Can biomarkers improve the rational use of antibiotics?

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Purpose of review
We aim to review recent literature about the use of biomarkers to guide the initiation and duration of antibiotic treatments for suspected bacterial infections.

Recent findings
Several good quality meta-analyses show that procalcitonin can be safely used to guide antibiotic-related decisions, especially for respiratory infections, thereby decreasing unnecessary antibiotic exposure. Furthermore, the use of CRP-based algorithms to guide antibiotic initiation in primary care patients with acute respiratory infections is associated with a reduction in antibiotic use without an increase in adverse events. Regarding neutrophil CD64 and serum amyloid A, more good-quality evidence is needed to assess their utility as biomarkers to tailor antibiotic use. Finally, transcriptomics, metabolomics and proteomics are promising tools for the development of tests to differentiate specific host responses to viral, bacterial and noninfectious stimuli, but such tests still need further validation.

Summary
Evidence shows that the use of biomarkers, procalcitonin and CRP, can safely reduce unnecessary antibiotic prescriptions in certain infectious syndromes. The clinical utility of host-based strategies such as transcriptomics, metabolomics and proteomics for the diagnosis of infectious diseases has yet to be evaluated, as well as considerations such as costs, technical complexity and result turnaround time.

Keywords
antibiotics, bacterial infection, biomarkers, C-reactive protein, metabolomics, neutrophil CD64, procalcitonin, proteomics, serum amyloid A, transcriptomics

INTRODUCTION

Improving the rational use of antibiotics is a worldwide priority. Studies show that antibiotic use and treatment duration drive bacterial resistance [1,2]. Initiation of antibiotic treatments is still commonly based on clinical syndromes and ancillary tests that are imperfect diagnostic tools. Furthermore, most recommendations about treatment duration still follow a 'one-size fits all' model because traditional diagnostic methods are unable to identify in real-time, the moment when infections have been eradicated and antibiotics are no longer needed [3]. To overcome this limitation, the use of biomarkers has been proposed to guide antibiotic treatment initiation and duration, with the goal of personalizing antibiotic use according to patient's needs.

In the last decades, many biomarkers have been proposed to guide antibiotic use. Among the biomarkers that were shown to be useful at bedside are procalcitonin, C-reactive protein (CRP), neutrophil CD64 (nCD64) and serum amyloid A. Most recently, the use of transcriptomics, metabolomics and proteomics has been studied to enhance the diagnosis of bacterial infection and guide antibiotic initiation.

PROCALCITONIN

Procalcitonin is a prehormone produced by the thyroid C cells [4]. In septic patients, it acts as a secondary mediator, further stimulating the release

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KEY POINTS

- There is good-quality evidence showing that procalcitonin can be safely used to guide antibiotic-related decisions, especially for respiratory infections.
- The use of CRP to guide antibiotic initiation in primary care patients with acute respiratory infections is associated with a reduction in antibiotic use without an increment in adverse events.
- More good-quality evidence is needed to assess the utility of nCD64 and serum amyloid A as biomarkers to tailor antibiotic use.
- Diagnostic strategies based on transcriptomics, metabolomics and proteomics show great promise in their ability to distinguish specific host responses to viral, bacterial and noninfectious stimuli, yet need further validation.

of pro-inflammatory cytokines (IL-6, IL-1β and TNF-α) and activating macrophages, which leads to the amplification of the initial inflammatory response [5–8]. Procalcitonin levels are associated with the severity of the infectious process and clinical picture; they peak between 6 and 13.5 h after the infectious insult and reach a plateau as of 24 h (half-life of 22.5 h). Sequential measurement of procalcitonin is recommended to guide antibiotic treatment duration. However, there is no consensus about the optimal cut-offs to be used for antibiotic-related decisions.

From January 2017 to March 2018, five good-quality meta-analyses on procalcitonin to tailor antibiotic use in critically ill adult patients were published [9–13]. Using different combinations of basically the same group of published studies, all meta-analyses showed that procalcitonin-based algorithms for both initiation and cessation of antibiotics are associated with a decrease in antibiotic exposure (from −1.26 days [95% CI −1.98 to −0.54] to −1.83 days [95% CI −2.51 to −1.15]). Importantly, three of the aforementioned meta-analyses focused on randomized controlled trials (RCTs) where procalcitonin was exclusively used to guide antibiotic cessation and found similar results (from −1.49 days [95% CI −2.27 to −0.71] to −1.66 days [95% CI −2.36 to −0.96]).[9–11] Finally, although four meta-analyses indicated that the use of procalcitonin algorithms is not associated with increased mortality, Huang *et al.* [10] showed that their use to tailor antibiotic cessation may actually decreases mortality risk [relative risk (RR) 0.86; 95% CI 0.76–0.98].

In 2017, Chu *et al.* [14▪▪] published a retrospective cohort study including 20 750 critically ill adult patients with sepsis to determine the effectiveness of procalcitonin algorithms to guide antibiotic treatment duration in the real world, i.e., outside a clinical trial setting. They showed that measuring procalcitonin was associated with longer duration of antibiotic treatments (RR 1.17; 95% CI 1.15–1.18) and with increased risk for *Clostridium difficile* infection [odds ratio (OR) 1.74; 95% CI 1.18–2.55]. These results can be explained by the fact that sequential measurement of procalcitonin was performed in less than one-third of patients for whom this biomarker was used. Procalcitonin was measured infrequently and most often checked only once, that is, no result trend was available to guide antibiotic use. It is, therefore, possible that physicians based their decisions on isolated (and probably abnormal) values. This underscores the need for careful real-world implementation of procalcitonin algorithms based on sequential measurements, as was successfully evaluated in clinical trials.

Schuetz *et al.* [15▪▪] published in 2018, a patient-level meta-analysis including 26 RCTs and 6708 patients to determine the safety of using procalcitonin algorithms to guide antibiotic decisions in adult patients with respiratory infections. Procalcitonin was associated with a reduction in antibiotic exposure (−2.43 days; 95% CI −2.71 to −2.15), antibiotic initiation (OR 0.27; 95% CI 0.24–0.32), antibiotic-related side effects (OR 0.68; 95% CI 0.57–0.82), and overall 30-day mortality (OR 0.83; 95% CI 0.70–0.99), without increasing the risk for treatment failure (OR 0.90; 95% CI 0.80–1.01). Similar results were observed in primary care, emergency departments and ICUs. Nevertheless, distinct effect mechanisms were observed in different settings: in primary care, the main mechanism was a decrease in antibiotic initiation, whereas in ICUs and in patients with pneumonia a shortening of treatment duration was seen.

Regarding newborns, Stocker *et al.* [16▪] published in 2017 the NeoPIns RCT in which 1710 neonates born after 34 weeks of gestational age with early-onset sepsis were randomized to procalcitonin-guided decision-making or standard care-based antibiotic treatment. The study algorithm proposed stopping antibiotics after two consecutive procalcitonin measurements within postbirth normal range (based on previous studies from the same group). Results of the intention-to-treat analysis showed that such an algorithm led to a statistically significant decrease in antibiotic treatment duration (−9.9 h; $P < 0.0001$), without a difference in the proportion of reinfection between groups (0.7 vs. 0.6%). Of note, protocol deviation was observed in approximately 25% of RCT participants; whereas the continuation of antibiotics despite protocol

recommendation was more commonly seen in the procalcitonin group (12 vs. 7%), stopping antibiotics earlier than recommended was more frequent in the standard care group (14 vs. 18%). Importantly, in the per-protocol analysis, a statistically and clinically relevant difference in antibiotic duration between groups was observed [40.0 h (95% CI 36.0–46.5) vs. 61.5 h (95% CI 59.0–64.5)], favoring the use of procalcitonin algorithms.

Finally, the before–after single-center study of Ross *et al.* [17▪] focused on the effectiveness of using procalcitonin to guide antibiotic treatments for critically ill children in the pediatric critical care setting. Similarly to the aforementioned results of Chu *et al.*, this study showed that procalcitonin was infrequently measured, with only 28% of patients having two or more measurements. Furthermore, it demonstrated that, in the absence of the implementation of a standardized algorithm to guide indication and frequency of procalcitonin testing and clinical decision-making, the use of procalcitonin led to an increase in antibiotic continuation after 72 h [39.9 vs. 51.1%; risk difference 11.1% (95% CI 4.9–17.3)]. Nevertheless, procalcitonin significantly decreased antibiotic initiation [85.2 vs. 64.9%; risk difference 20.2% (95% CI 15.4–24.9)].

C-REACTIVE PROTEIN

CRP is an acute-phase reactant synthetized mainly in the liver and secreted in response to inflammation [18]. Its secretion is regulated by cytokines, with levels starting to rise 6 h after the initial stimulus and reaching their peak at 48 h [18,19]. In cases of infection, CRP stimulates bacterial phagocytosis by binding bacterial polysaccharides and functioning as an opsonin for neutrophils and macrophages, and by activating the classical complement pathway [18,20–23]. Once the inflammation cause is eradicated, hepatocytes catabolize CRP and rapidly remove it from circulation [19,24–26]. In healthy adults, the median CRP concentration is 1.5 mg/l, with levels above 100 mg/l being associated with bacterial infections [27–29]. In healthy term neonates, CRP normal levels are associated with postnatal age, with median levels gradually increasing from birth (0.4 mg/l) to 48 h postpartum (2.7 mg/l), and then declining at 96 h (1.4 mg/l) [30,31]. Importantly, CRP values above 10 mg/l, the cut-off most often used to diagnose neonatal sepsis, are not uncommonly observed during the first 72 h after birth [30,31].

In 2014, Aabenhus *et al.* published a meta-analysis on six RCTs that studied the use of CRP to guide antibiotic initiation in primary care patients with acute respiratory infections [32]. They showed that CRP reduced the prescription of antibiotics in the index medical consultation (RR 0.78; 95% CI 0.66–0.92) and that this effect persisted 28 days postconsultation (RR 0.80; 95% CI 0.67–0.96). Furthermore, there were no differences between groups regarding adverse events, symptoms durations, severity at 7 days and re-consultation. Following this meta-analysis, Do *et al.* [33] published in 2016, a new RCT including 2037 adult and pediatric primary care patients with a suspected diagnosis of acute respiratory infection who were randomized to receive either routine care or CRP testing to guide initiation of antibiotics (cut-offs recommending against antibiotic prescription were $\leq$10 mg/l for children 1–5 years and $\leq$20 mg/l for patients 6–65 years). CRP testing led to an adjusted absolute risk difference of −12.5% (95% CI −16.6 to −8.6) in antibiotic use within 2 weeks of follow-up, without negatively affecting time to infection resolution (hazard ratio 0.92 and 95% CI 0.84–1.02) or adverse events. Of note, there was significant heterogeneity regarding the effect of the intervention in different centers.

Finally, Downes *et al.* [34] performed a prospective cohort study including 85 critically ill children less than 18 years of age to assess the performance of different biomarkers in identifying patients at low risk of bacterial infection. Results showed statistically significant differences between the median CRP levels of patients with and without bacterial infection, respectively, at time 0 (8.7 vs. 2.2 mg/dl; $P < 0.001$), 24 h (9.2 vs. 3.7 mg/dl; $P = 0.002$), and 48 h (6.1 vs. 2.8 mg/dl; $P = 0.05$). The combination of CRP (cut-off 5.0 mg/dl) and serum amyloid A (cut-off 15 μg/ml) at time 0 yielded a negative predictive value (NPP) of 96% (95% CI 88–100) and a specificity of 54% (95% CI 42–66). These results were superior to those obtained by combining CRP (cut-off 4.0 mg/dl) and procalcitonin (cut-off 1.75 ng/ml) at time 0 [NPP 90% (95% CI 79–100); specificity 43% (95% CI 30–55)]. The authors estimated that the use of the aforementioned combinations could have, respectively, reduced antibiotic treatment by 115 and 73 days for patients without a bacterial infection.

NEUTROPHIL CD64

nCD64 is a leukocyte surface molecule expressed mainly on monocytes and macrophages, but only to a very low extent on resting polymorphonuclear neutrophils [35]. It is shown to strongly upregulate within 4–6 h by cytokines such as interferon-c (IFN-c) and granulocyte colony stimulating factor (G-CSF) [36–43]. nCD64 expression is considered to be a very early phase of a host's immune response to bacterial infection. It starts to increase in a graded

manner about 1 h after invasion, but it substantially decreases within 48 h of cessation of IFN and returns to baseline levels by day 7 of antibiotic treatment [44–46]. Currently, nCD64 is quickly and precisely measured by flow cytometric technology using minimal blood volumes, but optimal cut-off values have yet to be determined [47,48].

In the last 8 years, two meta-analyses of observational studies have been published on the accuracy of nCD64 as an early diagnostic biomarker in bacterial infections [49,50]. Pooled sensitivity of nCD64 for the diagnosis of early onset of bacterial infections ranged from 76% (95% CI 74–78) to 79% (95% CI 70–86), whereas pooled specificity was between 85% (95% CI 83–86) and 91% (95% CI 85–95). In both studies, sensitivity and specificity improved in the culture proven infection subgroup. Importantly, both meta-analyses have pooled results from adult, pediatric and neonatal studies, which may explain their high level of heterogeneity.

Concerning newborns specifically, Shi *et al.* [51] published a meta-analysis in 2016 including 17 studies (2213 newborns) to determine the diagnostic accuracy of nCD64 in early neonatal sepsis. Pooled sensitivity and specificity for these studies were 77% (95% CI 74–79), and 74% (95% CI 72–75), respectively. Once again, a high level of heterogeneity was observed. Importantly, subgroup analyses showed that sensitivity and specificity in preterm infants and in the clinically diagnosed infection subgroups were lower. Furthermore, Lynema *et al.* [42] evaluated the use of nCD64 to guide antibiotic-related decisions in neonatal sepsis. In phase I of this single-center study, authors derived the nCD64 index cut-off (2.3) using a population of 108 patients less than 12 months old. This cut-off presented a sensitivity of 100% and a specificity of 93% for neonatal sepsis. In phase II, the nCD64 index cut-off was provided to neonatal ICU staff to be used as an adjunct tool to help with antibiotic-related decisions (initiation and duration of treatment). Crude results of 305 evaluations showed that the mean duration of antibiotic treatment in neonatal ICU patients with abnormal nCD64 index was longer compared with the group presenting normal nCD64 index (7.7 ± 5.1 vs. 4.3 ± 4.7 days; P not provided). Importantly, no information about the comparability or the clinical evolution of the above groups was presented. Thus, we cannot draw firm conclusions about the safety or efficacy of nCD64 to guide antibiotic use in neonates.

SERUM AMYLOID A

Serum amyloid A groups a family of apolipoproteins that are mainly produced by the liver. They are regarded as acute-phase proteins, whose expression is induced by inflammatory cues, including IL-1β and IL-6 [52,53]. The previously mentioned study of Downes *et al.* showed that the median levels of serum amyloid A of patients with and without bacterial infection were statistically different at time 0 (18.9 vs. 11.1 μg/ml; $P = 0.009$) and at 24 h (11.7 vs. 7.3 μg/ml; $P = 0.03$). Despite the superiority of the results obtained by combining CRP and serum amyloid A, these were disregarded in favor of the CRP and procalcitonin algorithm because serum amyloid A is not currently available for rapid clinical decision-making at most institutions.

TRANSCRIPTOMICS, PROTEOMICS AND METABOLOMICS

The development of advanced laboratory techniques that characterize an entire class of biological molecules has led to new fields of study (e.g. transcriptomics, metabolomics, proteomics) that can identify and quantify host responses to external stressors. On the basis of the premise that host responses to specific stresses may be stereotypical, advanced mathematical modeling tools such as machine learning techniques can be used to analyze the large amounts of data produced by 'omics' measurements to generate 'signatures' induced by different states of disease or health [54]. Omic signatures have thus been developed with the aim of personalizing therapy though improved diagnosis (e.g. early pathogen identification) and prognosis (e.g. disease severity classification).

During a discovery phase, analyses are run on biological samples from participants in human experimental challenge studies or from a population that has already been adjudicated regarding a diagnosis to derive omics signatures for infectious diseases [55]. The signature that distinguishes diseased from nondiseased state in the original population must then be validated in other cohorts to test its generalizability. To date, many omics signatures have been derived for the diagnosis of infection and to aid antimicrobial treatment decisions. Examples include proteomics to diagnose viral respiratory infection [56]; transcriptomics to distinguish viral, bacterial and noninfectious illness, to diagnose pulmonary tuberculosis, to diagnose bacterial infection in febrile neonates, and to discriminate between sepsis and infection-negative systemic inflammation [57–61]; and breath or urine metabolomics for respiratory infection [62,63]. The SeptiCyte LAB is the only the FDA-cleared omics-based test, but is not yet commercially available [61]. Prospective evaluations of clinical utility of such panels for

diagnosis and guidance of antimicrobial have not been published.

CONCLUSION

Traditional infectious disease diagnostic approaches have focused on pathogen detection and characterization. However, evidence shows that the use of biomarkers such as CRP and procalcitonin can safely reduce unnecessary antibiotic prescriptions in certain syndromes. Recently, host-based strategies such as transcriptomics, metabolomics, proteomics are being validated for infectious disease diagnosis. Their ability to discriminate between bacterial and other causes of disease (e.g. viral or noninfectious) opens exciting new avenues for antimicrobial stewardship. However, their clinical utility has not been evaluated and considerations such as costs, technical complexity and result turnaround time need to be addressed.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Chastre J, Wolff M, Fagon JY, *et al.,* PneumA Trial Group. Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial. JAMA 2003; 290:2588–2598.
2. Guillemot D, Carbon C, Balkau B, *et al.* Low dosage and long treatment duration of beta-lactam: risk factors for carriage of penicillin-resistant Streptococcus pneumoniae. JAMA 1998; 279:365–370.
3. Llewelyn MJ, Fitzpatrick JM, Darwin E, *et al.* The antibiotic course has had its day. BMJ 2017; 358:j3418.
4. Schneider H, Lam QT. Procalcitonin for the clinical laboratory: a review. Pathology 2007; 39:383–390.
5. Liappis AP, Gibbs KW, Nylen ES, *et al.* Exogenous procalcitonin evokes a proinflammatory cytokine response. Inflamm Res 2011; 60:203–207.
6. Wei JX, Verity A, Garle M, *et al.* Examination of the effect of procalcitonin on human leucocytes and the porcine isolated coronary artery. Brit J Anaesth 2008; 100:612–621.
7. Wiedermann FJ, Kaneider N, Egger P, *et al.* Migration of human monocytes in response to procalcitonin. Crit Care Med 2002; 30:1112–1117.
8. Steinwald PM, Whang KT, Becker KL, *et al.* Elevated calcitonin precursor levels are related to mortality in an animal model of sepsis. Crit Care 1999; 3:11–16.
9. Andriolo BN, Andriolo RB, Salomão R, Atallah ÁN, *et al.* Effectiveness and safety of procalcitonin evaluation for reducing mortality in adults with sepsis, severe sepsis or septic shock. Cochrane Database Syst Rev 2017; 1:Cd010959.
10. Huang HB, Peng JM, Weng L, *et al.* Procalcitonin-guided antibiotic therapy in intensive care unit patients: a systematic review and meta-analysis. Ann Intensive Care 2017; 7:114.
11. Iankova I, Thompson-Leduc P, Kirson NY, *et al.* Efficacy and safety of procalcitonin guidance in patients with suspected or confirmed sepsis: a systematic review and meta-analysis. Crit Care Med 2018; 46:691–698.
12. Lam SW, Bauer SR, Fowler R, Duggal A. Systematic review and meta-analysis of procalcitonin-guidance versus usual care for antimicrobial management in critically ill patients: focus on subgroups based on antibiotic initiation, cessation, or mixed strategies. Crit Care Med 2018; 46:684–690.
13. Zhang T, Wang Y, Yang Q, Dong Y. Procalcitonin-guided antibiotic therapy in critically ill adults: a meta-analysis. BMC Infect Dis 2017; 17:514.
14. ■■ Chu DC, Mehta AB, Walkey AJ. Practice patterns and outcomes associated with procalcitonin use in critically ill patients with sepsis. Clin Infect Dis 2017; 64:1509–1515.

This large retrospective cohort study shows the effect of using procalcitonin to guide antibiotic use in the real critical care setting. It highlights the importance of implementing standardized algorithms that use sequential measurement of procalcitonin to determine duration ot antibiotic treatment.

15. ■■ Schuetz P, Wirz Y, Sager R, *et al.* Effect of procalcitonin-guided antibiotic treatment on mortality in acute respiratory infections: a patient level meta-analysis. Lancet Infect Dis 2018; 18:95–107.

This large and well conducted meta-analysis provides important data on the effectiveness, and especially on the safety of using procalcitonin algorithms to guide antibiotic use in respiratory infections.

16. ■ Stocker M, van Herk W, El Helou S, *et al.* Procalcitonin-guided decision making for duration of antibiotic therapy in neonates with suspected early-onset sepsis: a multicentre, randomised controlled trial (NeoPIns). Lancet 2017; 390:871–881.

This is the largest RCT on the use of procalcitonin algorithms to guide antibiotic use in neonatal patients.

17. ■ Ross RK, Keele L, Kubis S, *et al.* Effect of the procalcitonin assay on antibiotic use in critically ill children. J Pediatric Infect Dis Soc 2018. [Epub ahead of print]).

This large retrospective study is another example of the importance of implementing standardized algorithms that use sequential measurement of procalcitonin to determine duration ot antibiotic treatment.

18. Ansar W, Ghosh S. C-reactive protein and the biology of disease. Immunologic Research 2013; 56:131–142.
19. Pepys M, Hirschfield G. C-reactive protein: a critical update. J Clin Invest 2003; 111:1805–1812.
20. Marnell LL, Mold C, Volzer MA, *et al.* C-reactive protein binds to Fc gamma RI in transfected COS cells. J Immunol 1995; 155:2185–2193.
21. Kilpatrick JM, Volanakis JE. Opsonic properties of C-reactive protein. Stimulation by phorbol myristate acetate enables human neutrophils to phagocytize C-reactive protein-coated cells. J Immunol 1985; 134:3364–3370.
22. Mortensen RF, Osmand AP, Lint TF, Gewurz H. Interaction of C-reactive project protein with lymphocytes and monocytes: complement-dependent adherence and phagocytosis. J Immunol 1976; 117:774–781.
23. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. J Immunol 1974; 112:2135–2147.
24. Ehl S, Gering B, Bartmann P, *et al.* C-reactive protein is a useful marker for guiding duration of antibiotic therapy in suspected neonatal bacterial infection. Pediatrics 1997; 99:216–221.
25. Luna C. C-reactive protein in pneumonia: let me try again. Chest 2004; 125:1192–1195.
26. Al-Zwaini E. C-reactive protein: a useful marker for guiding duration of antibiotic therapy in suspected neonatal septicaemia? East Mediterr Health J 2009; 15:269–275.
27. Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. Clin Chim Acta 1981; 117:13–23.
28. Morley JJ, Kushner I. Serum C-reactive protein levels in disease. Ann NY Acad Sci 1982; 389:406–418.
29. Rifai N, Ridker PM. Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. Clin Chem 2003; 49:666–669.
30. Chiesa C, Natale F, Pascone R, *et al.* C reactive protein and procalcitonin: reference intervals for preterm and term newborns during the early neonatal period. Clin Chim Acta 2011; 412:1053–1059.
31. Perrone S, Lotti F, Longini M, *et al.* Creactive protein in healthy term newborns during the first 48 h of life. Arch Dis Child Fetal Neonatal Ed 2017; 103:F163–F166.
32. Aabenhus R, Jensen JU, Jørgensen KJ, *et al.* Biomarkers as point-of-care tests to guide prescription of antibiotics in patients with acute respiratory infections in primary care. Cochrane Database Syst Rev 2014; (11):CD010130.
33. Do NT, Ta NT, Tran NT, *et al.* Point-of-care C-reactive protein testing to reduce inappropriate use of antibiotics for nonsevere acute respiratory infections in Vietnamese primary healthcare: a randomised controlled trial. Lancet Glob Health 2016; 4:e633–e641.

34. Downes KJ, Weiss SL, Gerber JS, *et al.,* Centers for Disease Control and Prevention Epicenters Program. A pragmatic biomarker-driven algorithm to guide antibiotic use in the pediatric intensive care unit: the Optimizing Antibiotic Strategies in Sepsis (OASIS) Study. J Pediatric Infect Dis Soc 2017; 6:134–141.

35. Egger G, Aigner R, Glasner A, *et al.* Blood polymorphonuclear leukocyte migration as a predictive marker for infections in severe trauma: comparison with various inflammation parameters. Intensive Care Med 2004; 30:331–334.

36. Layseca-Espinosa E, Pérez-González LF, Torres-Montes A. Expression of CD64 as a potential marker of neonatal sepsis. Pediatr Allergy Immunol 2002; 13:319–327.

37. Ng PC, Li G, Chui KM, *et al.* Neutrophil CD64 is a sensitive diagnostic marker for early-onset neonatal infection. Pediatr Res 2004; 56:796–803.

38. Bhandari V, Wang C, Rinder C, Rinder H. Hematologic profile of sepsis in neonates: neutrophil CD64 as a diagnostic marker. Pediatrics 2008; 121:129–134.

39. Groselj-Grenc M, Ihan A, Derganc M. Neutrophil and monocyte CD64 and CD163 expression in critically ill neonates and children with sepsis: comparison of fluorescence intensities and calculated indexes. Mediators Inflamm 2008; 2008:202646.

40. Streimish I, Bizzarro M, Northrup V, *et al.* Neutrophil CD64 as a diagnostic marker in neonatal sepsis. Pediatr Infect Dis J 2012; 31:777–781.

41. Du J, Li L, Dou Y, *et al.* Diagnostic utility of neutrophil CD64 as a marker for early-onset sepsis in preterm neonates. PLoS One 2014; 9:e102647.

42. Lynema S, Marmer D, Hall ES, *et al.* Neutrophil CD64 as a diagnostic marker of sepsis: impact on neonatal care. Am J Perinatol 2015; 32:331–336.

43. Nuutila J, Hohenthal U, Laitinen I, *et al.* Quantitative analysis of complement receptors, CR1 (CD35) and CR3 (CD11b), on neutrophils improves distinction between bacterial and viral infections in febrile patients: comparison with standard clinical laboratory data,. J Immunol Methods 2006; 315:191–201.

44. Schiff DR, Martin J, Davis TR, *et al.* Increased Phagocyte FcγRI expression and improved Fcγ-receptor–mediated phagocytosis after in vivo recombinant human interferon-γ treatment of normal human subjects. Blood 1997; 90:3187–3194.

45. Hoffmeyer F, Witte K, Schmidt KE. The high-affinity FcγRI on PMN: regulation of expression and signal transduction. Immunology 1997; 92:544–552.

46. van der Meer W, Pickkers P, Scott CS. Hematological indices, inflammatory markers and neutrophil CD64 expression: comparative trends during experimental human endotoxemia. J Endotoxin Res 2007; 13:94–100.

47. Ng PC, Lam HS. Diagnostic markers for neonatal sepsis. Curr Opin Pediatr 2006; 18:125–131.

48. Nuutila J, Hohenthal U, Laitinen I, *et al.* Simultaneous quantitative analysis of FcgammaRI (CD64) expression on neutrophils and monocytes: a new, improved way to detect infections. J Immunol Methods 2007; 328:189–200.

49. Li S, Huang X, Chen Z, *et al.* Neutrophil CD64 expression as a biomarker in the early diagnosis of bacterial infection: a meta-analysis. Int J Infect Dis 2013; 17:e12–e23.

50. Cid J, Aguinaco R, Sánchez R, *et al.* Neutrophil CD64 expression as marker of bacterial infection: a systematic review and meta-analysis. J Infect 2010; 60:313–319.

51. Shi J, Tang J, Chen D. Meta-analysis of diagnostic accuracy of neutrophil CD64 for neonatal sepsis. Ital J Pediatr 2016; 42:57.

52. Cetinkaya M, Ozkan H, Köksal N, *et al.* Comparison of serum amyloid A concentrations with those of C-reactive protein and procalcitonin in diagnosis and follow-up of neonatal sepsis in premature infants. J Perinatol 2009; 29:225–231.

53. Arnon S, Litmanovitz I, Regev R. The prognostic virtue of inflammatory markers during late-onset sepsis in preterm infants. J Perinat Med 2004; 32:176–180.

54. Tsalik EL, Bonomo RA, Fowler VG Jr. New molecular diagnostic approaches to bacterial infections and antibacterial resistance. Annu Rev Med 2018; 69:379–394.

55. Barton AJ, Hill J, Pollard AJ, Blohmke CJ. Transcriptomics in human challenge models. Front Immunol 2017; 8:1839.

56. Burke TW, Henao R, Soderblom E, *et al.* Nasopharyngeal protein biomarkers of acute respiratory virus infection. EBioMedicine 2017; 17:172–181.

57. Sweeney TE, Wong HR, Khatri P. Robust classification of bacterial and viral infections via integrated host gene expression diagnostics. Sci Transl Med 2016; 8:346ra91.

58. Tsalik EL, Henao R, Nichols M, *et al.* Host gene expression classifiers diagnose acute respiratory illness etiology. Sci Transl Med 2016; 8:322ra11.

59. Sweeney TE, Braviak L, Tato CM, Khatri P. Genome-wide expression for diagnosis of pulmonary tuberculosis: a multicohort analysis. Lancet Respir Med 2016; 4:213–224.

60. Mahajan P, Kuppermann N, Mejias A, *et al.* Association of RNA biosignatures with bacterial infections in febrile infants aged 60 days or younger. JAMA 2016; 316:846–857.

61. McHugh L, Seldon TA, Brandon RA. A molecular host response assay to discriminate between sepsis and infection-negative systemic inflammation in critically ill patients: discovery and validation in independent cohorts. PLoS Med 2015; 12:e1001916.

62. Ahmed WM, Lawal O, Nijsen TM. Exhaled volatile organic compounds of infection: a systematic review. ACS Infect Dis 2017; 3:695–710.

63. Adamko DJ, Saude E, Bear M, *et al.* Urine metabolomic profiling of children with respiratory tract infections in the emergency department: a pilot study. BMC Infect Dis 2016; 16:439.