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BIOMARKERS: CAN THEY REALLY GUIDE OUR DAILY PRACTICE?

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ABSTRACT—Optimal management of septic patients requires accurate assessment of both current severity status and prognosis. Since the 1990s, substantial advances have been made in the use of circulating biomarkers for such assessments. This summary of the session on “Biomarkers: can they really use guide our daily practice?” presented at the 2021 WEB-CONFERENCE OF THE EUROPEAN SHOCK SOCIETY, 6 November 2021. These biomarkers include ultrasensitive detection of bacteremia, circulating soluble urokinase-type plasminogen activator receptor (suPAR), C-reactive protein (CRP) and ferritin and procalcitonin. In addition, the potential application of novel multiwavelength optical biosensor technology allows noninvasive monitoring of multiple metabolites that can be used to assess severity and prognosis in septic patients. The application these biomarkers and improved technologies provide the potential for improved personalized management of septic patients.

KEYWORDS—C-reactive protein (CRP), ferritin, procalcitonin (PCT), and soluble urokinase plasminogen activator receptor (suPAR), sepsis, biomarkers

INTRODUCTION

Most of the attention of translational research of severe infections has been focused on the use of biomarkers. As far back as the early 90s, research was aiming to compare the circulating levels of various biomarkers in different stages of severity and to calculate in terms of sensitivity and specificity the diagnostic and prognostic role of each biomarkers. In the last decades, the methodology of research in the biomarker field has changed dramatically. Instead of simplistic measurements, randomized clinical trials have been done where decision making is judged based on algorithmic changes of biomarkers. The real question is where we are heading today. The current chapter aims to described the status of some of the broadly studied biomarkers, specifically C-reactive protein (CRP), ferritin, procalcitonin (PCT), and soluble urokinase plasminogen activator receptor (suPAR), in guiding therapeutic decisions and the resultant impact on outcomes.

suPAR for COVID-19

suPAR stands for soluble urokinase-type plasminogen activator receptor. It is the circulating form of a membrane protein that is expressed by immunologically active cells, endothelial cells,

and podocytes. Both the circulating and membrane forms are involved in the regulation of cell adhesion and cell migration. The circulating form can be easily assayed in plasma and other biological fluids. Elevated levels of suPAR have been associated with poor prognosis in several patient populations with a broad spectrum of neoplastic, infectious, and inflammatory diseases, both acute and chronic (1–6). In a paper by Hayek et al. (7), elevated suPAR levels were independently associated with the occurrence of chronic renal failure in a cohort of 3,683 people. A systematic review pointed out that, precisely because of its nonspecificity, suPAR levels have a poor diagnostic value in patients with inflammatory and infectious diseases, whereas its prognostic value was demonstrated with high levels being associated with increased mortality (8). In the same work, it was hypothesized that suPAR could be used to identify patients who were candidates for specific therapeutic strategies, which indeed was successfully applied years later to patients with coronavirus disease 2019 (COVID-19).

One area where the value of suPAR has been particularly studied is in patients with sepsis. In a paper by Giamarellos-Bourboulis et al. (9), a prognostic stratification system for patients with sepsis was developed, which combines the Acute Physiology and Chronic Health Evaluation II score with the suPAR assay. In this paper, it was shown that using an Acute Physiology and Chronic Health Evaluation II score cutoff of 17, the suPAR level (with a cutoff of 12) could significantly indicate patients at higher risk of death. This showed that both parameters were independent prognostic indicators and could be used in the construction of a predictive model. These observations were subsequently confirmed in another work on a smaller population of Chinese patients with sepsis (10). Based on these observations, the prognostic value of suPAR

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levels in patients with respiratory failure was studied when the COVID-19 epidemic broke out. The theoretical assumption stemmed from the fact that, because respiratory failure in COVID-19 was in part caused by intense endothelial activation, the suPAR that is bound to the endothelium may be cleaved early during the disease leading to an increase in suPAR, its soluble counterpart. If this is true, suPAR can be used as an early predictor of respiratory failure. Indeed, this is what Rovina et al. (11) demonstrated in a small cohort of patients, suPAR levels of 6 ng/mL served as a cutoff above which progression to respiratory failure was much more rapid. The same group then identified in the most severe patients an association between the presence of high levels of suPAR and the presence of danger-associated molecular patterns that induce an inflammatory tissue response through a mechanism mediated by interleukin (IL)-1 (12).

With these theoretical premises established in COVID-19, two important studies were carried out that used suPAR levels >6 to select patients who would receive treatment with anakinra, which is a specific inhibitor of IL-1. The first, the SAVE (suPAR-guided anakinra treatment for validation of the risk and early management of severe respiratory failure by COVID-19) study, was an open-label study, which showed very encouraging results, both for the use of suPAR as a prognostic marker and for anakinra as a specific treatment (13). Based on these results, a pivotal phase 3 randomized, double-blind study was performed in which nearly 600 patients were studied (14). In the SAVE-MORE (suPAR-guided anakinra treatment for validation of the risk and early management of severe respiratory failure by COVID-19) study, the risk of having a worse clinical status (measured by the 11-point World Health Organization Clinical Progression Scale) with anakinra compared with placebo was 0.36, whereas the risk of 28-day mortality was 0.45. The SAVE-MORE study, therefore, provided very important information on the use of suPAR. By using suPAR levels for the early identification of patients at higher risk of progression, the use of an IL-1 inhibitor in these patients provides a great advantage in terms of clinical outcome. In conclusion, we can state that suPAR is a valuable tool for the practice of personalized medicine, helping to identify the right patient and the right timing for targeted therapy (14).

Ultrasensitive detection of bacteremia to prevent or early start therapeutic management of sepsis

Over the past two decades, the incidence of sepsis has been continuously growing, possibly in correlation with human population increase and aging. Thus, sepsis has reached almost 50 million people in 2018, and fatal case numbers can vary from 20% to 80% of hospitalized cases. Septic pathogenic progression is rapid (it takes only very few hours) and is recognized as the most common cause of deaths in intensive care units. Sepsis is considered as a significant medical emergency, and in 2017, World Health Organization launched a call to combat sepsis. The main threat is in patients exhibiting a high bacteremia level, including those with meningitis (purpura fulminans in young people). Moreover, nonpathogenic bacteria, naturally present in the human microbiome, would also be able to cause sepsis and potential conditions for a fatal outcome. In addition, fungi and viruses (respiratory and hemorrhagic viruses) can be associated with similar symptoms. The microbiological status is one of the essential diagnostic elements to identify the infection and accordingly adopt the most appropriate therapy.

This approach together with robust biomarkers provides a positive impact on both the individual level and the population level in terms of both proper use of anti-infectious drugs and proper control of antibiotic resistance.

To contribute to the World Health Organization's call to combat sepsis, ApoH Technologies has developed a method to provide an ultrasensitive detection of bacteremia (or viral or fungi replication) from whole blood, to detect it as early as possible, and to enable timely adoption of the appropriate antibiotic management. This method is based upon the capacity of the apolipoprotein H (ApoH) or its synthetic derivatives to bind with high affinity (nanomolar scale) to non-self-motifs, including bacteria, viruses, fungi, and prions. The capture and concentration of pathogens are done with nanomagnetic beads coated with synthetic ApoH peptides in the presence of a specific ApoH-activation buffer. In these conditions, ApoH allows detection of a single Colony Forming Units in 5 mL of whole blood. In a starting clinical study, whole blood clinical samples from patients suspected of sepsis are processed with ApoH-magnetic beads to capture and concentrate bacteria to be detected with a 16s quantitative polymerase chain reaction to provide a diagnosis within 3 to 6 hours (15).

CRP and ferritin: Can they really guide our daily practice

C-reactive protein is a highly conserved protein involved in early defense against infection and is the prototype of acute phase protein (APP) in humans. C-reactive protein is a pentraxin with a recognition binding site for phosphocholine expressed on the membranes of various extracellular bacteria or damaged cells, and an effector site to bind to C1q or Fc γ receptors inducing subsequent bacteria or cell phagocytosis by immune cells (16). The normal circulating concentrations of CRP is around 0.8 mg/L but increases up to 1,000-fold during inflammation due to increased transcription in liver cells induced by IL-6 (16). Ferritin is a shell protein composed of 24 light or heavy monomers and is the main intracellular iron storage molecule, preventing free iron from catalyzing oxygen free radical formation (17). Ferritin behaves as an APP since its transcription in macrophages and hepatocytes is increased by IL-1, tumor necrosis factor, interferon γ (IFN γ), and various oxidants. During inflammation, increased hepcidin synthesis prevents iron exit from the cell through interaction with ferroportin, inducing iron accumulation by macrophages or hepatocytes, thus reducing its availability for the growth of invading pathogens. Elevated intracellular iron concentrations also increase ferritin translation via decreased interactions of iron responsive proteins with iron responsive elements on its messenger RNA. Normal circulating concentrations of ferritin are less than 250 μ g/L but increase during inflammation reflecting macrophage activation mainly through cytoplasmic leakage from damaged cells. Circulating ferritin appears poorly loaded with iron but demonstrates both proinflammatory and immunosuppressive functions (18).

Although CRP and ferritin are both APP, they may constitute biomarkers of different cytokine and defense pathways. Although imperfect, CRP is a common biomarker of inflammation due to extracellular bacterial infection and sepsis (19). C-reactive protein is mainly induced by IL-6, whose synthesis is itself induced by IL-1. Interleukin-1 also induces prostaglandin E2 secretion, which downregulates IFN γ synthesis and Th-1 (CD4 positive T

helper cell-1) polarization (20). Conversely IL-1 in combination with IL-6 favors Th-17 (CD4 positive T helper cell-1) polarization. Th-17 (CD4 positive T helper cell-1) response is very efficient in antimicrobial defenses against extracellular bacteria and fungi but not against viral and intracellular bacteria (21). Ferritin is found to be more elevated during intracellular infections, notably because of viruses like Epstein-Barr or dengue in which TH-1 T-cell polarization and IFN γ are known to be protective (22). In addition, much higher ferritin concentrations (10,000 $\mu\text{g/L}$) have been reported in severe conditions such as hemophagocytic lymphohistiocytosis or macrophage activation syndrome characterized by an important IL-18 and IFN γ signature (23). Interleukin-18 is a member of the IL-1 family, which similarly to IL-1 β is processed in its bioactive mature form by activation of the NLRP3 inflammasome and caspase 1. Interleukin-18 drives TH-1 polarization and IFN γ production by T and NK cells (24).

Thus, both CRP and ferritin may constitute helpful APP to discriminate between an IL-1/IL-6/IL-17 signature directed against extracellular bacterial infection and an IL-18/IFN γ signature directed against virus infection (25). However, this may be too simplistic as recently exemplified by Severe Acute Respiratory Syndrome-Coronavirus 2 infection, because coronavirus among others can directly activate the NLRP3 inflammasome and induce both an IL-1/IL-6 and an IL-18/IFN γ signature along disease evolution (26). Moreover, these different cytokine signatures may also occur over time in the same patient suffering a noninfectious disease such as Still disease (27). However, future progress may help identify disease pathogenic pathways and decide of the best treatment with the appropriate timing.

Optical biosensor technology

Sepsis is one of the leading causes of mortality worldwide, with a life claim of almost 11 million humans annually, based on data before the COVID-19 pandemic (28). Even in this long period of COVID-19 pandemic, sepsis contributes significantly to the death toll because the vast majority of COVID-19 patients eventually pass away owing to respiratory and multiorgan failure, manifestations of sepsis according to Sepsis-3 alliance definitions. Early detection of patients prone to sepsis or already septic is of vital importance.

To date, the diagnosis of sepsis is made after clinical evaluation using clinical scores (like quick Sequential Organ Failure Assessment) along with more specific biomarkers and rapid tests, especially for early detection of Severe Acute Respiratory Syndrome-Coronavirus-2. In health care settings, routine laboratory measurements (like blood cell count and biochemistry parameters) and microbiological procedures (cultures or Polymerase Chain Reaction for the rapid isolation of the pathogens) along with imaging can additionally assist in the diagnosis. The main disadvantage of all paraclinical procedures mentioned is the fact that they are time-consuming. Therefore, decision making is mostly based on clinical judgment and on quick point-of-care testing. Any portable device easy to use, capable of providing quick results with a minimal procedure (noninvasive) that can accelerate the diagnosis of sepsis, is a necessary addition to the arsenal of the clinician. The latest generation of optical biosensors fulfills these requirements.

Photoplethysmography is a technique studied as far back as the 1940s, and in the 1970s, its application for the calculation of blood oxygen saturation made the pulse oximeter a common tool during the past decades (29). More recent applications include blood pressure measurement and continuous glucose monitoring. Vascular stiffness and endothelial function are additional parameters that can be quantified by photoplethysmography. A novel optical biosensor point-of-care device was developed by Trilibeat Ventures Corporation (Huntsville, AL), measuring optical absorptions at five wavelengths; more specifically at 940 nm (infrared), 660 nm (red color), 530 nm (green color), 465 nm (blue color), and 395 ± 10 nm (ultraviolet). The novel optical biosensor was validated through two different phases during the pulse Photoplethysmography as an early tool for the diagnosis of sepsis through a two-stage Development approach (PROUD) study, a clinical study conducted in four study sites (two departments of internal medicine and two intensive care units) participating in the network of the Hellenic Sepsis Study Group (30).

In phase 1 of the PROUD study, participants were patients at high risk for sepsis. Recordings of the optical biosensor, performed at serial time intervals, were used for the development of an algorithm capable of performing efficient diagnosis. Blood samples were obtained for nitric oxide (NO) measurements, blood cell count, and standard biochemistry.

Based on the optical biosensor recordings, the algorithm incorporated the absorption ratio of 660/940 nm of oxygenated versus deoxygenated hemoglobin, the difference in time between the systolic points in 395 to 940 nm providing an approximation of the vessel diameter, the absorption ratio 530/940 nm reflecting to creatinine levels, the absorption ratio 395/940 nm reflecting to the NO levels, and the absorption ratio 530/660 nm expressing poor oxygen absorption due to inflammatory interferences. NO blood measurements corroborated the findings from the 395/940 nm absorption ratio, whereas serum creatinine levels and the algorithm of the optical biosensor resulted in a positive correlation between them. With algorithm values ranging from 0 to 100, receiver operating characteristic curve analysis identified that a cutoff greater than 18 that could provide diagnosis of sepsis with 70.6% sensitivity, 100% specificity, 100% positive predictive value, and 93.2% negative predictive value.

In phase 2 of the PROUD study, patients with viral infections were enrolled. On each patient, one single test with the forefinger optical biosensor device was performed for 5 minutes. In addition, blood samples were obtained for the measurements described previously with a single blood draw. The algorithm was applied to diagnose COVID-19 and the clinical severity. At the same cutoff value of 18, COVID-19 was diagnosed with 57.9% sensitivity, 87.5% specificity, 91.7% positive predictive value, and 46.7% negative predictive value. Moreover, this algorithm value could discriminate severe COVID-19 with 83.8% sensitivity and 87.5% negative predictive value.

Based on the results of the PROUD study, the algorithm of the novel optical biosensor can perform better than biomarkers for the diagnosis of sepsis. Moreover, it can identify patients prone to severity and with high suspicion of COVID-19 before the Polymerase Chain Reaction results. Larger clinical trials need to be conducted. Nonetheless, this technology is very promising for quick and easy detection of septic patients.

PCT use and sepsis survival

Procalcitonin is a peptide produced by all tissues with mesenchymal origin during bacterial infection (31). Apart from its well-known role in sepsis diagnosis and prognosis, PCT has been studied during the last decade as a guide for the duration of antimicrobial treatment. Most of our experience with PCT guidance comes from trials recruiting patients with lower respiratory tract infections. The largest sepsis trials so far are the procalcitonin to reduce patients' exposure to antibiotics (PRORATA) and the Stop Antibiotics on guidance of Procalcitonin Study (SAPS) trial. In PRORATA, enrolling 621 patients, PCT guidance was associated with a shorter antimicrobial treatment, whereas relapse, reinfection, and 28-day mortality rate were similar between the two arms of treatment (32). There was, however, a signal for higher 60-day mortality in the PCT arm. Thus, the SAPS trial was conducted to prove mainly the safety of the strategy (33). The SAPS trial was successful in the primary endpoint; PCT guidance shortened antimicrobial treatment, but the investigators came across a paradoxical result. Procalcitonin guidance in sepsis provided a survival benefit after 28 days, which remained significant over a year. Apart from PRORATA and SAPS, more than 10 sepsis trials reported results regarding mortality. All these trials were conducted before implementation of the Sepsis-3 definitions, but through median/mean baseline Sequential Organ Failure Assessment score values, we extrapolated that the population investigated fulfilled the new definition (34,35). Two meta-analyses summarized the results of these trials and ended that PCT guidance decreased 28-day mortality (odds ratio, 0.89; 95% confidence interval, 0.80–0.99) in total but also in the subgroup of patients meeting the Sepsis-3 criteria (36,37). A major cause of heterogeneity in these trials is the different algorithm used; some used a start, a stop, or a mixed rule (35). Two meta-analyses support that PCT guidance favors survival only when a stopping algorithm is applied (38,39).

A hypothesis of the underlying mechanism of favorable outcome when a PCT-guided antimicrobial strategy is followed is the reduction of adverse events related with antimicrobials, such as allergic reactions or subsequent infections by *Clostridiodes difficile* or multidrug-resistant organisms (MDRO). This hypothesis was first tested in the procalcitonin-guided antimicrobial therapy to reduce long-term sequelae of infections trial, designed and conducted by the Hellenic Institute for the Study of Sepsis (40). In procalcitonin-guided antimicrobial therapy to reduce long-term sequelae of infections, 256 patients with sepsis according to Sepsis-3 definition and community- or hospital-acquired pneumonia, acute pyelonephritis or primary bacteremia were randomized to standard-of-care (SOC) or PCT guidance. The trial used the same stopping rule for antimicrobials as the SAPS trial (at least 80% decrease from baseline or PCT <0.5 ng/mL); PCT was measured at baseline and repeated after 5 days. The median length of treatment was 5 days for the PCT group compared with 10 days in the SOC group ($P < 0.001$). This reduction in the LOT was similar for all types of infections. Mortality after 28 days was lower in the PCT arm compared with SOC (15.2% vs. 28.2%; $p = 0.02$) associated to a decreased incidence of antibiotic-associated adverse events, in particular, diarrhea and acute kidney injury. The primary endpoint of the trial was a composite and comprised all elements of the incidence of long-term infectious complications after 6 months, that is, new infection by *C. difficile* and/or

MDRO or death due to baseline MDRO infection. This incidence was 7.2% in the PCT and 15.3% in the SOC group ($p = 0.045$). The study was designed to follow up the incidence of gut colonization by MDRO and *C. difficile* and the association with the incidence of infections by these species. The risk for infection was significantly higher in colonized patients in the SOC but not in the PCT arm. These results indicate that long-term antibiotic exposure in the SOC arm could affect the integrity of the mucosal barrier resulting in the increased incidence of infections by MDRO and *C. difficile*. Real-world data of antimicrobial stewardship programs are in full alignment with these results (41). In conclusion, a combination of PCT guidance with a wise clinical judgment may reduce unnecessary antimicrobial use and improve both short- and long-term sepsis outcome.

CONCLUSIONS

The previous analysis clearly elucidates that each of the studied biomarkers has clear-cut position nowadays for therapeutic decision making. C-reactive protein and ferritin are positioned for the diagnosis of hyperinflammation, PCT for the early stop of antibiotics, and suPAR as companion biomarker to properly use the early treatment with anakinra in hospitalized COVID-19 patients. These biomarkers, alongside the use of sophisticated point-of-care tools of artificial intelligence and early microbiological diagnosis, pave the way for a new era for the personalized clinical management of the patient exhibiting severe infections.

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