UNIVERSITÀ DEGLI STUDI DI TRIESTE

Derivation and Validation of a Biomarker-Based Clinical Algorithm to Rule Out Sepsis From Noninfectious Systemic Inflammatory Response Syndrome at Emergency Department Admission: A Multicenter Prospective Study

This is the peer reviewed (post-print) version of the following article:

Original
Derivation and Validation of a Biomarker-Based Clinical Algorithm to Rule Out Sepsis From Noninfectious Systemic Inflammatory Response Syndrome at Emergency Department Admission: A Multicenter Prospective Study / Mearelli, F., Fiotti, N., Giansante, C., Casarsa, C., Orso, D., De Helmersen, M., Altamura, N., Ruscio, M., Castello, L.M., Colonetti, E., Marino, R., Barbati, G., Bregnocchi, A., Ronco, C., Lupia, E., Montrucchio, G., Muiesan, M.L., Di Somma, S., Avanzi, G.C., Biolo, G.. - In: CRITICAL CARE MEDICINE. - ISSN 0090-3493. - 46:9(2018), pp. 1421-1429. [10.1097/CCM.0000000000003206]

Availability:
This version is available at: 11368/2932070 since: 2018-12-02T15:29:52Z
Publisher:

Published
DOI:10.1097/CCM.0000000000003206
Terms of use:

Some rights reserved. More details in the PostPrint rights section below.

License:
Digital Rights Management not defined

Publisher copyright

-.-

(Article begins on next page)

06 October 2026

arTs Università degli Studi di Trieste
Archivio della ricerca – postprint

Derivation and Validation of a Biomarker-Based Clinical Algorithm to Rule Out Sepsis From Noninfectious Systemic Inflammatory Response Syndrome at Emergency Department Admission: A Multicenter Prospective Study

Filippo Mearelli, MD[1]; Nicola Fiotti, MD[1]; Carlo Giansante, MD[1]; Chiara Casarsa, MD[1]; Daniele Orso, MD[1]; Marco De Helmersen, MD[1]; Nicola Altamura, MD[1]; Maurizio Ruscio, PhD[6]; Luigi Mario Castello, MD[3]; Efrem Colonetti, MD[4]; Rossella Marino, MD[5]; Giulia Barbati, PhD[1]; Andrea Bregnocchi, MD[7]; Claudio Ronco, MD[8]; Enrico Lupia, MD[9]; Giuseppe Montrucchio, MD[9]; Maria Lorenza Muiesan, MD[4]; Salvatore Di Somma, MD[5]; Gian Carlo Avanzi, MD[3]; Gianni Biolo, MD[1]

Objectives: To derive and validate a predictive algorithm integrating a nomogram-based prediction of the pretest probability of infection with a panel of serum biomarkers, which could robustly differentiate sepsis/septic shock from noninfectious systemic inflammatory response syndrome.

Design: Multicenter prospective study.

Setting: At emergency department admission in five University hospitals.

Patients: Nine-hundred forty-seven adults in inception cohort and 185 adults in validation cohort.

Interventions: None.

Measurements and Main Results: A nomogram, including age, Sequential Organ Failure Assessment score, recent antimicrobial therapy, hyperthermia, leukocytosis, and high *C*-reactive protein values, was built in order to take data from 716 infected patients and 120 patients with noninfectious systemic inflammatory response syndrome to predict pretest probability of infection. Then, the best combination of procalcitonin, soluble phospholipase A_2 group IIA, presepsin, soluble interleukin-2 receptor α, and soluble triggering receptor expressed on myeloid cell-1 was applied in order to categorize patients as "likely" or "unlikely" to be infected. The predictive algorithm required only procalcitonin backed up with soluble phospholipase A_2 group IIA determined in 29% of the patients to rule out sepsis/septic shock with a negative predictive value of 93%. In a validation cohort of 158 patients, predictive algorithm reached 100% of negative predictive value requiring biomarker measurements in 18% of the population.

Conclusions: We have developed and validated a high-performing, reproducible, and parsimonious algorithm to assist emergency department physicians in distinguishing sepsis/septic shock from noninfectious systemic inflammatory response syndrome.

Key Words: biomarkers; emergency department; phospholipase A_2 group IIA; procalcitonin; sepsis; systemic inflammatory response syndrome

[1]Unit of Internal Medicine, Department of Medical Surgical and Health Sciences, University of Trieste, Trieste, Italy.

[2]Department of Chemical and Pharmaceutical Sciences, University of Trieste, Trieste, Italy.

[3]Unit of Emergency Medicine, Department of Translational Medicine, Eastern Piedmont University, Novara, Italy.

[4]Unit of Internal Medicine, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.

[5]Unit of Emergency Medicine, Department of Medical Surgery Sciences and Translational medicine, University "Sapienza" of Rome, Rome, Italy.

[6]Biostatistics Unit, Department of Medical Sciences, University of Trieste, Trieste, Italy.

[7]Unit of Internal Medicine, General Hospital of Susa, Susa (TO), Italy.

[8]Unit of Nephrology, Department of Nephrology, Dialysis and Transplantation International Renal Research Institute St Bortolo Hospital, Vicenza, Italy.

[9]Unit of Emergency Medicine, Department of Medical Sciences, University of Turin, Turin, Italy.

Supported, in part, by the Italian Ministry of Health.

Drs. Mearelli, Fiotti, Giansante, Casarsa, De Helmersen, Altamura, Barbati, and Bregnocchi disclosed government work. Dr. Orso received support for article research from the Italian Ministry of Education, Universities and Research. The remaining authors have disclosed that they do not have any potential conflicts of interest.

For information regarding this article, E-mail: filippome@libero.it

One of five patients with suspected sepsis at admission to emergency department (ED) may not be infected (1). In Italy, the decision to start antibacterial therapy is generally based on fever, leukocytosis, and increased *C*-reactive protein values combined with clinical signs of infection. This "Usual" approach is not specific and only partially reproducible (2). Even when clinicians reach a definitive diagnosis of sepsis, more than 40% of these cases are culture negative (3). It is no surprise that, in patients with suspected sepsis and culture negative status, the lack of a gold standard leads to a variable percentage uncertain diagnosis with low interobserver agreement, even after careful workup (1, 4). This hinders early application of therapeutic bundles in sepsis/septic shock, whereas in noninfected patients, it prevents prompt start-up of specific and equally life-saving therapies. Over the last decade, there has been great interest in procalcitonin to assist clinicians in their diagnosis of infection. However, procalcitonin alone does not have an optimal level of performance for ruling out sepsis/septic shock (5). Given the complexity of immune response to infection, more recent research has increasingly focused on a combination of biomarkers (6–8); however, only one study, conducted in two ICUs, validated the combination identified as highly predictive of infection in an external cohort (8). In ED setting, there are no studies about a combination of biomarkers for early diagnosis of sepsis based on the same rigorous approach. Therefore, the objectives of this study were as follows:

1) to derive and validate a nomogram to stratify the pretest probability of infection and
2) to derive and validate the accuracy of a panel of biomarkers to rule out sepsis/septic shock on the basis of nomogram prediction strata at ED admission.

METHODS

Setting and Participants

Patients were enrolled at ED admission in five Italian University hospitals. The centers of Novara, Rome, and Trieste recruited, from March 15, 2013, to March 15, 2015, consecutive adults who fulfilled two or more criteria of community-acquired systemic inflammatory response syndrome (SIRS). These patients constituted the inception cohort. The centers of Brescia and Turin enrolled patients using the same criteria and in the same period to constitute the validation cohort. The study was approved by each local institutional review board. Complete inclusion and exclusion criteria are listed in the **supplementary data** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587)

Data Collection, Adjudication, and Follow-Up

Clinical data were registered prospectively without interfering with usual clinical practice. At discharge or in the case of hospital death, the attending physician established a preliminary diagnosis that was recorded using a predefined classification system (PDCS). The PDCS was based on guidelines compiled by the main authorities in the field of infection and was approved, before the start of enrollment, after discussions among the principal investigators. The preliminary diagnosis categorized patients as infected SIRS or noninfected SIRS (ni-SIRS). The latter group was defined as SIRS associated with a noninfective diagnosis, whereas the former was defined as SIRS associated with an underlying infective diagnosis. An independent physician retrospectively evaluated all available records from clinical workup prescribed by the attending physician and established the diagnosis according to the same explicit criteria (PDCS): the main objective of the use of identical predefined explicit criteria was to limit interobserver variability in infection diagnosis. When the attending physician and independent physician were in agreement, a definitive diagnosis was reached as mentioned above. In cases of disagreement, the patients were considered debatable SIRS (d-SIRS). d-SIRS include unclear SIRS cases that could not be robustly characterized as infected. Patients with definitive diagnosis of infection were further subdivided in clinically documented infections (c-infections) and microbiologically documented infections (m-infections). c-infections comprised SIRS cases with a clinical history and course suggestive of infection without supporting gram stain or cultures. c-infections in whom blood cultures were drawn and resulted negative were defined culture-negative infections. m-infections included SIRS cases with microbiological confirmation of infection. m-infections were further subdivided into bacterial and nonbacterial infections depending on the type of germ cultured in sterile biological fluids or identified by ancillary diagnostic examinations. Uncomplicated infection was a SIRS with a definite infective etiology and absence of organ dysfunction. Sepsis was defined as infection associated with signs of hypoperfusion or organ dysfunction. Septic shock was sepsis plus one or both of the following conditions: mean systemic arterial pressure less than 60 mm Hg (or < 80 mm Hg compared with usual pressure rates) despite adequate fluid resuscitation strategy and need for dopamine, norepinephrine, or epinephrine despite the administration of fluids required to maintain mean arterial pressure greater than 60 mm Hg (or > 80 mm Hg in hypertensive patients).

Further details about PDCS, data collection, adjudication, and follow-up are provided in the specific section of the supplementary data (Supplemental Digital Content 1, http://links.lww.com/CCM/D587).

Biomarker Analysis

A preliminary pilot study (9) and a thorough literature review (10) guided the selection of biomarker analysis in this study. The final list of biomarkers determined in frozen serum samples included procalcitonin, soluble phospholipase A_2 group IIA ($sPLA_2GIIA$), presepsin, soluble interleukin-2 receptor α (sCD25), and soluble triggering receptor expressed on myeloid cell (sTREM-1). Details about biomarker analysis are reported in the supplementary data (Supplemental Digital Content 1, http://links.lww.com/CCM/D587).

Sample Size

Using data from previously published studies (9–11), we proposed a sample size of at least 845 patients in the inception cohort. These patients were enrolled to construct a predictive

TABLE 1. Inception Cohort: Clinical Characteristics of the Patients With Definitive Diagnosis at Emergency Department Admission

Characteristics	Infections, *n* = 716 (%)	Noninfectious Systemic Inflammatory Response Syndrome, *n* = 120 (%)	*p*
Male, *n* (%)	375 (52)	61 (51)	0.768
Median age (IQR)	81 (73–87)	72 (59–83)	0.000
Median Charlson Index (IQR)	3 (1–4)	2 (1–4)	0.008
Median Sequential Organ Failure Assessment score (IQR)	3 (1–4)	2 (1–3)	0.000
Antibacterial therapy within 30 d, *n* (%)	225 (31)	17 (14)	0.000
Clinical variables, *n* (%)			
Body temperature			
> 38°C	348 (49)	20 (17)	0.000
< 36°C	33 (5)	4 (3)	0.639
WBC count			
> 12,000/mm^3	401 (56)	48 (40)	0.001
< 4,000/mm^3	27 (4)	4 (3)	1.000
Respiratory rate > 20/min or Pco_2 < 32 mm Hg	539 (75)	94 (78)	0.565
Heart rate > 90/min	527 (74)	105 (87)	0.001
C-reactive protein > 21.75 mg/dL	609 (85)	45 (37)	0.000
Median biomarker (IQR)[a]			
Procalcitonin (ng/mL)	0.525 (0.200–3.617)	0.127 (0.077–0.2170)	0.000
Soluble interleukin-2 receptor α (pg/mL)	14,189 (8,949–23,109)	9,079 (5,340–16,613)	0.000
Soluble triggering receptor expressed on myeloid cell-1 (pg/mL)	399.5 (270.2–649.5)	283.9 (215.1–499.1)	0.000
Soluble phospholipase A_2 group IIA (ng/mL)	31.60 (25.88–35.85)	11 (4.15–25.62)	0.000
Presepsin (pg/mL)	500.5 (313–970.5)	308 (194.5–529.5)	0.000
Severity of infection, *n* (%)			
Uncomplicated infection	134 (19)	–	–
Sepsis	533 (74)	–	–
Septic shock	49 (7)	–	–
Mortality[b]	133 (19)	9 (7)	0.002

IQR = interquartile range.

[a]Procalcitonin was not available for nine patients; soluble interleukin-2 receptor α, soluble phospholipase A_2 group IIA, and presepsin were not available for five patients.

[b]Mortality at 30 d was not available for 45 patients.

Dashes indicate data not applicable.

algorithm, combining a nomogram-based prediction of the pretest probability of infection with the above biomarkers, which was then validated in an independent multicenter prospective cohort of at least 100 patients (see Sample Size paragraph of the supplementary data, Supplemental Digital Content 1, http://links.lww.com/CCM/D587).

Statistical Analysis

To construct the nomogram in the inception cohort, we first built a multivariable clinical logistic regression model (MCLRM) by identifying the most powerful subset of independent clinical predictors of infection diagnosis at the end of clinical workup. MCLRM provided the equation used to generate the nomogram. In agreement with the nomogram output, patients were categorized in three groups with less than 50%, 50–80%, and greater than 80% estimated probability of infection, respectively. Second, to evaluate the nomogram and biomarker diagnostic performance, receiver operating characteristic (ROC) curves were constructed and areas under receiver operating characteristic curves (AUC) were estimated. Third, the best combination of

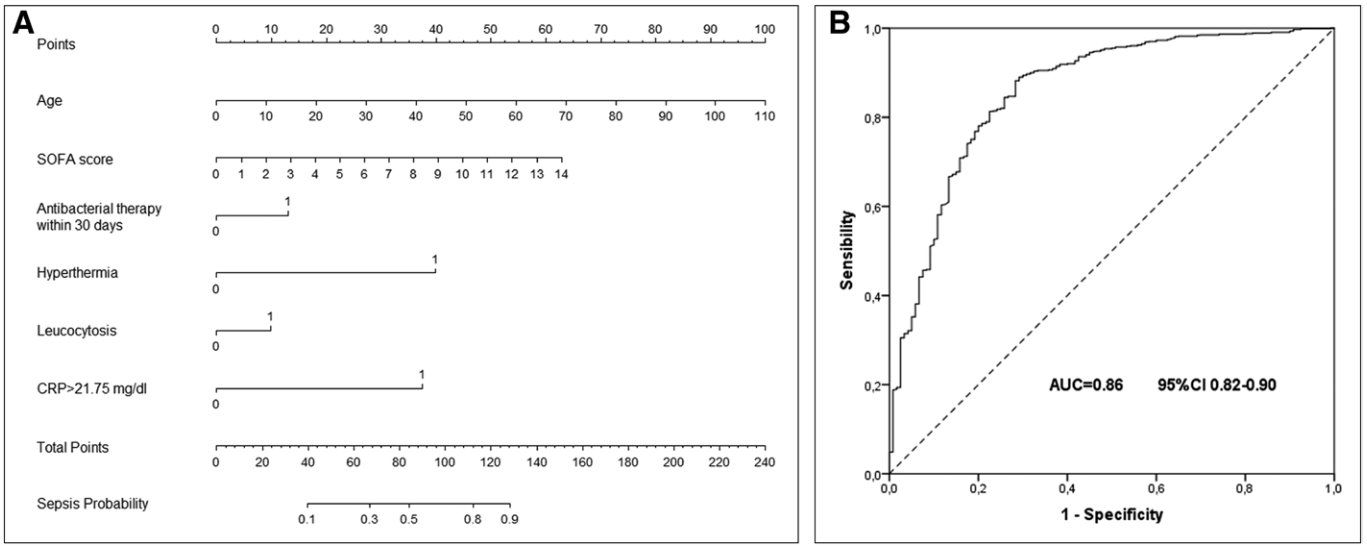

Figure 1. Characteristics (**A**) and performance (**B**) of the nomogram to predict pretest probability of infection. AUC = area under the receiver operating characteristic curve, CRP = *C*-reactive protein, SOFA = Sequential Organ Failure Assessment.

biomarkers, which resulted significantly associated with definitive diagnosis of infection, was assessed in each of the three groups. A Multiple Biomarker Score (MBMS) was generated attributing 1 point for each biomarker with a value above the optimal cutoff point. Finally, the MBMS allowed us to categorize patients in "likely" to be infected or "unlikely" to be infected in each group (i.e., MBMS 1, MBMS 2, and MBMS 3). To determine the negative predictive value (NPV) and positive predictive value (PPV) of the combined use of the nomogram and MBMS (i.e., predictive algorithm) in diagnosis of infection in the full spectrum of severity and in sepsis/septic shock, the definitive categorization of the patient at the end of clinical workup was compared with the output of MBMS in traditional 2×2 tables. To validate the results obtained in the inception cohort, the predictive algorithm was applied in an independent validation cohort. The proportion of false positive (FP) and false negative (FN) results was compared with those of the inception cohort by the *z* score test for two population proportions. To determine the performance of the Usual approach, the prescription of antimicrobial therapy at ED admission was compared with definitive diagnosis of sepsis/septic shock in traditional 2×2 tables for all the patients enrolled in the study. To compare the NPV and PPV of Usual approach and predictive algorithm in diagnosing sepsis/septic shock, we used a generalized score statistic for comparison of values. d-SIRS patients were reclassified using the predictive algorithm both in the inception and validation cohorts. An example of nomogram application and other details about the statistical analysis are provided in the supplementary data (Supplemental Digital Content 1, http://links.lww.com/CCM/D587).

RESULTS

Clinical Characteristics of Inception and Validation Cohorts

A flow chart of enrollment and adjudication in the inception cohort is shown in **Supplementary Figure 1** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587), whereas **Supplementary Figure 2** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587) reports that of the validation cohort. The clinical characteristics of definitively diagnosed patients enrolled in the inception and validation cohort are summarized in **Table 1** and in **Supplementary Table 1** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587), respectively. Patients in the validation cohort were younger than those of the inception cohort ($p < 0.007$), with lower median Charlson Index and Sequential Organ Failure Assessment score ($p < 0.000$ and $p < 0.001$, respectively). The most common source of infection, both in the inception and validation cohorts, was the lower respiratory tract (LRT). The etiology of m-infections is reported according to source of infection in **Supplementary Table 2** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587).

Definitive Diagnosis Prediction by the Predictive Algorithm in the Inception Cohort

Clinical variables independently associated with definitive diagnosis of infection are reported in **Supplementary Table 3** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587). The nomogram based on their individual regression weight is provided in **Figure 1*A***. As shown in **Figure 1*B***, the AUC of the nomogram was 0.86 (95% CI, 0.82–0.90). Out of the five biomarkers tested in group 1, only $sPLA_2GIIA$ entered the MBMS 1 with a cutoff of 11.25 ng/mL. In group 2, $sPLA_2GIIA$ and procalcitonin entered the MBMS 2 with a cutoff of 25.39 ng/mL and 0.1787 ng/mL, respectively. The distribution of the patients with definitive diagnosis according to number of positive biomarkers in MBMS 2 is provided in **Supplementary Table 4** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587). The best cutoff value of number of positive biomarkers for MBMS 2 was set between 0 and 1. In group 3, none of the biomarkers, alone or in combination, improved the nomogram classification. ROCs for MBMS 1 and MBMS 2,

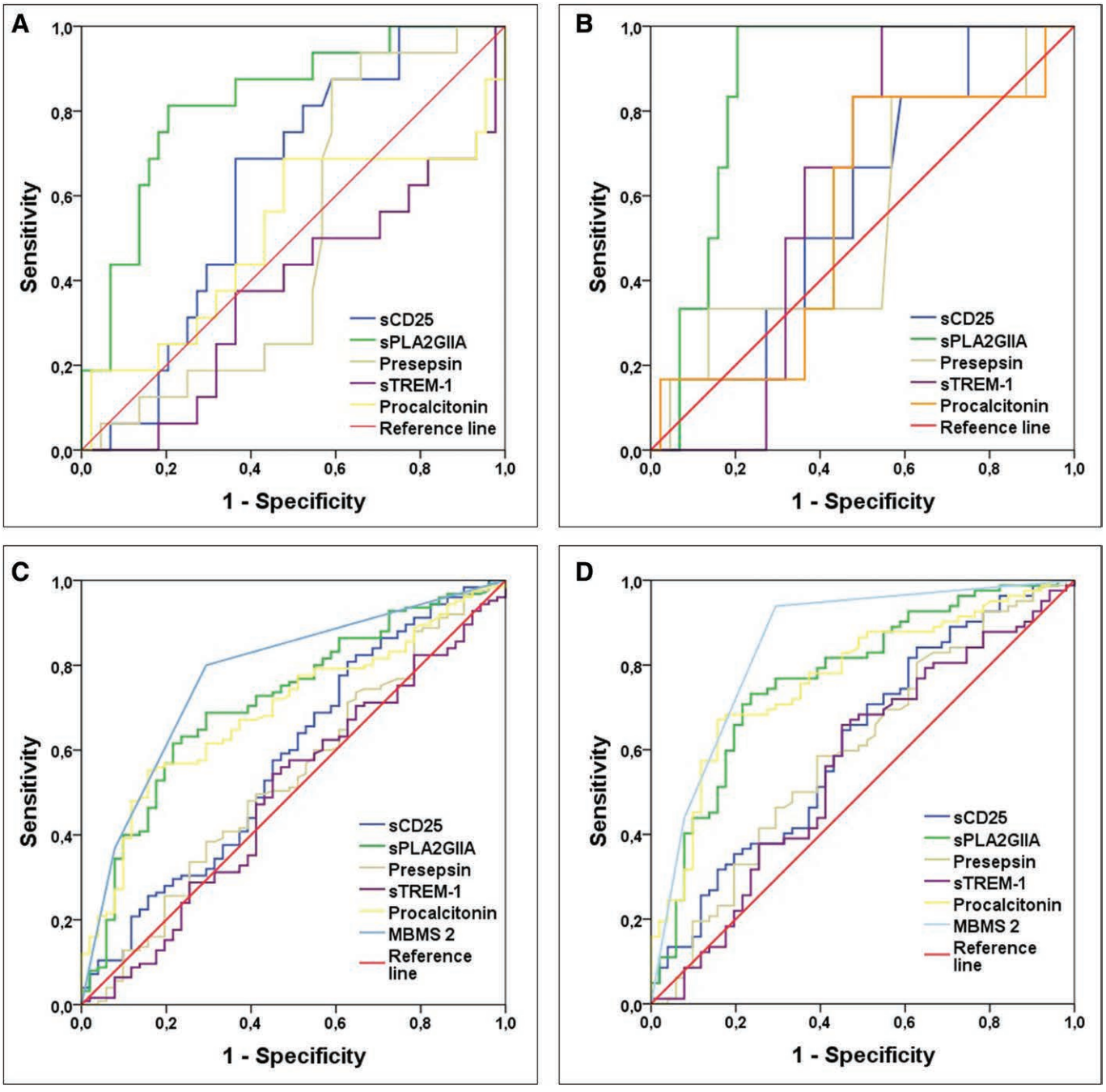

Figure 2. Inception cohort: receiver operating characteristic curves of biomarkers to diagnose infection according to its severity in group 1 and group 2. Group 1: receiver operating characteristic curves of biomarkers in diagnose infection in the full spectrum of severity (**A**) and in sepsis/septic shock (**B**). Group 2: receiver operating characteristic curves of biomarkers in diagnose infection in the full spectrum of severity (**C**) and in sepsis/septic shock (**D**). MBMS 2 = Multiple Biomarker Score 2, sCD25 = soluble interleukin-2 receptor α, sPLA$_2$GIIA= soluble phospholipase A$_2$ group IIA, sTREM-1 = soluble triggering receptor expressed on myeloid cell-1.

both in diagnosis of infection in the full spectrum of severity or in diagnosis of sepsis/septic shock, are shown in **Figure 2**, and their AUC in **Table 2**. In group 1, none of the patients with sepsis and nine ni-SIRS were misclassified (**Supplementary Fig. 3**, Supplemental Digital Content 1, http://links.lww.com/CCM/D587); the corresponding figures for group 2 were five and 15 patients, respectively (**Supplementary Fig. 4**, Supplemental Digital Content 1, http://links.lww.com/CCM/D587). In group 3, sole use of the nomogram misclassified 24 patients with ni-SIRS (**Supplementary Fig. 5**, Supplemental Digital Content 1, http://links.lww.com/CCM/D587).

The NPV and PPV of the predictive algorithm for the diagnosis of sepsis/septic shock were 93% and 92%, respectively.

Definitive Diagnosis Prediction by the Predictive Algorithm in the Validation Cohort

AUC for the nomogram in the validation cohort was 0.95 (95% CI, 0.92–98) (**Supplementary Fig. 6**, Supplemental

TABLE 2. Inception Cohort: Areas Under Receiver Operating Characteristic Curves of the Biomarkers to Diagnose Infection According to Its Severity in Group 1 and Group 2

Groups and Severity of Infection	Soluble Interleukin-2 Receptor α	Soluble Phospholipase A_2 Group IIA	Presepsin	Soluble Triggering Receptor Expressed on Myeloid Cell-1	Procalcitonin	Multiple Biomarker Score 2
Group 1						
Full spectrum of severity	0.626 (0.483–0.768)	0.821 (0.703–0.94)	0.495 (0.343–0.647)	0.376 (0.213–0.54)	0.509 (0.321–0.696)	NA
Sepsis/septic shock	0.581 (0.376–0.787)	0.864 (0.764–0.963)	0.542 (0.294–0.79)	0.617 (0.467–0.768)	0.557 (0.321–0.793)	NA
Group 2						
Full spectrum of severity	0.58 (0.484–0.676)	0.715 (0.632–0.798)	0.532 (0.436–0.628)	0.496 (0.4–0.592)	0.693 (0.612–0.774)	0.776 (0.699–0.852)
Sepsis/septic shock	0.617 (0.518–0.716)	0.771 (0.687–0.855)	0.596 (0.495–0.697)	0.563 (0.460–0.666)	0.77 (0.688–0.852)	0.85 (0.778–0.923)

NA = not applicable.

Digital Content 1, http://links.lww.com/CCM/D587). Categorization of the patients using the predictive algorithm is shown in **Supplementary Figure 7** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587). The NPV and PPV of the predictive algorithm for the diagnosis of sepsis/septic shock were 100% and 93%, respectively.

Definitive Diagnosis Prediction by the Predictive Algorithm in Both Cohorts

The final categorization of both inception and validation cohorts using the predictive algorithm is shown in **Figure 3**. In the whole population, five patients (0.7%) with sepsis/septic shock were misclassified by the predictive algorithm. The proportion of FN did not differ between the inception and validation cohorts whether in diagnosis of infection in the full spectrum of severity (z score = 0.28; $p = 0.28$) or in sepsis/septic shock (z score = –1.45; $p = 0.14$). Furthermore, we found no statistically significant difference in the proportion of FP between the cohorts (z score = –1.4516; $p = 0.14$) (**Supplementary Table 5**, Supplemental Digital Content 1, http://links.lww.com/CCM/D587). From the comparison of predictive values, predictive algorithm resulted significantly better than the Usual approach in ruling out sepsis/septic shock ($p < 0.000$); we found no statistically significant dissimilarity between the predictive algorithm and Usual approach in ruling in sepsis/septic shock ($p = 0.11$).

d-SIRS Versus Definitive Diagnosis

The definitive categorization of d-SIRS using the predictive algorithm and comparison with patients with definitive diagnosis are shown in Figure 3 and **Supplementary Table 6** (Supplemental Digital Content 1, http://links.lww.com/CCM/D587), respectively.

DISCUSSION

In most of the studies about sepsis, the prescription of blood cultures and antibacterial therapy at ED admission have been considered a signal of when sepsis is suspected by the treating physician (10–15). Our study clearly highlights the limitations of this approach: blood cultures were drawn in 82% of the patients with a definitive diagnosis of ni-SIRS, and antibiotic treatment was improperly administered to 52% of them. Conversely, 4% of those with a definitive diagnosis of sepsis/septic shock, at the end of clinical workup, did not receive antibacterial therapy at ED admission despite that fact that early treatment has been demonstrated to decrease patient mortality (16). Further significant disadvantages of the usual practice include the fact the combined use of SIRS criteria with suspect infection has given disappointing results in terms of sensitivity and specificity for the diagnosis of sepsis in ED setting (17). This approach, therefore, is neither accurate nor reproducible as it is subject to further level of adjudication by expert opinion before patients might be included in sepsis studies (18).

The predictive algorithm may contribute in settling major concerns in usual practice to rule out of sepsis/septic shock. On the one hand, the proposed nomogram is fully replicable, on the other, its association with multiple biomarkers misclassified only five of 700 patients (0.7%) with sepsis/septic shock. In all of these FN cases, the source of infection was LRT, and three of five patients were c-infections. Interestingly, median concentrations of procalcitonin and $sPLA_2GIIA$ were significantly inferior in c-infections than in m-infections ($p < 0.000$ and $p < 0.003$, respectively) and for procalcitonin, as previously reported in other studies, in LRT infections than non-LRT infections ($p < 0.000$) (19). It is not surprising that, when the performance of predictive algorithm in ruling out sepsis/septic shock was compared with that of the Usual approach, based on the physician decision to prescribe antibiotics, the NPV of the predictive algorithm resulted significantly better than that of the Usual approach ($p < 0.000$).

Furthermore, the predictive algorithm would have discouraged the use of antibacterial therapy in 44% of ni-SIRS patients, classified as "unlikely" to be infected and treated at

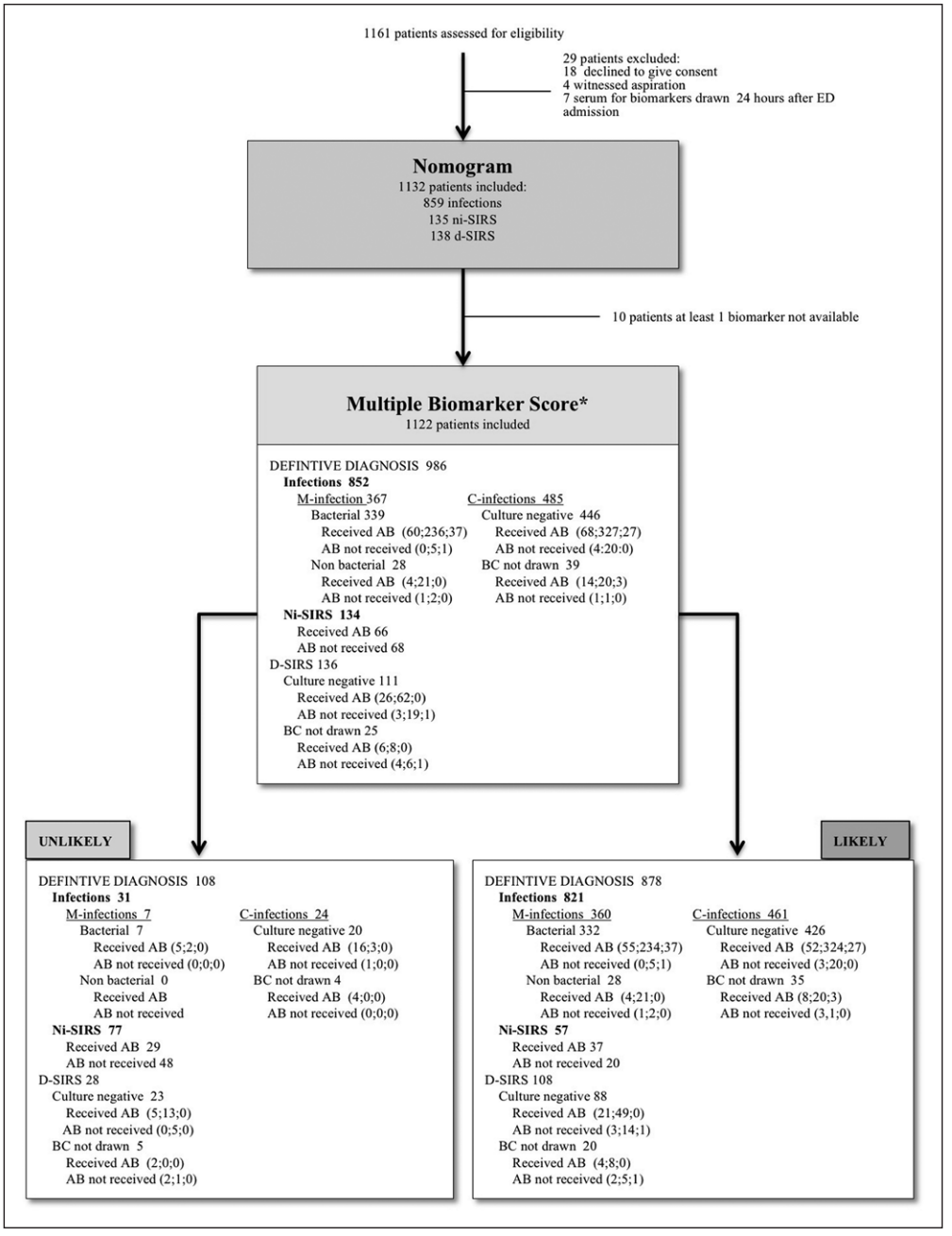

Figure 3. Predictive algorithm: final adjudication of the patients enrolled in inception and validation cohort. *Biomarkers were not available for seven infections, one noninfectious systemic inflammatory response syndrome (ni-SIRS), and two debatable systemic inflammatory response syndrome (d-SIRS). AB = antibacterial therapy, BC = blood cultures, c-infections = clinically documented infections, ED = emergency department, MBMS = Multiple Biomarker Score, m-infections = microbiologically documented infections.

ED admission following the Usual approach. Conversely, the predictive algorithm misclassified 29% patients with ni-SIRS not receiving antimicrobial therapy at ED admission as "likely" to be infected. These patients represent a major concern. FP did not differ from true negative (TN), in terms of age, comorbidities, and disease severity, but in terms of etiology, since new diagnosis or progression of underlying solid cancer was at least seven times more common in FP than in TN. In ni-SIRS patients diagnosed with solid cancer, median concentrations of procalcitonin and $sPLA_2$GIIA were not significantly different from that of infected patients ($p = 0.326$ and $p = 0.329$, respectively). We speculate that in these FP patients classified by the nomogram in the groups 1 and 2 as "unlikely" to be infected, serial biomarker determination might induce prompt discontinuation of antibiotics.

To our knowledge, this is the largest multicenter study aimed at evaluating the role of multiple biomarkers in the diagnosis of infection both in an inception cohort and in a validation cohort. Our study includes patients routinely managed at ED admission but usually excluded from clinical trials because of age, comorbidities, and recent antimicrobial exposure. This "real-life" context is the complex ground that might benefit most from the use of biomarkers, and yet this is the context where it is difficult to identify the role biomarkers may play.

Furthermore, in the group most likely to be infected (i.e., group 3), biomarkers do not improve the diagnostic performance of the nomogram for ruling out infection. These results indicate that biomarkers should be applied only to selected patients with a lower likelihood of infection according to the nomogram (groups 1 and 2), which account for about 30% of our population. This selective use of biomarkers in infection diagnosis has never been highlighted in previous studies and may potentially reduce the costs of biomarker employment in clinical practice.

In agreement with the results of our pilot study (9), $sPLA_2$GIIA exhibited a performance at least comparable with procalcitonin: both contributed to infection diagnosis in the patient cohort with intermediate infection probability (group 2), but only $sPLA_2$GIIA contributed to infection diagnosis in the cohort of patients with the lowest infection probability (group 1). In addition, even if nomogram is not implemented for all the patients with a definitive diagnosis, the

performance of $sPLA_2$GIIA in diagnosing infection, regardless of its spectrum of severity, is better than that of presepsin, sCD25, and sTREM-1 (**Supplementary Fig. 8**, Supplemental Digital Content 1, http://links.lww.com/CCM/D587). The latter result seems to be generalizable to ED since sepsis prevalence in the whole population enrolled in our report is similar to that found in previous studies conducted in the same setting (1).

Finally, the predictive algorithm may assist physicians in ruling out sepsis/septic shock in d-SIRS. It could be hypothesized that in view of the small proportion of sepsis/septic shock misdiagnosed by the predictive algorithm in patients with a definitive diagnosis, 13 blood cultures negative d-SIRS (14%) treated at ED admission for suspect sepsis/septic shock and classified as "unlikely" to be infected, have a negligible risk of infection (Fig. 3). This aspect could constitute a further benefit of the predictive algorithm since our new method would have prevented the use of empirical antibacterial therapy in these patients.

Our study has several limitations. First, SIRS criteria were used to enroll the patients in this study: this may constitute a major concern as one of eight patients had SIRS negative sepsis (20). A debate is ongoing as to whether to include or abandon the combined use of SIRS criteria with suspected infection to diagnose sepsis. Nonetheless, SIRS criteria are still widely used in clinical practice and in observational and interventional trials (21–23). Second, in this study, trauma was infrequent (3% of ni-SIRS and 7% of suspect ni-SIRS in d-SIRS), whereas burns were absent limiting the use of our algorithm to these cohorts of patients. Third, on the basis of retrospective studies, there is a clear relationship between patient outcome and early diagnosis of sepsis (24, 25). Of the biomarkers included in the predictive algorithm, *C*-reactive protein and procalcitonin are available in prime time, whereas $sPLA_2$GIIA is not: this may constitute an obstacle to the widespread application of our strategy. However, the optimal time window between the start of the appropriate antibacterial therapy and outcome is still subject to debate (26). In the absence of prospective studies, it is impossible to be certain as to the exact collocation of the predictive algorithm; however, we cannot a priori exclude its immediate use in clinical practice, especially after a point of care measurement of $sPLA_2$GIIA (27).

In conclusion, this multicenter prospective study supports the usefulness of a reproducible, parsimonious predictive algorithm in ruling out sepsis/septic shock from noninfectious SIRS at ED admission.

ACKNOWLEDGMENTS

We thank Mario Calci (Unit of Emergency Medicine, Palmanova General Hospital, Via Natisone 11, Jalmicco, Udine, Italy), Margherita De Nardo, Andrea Breglia, Silvia Castelletto, Silvia Marizza, Denis Valentini, Alessandra Iudicello, Daniele Peric, Alessandro Occhipinti, Enrico Chendi, Irene Paoli, Lorenza Mamolo, Annalisa Spalluti, Giulia Pivetti, Giovanni Fernandes, Marina De Roia, Michela Zanetti, Pier Andrea Vinci, Andrea Spica, Silvia Matta, Jacopo Lombardi, Selene Degrassi, Anna Pepe, Alberto Peretti, Irene Paoli, Alessia Degrassi, Francesca Spagnol, Valeria Baglio, and Giulia Montanari (Unit of Internal Medicine, Department of Medical Surgical and Health Sciences-University of Trieste, Strada di Fiume 447, Trieste, Italy).

REFERENCES

1. Heffner AC, Horton JM, Marchick MR, et al: Etiology of illness in patients with severe sepsis admitted to the hospital from the emergency department. *Clin Infect Dis* 2010; 50:814–820
2. Rhee C, Kadri SS, Danner RL, et al: Diagnosing sepsis is subjective and highly variable: A survey of intensivists using case vignettes. *Crit Care* 2016; 20:89
3. Phua J, Ngerng W, See K, et al: Characteristics and outcomes of culture-negative versus culture-positive severe sepsis. *Crit Care* 2013;17:R202
4. Llewelyn MJ, Berger M, Gregory M, et al: Sepsis biomarkers in unselected patients on admission to intensive or high-dependency care. *Crit Care* 2013; 17:R60
5. Wacker C, Prkno A, Brunkhorst FM, et al: Procalcitonin as a diagnostic marker for sepsis: A systematic review and meta-analysis. *Lancet Infect Dis* 2013; 13:426–435
6. Pierrakos C, Vincent JL: Sepsis biomarkers: A review. *Crit Care* 2010; 14:R15
7. Kim H, Hur M, Moon HW, et al; GREAT Network: Multi-marker approach using procalcitonin, presepsin, galectin-3, and soluble suppression of tumorigenicity 2 for the prediction of mortality in sepsis. *Ann Intensive Care* 2017; 7:27
8. Gibot S, Béné MC, Noel R, et al: Combination biomarkers to diagnose sepsis in the critically ill patient. *Am J Respir Crit Care Med* 2012; 186:65–71
9. Mearelli F, Fiotti N, Altamura N, et al: Heterogeneous models for an early discrimination between sepsis and non-infective SIRS in medical ward patients: A pilot study. *Intern Emerg Med* 2014; 9:749–757
10. Mearelli F, Orso D, Fiotti N, et al: Sepsis outside intensive care unit: The other side of the coin. *Infection* 2015; 43:1–11
11. Collins GS, Ogundimu EO, Altman DG: Sample size considerations for the external validation of a multivariable prognostic model: A resampling study. *Stat Med* 2016; 35: 214–226
12. Klouche K, Cristol JP, Devin J, et al: Diagnostic and prognostic value of soluble CD14 subtype (Presepsin) for sepsis and community-acquired pneumonia in ICU patients. *Ann Intensive Care* 2016; 6:59
13. Linder A, Christensson B, Herwald H, et al: Heparin-binding protein: An early marker of circulatory failure in sepsis. *Clin Infect Dis* 2009; 49:1044–1050
14. Lang Y, Jiang Y, Gao M, et al: Interleukin-1 receptor 2: A new biomarker for sepsis diagnosis and gram-negative/gram-positive bacterial differentiation. *Shock* 2017; 47:119–124
15. Kelly BJ, Lautenbach E, Nachamkin I, et al; Centers for Disease Control and Prevention (CDC) Prevention Epicenters Program: Combined biomarkers discriminate a low likelihood of bacterial infection among surgical intensive care unit patients with suspected sepsis. *Diagn Microbiol Infect Dis* 2016; 85:109–115
16. Rhodes A, Evans LE, Alhazzani W, et al: International guidelines for management of sepsis and septic shock. *Crit Care Med* 2017;45:486–552.
17. Jaimes F, Garcés J, Cuervo J, et al: The systemic inflammatory response syndrome (SIRS) to identify infected patients in the emergency room. *Intensive Care Med* 2003; 29:1368–1371
18. Freund Y, Lemachatti N, Krastinova E, et al; French Society of Emergency Medicine Collaborators Group: Prognostic accuracy of sepsis-3 criteria for in-hospital mortality among patients with suspected infection presenting to the emergency department. *JAMA* 2017; 317:301–308
19. Wong HR, Liu KD, Kangelaris KN, et al: Performance of interleukin-27 as a sepsis diagnostic biomarker in critically ill adults. *J Crit Care* 2014; 29:718–722

20. Kaukonen KM, Bailey M, Pilcher D, et al: Systemic inflammatory response syndrome criteria in defining severe sepsis. *N Engl J Med* 2015; 372:1629–1638
21. Quinten VM, van Meurs M, Ter Maaten JC, et al: Trends in vital signs and routine biomarkers in patients with sepsis during resuscitation in the emergency department: A prospective observational pilot study. *BMJ Open* 2016; 6:e009718
22. Peake SL, Delaney A, Bailey M, et al: Goal-directed resuscitation for patients with early septic shock. *N Engl J Med* 2014; 371:1496–1506
23. Churpek MM, Snyder A, Han X, et al: Quick sepsis-related organ failure assessment, systemic inflammatory response syndrome, and early warning scores for detecting clinical deterioration in infected patients outside the intensive care unit. *Am J Respir Crit Care Med* 2017; 195:906–911
24. Kumar A, Roberts D, Wood KE, et al: Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; 34: 1589–1596
25. Liu VX, Fielding-Singh V, Greene JD, et al: The timing of early antibiotics and hospital mortality in sepsis. *Am J Respir Crit Care Med* 2017; 196:856–863
26. Singer M: Antibiotics for sepsis: Does each hour really count, or is it incestuous amplification? *Am J Respir Crit Care Med* 2017; 196:800–802
27. van Houten CB, de Groot JAH, Klein A, et al: A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): A double-blind, multicentre, validation study. *Lancet Infect Dis* 2017; 17:431–440