Biomarkers of infection – user's guide at the bedside

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Abstract (N=257)

Severe infections and sepsis are a major public health problem worldwide with high morbidity and mortality. The definite and timely diagnosis of these infections remains difficult and frequently elusive since there is no gold standard diagnostic test. Delayed diagnosis is coupled with delayed antibiotic therapy that is associated with worse prognosis. The fear of missing an infection due to the lack of an accurate diagnostic test remains one of the main reasons for unnecessary antibiotic overuse, which is associated with increased risk of side-effects, pressure for the development of multi-drug resistance, and *Clostridium difficile* colitis. With all these problems and limitations, researchers and clinicians have tried to use biomarkers as surrogate markers of infection. More than 250 biomarkers have been identified and evaluated in the last decades, but unfortunately the "perfect" biomarker is yet to be found. The aim of this review is to summarize the available evidence, describe the newly discovered strategies (omics), and discuss the current evidence regarding the clinical utility of biomarkers in the Intensive Care Unit. Published data support the use of biomarkers for pathogen identification, clinical diagnosis, and optimization of antibiotic treatment. Biomarker kinetics are more useful than single values in the prediction of infection, diagnosis, and in the assessment of response to antibiotic therapy. Last, integrated biomarker-guided algorithms may hold promise to improve both diagnosis and prognosis of severe infectious diseases. This narrative review aims to provide information on the clinical usefulness of current biomarkers, offer a guidance on how to optimize their use, and propose the needs for future research.

Keywords – infection, sepsis, intensive care unit, biomarkers, diagnosis, antibiotic stewardship

Take home message

This narrative review presents the available evidence, the strengths and limitations of biomarkers of infection in the clinical decision-making process at the bedside in ICU, namely in prediction of infection, its diagnosis, in the assessment of response to antibiotic therapy, and integrated biomarker-guided algorithms in antibiotic stewardship

1. Introduction

Worldwide sepsis incidence and mortality seem to be decreasing over the last decade. However, sepsis remains a major public health problem, with an estimated incidence around 50 million in 2017, and 1 in 5 deaths was attributed to sepsis [1]. Unfortunately, the definite and timely diagnosis of sepsis is difficult and frequently elusive, since there is no gold standard diagnostic test that alone could be used to "rule-in" or "rule-out" sepsis, nor a mechanistic aspect that is pathognomonic of sepsis [2].

The diagnosis of sepsis results from the intersection of three vectors: systemic manifestations, organ dysfunction, and microbiological etiology. But the last one takes at least 2-3 days to have the results and in roughly half of the cases the microbiology results negative.

The current Surviving Sepsis Campaign (SSC) guidelines recommend starting antibiotic within one hour in patients in septic shock and within three hours in those in sepsis since a delay in prescribing antibiotics is associated with poor outcomes [3]. To achieve that goal, we need reliable definitions of sepsis to both treat true-positives, and prevent false-negatives, since delayed therapy is associated with worse prognosis. Another needed property for a sepsis definition is to prevent false-positive results, which lead to an inappropriate antibiotic use and overuse, additional drug side-effects, development of multi-drug resistance, and higher rate of *Clostridium difficile* colitis, all associated with worsening outcomes. Additionally, with false-positive results the underlying condition that is mimicking sepsis may not be adequately treated and further worsen patient outcomes [4].

Currently, in our daily clinical practice at the bedside we use a combination of data, frequently sequential data, from clinical evaluation, standard laboratory values, radiology, and "old" biomarkers to help us in the management of sepsis and antibiotic stewardship. Yet, we know that these tools are very sensitive and poorly specific. All these problems and limitations lead researchers and clinicians to use molecular tests, in particular, biomarkers, as surrogate markers of infection [5]. More than 250 biomarkers have been studied and evaluated in the last decades, but the "perfect" biomarker is yet to be found [6-8]. The aim of this review is to provide information to clinicians about the clinical usefulness of current biomarkers as well as guidance on how to optimize their use.

2. What are the currently available biomarkers?

Although detection of microbial nucleic acids is becoming more common, their place in the management of infections in general and in specific bacterial infections remains uncertain and has been recently reviewed elsewhere [9]. Direct antigen tests, however, are in widespread use amongst critically ill patients. The pooled diagnostic performance of the major tests is shown in table 1.

Most rapid antigen-based tests are based on immunochromatographic assays and have the potential for bedside use. Influenza or SARS-CoV-2 respiratory antigen tests and *Streptococcus pneumoniae* and *Legionella* spp. urinary antigen tests are used in severe community-acquired pneumonia (CAP). They exhibit a high specificity, but moderate sensitivity. Therefore, notwithstanding improvements through automated reading, a negative test cannot be reliably considered a rule-out result [10]. Legionella antigen tests detect *Legionella pneumophila* serogroup 1. Whilst this is the predominant cause of legionellosis, false negatives occur when other serogroups or species are involved [10]. The diagnostic accuracy of pneumococcal antigen tests is also highly dependent on serotype, and lower sensitivity has been noted due to antigenic shift following the introduction of the 13-valent polysaccharide conjugate vaccine. Currently, few rapid diagnostic tests, such as the mariPOC® test, utilize multiplex testing for several pathogens in a single sample.

Clostridium difficile infection (CDI) can be diagnosed in patients with compatible symptoms, using a two-step algorithm, using stool-based rapid enzyme immunoassays detecting glutamate dehydrogenase (GDH) and free toxins A and B. Low positive predictive values at low CDI prevalence prevents either test of being used alone [11]. The first test is highly sensitive, and if positive, is combined with the more specific toxin A/B detection test. Careful evaluation of patients with positive GDH but a negative toxin A/B detection is needed, as it may indicate CDI with toxin levels below the detection threshold in patients with diarrhea, or non-toxigenic *Clostridium difficile* carriage.

Fungal antigens are structural polysaccharides derived from fungal cell walls. (1,3)-β-D-glucan (BDG) is a panfungal serum biomarker commonly used to detect invasive candidiasis. With a high sensitivity, but poor specificity, BDG is a valuable tool to rule-out the diagnosis in Intensive Care Unit (ICU) settings with a low prevalence of

invasive candidiasis [12]. However, a recent randomised clinical trial (RCT) failed to demonstrate benefits on survival of BDG-guidance for early initiation of antifungal therapy in septic critically ill patients with a low to intermediate risk for invasive candidiasis, at the cost of a substantial overuse of antifungals [13]. Similarly, BDG has a high negative predictive value for the diagnosis of *Pneumocystis jirovecii* pneumonia among patients without HIV, and a low/intermediate likelihood of the disease [14]. Specificity and positive predictive value can be increased by repeating the test and/or increasing the cut-off value. Galactomannan (GM) can be measured in serum and broncho-alveolar lavage (BAL), and shows high specificity for the diagnosis of invasive pulmonary aspergillosis (IPA). BAL GM is more sensitive than serum GM in diagnosing IPA in non-neutropenic patients, and plays a central role in diagnostic criteria for IPA amongst the critically ill [15]. Rapid, bedside *Aspergillus* lateral-flow device tests for BAL samples have been developed and research is ongoing (Trial ISRCTN 43895480). Cryptococcal antigen detection in serum is highly predictive of cryptococcal meningitis in HIV-patients with central nervous system symptoms and can rule-out the diagnosis when negative [16].

Blood based biomarkers lack specificity for specific sites of infection, and do not indicate the causative organisms. Markers include the widely used C-reactive protein (CRP), an interleukin (IL) 6 dependent, hepatically produced acute phase protein. Whilst CRP elevation is not specific to infection, rapidly rising or persistently elevated levels are highly predictive of subsequent diagnosis of infection amongst ICU patients [17]. Notable false negatives can occur in the context of prior anti-IL6 therapy, such as tocilizumab, and in severe acute hepatic failure. Trajectories of CRP may be helpful not only in predicting and diagnosing infection but also in limiting antibiotic duration. Two small ICU-based RCT suggest CRP may be able to achieve this safely [18, 19], and similar results from a large RCT in bacteraemic patients although in this latter study critically unwell patients were excluded [20]. A large trial of this strategy in ICU is ongoing (ISRCTN47473244).

Procalcitonin (PCT) is notable for its apparent greater specificity for bacterial infection. However, several non-infectious processes such as pancreatitis, major surgery and burns can induce significant elevation. Procalcitonin lacks sensitivity early in the course of infection and cannot reliably distinguish viral from bacterial infections at that

point [21], therefore its major role is in shortening the duration of antibiotics rather than in their initiation [22].

By contrast, organ specific markers may help indicate site of infection. Alveolar cytokine and inflammatory marker levels have been investigated for diagnosis of pneumonia [23], and whilst these tests may be highly sensitive, their low specificity and lack of standard prescribing habits suggests that antimicrobial use was not affected by that [23]. In the urinary tract, urinary leucocyte levels and bacterially produced nitrites are commonly used to screen for infection, but these are of limited use in catheterised patients in ICU as the catheter itself induces pyuria [24]. The use urinary cytokines to aid diagnosis of CA-UTI remains experimental [24].

3. How to use biomarkers?

Faced with a suspicion of infection the clinician has several questions to address: 1) does the patient actually have an infection? 2) what is the most likely pathogen? 3) what is the predicted severity – will the infection evolve into sepsis? and 4) what, if any, antimicrobial treatment is appropriate? A subsequent requirement is to gauge the response to treatment; is the patient improving or not, and if not, why not? We frequently try to answer these questions with biomarkers, but a single biomarker cannot answer all these questions [5]. Encouragingly, the field is rapidly expanding with novel molecular or light-based technologies that will offer faster results, though this expanded choice will generate new challenges.

Biomarkers for infection/sepsis can be divided into prognostic, predictive and theranostic (Figure 1). The clinical utility of a *prognostic biomarker* is limited. Other than identifying patients with an increased risk of death, it provides no information as to whether a therapeutic intervention will modify the prognosis. Such biomarkers may however be useful for trial enrichment, restricting enrolment to high-risk patients. *Predictive biomarkers* are more useful in clinical practice but must possess certain characteristics to aid decision-making: 1) be absent or present at very low values in non-infected patients, 2) markedly increase before or concurrent with clinical manifestations of infection, 3) respond quickly to effective antimicrobial therapy, or remain elevated if not. *Theranostic biomarkers* indicate which therapy will suit a particular patient and enable dose titration to optimal response. They are well established in oncology, e.g.

transtuzumab for those with HER2-positive breast cancer, and will definitely be needed in critical care. Septic patients have different biological signatures, even to the same infection, and respond differently to the same host response modulating treatment [25].

Information obtained from biomarker kinetics are more sensitive and specific than single measurements, either in forming a diagnosis of infection or in evaluating the response to treatment [17]. Similarly, combination panels or transcriptomic analyses may enhance differentiation between infectious and non-infectious systemic conditions, enabling better antimicrobial stewardship [26].

4. Biomarkers in the prediction of infection

Biomarkers to Predict Sepsis

The usual dilemma in the ICU is to underuse or overuse antibiotics. Biomarkers kinetics may help guide predict nosocomial sepsis (Figure 2) [4, 5, 27]. Povoa et al, demonstrated that CRP kinetics (days before ICU-acquired infection) accurately predicted its diagnosis when a maximum daily CRP increase above 4.1mg/dL (associated with a concentration above 8.7mg/dL) was present [17]. A similar finding was observed in a large study of community-acquired bloodstream infections (BSI). Garvik et al observed that CRP concentrations increased in the 3 days preceding a definitive diagnosis of BSI [28].

Regarding PCT, recent meta-analyses and systematic reviews showed that PCT-guided algorithms were associated with reductions of antibiotic exposure, however with no impact on mortality and a low-certainty evidence with a high risk of bias for critically ill patients [29-32]. However, individual studies of PCT kinetics in the ICU showed poor diagnostic accuracy and a low impact regarding guidance for initiation of therapy [30]. Thus, although associated with decreased antibiotic exposure in selected settings, the usefulness of PCT to predict infection in the ICU is limited.

Finally, regarding novel biomarkers, pancreatic stone protein, a C-type lectin protein has been evaluated and in preliminary studies it was better than PCT for an early and accurate identification of infection and sepsis [33-36].

Biomarkers to predict pneumonia

In patients with CAP, PCT levels vary during the course of illness, with levels higher in those presenting within 3 days from symptom onset and lower thereafter [37].

This period of time has to be taken into account in CAP and there is evidence from preliminary studies in documented influenza cases, where PCT levels could present a higher negative predictive value and may help rule-out bacterial coinfections [38], although these findings have not been replicated by others [39, 40]. As a result, more studies are needed before a wider use of this strategy, especially in other viral (non-Influenza) infections.

In addition, PCT measurements may be inaccurate in renal failure, as low glomerular filtration can falsely elevate PCT levels. Moreover, certain dialysis membranes can remove PCT, which can lead to falsely low measurements. Considering these caveats, both PCT measurements and clinical judgement should be included in the initial management of CAP, including severe CAP [41].

CRP kinetics has been shown to be associated with response to antibiotic treatment. Although it is less expensive, a paucity of large studies performed in pneumonia preclude CRP use for withholding antibiotics or shorten antibiotic duration. However, it has been successfully used to stratify patients in RCT to search for an inflammatory phenotype [42].

PCT is the most studied biomarker in the setting of ventilator-associated pneumonia (VAP). The lack of utility of PCT measurements, either single or serial measurement, in VAP diagnosis has been shown in several observational studies. Both the 2016 IDSA/ATS guidelines [43] and the 2017 ERS/ESICM/ESCMID/ALAT guidelines [44] do not recommend the use of any biomarker for the diagnosis of VAP. However, measuring the delta variations of CRP over time, some groups have found a good evolution prediction [44]. The BioVAP multicenter study investigated the kinetics of biomarkers to predict VAP and found that CRP and CRP slope over time were good predictors of VAP occurrence. This finding was not shown with PCT and proadrenomedullin [45]. Finally, the soluble urokinase plasminogen receptor was also investigated in the same cohort and although its levels were elevated 3 days before VAP, its predictive value was moderate [46].

5. Biomarkers in the diagnosis of infection

Single biomarker measurements at time of presentation.

White blood cell count

The measurement of WBCs in circulating blood is standard procedure in the workup of a patient in the emergency department or the ICU who is suspected of being infected. And although leukopenia (<4x10^9/L) compared to leukocytosis (>12x10^9/L) is a more specific marker of infection (96% vs 59%), neither finding can be used to either diagnose or exclude infection [47]. The utility of WBC count analysis is improved, however, if >10% bands are present along with leukocytosis thereby increasing the post-test probability of infection (+likelihood ration [LR] 8.99) [47]. Unfortunately, when the band count is <10%, infection cannot be ruled out (-LR 0.62).

C-reactive protein

Serum CRP levels rise within 4-6 hours after onset of inflammation with a half-life of approximately 19 hours [48]. Interestingly, among infected patients, CRP levels are not significantly different between individuals with and without cirrhosis [49]. The use of a single CRP measure to diagnose infection on clinical presentation has not been consistently demonstrated by two meta-analyses (one analysis of adult only trials and the other analysis performed with trials involving both pediatric and adult patient populations) noting a sensitivity and specificity range from 78%-80% and 60%-61% respectively [50, 51]. The variable accuracy of CRP in clinical studies is also impacted by the use of different cutoff points typically ranging between 2 to 10 mg/dL [52].

Procalcitonin

There is no agreed upon PCT cutoff value for infection diagnosis as published studies have either not reported the cutoff value used or used values ranging from 0.5 ug/L to 2.0 ug/L [53]. Numerous noninfectious inflammatory states have been identified that are associated with elevated PCT serum levels: abdominal surgery, burn injury, methamphetamine toxicity, pancreatitis, trauma and kidney and liver failure due to impaired clearance [54]. When patients with these conditions are excluded, three separate meta-analyses of PCT for the diagnosis of sepsis revealed a sensitivity and specificity range of 77-85% and 75-83% respectively [50, 55, 56]. Caution should be exercised when evaluating these results as there was substantial heterogeneity (I^2 >80%) identified among the pooled estimates of sensitivity and specificity which could not be fully explained by sensitivity analysis (including variability of cutoff test values) [55, 56].

And while PCT may be superior to CRP for diagnosing a *de novo* infection, a negative test cannot be used as sole justification for withholding antibiotic treatment.

Biomarker panels and biomarker-based algorithms to diagnose infection

The combination of various biomarkers to construct a diagnostic panel has not been shown to be consistently superior to any individual biomarker for the purpose of diagnosing an infection [57]. This is a consequence from the fact that sepsis biomarkers are usually highly correlated, and their combination do not improve the diagnostic accuracy. The way biomarkers are combined is also important. If it is needed at least one positive to have a positive result, it increases the sensitivity and decreases specificity. If both must be both positive to have a positive result, it increases the specificity at the expense of sensitivity. However, algorithms that combine biomarkers with clinical data have shown promise for identifying patients with sepsis in the emergency room. An algorithm combining clinical variables and a panel of biomarkers was recently developed and validated in a cohort of 158 patients (negative predictive value of 100% and positive predictive value of 93%) [58]. Two limitations of this study should be noted. Firstly, all study participants had at least two systemic inflammatory response syndrome (SIRS) criteria; thus, how this algorithm would perform in SIRS-negative sepsis is not known. Secondly, the speed with which the results of this algorithm can be finalized is not described.

6. Biomarkers in the assessment of infection response to therapy

After diagnosis of infection and prescription of antibiotics, the assessment of clinical response is based on either clinical or microbiological criteria, mostly the same used in diagnosis. Usually from 48 to 72h after the start of antibiotic therapy, several questions arise concerning the patient's clinical course monitoring: 1) Is the treatment being effective? 2) If the clinical course is not the expected one, is it due to a complication of the initial infection (e.g., empyema, pulmonary abscess), to a new infection (in the same or in another focus), or to inadequate initial antibiotic therapy? 3) Or due to another diagnosis unrelated to infection?

A biomarker useful in the assessment of infection response to antibiotic therapy should return to baseline levels with successful therapy or remain elevated if infection

is refractory to treatment [59]. Also, to evaluate the clinical course, the biomarker should exhibit large amplitude of variation and have a non 'exhaustion' or 'fatigue' behavior in prolonged infection episodes [60].

CRP has been extensively studied in monitoring of clinical response in several infections, namely VAP, BSI, CAP, showing that the course CRP after prescription of antibiotics correlates with clinical course and prognosis [61-63]. It is possible to monitor absolute CRP changes over time, but the use of relative CRP variations (CRP-ratio) has shown to be more informative. CRP-ratio is defined has the ratio of each day CRP concentration in relation to day 0 (D0) level to assess the dynamic changes of CRP instead of its absolute values. A sharp decrease in CRP-ratio is a surrogate marker of infection resolution whereas a persistently elevated or an increasing CRP-ratio suggests that infection is refractory to therapy. In patients with microbiological documented VAP, Póvoa et al demonstrated that, at D4, a CRP >60% of the initial value was a marker of poor outcome [63]. Similar results were observed in severe CAP [64]. Moreno et al, in patients with nosocomial pneumonia, found that daily measurements of CRP were useful, as early as D4, in the identification of patients with poor outcome and in detecting patients with inappropriate antimicrobial therapy [65].

Using the concept of CRP-ratio, four individual patterns of CRP-ratio response to antibiotic therapy were defined [63]. The first, a fast response pattern, consists of a rapid decline of CRP-ratio to <0.4 by D4. The second, slow response pattern, is a continuous decline of CRP-ratio, being its value, by D4, <0.8 (but >0.4). The third is a nonresponse pattern, which is defined by a CRP-ratio course persistently >0.8 (and sometimes even increasing), and the last, a biphasic response pattern, characterized by an initial drop of the CRP-ratio <0.8, followed by a secondary rise, to a value above that threshold. In severe CAP, VAP and BSI, patients with the patterns of fast and slow response had significantly lower mortality than patients with a nonresponse and biphasic patterns [61-63, 66]. In VAP patients, PCT measured at onset and on D4 of treatment was able to predict survival, differentiating patients with good and bad outcome [67]. Persistent high levels of PCT at D4 of antibiotic therapy were indicative of failure of infection control [68].

In clinical practice, patients who present persistent elevated levels of biomarkers by D3/D4 after initiation of antibiotic therapy should raise suspicion of treatment failure

and should prompt an aggressive diagnostic and therapeutic approach, with source control efforts (e.g. removing central lines, debriding necrotic tissues) and performance of a full diagnostic approach (consider repeating microbiological cultures, performing ultrasound or CT scan) and bearing in mind that the patient could have a non-infectious disease process. However, we should be careful in using biomarkers as a stand-alone criterion to decide when to escalate diagnostic and treatment interventions since this could increase morbidity [69].

7. Biomarker-guided antimicrobial stewardship

While fixed antibiotic durations provide clinicians with clear and simple guard rails, they do not, by definition, consider the vast differences in pathogen and host characteristics, their interactions and responses to therapy. Because of these limitations, and because of a relative abundance of antibiotic options in earlier decades, classic fixed durations tended to play it safe for the individual patient, generally overshooting the mark defining the minimum duration needed to protect from clinical relapse. The double hit of a drying antibiotic pipeline with rapid spread of antibiotic-resistance genes on a global level spurred an intense reassessment of the utilization of fixed antibiotic durations that is still ongoing.

While one strategy is simply to convert fixed long durations to fixed short ones, an increasingly popular approach is to use biomarkers to personalize antibiotic treatment duration. This approach includes the individual patient's response to therapy, matching the antibiotic discontinuation to the patient's actual clinical recovery. While it was initially unknown whether such an approach would be antibiotic-sparing, increasing evidence confirms there is reduction in overall antibiotic use, as with CRP [20, 70]. Another classic biomarker is PCT, which has been studied in more than 20 RCT to guide durations of antibiotic therapy in severe infections [56, 70]. Observational and randomized studies have demonstrated no substantial differences in the ability of these two biomarkers to reflect improvement (or worsening) in the clinical course of severe infections [18, 71]. Indeed, a RCT comparing the two markers head-to-head for guiding antibiotic therapy duration in sepsis found that CRP was non-inferior to PCT for duration guidance, with no difference in morbidity or mortality observed [18].

How to integrate biomarker guidance for antibiotic therapy with a clinical course and fixed duration?

The first trials conducted to investigate the usefulness of biomarkers to guide antibiotic durations enrolled in their control groups patients treated according to standard practices for bacterial infections [70, 72, 73]. Given the lack of evidence for treatment durations for most infection types, these previous control groups ultimately received what would today be considered excessively long therapies, resulting in a potentially biased conclusion that a biomarker-based strategy is associated with reduced antibiotic exposure [8, 32]. To overcome these limitations, more recent trials have used shorter, fixed control durations based on new data [74-76]. For instance, in a single-center RCT with 130 patients admitted to a tertiary-care hospital's ICU in Brazil, Borges *et al*. compared a CRP-based protocol of antibiotic therapy with a pre-specified maximum duration of 5 to 14 days treatment, depending on the site of infection [19]. In addition, to avoid overuse of antibiotics in patients without an expected drop of CRP levels despite clinical improvement, they set a ceiling of 7 days for the length of therapy in the intervention group. In the per-protocol analysis, the biomarker-guided strategy led to shorter antibiotic durations (6.0 [5.0-8.0] days *vs*. 7.0 [7.0-10.0]; p=0.011), with no difference in relapse and hospital mortality. In the same year, von Dach et al published the results of a noninferiority RCT carried out in three tertiary Swiss hospitals [20]. In this trial of 504 patients with uncomplicated gram-negative bacteremia, a CRP-guided strategy was tested against two fixed antibiotic durations (7 or 14 days). Clinical failure rates at D30 did not exceed the non-inferiority margin of 10% (2,4%, 6,6%, 5,5%, respectively), and CRP guidance proved to be antibiotic-sparing (median duration 7 days). Altogether, these two trials suggest that a biomarker-based strategy of antibiotic therapy is safe and can add to consolidated practices of rational antibiotic use (Figure 3) [77].

8. The future of biomarkers

Despite thousands of publications, the only biomarkers that have become routinely useful in clinical care in patients with infections are those we have been using for decades, that is, the WBC count, CRP, and PCT. In outlining future directions for

biomarker research in sepsis, it is important to understand why research to date has failed to translate into more clinically useful tools.

At least three common problems exist in existing biomarker research. The first is the repetition of studies focused on evaluating if biomarker x correlates with outcome y, and comparing if the studied marker is better than some other one. While scientifically valid, this approach has little clinical utility. To be useful a biomarker must inform a clinician something they don't already know which leads to a beneficial change in the therapeutic approach. One example of the types of studies we need, albeit a negative result, was the trial to assess whether PCT guided to initiate/escalate antibiotic therapy improved outcomes in patients in ICU [69].

The second problem is the failure to define clinically meaningful endotypes by considering all patients with the same disease as part of a single group. For example, it is usual for studies to evaluate "CAP", despite there clearly being significant differences between viral and bacterial pneumonia, rapidly progressive versus slowly progressive disease, or patients with and without chronic organ failure etc.

The third and final problem is the piecemeal approach, studying one biomarker at a time. While this may be useful for feasibility and publication, the inability to compare the performance of putative biomarkers, and the interaction between different biomarkers is a major barrier to progress. Now omics platforms allowed us to study large sets of genomic, transcriptomic, proteomic and metabolomic biomarkers (Table 2) increasing our ability to study at the same sample multiple concomitant biomarkers.

Given the complexity of the above-described interactions (such as multiple different treatment approaches, multiple endotyping, thousands of biomarkers) machine-learning approaches and bioinformatic tools (Table 2) across large cohorts of patients would be crucial to the optimal exploration of these data [78]. Several of these problems that we face with sepsis were partially solved secondary to the intense research done in COVID-19 [79].

In summary, to move forward from where we are with biomarkers of infection to a point where we have clinically useful markers driving patient treatment pathways to improve outcomes will require a significant change in approach. Single center,

unidimensional studies will unlikely bring much progress. Large multi-center cohort studies, utilizing state of the art omics, bioinformatic and machine learning algorithms to identify biomarkers that predict differential response to interventions in specific clinical endotypes is what is needed. The combination of the tools we already have with multicenter and multidisciplinary collaborations will be the most effective way to discover new biomarkers that can be implemented into clinical practice to optimize patient care.

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Figure Legends

Figure 1 – Clinical utility of Biomarkers; prognosis – identifying patients with an increased risk of death; predictive – useful in clinical practice in identifying patients with (and without) infection, ideally before clinical manifestations, and in the assessment of response to therapy; theranostic – indicating which therapy will suit a particular patient.

Figure 2 – Usefulness of biomarker kinetics in the prediction of the likelihood of infection in different clinical settings, community-acquired as well as nosocomial infections. PSP – pancreatic stone protein; PCT – procalcitonin; CRP – C-reactive protein; BSI – bloodstream infection; CAP – community-acquired pneumonia

Figure 3 – User's guide for biomarker-guided antibiotic therapy. Starting antibiotics in critically ill patients with suspicion of infection should be done irrespective of any biomarker level. But this should be reassessed daily. Use the clinical course, the organ dysfunction course (with SOFA score), the kinetics of biomarkers and the duration of antibiotic therapy to ascertain of the optimal duration of therapy. PCT – procalcitonin; CRP – C-reactive protein; SOFA, sequential organ failure assessment
NOTE – These recommendations do not apply to immune-compromised patients nor to patients with infections requiring long-term antibiotic therapy, like endocarditis or osteomyelitis. Adapted from Salluh Crit Care 2014; 18:142 [77].