but failing immunity that appears as maternally derived immunity expires. Some studies of this age group have suggested that *Kingella kingae* is frequently the micro-organism responsible for PSI [8,21,22,27]. The classic clinical presentation of a *K. kingae* osteoarticular infection (OAI) is mild and characterized by a moderate biological inflammatory response; children present with few or no symptoms suggestive of an OAI [32–35]. Most appear in excellent general condition, usually with symptoms only discreetly suggestive of a musculoskeletal infection. Finally, in the forms of PSI affecting children above 4 years old, patients are more prone to being febrile, appearing ill, and suffering from vertebral osteomyelitis due to *S. aureus*. Except for the neonatal forms, childhood PSIs affect children who, for the most part, present without co-morbidities.

Clinical forms of PSI in adults

Adults with a PSI face a totally opposite paradigm, with most patients presenting with serious comorbidities. Indeed, there are many factors predisposing adult patients to a PSI, including advanced age, malnutrition, diabetes mellitus, hepatic cirrhosis, renal failure, chronic steroid use, immunocompromised status, malignancy, HIV/AIDS, infective endocarditis, septicemia, intravenous drug use, intravascular devices, and prior nonspine surgery [6,33].

Interestingly, most PSIs are caused by a single micro-organism rather than multiple pathogens [34]. *S. aureus* is probably the most common pathogen responsible for PSIs in adults, accounting for more than 50% of cases in most case series from Western countries [35–37]. Far behind *S. aureus*, it is estimated that 5% to 20% of PSIs are caused by *Streptococci* and *Enterococci*, whereas less than 5% of cases are caused by anaerobic microorganisms [35–37]. Group B, C, and G pyogenic streptococci are found predominantly in patients suffering from diabetes mellitus. *Enterobacteriaceae* spp. are considered responsible for 7% to 33% of PSIs, with *E. coli* being the most common microorganism from this group, followed by *Proteus* and *Klebsiella* spp. The latter micro-organisms are common causes of urinary tract infections (especially following genitourinary procedures) and gastrointestinal infections (especially among diabetic or immunosuppressed patients) [17,38]. Intravenous drug users sometimes sustain vertebral osteomyelitis caused by *Pseudomonas aeruginosa* because of the poor hygienic conditions in which they inject, even though *S. aureus* remains the most frequently encountered micro-organism among this population too [39,40].

Brucellar and tuberculous PSI

A Brucellar PSI should be kept in mind in cases involving the specific risk factors for *Brucella* spp, such as consuming unpasteurized dairy products, working in livestock farming, the meat industry, abattoirs, or as veterinary or laboratory personnel in endemic areas. Finally, tuberculosis osteomyelitis of the bone is a rare condition caused by *Mycobacterium tuberculosis*. Its incidence has increased in Western countries in recent years due to an influx of refugees from countries where tuberculosis is endemic, HIV infection, increasingly elderly populations, and emerging resistant strains [41,42]. Thus, the incidence of spinal tuberculosis is increasing across developed nations, especially among children

and young adults. In conclusion, the pathogens responsible for PSIs are closely correlated with the risk factors presented by the patients as the potential origin of the bacteremia responsible for the spinal infection.

Blood tests

Recognizing that there is no single routine marker for diagnosing a PSI, a laboratory approach should include blood tests. An initial work-up should include the patient's white blood cell (WBC) count and inflammatory marker measurements such as C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). Interpreting blood test results must be done with great caution as these parameters can vary significantly according to patient age, the germ incriminated, and the site of the primary infection. It is, therefore, difficult, or perhaps even impossible, to have a single, standardized approach to examining these parameters without considering the limitations mentioned above.

In children, since laboratory test results related to PSIs—like the WBC count, CRP, and ESR—are usually normal or slightly elevated, they only provide nonspecific information [8,21,22,43–45]. Indeed, it is currently recognized that few laboratory data for children with a PSI show significant results. There are normally only slight-to-moderate increases in the levels of inflammatory markers, with the confusing consequence that children with PSIs present with few, if any, of the usual symptoms suggestive of an OAI [8,21,22].

In fact, WBC counts above 12,000 per mm^3 are rare and present in only about 35% to 40% of cases, and 60% to 65% of patients with a PSI have normal or near-normal CRP levels [8,21,22,44,46]. The most sensitive markers of inflammation in the presence of a PSI appear to be the ESR (abnormal values in > 80% of cases) [8,17,21,22,27] and the platelet count (abnormal values in 50% to 60% of cases) [8,21,22].

The situation is quite similar among adults, although there are small particularities specific to certain clinical forms of PSI in different age groups. Unlike what we see for children, CRP is considered to have the highest sensitivity (98%) among adults with a PSI—higher than other blood tests, such as the ESR, which appears elevated in 75% of cases [23,47,48]. Both markers are used for monitoring therapeutic response [49]; however, the ESR is only a rough marker of therapeutic response since it remains high in 50% of patients with a good clinical outcome following treatment for their PSI [49]. Thus, CRP is currently considered the most specific marker for treatment response since it rapidly returns to normal levels after successful therapy [2,47]. Procalcitonin (PCT) raised many hopes, and it initially appeared to be a promising marker for distinguishing between bacterial and nonbacterial spinal infections. However, this promise did not materialize clinically, and PCT is currently considered to exhibit less sensitivity than CRP for recognizing PSIs [2]. Among adults with a PSI, the WBC count is a relatively unhelpful laboratory parameter, as it can appear normal in up to 55% of patients with a proven infection [6,48]. Thus, a normal WBC count does not exclude the diagnosis of a PSI. In summary, the WBC count, PCT, CRP, and ESR parameters appear insufficiently discriminatory and not particularly helpful for reliably determining etiology; only the CRP level is interesting as it makes it possible to precisely monitor the patient's response to infection [1].

Microbacteriological investigations

As with most infectious diseases, clinicians should confirm the bacteriological etiology of a PSI before starting an antibiotherapy. This is particularly important for vertebral osteomyelitis given its required treatment's length and increasing morbidity the longer effective treatment is delayed. In addition, growing rates of antibiotic resistance among adults make identifying the causative pathogen and its weaknesses even more essential. However, here again, the results of bacteriological investigations can differ greatly between pediatric and adult populations. Thus, these differences should be integrated into the clinician's reflections and closely considered when making treatment decisions. The present work will thus evoke all the currently available investigations, whether traditional or innovative, which enable us to establish the bacteriological diagnosis of a PSI. The emphasis will be on their performance.

Blood cultures

There is a consensus that blood cultures should be made for all patients with a suspected PSI before starting an empiric antibiotic therapy [50]. Some authors even suggest that at least 2 or 3 sets of blood cultures should be made with samples from different sites, including aerobic and anaerobic samples. However, there are substantial differences between pediatric and adult populations with regard to the effectiveness of blood cultures for identifying the microorganisms responsible for a PSI. In children, for example, blood cultures show high percentages of negative results, ranging from 88% to 100% [43–45,51,52]. The low rate of positive blood cultures among children can be explained by the high frequency of PSIs in children under 4 years old, an age group in which infections seem mainly to be caused by *K. kingae* [21]. Several large epidemiological studies of *K. kingae* OAIs have shown that the detection of this pathogen through blood cultures is the exception rather than the rule [32,53–58]. Among adults, the situation is quite different, as the rate of positive blood cultures varies from 30% to 89% [1,59]. This variability in the rate of positive blood cultures in cases of adult PSI can be explained by adults' previous use of antibiotics, by the concentrations of organisms in their bloodstream, and, above all, by the duration of the bacteremic phase [1]. In addition, blood culture isolates do not always correlate with the micro-organism responsible for the PSI, as an alternate source of the bacteremia may also be present [60]. Even though their results can fluctuate according to the patient's age, blood cultures are essential and should be carried out systematically in cases of PSI.

Cultures from biopsy or needle aspirations

The questions surrounding biopsy or needle aspirations concentrate the controversies over bateriological investigations. These are fueled not only by which technique should be used—a needle biopsy versus an open biopsy—but also by whether these procedures are even legitimate. Here again, it appears that the patient's age is the decisive factor in whether or not to recommend taking a sample for a bacteriological examination. In the pediatric population, the indication for performing invasive procedures, such as a biopsy or needle aspiration, is not currently established, and most authors consider it heretical [8,21,28]. Although some authors believe that a spinal specimen should be obtained using a closed percutaneous or open surgical biopsy, a critical review of the literature shows that the needle aspiration and open biopsy success rates for identifying causative organisms range from 0% to 63% for spondylodiscitis [8,27,52,61–63]. Using spinal specimens for patients with vertebral osteomyelitis, de Lucas et al. [64] reported that the positive culture rate reached 43%. However, due to the surgical and anesthetic risks involved, most authors do not consider these interventions to be standard diagnostic procedures [8,27].

Is there a real need for biopsy in PSI in pediatric populations?

We mentioned previously that 60% to 80% of PSIs in pediatrics populations are probably caused by *K. kingae*, a germ for which an indirect diagnosis now exists (see next sections). Furthermore, this pathogen is relatively easy to treat and its proven resistance to antibiotics has been rare, thus justifying taking a sample for bacteriological examination. It should also be noted that most pediatric PSIs present in the form of spondylodiscitis, for which a disc puncture is known to be deleterious. Indeed, animal studies have demonstrated that even relatively minor damages to the disc resulting from a needle puncture injury could have immediate and progressive mechanical and biological consequences resulting in slow but progressive degeneration [65].

Should one reconsider routine percutaneous biopsy in adult primary spinal infections?

The situation appears quite different among adult populations and taking a sample for bacteriological diagnosis is important or even essential. Most authors consider that a radiologically guided biopsy probably constitutes the best method for obtaining a sample for bacteriological analysis [1]. Computed tomography (CT) and fluoroscopic guidance [1] are used most commonly, but biopsies guided by magnetic resonance imaging [66] or endoscopy [67,68] have been described. These different techniques differ not only because of their rate of complications but also in terms of adequacy and accuracy. Indeed, the rate of diagnosis is probably lower in cases using percutaneous biopsy when compared with the results of other sampling techniques.

The rates of bacteriological diagnoses obtained using samples from percutaneous image-guided biopsies range from 14% to 76% for pyogenic osteomyelitis [1,37,39,59,64,69–71] and from 42% to 76% for tuberculous osteomyelitis [72]. Open biopsies have significantly higher rates of positive cultures than percutaneous biopsies: in pyogenic vertebral osteomyelitis, positive cultures for percutaneous biopsies ranged from 48% to 53%, and positive cultures for open biopsies ranged from 91% to 93% [73,74]. Open surgical biopsies are considered longer, more invasive, more expensive, and require prolonged hospitalization, putting patients at risk of general anesthaesia and major surgery [75]. Thus, percutaneous CT-guided biopsy has become the procedure of choice owing to availability, cost, better spatial resolution, and relative safety.

However, recent evidence has shown that the positive culture yield of CT-guided biopsy in suspected spinal infection ranged between 30% and 35% [63,75–77]. In addition, recent evidence also have demonstrated that percutaneous image-guided biopsy rarely adds any new information when blood cultures have positive findings [78]. For this reason, some authors have suggegsted that, rather than subjecting all patients with acute spondylodiscitis to this procedure, only those with blood cultures with negative findings should be eligible for CT-guided biopsy [77].

Technical and theoretical aspects of percutaneous biopsy

Since PSIs may affect the vertebral endplate, the disc, and the paravertebral tissues, the first question will be therefore to define which should be the target tissue type for biopsy. It is currently recognized that fluid collections, when present, should be targeted for aspiration and/or biopsy. In fact, a study showed higher microbiologic yield from soft-tissue targets, a category that included disc material, psoas abscess, and epidural abscess [79]. Some researchers advice targeting bone for biopsy because samples may also be sent for pathologic evaluation, which may help in diagnosis during PSIs with false-negative cultures [80,81].

Larger core needles and multiples core samples are favored when feasible without exposing the patient to undue risk [82]. Ideally, antimicrobial therapy should be interrupted for 1 to 2 weeks before biopsy if possible, but biopsy should be performed, if needed, without discontinuing antimicrobial therapy [23]. With the larger use of NAAAs for investigating percutaneous biopsy samples, all the problems that revolved around the specimen transfer method, the inoculation of the material immediately after sampling at the patient's own bedside and the transfer time in the microbacteriology laboratory are factor that are much less important now. That being said, it is strongly recommended however to use sterile anaerobic and aerobic transport container, to maintain the samples at room temperature, and to send them within 2 hours to the microbiology laboratory [83].

Most of the disagreements between authors involve the circumstances surrounding sample taking. Multiple biopsy procedures are probably the most controversial topic [1]. Some authors consider it legitimate either to take multiple samples at once to increase the yield of micro-organisms [67] or to make repeated biopsies, whether open or percutaneous, when the first phase of bacteriological investigations (blood cultures and biopsy) ends with no causative microbiological agent [68,84,85]. If a second percutaneous biopsy is required, it is advisable in this regard to perform it at least 3 days after the initial biopsy [86]. Other authors, however, conclude that this way of doing things is unjustifiable [59,87] as a second percutaneous biopsy will not significantly improve the chances of a positive culture [88] and repeated sampling is more likely to lead to complications [71,84]. Finally, a new technique is currently being used to improve the yields from spinal sampling, and it is based on injecting saline and collecting the reflux using one or 2 needles. This technique is expected to result in more than 90% of cultures being positive [89].

Nucleic acid amplification assays (NAAAs)

Since the early 2000s, nucleic acid amplification assays (NAAAs) have given bacteriologists the ability to highlight infinitesimal quantities of bacterial DNA and have provided clinicians with an effective and potent set of tools for detecting traces of bacteriological agents in clinical samples [63,90]. During the last few years, NAAA technology has progressed significantly, and continuing improvements have been recorded both in terms of efficiency (the development of primers amplifying species-specific targets better than universal primers) and specificity (because the assays were less prone to contamination) [90,91]. The use of NAAAs now makes it possible to detect either nucleic acid sequences or specific antigens in clinical samples. The crucial contribution of these diagnostic assays has drastically improved clinicians' ability to reliably recognize infectious diseases. Indeed, the wide-scale use of NAAAs has remarkably improved the identification of OAIs and thus changed both the incidence of pediatric OAIs and their observed bacteriological epidemiology [32,54,90]. Physicians can now identify the germ responsible for most of the OAIs that had remained culture-negative before the advent of NAAAs.

Initially, NAAA's provided only limited information on the activity and the physiological state of microorganisms detected in samples. For this reason, there was an urgent need to develop a system able of differentiating nucleic acids associated with viable cells from those associated with inactivated cells [92]. In order to address this limitation, 2 new PCR-based strategies have been developed. The first one, called viability PCR (vPCR), correlates viability of the cell or mico-organims with its envelope impermeability. Nonviable cells/micro-organisms with damaged membranes, and therefore free nucleic acids, are not protected from the reagents, and their amplification is inhibited when the reagent-DNA complex photoactivated [93]. The second strategy, termed as "molecular viability testing" (MST), correlates viability with the ability to quickly synthetise a specific macromolecule in response to a brief nutritional stimulus [92,94,95].

Application of NAAAs to biopsy samples

Using NAAAs for investigating percutaneous biopsy samples has been demonstrated to be more sensitive than using conventional cultures for diagnosing PSIs [96]. The advantages of using NAAAs are exemplified in cases of PSIs due to fastidious, slow-growing pathogens or when an antimicrobial therapy has already been initiated, which would undoubtedly diminish the positivity of blood cultures and biopsy cultures. It is recognized that more than 4 days of prior antibiotic exposure significantly decreases the rate of positive cultures [97]. However, most of the publications mentioning the contribution of NAAAs in the microbiological diagnosis of PSIs have used a broad-range 16S rRNA polymerase chain reaction (PCR) assay, that is, the latest, most efficient version of this technology. Notwithstanding this, the use of 16S rRNA PCR assays significantly improved pathogen recognition in 53% to 60% of cases, whereas traditional culture methods had only identified them in 28.9% to 50% of cases [96,98].

A new approach involving nucleic acid amplification is now available based on a multiplex PCR technique. The basic principle of multiplex PCR is the same as that of conventional PCR, except that more than 1 pair of primers are required in the same reaction. The primers combine specifically with their corresponding DNA template, and more than one DNA fragment can be amplified in one simultaneous reaction. Thus, this assay improves test sensitivity while extending recognition to a panel of pathogens known to be responsible for PSIs. Multiplex real-time PCRs performed on vertebral tissue have made it possible to establish a diagnosis of spinal brucellosis in 90.9% of cases and a diagnosis of spinal tuberculosis in 83.3% of cases [72].

Histopathological assessment

It is unanimously recognized that the histopathological examination of samples taken by open or CT-guided percutaneous biopsies can be a valuable aid to establish the diagnosis of PSIs. Usually, pyogenic infection is considered when acute neutrophilic infiltration is noted with widespread necrosis. Tuberculous vertebral infection is evoked when granuloma or caseous necrosis with chronic inflammation is noted, whereas it is considered as definitive when acid-fast bacillus bacteria is positive [99]. Many studies have shown the superiority of cytopathology over microbiological investigations in establishing the diagnostic of PSI [100], since it was demonstrated that histology could provide the diagnostic even when no specific infectious agent was isolated [63,80,99–101]. Two papers reported of a sensitivity ranging between 81% and 91%, and a specificity of 100% for histologic examinations [100,101]. Thus, adding cytopathologic analysis to microbiologic investigations provides high diagnostic accuracy, especially in countries in which tuberculous is still endemic [100,101].

Indirect diagnosis of PSIs using oropharyngeal RT PCR assays

An interesting paper published in 2022 tried to demonstrate that performing an oropharyngeal swab PCR could detect the *K. kingae* RTX toxin gene in almost 90% of toddlers with confirmed spondylodiscitis [21]. This was robust support for previous studies suggesting that *K. kingae* DNA could be found in the oropharynx of children with an OAI, and it indicated that a throat swab could provide strong evidence that this micro-organism was responsible for an infection. Together, these form a solid argument for suggesting that *K. kingae* should be the primary etiological pathogen suspected in children from 6 to 48 months old with spondylodiscitis. The indirect diagnosis of *K. kingae*'s involvement in spinal infections by searching for this specific pathogen's DNA in infants' oropharynges is of great interest in this particular situation and performing discal needle aspirations or biopsies to investigate spondylodiscitis in this specific age group should probably no longer constitute the gold standard.

Next-generation sequencing

Recent advances in next-generation sequencing (NGS) have built a strong foundation on which to develop more efficient methods of identifying micro-organisms. Next-generation sequencing is a massively parallel sequencing technology that offers ultra-high throughput, scalability, and speed. It represents the targeted sequencing of all the genomes present in a clinical sample and, thus, does not depend on pathogens growing in cultures. Metagenomic NGS (mNGS) is based on high-performance DNA sequencing technologies, procuring millions of DNA strands, and thus reducing the need for the traditional cloning methods used in other genome sequencing techniques. This method can thus arbitrarily amplify and detect all the micro-organisms present in a clinical sample, thus theoretically enabling the recognition of any and every pathogen present.

In reality, mNGS can currently unambiguously identify more than 1,400 species with a turnaround time that has been substantially shortened to 1 to 2 days [102]. Indeed, species detection and identification can also be done with no a priori knowledge of the etiological agent, and, providing a comprehensive database of single nucleotide polymorphisms is available, identification resolution can go down to the subspecies or strain level [103]. This unbiased, hypothesis-free strategy is particularly suitable for establishing a microbiological diagnosis for infections for which standard cultures and NAAAs have failed to detect the culprit [104]. This selective sequencing will provide increased sensitivity, improved specificity, and faster identification of pathogens of interest; it will also help to keep costs under control since more samples can be tested in the same run [105].

This new technology is beginning to be used in clinical practice, but despite its conventional use on biopsy samples, one might legitimately ask whether future diagnoses could not be established indirectly from plasma samples using plasma mNGS. When mNGS is performed using plasma (known as "liquid biopsy"), this method detects not only the pathogens circulating in the bloodstream but also those causing focal infections. Sequences of a pathogen's DNA leak from infected sites such as bones, joints, or the spine into the patient's blood, potentially allowing clinicians to dispense with the need for source sample collection using costly, invasive surgical procedures [106].

In a recent interesting multicenter study, mNGS performed using plasma detected *K. kingae* in 10 young children (median age 16.5 months; range 10–23 months) with spondylodiscitis [106]. Despite negative blood cultures in all 10 patients, detecting *K. kingae* using mNGS from plasma enabled narrow antimicrobial coverage for 9 patients and established diagnoses without a biopsy for 8 of them. mNGS is a novel, promising, versatile tool that is expected to drastically change how infectious diseases will be diagnosed in the future. The benefits to patients include avoiding invasive diagnostic procedures and the identification of both common and novel pathogens that would otherwise remain undetected by culture or NAAA methods. However, the mNGS approach has yet to fulfill all of its theoretical potential [106] (Tables 1 and 2).

Serology

In any particular case of vertebral osteomyelitis, each of which occurs in very specific circumstances, using serological reactions to establish a bacterial diagnosis remains relevant for many pathogens. Serological methods currently play a key role, for example, in the routine diagnosis of brucellosis vertebral osteomyelitis. The serological reactions used in bacteriological diagnoses are numerous: the most popular serological tests for diagnosing human brucellosis are the serum agglutination test (SAT), the Rose Bengal test (rapid agglutination detecting IgG), the immunocapture-agglutination test, the Coombs test, and ELISA. By examining their overall accuracy in clinical settings, these test systems can be ranked as follows: ELISA = immunocapture test > RBT > SAT > Coombs test [107]. ELISA is thus the most sensitive test for reliably diagnosing human brucellosis [108–110], but its sensitivity and specificity for detecting antibodies against *Brucella* spp. differ substantially between studies. Araj et al. [109] demonstrated that ELISA's sensitivity levels for detecting IgG and IgM were 91% and 100%, respectively, whereas its specificity levels were 100% for both. In a study conducted by Memish et al. [111], sensitivity and specificity levels were 45.5% and 97.1% for IgM and 79% and 100% for IgG, respectively. When the 2 ELISA results were evaluated together, sensitivity and specificity climbed to 94.1% and 97.1%, respectively [111]. Finally, Xu et al. [110] noted that ELISA's sensitivity for IgG detection (88.37%) was higher than for IgM detection (74.42%) and that its combined IgG and IgM results significantly improved the level of sensitivity (98.84%) but decreased its specificity (84.13%). Thus, ELISA can detect human brucellosis with very high sensitivity [107] but may not have sufficient specificity to be used as a diagnostic tool [112].

For this reason, in focal complications of brucellosis, at least 2 serological tests (eg, ELISA and the immunocapture-agglutination test) should be used to increase serological diagnostic sensitivity

Table 1
Incriminated micro-organisms according to the age of patients and to predisposing medical conditions.

Age group	Predisposing medical conditions	Exoected pathogens
Neonates and infants < 6 mo	Prematurity	*S. aureus*
	Stay in ICU	*E.coli*
	Multiple invasive procedure	Gram negative bacilli
		Streptococcus pyogenes
		Sterptococcus pneumoniae
Infants ranging between 6 and 48 mo	Oropharyngeal viral infections	*K. kingae*
	Viral gastroenteritis	
Children > 4 y and adolescents	No specific co-morbidities	*S. aureus*
Young adults	Intravenous drugs users	*S. aureus*
		P. aeruginosa
		Fungal infections
	HIV/AINS	*S. aureus*
	Immunosuppression	*M. tuberculosis*
		Fungal infections
	Origin from endemic countries	*M. tuberculosis*
		B. melitensis
Older adults	Malnutrition	*S. aureus*
	Diabetes mellitus	*Streptococci*
	Hepatic cirrhosis	*Enterococci*
	Renal failure	
	Immunosuppression	
	Chronic steroids use	
	Malignancy	
	Urinary tract problems	
	Intravascular devices	
	Non spinal prior surgery	
	Bacteremia due to concomitant infection	
	Diabetes mellitus	Groupe B, C, G *Streptococci*
	Urinary tract infections	*Entorobacteriaceae*
	Genitourinary procedures	*-E.coli*
		-Proteus spp
		-Klebsiella ssp
	HIV/AINS	*S. aureus*
	Immunosuppression	*M. tuberculosis*
		Fungal infections
	Origin from endemic countries	*M. tuberculosis*
		B. melitensis

Table 2
Contribution of blood tests and bacteriological investigations during PSI.

Laboratory investigations	Contribution
WBC	Few contributive
	Children: abnormal values in 35%–40% of cases.
	Adults: abnormal values in 45% of cases
CRP	Children: few contributive
	Abnormal in 60%–65% of cases
	Adults: contributive
	Highest sensitivity (98%)
SR	Children: contributive
	Abnormal value in > 80% cases
	Adults: contributive
	Abnormal in 75% of cases
Blood cultures	Children: non contributive
	Positive in 0%–12% of cases
	Adults: moderately contributive
	Positive in 30%–89% of cases
Cultures from biopsies	Children: moderately contributive
	Positive in 0% to 67% of cases
	Adults: moderately contributive
	Positive in 30%–35% of cases
Application of NAAAs to biopsies	Contributive in both populations
	rRNA PCR: positive in 60% of cases
	Real-time PCR: positive in 80%–90% of cases
Serology Brucella	Contributive
	Sensitivity ELISA IgM: 45.5%–100%
	Sensitivity ELISA IgG: 79%–100%
	Sensitivity ELISA IgM & IgG together: 94%–98.8%
Histopathological assessment	Contributive in both populations
	Sensitivity: 81%–91%

[109,110,113]. The combined use of blood cultures and 2 serological tests enable diagnosis in > 95% of cases, which explains why it is not often necessary to resort to a vertebral biopsy to reach a correct diagnosis of brucellosis vertebral osteomyelitis [112].

Conclusions

This review critically examined the roles and usefulness of traditional and modern laboratory diagnostics in supporting clinicians' decision-making in cases of pediatric and adult PSI.

1. It appears impossible to compare PSIs in children and adults, whether from an epidemiological, clinical, bacteriological, or biological perspective.
2. The interpretation of traditional laboratory blood tests appears to contribute little guidance for clinicians attempting to diagnose a PSI.
3. As with most infectious diseases, confirming a PSI's bacteriological etiology before initiating an antibiotherapy is highly recommended.
4. Blood culture results can differ according to patients' ages, but we nevertheless consider them essential, and they should be performed systematically in cases of PSI.
5. Whether biopsy or needle aspiration should be used for bacterial identification remains the most controversial subject in this area, as the success rates of these procedures for identifying causative organisms are relatively uncertain.
6. Most authors consider that biopsy or needle aspiration should not be considered standard diagnostic procedures for pediatric populations and should be avoided.
7. In adult populations, however, the situation appears quite different, and taking a sample for a bacteriological diagnosis seems important, perhaps even essential.
8. Using nucleic acid amplification assays (NAAAs) on biopsy samples has been demonstrated to be more sensitive than conventional cultures for diagnosing PSI.
9. An indirect diagnosis of *K. kingae*'s involvement in the PSIs of children under 4 years old can be made by searching for this pathogen's DNA in their oropharynx.
10. Recent advances in next-generation sequencing (NGS) are particularly suitable for establishing a microbiological diagnosis of a PSI when standard cultures and NAAAs have failed to detect the culprit.
11. Plasma metagenomic NGS can be performed using plasma (known as "liquid biopsy"), and this assay can detect not only pathogens circulating in the bloodstream but also those causing focal infections.
12. Plasma metagenomic NGS using plasma is a diagnostic approach that may eliminate the need for source sample collection using costly invasive surgical procedures.

Declaration of Competing Interest

One or more authors declare potential competing financial interests or personal relationships as specified on required ICMJE-NASSJ disclosure forms.

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