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Diagnostic stewardship based on patient profiles: differential approaches in acute versus chronic infectious syndromes

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ABSTRACT

Introduction: New diagnostics may be useful in clinical practice, especially in contexts of high prevalence of multidrug-resistant organisms (MDRO). However, misuse of diagnostic tools may lead to increased costs and worse patient outcome. Conventional and new techniques should be appropriately positioned in diagnostic algorithms to guide an appropriate use of antimicrobial therapy.

Areas covered: A panel of experts identified 4 main areas in which the implementation of diagnostic stewardship is needed. Among chronic infections, bone and prosthetic joint infections and subacute-chronic intravascular infections and endocarditis represent common challenges for clinicians. Among acute infections, bloodstream infections and community-acquired pneumonia may be associated with high mortality and require appropriate diagnostic approach.

Expert opinion: Diagnostic stewardship aims to improve the appropriate use of microbiological diagnostics to guide therapeutic decisions through appropriate and timely diagnostic testing. Here, diagnostic algorithms based on different patient profiles are proposed for chronic and acute clinical syndromes. In each clinical scenario, combining conventional and new diagnostic techniques is crucial to make a rapid and accurate diagnosis and to guide the selection of antimicrobial therapy. Barriers related to the implementation of new rapid diagnostic tools, such as high initial costs, may be overcome through their rational and structured use.

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1. Introduction

Multidrug resistant organisms (MDRO) represent a threat to healthcare systems and the containment of their spread represents a global priority [1]. Epidemiology may greatly vary among different countries, but several mechanisms of resistance in Gram-positive and Gram-negative bacteria are emerging worldwide in both community and nosocomial settings, greatly impacting on patient mortality and morbidity [2]. New rapid diagnostics may represent a potent weapon against MDRO. A great number of new advanced diagnostic tests have been developed during the last decade, offering the opportunity to achieve rapid and precise laboratory diagnosis which was not possible previously with conventional microbiology [3,4]. Since cost considerations and over-utilization of new diagnostic techniques may have a negative impact on healthcare system and patient outcome, the concept of diagnostic stewardship is increasingly used. Diagnostic stewardship indicates the role of diagnostic tests in improving the use of antibiotics and promoting the appropriate use of microbiology diagnostic methods. A reconsideration of current practices is needed to improve a diagnostics-guided therapy.

The aim of this position paper is to provide a practical diagnostic guide for the appropriate use of old and new diagnostic tools in some chronic and acute syndromes. These syndromes have been chosen by the panel of experts since they may represent a diagnostic challenge for clinicians and require a well-structured diagnostic approach.

2. Diagnostic approach to chronic infections

2.1. Bone and prosthetic joint infections (PJIs)

The global rise in life expectancy has led to an increased number of chronic osteoarticular degenerative diseases and joint prosthesis replacements. Infections represent a significant complication of implant surgery, resulting in major challenges regarding the diagnosis and treatment [5]. Prosthetic joint infections (PJIs) account for 30% of osteoarticular infections and are responsible for prolonged hospitalization, and significant morbidity [6]. PJIs are classified in relation to the time of onset after surgery: (1) early PJIs, generally

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Article highlights

- New rapid diagnostics may be useful against multidrug resistance and should be appropriately combined with conventional techniques.
- Chronic bone/prosthetic joint infections and chronic endovascular infections are difficult-to-manage infections that require well-structured diagnostic algorithms for definitive antibiotic therapy.
- Bloodstream infections at Emergency Department need well-structured diagnostic algorithm to guide early antibiotic therapy.
- Community-acquired pneumonia may be due to several causes, and predicting the etiological pathogens remains an unmet need.

caused by highly virulent and often nosocomial bacteria; (2) delayed onset PJIs, which are usually primary chronic infections and involve low virulent or slow-growing small-colony-variant (SCV) bacterial strains; (3) late hematogenous high-grade infections [7]. The most frequent etiological agents are *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS). PJIs caused by CoNS tend to occur relatively late due to their low pathogenicity and tendency to produce biofilm. Low-grade pathogens such as streptococci and enterococci, *Corynebacterium* spp, anaerobic bacteria, *Enterobacterales,* and *Pseudomonas aeruginosa* may also cause PJIs [7]. *Kingella Kingae* and *Cutibacterium acnes* (formerly *Propionibacterium acnes*) may be responsible for postoperative spinal implant infection [8]. *Candida, Brucella* spp and *Mycobacterium* are rare cause of PJIs, but should not be forgotten [8,9].

The standard care for PJI involves the surgical removal of the infected device and the surrounding tissue in one-stage or two-stage revision. Accurate diagnosis and identification of pathogens remain the two most important steps for the optimal management of patients with PJIs. Unfortunately, an accurate diagnosis of PJI poses several problems. Thus, diagnostic stewardship should be implemented in this setting and should consist of a coordinated multifaceted approach. We focused on the diagnostic management of delayed PJIs because diagnosis may be particularly challenging for the following reasons: 1) delayed PJIs are usually characterized by nonspecific symptoms, false-negative cultures, and low values of serum biomarkers, factors that may lead to misinterpreting the PJIs as an aseptic phenomenon; 2) the presence of polymicrobial biofilm may complicate the diagnosis of PJIs because of the difficulty to identify pathogens in cultures media; 3) some strains can grow on the surface of foreign bodies and persist in SCVs or intra-cellular conditions that significantly increase the detection time [10,11].

Considering that there is no single accepted set of diagnostic criteria for chronic-bone infections and PJI, there is an urgent need for a diagnostic algorithm (Figure 1). In the pre-operative phase, no effort should be spared to obtain cultures from periprosthetic tissue and joint fluids [12]. Although Gram-staining has high specificity but low sensitivity, Gram-staining and microbiological culture of synovial fluid, obtained by percutaneous joint aspiration under ultrasound guidance, should be performed [5]. Pre-operative diagnostic tools should also include biochemical, and serological analyses, and blood cultures in patients with fever and/or acute onset of symptoms. The role of C-reactive protein (CRP) in late PJIs is debated: using serum CRP as a screening tool to rule out late PJI, a great proportion of infected prostheses would be misdiagnosed as aseptic loosening [13]. Recent efforts aimed to improve the accuracy of PJI diagnosis and focused on synovial fluid biomarkers. The assessment of inflammatory biomarkers in synovial fluid, such as alpha defensin, leukocyte esterase (before centrifugation to avoid blood contamination) and calprotectin, could facilitate the diagnosis of PJIs and had better diagnostic efficacy than routinely available clinical laboratory test [14]. The α-defensin detection on synovial fluid in typically challenging situations such as culture negative infections, systemic inflammatory conditions, and antibiotic therapy should be considered and implemented as diagnostic tool for PJIs [15].

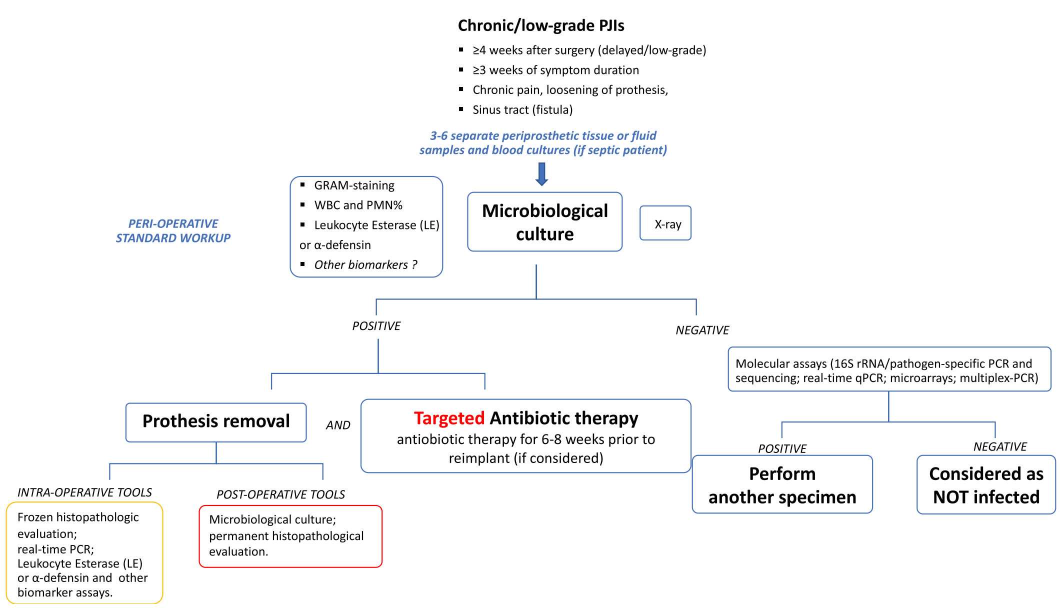

Figure 1. Diagnostic algorithm in patients with chronic prosthetic joint infections (PJIs).

Imaging techniques, including X-ray, computed tomography (CT), ultrasonography (USG), magnetic resonance imaging (MRI), and fluorescence imaging, may support the PJI diagnosis but have several limitations: fluorescence modality is limited to *ex vivo* and *in vivo* preclinical models due to limitations in light penetration depth; conventional CT and MRI may be affected by the presence of metallic implants and have low sensitivity, especially in early infection stages; USG cannot visualize bones and is limited to soft tissue abnormalities. We suggest that a conventional radiographic imaging of the implant/bone should be performed in case of suspected PJI [16].

Although serological, synovial, and radiological investigations may help clinicians, microorganism culture and histopathological diagnosis remain the 'gold standard' for the diagnosis of PJI [17]. This is highlighted by the central role of microbiological culture in the proposed algorithm (Figure 1). Unfortunately, the positivity rate of microbial culture ranges between 60 and 70% [7]. Thus, molecular rapid diagnostic testing (mRDT) may be useful in case of a first negative culture (Table 1). Various molecular-based diagnostic strategies, including polymerase chain reaction (PCR) amplification, both with specific primers or multiplex-PCR, microarrays, qRT-PCR and sequencing analysis of 16S rRNA, have been used in the diagnosis of PJI [18]. The commercial FilmArray Blood Culture ID (BCID) panel (BioFire Diagnostics), an FDA-cleared multiplex PCR panel for pathogen identification from positive blood culture, showed a good performance also in sonicate fluids [19].

The most relevant disadvantages of mRDT in the PJIs diagnosis are represented by costs and low specificity (uncertainty of whether the bacterial DNA revealed in the final analysis of a sample actually represents DNA in the original sample and whether it represents an organism causing a significant infection). Any result should be interpreted in the light of the other clinical, microbiological and histopathological data with multidisciplinary involvement. To date, no PCR-based method has been incorporated into routine laboratory diagnostic workflows due to their higher cost and lower sensitivity values with respect to the conventional culture methods, but they are a promising alternative for specific pathogen identification, especially in culture-negative infections, or in the presence of biofilm-growing bacteria, previous antibiotic therapy, and presence of fastidious microorganisms [18].

In case of positive microbial cultures or if prosthesis is removed, intra-/post-operative diagnostics, including microbiological investigations and histopathological analysis from the removed prosthetic components, should complete the diagnostic workup. In late and chronic infections, antibiotic therapy should be discontinued, if possible, at least 2 weeks before the prosthesis removal [5,20]. To increase the sensitivity of culture analysis, from 3 to 6 samples of periprosthetic tissue or, alternatively, 4 periprosthetic samples or 3 specimens from periprosthetic tissue in homogenate cultures (inoculated in blood culture bottles) should be obtained. All explanted prosthetic components are essential for microbiological isolation [5,20]. Since PJIs are characterized by the presence of biofilm, detecting the infecting microorganism in standard cultures may be challenging. Thus, the dislodgement of the biofilm should always precede the standard cultivation methods in solid or liquid growth media. The biofilm's dislodgement may be achieved by chemical (chelating agent

Table 1. Role of molecular rapid diagnostic testing in clinical syndromes.

Clinical syndrome	Type of mRDT	Advantages	Disadvantages	Clinical application
Prosthetic joint infections	Broad-range 16S rDNA PCR and sequencing of PJI samples	High positive predictive value, even from a single sample	Low sensitivity	Infection suspected and cultures negative Any result must be interpreted in the light of the other clinical, microbiological and histopathological data
	PCR and mass spectroscopic detection of PJI samples	High sensitivity, multiple species can be identified from a single sample	Potential contaminants identified	
	Species-specific real-time PCR	High sensitivity	Limited range of organisms detected	
	Multiplex PCR on sonicate fluids	High sensitivity	Potential contamination	
Intravascular infections and endocarditis	Real-time PCR or 16S rRNA PCR on excised tissue (heart valve/ tissue from embolectomy)	High sensitivity	Specialized laboratory and expertise in the interpretation of test	Blood culture negative endocarditis
Bloodstream infections	Multiplex PCR panels for pathogens identification from positive blood culture bottles Molecular detection of carbapenemase, vanAB and mec A/mecC on rectal swab, nasal swab, blood cultures Direct detection of *Candida* spp. and bacteria from blood by T2 magnetic resonance	Guide empirical therapy in septic patients Improve infection control	Need of organized surveillance program and low turn-around time from laboratory Costs	Patients with septic syndrome
Community-acquired pneumonia	Real-time PCR for SARS-CoV2 Molecular detection of carbapenemase, vanAB and mec A/mecC on respiratory samples	Identify patients at high risk of MDR-etiology	Need of organized surveillance program and low turn-around time from laboratory Costs	Patients with severe CAP or risk factors for MDR-etiology

mRDT molecular rapid diagnostic test

ethylenediaminetetraacetic acid [EDTA] and the reducing agent dithiothreitol [DTT]) and mechanical (sonication) techniques [19]. The sonication procedure of adequate samples for microbial detachment should be performed in Ringer's solution or sterile physiological solution to cover 90% volume of explanted component [21]. It represents a reliable technique and its implementation as part of the diagnostic algorithm for diagnosis of PJI may improve the diagnostic sensitivity of PJI.

2.2. Subacute-chronic intravascular infections and endocarditis

The spectrum of intravascular infections presenting a chronic and indolent course includes native valve endocarditis (NVE) and a miscellanea of infections of foreign bodies, including prosthetic valves, vascular grafts, transcatheter aortic valve implants, pacemakers, implantable cardiac defibrillators, or left ventricular assist devices and ventricular-atrial shunts for the treatment of hydrocephalus [22].

The identification of etiological agents is a critical point in patients with subacute-chronic intravascular infections and endocarditis, because atypical pathogens may be involved in these infections. Typical agents are represented by viridans group streptococcal species (VGS), *Streptococcus gallolyticus* and *Enterococcus faecalis* [23]; less frequent agents are other non-VGS streptococcal and non-*E. faecalis* enterococcal species, *Abiotrophia/Granulicatella* species and Gram-negative bacilli of the HACEK (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella)* group [24–27]. While *Erysipelothrix rhusiopathiae, Bartonella* spp., *Coxiella burnetii, Brucella* spp. and *Tropheryma whipplei*, are rare agents, *Mycoplasma* and *Chlamydia* spp should be considered as exceptional ones [28–32]. Except for *E. rhusiopathiae*, all these organisms may be responsible for the so-called Blood-Culture-Negative Endocarditis (BCNE), defined as endocarditis with negative blood cultures after 7 days of incubation. Thus, they should be considered when prior antibiotic therapy is excluded as the cause of negative cultures results [27]. Modern techniques and prolonged blood culture incubation are instruments to increase the chance of organism identification: modern conventional automated blood culture systems may support the identification of some fastidious or slow-growing organisms historically known as cause of BCNE (including HACEK Gram-negative bacilli and *Abiotrophia/Granulicatella* species) [26], while prolonged incubation up to 3 to 6 weeks is suggested in patients with risk factors and exposures to *Brucella* species [33,34].

Noteworthy, *Candida* species may cause prosthetic valve infections with a very prolonged and indolent course that becomes clearly clinically evident even 7 to 12 months after the initial episode of post-surgery candidemia [35,36]. *Mycobacterium chimaera* is an emerging agent of prosthetic valve infection, especially in presence of lymphocytopenia and exposure during previous cardiac surgery: in cases of intravascular infections of foreign bodies, together with *Mycoplasma hominis* this organism should be considered after the exclusion of other common agents [31,37].

Figure 2 summarizes the diagnostic approach to subacute and chronic cardiovascular infections. The classical endocarditis syndrome, including fever, embolic and vascular phenomena, cardiac murmur, splenomegaly, and digital clubbing [23,24], may be corroborated by advanced imaging techniques (such as fluorodeoxyglucose positron emission tomography [FDG/PET]), especially when proper transthoracic/transesophageal (TTE/TEE) exams resulted inconclusive [38].

Considering that antibiotic therapy is the main cause of negative blood cultures [27], blood cultures should be repeated after a proper 'wash out' time interval from antibiotics, unless hemodynamic decompensation or major embolisms. Most guidelines recommend collecting at least two sets of blood cultures. However, evidences regarding the optimal number of bottles and venipunctures are limited and contrasting [39,40]. Two main sampling strategies currently used: multi-sampling strategy (MSS), with sampling from two venipuncture sites, and the single-sampling strategy (SSS),

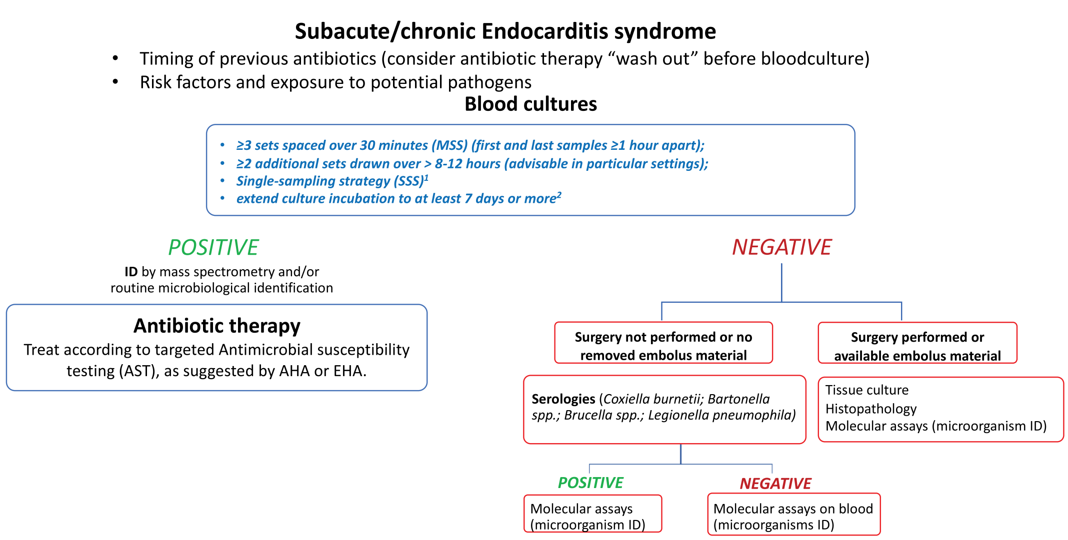

Figure 2. Diagnostic algorithm in patients with subacute-chronic intravascular infections and endocarditis.

[1] Most guidelines recommend collecting 40 ml of blood from separate venipuncture sites, i.e. multi-sampling strategy (MSS). Sampling through a single venipuncture site (single-sampling strategy [SSS]), the whole desired volume of blood is collected from one venipuncture site [39,40,63][2] Occupational exposures to farm animals (sheep, cattle, goats) may be indicative of *C. burnetii, T. whipplei, Bartonella* spp. and *Brucella* spp.

with the whole desired volume of blood collected from one venipuncture site (Figure 2). The MSS may allow a better discrimination between true bacteremia and contamination but is also associated with higher risk of contamination. MSS is the predominant approach, but SSS is gaining approval as a safe alternative. In the setting of subacute-chronic intravascular infections and endocarditis, no specific recommendations are available. The collection of blood cultures over longer time may be reasonable in this specific setting to demonstrate persistent bacteremia and increase the possibility to detect etiological pathogen in case of previous antibiotic therapy.

Efforts to identify the etiological pathogen have pivotal importance also to allow further diagnostic strategies in particular cases (such as the screening for colon carcinoma in the case of *S. gallolyticus*, formerly *S. bovis* biotype 1, isolation) [41].

In the presence of BCNE, proper investigations include cultures of excised valves and/or *embolus* material [26,27,42,43]. Since valve cultures may yield false positive results, caution is required to correct the interpretation of microbiology results [44]. The isolation of the involved pathogen from the valve also guides the duration of postoperative antibiotic therapy (with a longer recommended duration when the organism is grown from the valve) [45]. Histology examination of the valve and/or embolus material might also have value: specific stains can be used to look for mycobacterium (Ziehl-Neelsen stain), fungi (silver stain, appropriate for both yeast and hyphae) *Bartonella* species (Warthin-Starry stain) and *T. whipplei* (periodic acid Schiff positive macrophages) [43].

Molecular methods from both blood and excised valve tissue may increase the probability to detect the offending pathogen in the BCNE. In particular, targeted PCR and 16S ribosomal ribonucleic acid (rRNA) might be adopted as supplementary tests. Unlike the pan-bacterial PCR, targeted PCR looks for a well-defined range of molecular targets [46]. Specialized reference laboratories and large clinical laboratories are currently increasingly purchasing laboratory-developed tests for *C. burnetii, Bartonella species, T. whipplei, C. acnes*, and *M. hominis* [47]. Pan-bacterial 16S rRNA on homogenized tissue with organism-specific PCR assays seems very promising and more sensitive, due to the relative abundance of bacterial DNA in valve tissue *versus* blood or serum. Since 16S rRNA material is conserved in hypervariable segments of all bacteria, it can allow the identification of organisms down to a species level [27]. However, molecular techniques should be interpreted with caution: cross-contamination of tissue may occur, and microbial deoxyribonucleic acid can persist for months following infection. Thus, the presence of bacteria from PCR analysis does not necessarily imply ongoing infection, and clinical judgment remains critical to draw definite conclusions [27].

Once the etiology of the intravascular infection has been established, pharmacokinetics/pharmacodynamics (PK/PD) considerations should be taken into account. First, the antibiotic should be bactericidal, the ideal activity should be rapid and adequate exposure should be maintained over time within the vegetations [48,49]. Example of ideal rapid concentration-dependent bactericidal antibiotics are daptomycin and gentamicin, administered as once daily pulse dose [50], ensuring high concentrations in the serum and sustained concentrations in the vegetations [49]. Second, high penetration and diffusion of antibiotics inside the vegetation should be achieved. In the deeper areas of vegetation the paucity of polymorphonuclear leukocytes contrasts with the highest bacterial densities of organisms that are in an inactive metabolic state, especially in subacute-chronic infections [49]. Some organisms with abnormal growth patterns exhibit a SCV phenotype and are recalcitrant to conventional antibiotics with an increased risk of recurrent infection [51]. In these cases, antibiotics (such as beta-lactams, active only against growing microorganisms) can reduce a small proportion of the microbial population growing at the time of drug administration, leading to a slow rate of bactericidal action [51]. The penetration of some hydrophilic antibiotics may be very heterogeneous inside the vegetations with penetration rate into heart valve below 50% [51]. Since beta-lactams are time-dependent antibiotics, sustained concentrations inside the vegetation may be granted by the use of high dosages and continuous infusion administration. This approach has been associated with a better clinical response rate than intermittent infusion in the treatment of infective endocarditis [52]. Other agents, such as quinolones, aminoglycosides and daptomycin, show homogeneous diffusion throughout the whole vegetative lesion [53,54]. Conversely, teicoplanin remains concentrated at the periphery and does not diffuse inside the core of the vegetation [54]. Penetration of antibiotics inside the biofilm is important in the presence of foreign body materials. Some antibiotics may penetrate more rapidly and effectively inside the biofilm, as showed for daptomycin compared with vancomycin and other antibiotics [55–59].

Further diagnostic stewardship considerations include appropriate use of TTE/TEE and FDG/PET. International guidelines recommend that both TTE and TEE should be performed in patients with moderate to high risk of endocarditis, prosthetic valves and in patients with endocarditis diagnosed on TTE alone to look for peri-valvular lesions as abscess, pseudoaneurysm, fistula and/or large and mobile vegetations at high risk of embolism [60,61]. In the case of NVE, FDG/PET may be limited by its relative insensitivity with a primary benefit being the high specificity of positive test results. However, in carefully selected patients, FDG/PET may be either a useful adjunct in the diagnosis and monitoring of response to antimicrobial therapy of chronic intravascular infections (especially in the setting of foreign body infections where the TTE/TEE may give equivocal results) [62]. Thus, use of this diagnostic tool requires a multidisciplinary decision involving infectious diseases and radiology specialists, as well as the cardiac surgeon.

3. Diagnostic approach to acute infections

3.1. Bloodstream infections at the emergency department

Community onset bloodstream infections (BSIs) are associated with high morbidity and mortality [63]. Sepsis and septic shock require appropriate management in the initial hours after onset, preferably directly at the emergency department (ED) [64,65]. About 80% of sepsis is the result of one of the following infections: pneumonia, intra-abdominal infections, infections of the genitourinary tract, skin and soft tissue infection and primary BSI [66]. The rational use of new diagnostic techniques may lead to a definitive switch from empiricism to diagnostic-guided antibiotic therapy in the setting of sepsis and septic shock. The first step for an appropriate diagnostic approach to patients presenting at the ED with suspected BSIs is the accurate identification of pathogens [67]. The gold standard for BSIs etiologic diagnosis is represented by the blood cultures followed by conventional pathogen identification and antimicrobial susceptibility testing. Unfortunately, even accelerating and tightly monitoring the pre-analytic and analytic phases [68], blood cultures turnaround-time is often long. In the last decades, the average reporting time of blood cultures results has been significantly shortened by the introduction of blood cultures automated monitoring systems and MALDI-TOF-based microbial identification [69,70]. Together with these two technologies, several other mRDT are currently revolutionizing clinical microbiology. The adoption of mRDT for BSIs diagnosis, coupled with an antimicrobial stewardship program, represents a clear advantage in terms of reduction of mortality risk, decrease in the time to effective therapy and the length of stay [71]. As shown in Table 1, among mRDT, those that have demonstrated a greater impact in terms of improved clinical outcome in patients with BSI are:

- multiplex PCR panels for pathogen identification from positive blood culture bottles [72];
- gene-based detection of antimicrobial resistance determinants [73–75];
- direct detection of *Candida* spp. and bacteria from blood by T2 magnetic resonance [76].

A careful introduction into the laboratory workflow of these techniques is recommended and a diagnostic stewardship approach is needed to appropriately implement these mRTD [77]. A workflow for diagnosis and management of BSIs originating from intra-abdominal or skin and soft tissues infections (but potentially applicable to all other sources) incorporating the use of mRDTs is shown in Figure 3. It is particularly useful in settings with high prevalence and incidence of MDRO infections. Septic patients at higher risk of infection by MDRO, especially carbapenemase-producing *Enterobacterales* (CPE), vancomycin-resistant *Enterococcus* (VRE) and/or methicillin-resistant *S. aureus* (MRSA), should be promptly identified. The majority of BSIs caused by MDRO occurs in previously colonized patients [78,79]. For this reason, as first step, a screening by mRDTs for intestinal colonization by VRE and/or CPE and nasal colonization by MRSA may provide relevant information (Figure 3) [80–82]. Following this first phase we introduced a second evaluation step, aimed to exclude the presence of a fungemia. It is important considering that antifungal therapy is not automatically recommended as first line empiric regimens in patients with BSIs presenting at ED. Patients with risk factors for candidemia should be subjected to T2 RM, where available, or to Beta-D-glucan as alternative (Figure 3) [83,84]. After this evaluation, the workflow differentiates depending on information derived from Gram-stain of positive blood-culture broth. For polymicrobial infections, we

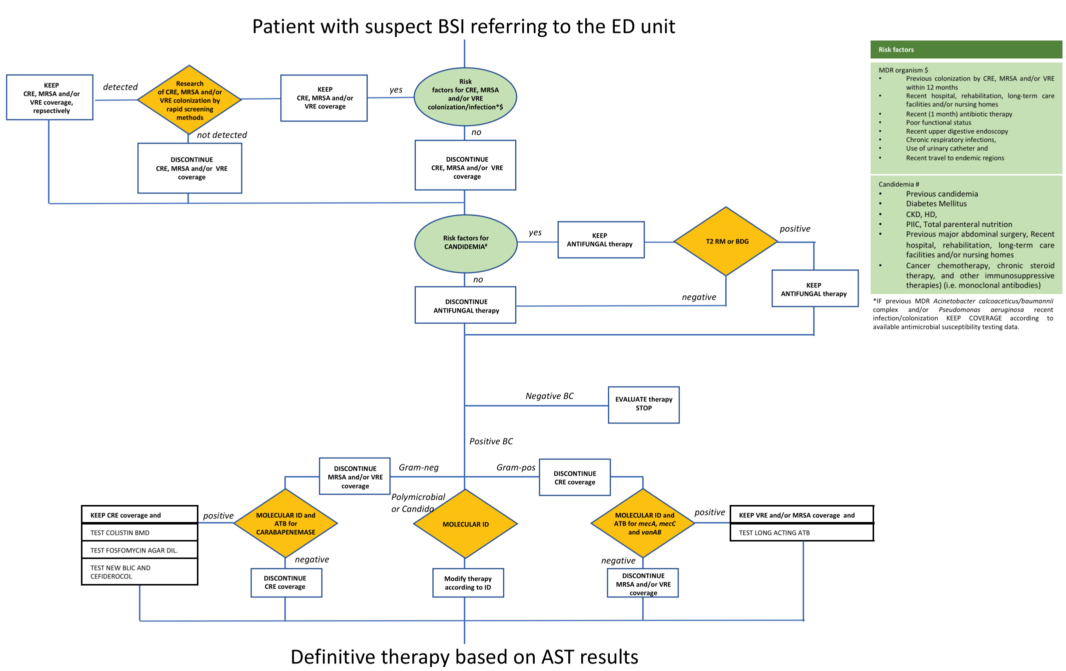

Figure 3. Diagnostic algorithm in patients with bloodstream infections presenting at emergency department.

AGAR DIL.: agar-dilution method; ATB: antibiotic; BC, blood cultures; BDG, Beta-D-glucan; BLIC, β-lactam inhibitor combination; BMD, broth micro-dilution; BSI: bloodstream infection; CRE, carbapenem-resistant *Enterobacterales*; CKD, Chronic kidney disease; ED, Emergency department; HD, hemodialysis, ID, identification; MRSA, methicillin-resistant *Staphylococcus aureus*; T2 RM, T2 magnetic resonance; VRE, vancomycin-resistant *Enterococcus* spp.Legend: in orange key-points for introduction of new microbiological assays and in green risk factors evaluation steps.

suggest the use of a syndromic panel for the identification of pathogens responsible for BSIs [85].

In the case of Gram-positive identification, we support the use of a rapid identification method able to differentiate *S. aureus* from CoNS and *Enterococcus* spp and the direct detection of *mecA, mecC* and/or *vanA/B* genes. The presence of one or more of these resistance genes should trigger the implementation of susceptibility testing for long-acting antibiotics (especially for BSIs originating from skin and soft tissue infections). By contrast, the observation of Gram-negative bacilli at the Gram-stain should be followed by rapid molecular identification and detection of carbapenemase genes from positive BC broth (at least KPC-type, VIM-type, OXA-48-like and NDM-type). In this case, mRTDs work as companion test for the correct place in therapy of new antibiotics (ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam and imipenem-relebactam) [86].

At the end of the workflow, the therapy must be optimized depending on the results of conventional antimicrobial susceptibility testing. A continuous collaboration between the laboratory and clinicians may greatly impact on therapeutic appropriateness and, of consequence, on patient outcome. Ideally, infectious disease physicians and pharmacologists should work alongside microbiologists within a diagnostic management team that assists clinicians with the interpretation of complex test results.

3.2. Community-acquired pneumonia

Community-acquired pneumonia (CAP) is a leading infectious cause of hospitalization with an estimated incidence of 2–11 cases per 1000 adults in the developed world and a mortality rate of 2–14% [87–89]. Detection of bacterial pathogens responsible for CAP is not usually achieved [90] and antibiotic therapy is commonly selected based on epidemiological data and host risk factors. *Streptococcus pneumoniae, Haemophilus influenzae*, and *Legionella* spp represent major causes of CAP. *S. aureus* causes approximately 2to 8% of hospitalized CAP, but most studies highlighted that MRSA is becoming an emerging cause of CAP. Identifying patients with CAP at high risk of MRSA etiology is crucial, because MRSA-induced CAP is associated with high mortality rates [91]. Over the past decades, organisms traditionally associated with the healthcare setting, such as *Pseudomonas aeruginosa*, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacterales* and MRSA (so-called PES pathogens [92]), have emerged as causes of CAP [93]. The spread of microbial resistance has been identified as a priority for the World Health Organization. Several scoring systems have been validated to help clinicians to select patients with CAP at high risk of resistant etiology [94]. Since patients with severe pneumonia have a higher risk of resistant etiology [95], clinical presentation is one of the items of the risk scores and should be considered to guide the choice of antibiotic therapy.

The SARS-CoV-2 pandemic has further complicated the approach to patients with CAP [96,97]. Reshaping of diagnostic strategies in patients with CAP presenting at the ED is needed in the pandemic context, because the rapid discrimination between viral (SARS-CoV-*2* or other respiratory viruses) and bacterial etiology may be challenging based on clinical and laboratory findings. Molecular techniques based on multiplex real-time PCR are useful to simultaneously identify and quantify multiple respiratory pathogens from different types of samples (Table 1). Nasopharynx swabs are now routinely performed directly in the ED to exclude the presence of *SARS-CoV-2*. This increased use may be useful to identify other respiratory pathogens such as in the so-called syndromic panels. However, the use of PCR assays for the detection of resistant bacterial pathogens is more challenging. Some issues, such as costs, risk of false-negative results or the ability to detect genotypic markers which phenotypically do not show clinically significant resistance, may limit their widespread use [98]. The use of molecular techniques should be implemented because they may improve the ability to detect organisms responsible for CAP more precisely and rapidly.

Combining clinical judgment and currently available molecular instruments, an algorithm for the etiological diagnosis of CAP in the context of Covid-19 pandemics is shown in Figure 4. As shown, after the exclusion of SARS-CoV-2 infection, in patients with severe CAP urinary antigen test for *Legionella* or *S. pneumoniae* should be performed, because this determination may guide antibiotic therapy [99]. In patients with negative urinary antigen test for *Legionella* or *S. pneumoniae* and risk factors for MDRO, rapid multiplex PCR on respiratory specimens for microbial identification and resistance markers should include both carbapenemases and *mecA/C* detection, that may guide the choice of antimicrobial therapy. In patients with non-severe CAP the presence of risk factors for MDRO and the need for hospitalization should be considered.

4. Expert opinion

Developing diagnostic algorithms that combine old and new diagnostic tools may help clinicians in the prompt diagnosis of some challenging clinical syndromes and improve patient outcome. It has particular importance since reducing the spread of MDRO represents one of the priorities worldwide [100]. New mechanisms of resistance are continuously emerging, leading to delayed diagnosis and worse patient outcome [101]. This paper has the following goals:

- to provide a practical guide for the diagnosis of common acute and chronic syndromes, that may represent a challenge for clinicians;

- to highlight the importance of new rapid diagnostic techniques, that may facilitate the diagnostic process of common infectious disease and allow a rapid identification of causative pathogens;

- to promote evidence-based utilization of diagnostic tests and favor their rational use;

- to highlight the role of new diagnostic tools in facilitating the targeted antibiotic therapy and reducing duration of unnecessary antibiotic use.

The implementation of diagnostic tools may impact on real-life clinical practice, supporting the diagnosis and increasing the probability of clinical success. Advances in the field of diagnostic stewardship may lead to more accurate diagnosis

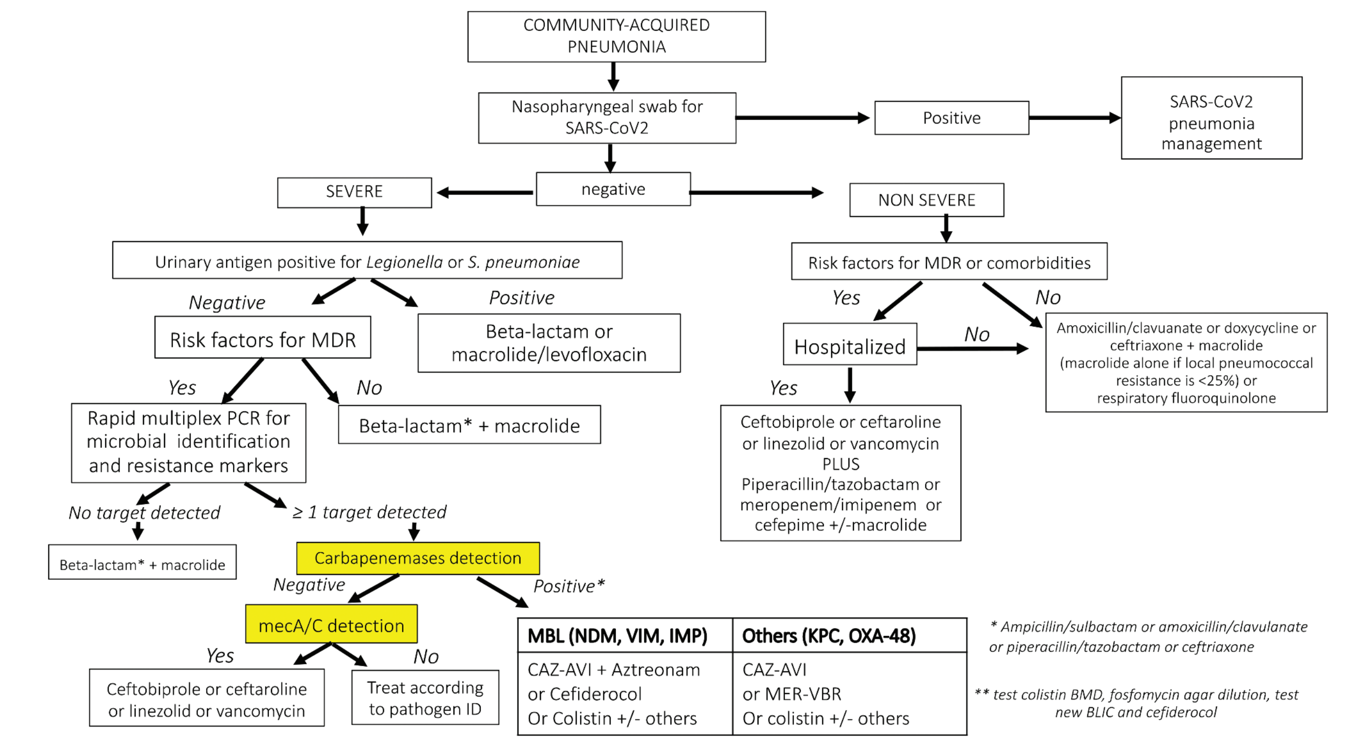

Figure 4. Diagnostic algorithm in patients with community-acquired pneumonia.
MBL: metallo-beta-lactamases; MDR: multidrug resistance

and should be realistically implemented into clinical practice [102]. Implementing new diagnostic tools is not free from challenges: specialized laboratories, technical expertise, technological systems are required. However, they may greatly impact on patient management and shorten the length of treatment, reducing costs and unnecessary antibiotic use. In this position paper, we focus on specific clinical syndromes since physicians may have difficulty in their accurate diagnosis and management. Among chronic infections, chronic bone infections, PJIs, and chronic/subacute intravascular infections are chosen, while among acute infections BSIs and CAP are selected. The proposed algorithms only work as guide that may support physicians, but the integration of new diagnostics in well-defined diagnostic process may be implemented in all type of infectious disease. It appears evident that, in all proposed clinical scenarios, a systematic and well-structured diagnostic approach, combining old and new tools, should be adopted in clinical practice. The implementation of new rapid diagnostic techniques and specialized structures able to perform and interpret them may facilitate the diagnostic process and impact on patient outcome.

Conventional diagnostic tools may be useful as a first step, but in specific contexts mRDTs may help physicians to obtain an etiological diagnosis. These techniques may have pros and cons and should be critically used in each clinical syndrome. In the setting of PJIs, the role of molecular diagnosis in patients with aseptic loosening is uncertain because detected bacterial DNA may not be indicative of infection. Thus, mRDT may be useful in case of high clinical suspicion of PJI, based on clinical, instrumental and standard perioperative workup, and negative culture results from standard techniques. Moreover, it has been demonstrated that the rational use of mRDT reduced the proportion of BCNE. However, these assays should be included in a global diagnostic strategy involving other methods such as serology, broad range PCR, and valve culture. Finally, in the setting of acute syndromes, the role of mRDT may have several advantages: 1) the knowledge of rectal colonization status may allow the early start of an empirical therapy targeting the colonizing organism in rectal carriers with septic syndrome and reducing the time to appropriate therapy; 2) a prompt de-escalation in case of not confirmed infection due to the MDR colonizing bacteria may be applied as soon as microbiological results are available; 3) the knowledge of rectal colonization status is crucial for implementing infection control measures.

Of importance, new techniques, such as metagenomic next-generation sequencing (mNGS) of plasma cell-free DNA, emerged as attractive diagnostic tools allowing broad-range pathogen detection, noninvasive sampling, and earlier diagnosis [103,104]. The Karius test is a commercially available mNGS for the diagnosis of BSIs. However, its value as a diagnostic tool is debated and may be limited by low sensitivity compared to conventional methods. mNGS may be useful if all other routine tests have failed to yield a diagnosis and in the case of high pretest probability. The complementary role of mNGS to conventional microbiological methods and the integration of mNGS into current testing algorithms need further studies and implemented methodology. Future studies should address the efficacy and the cost-effectiveness of new diagnostic tools in context with high prevalence of MDRO and in patients with difficult-to-manage infections.

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