J Antimicrob Chemother 2011; **66** Suppl 2: ii33–ii40
doi:10.1093/jac/dkq523

Journal of Antimicrobial Chemotherapy

Diagnostic and prognostic biomarkers of sepsis in critical care

Savitri Kibe[1], Kate Adams[1] and Gavin Barlow[1,2]*

[1]*Department of Infection & Tropical Medicine, Castle Hill Hospital, Hull and East Yorkshire Hospitals NHS Trust, Cottingham, East Yorkshire, UK;* [2]*Hull York Medical School, Hull, UK*

*Corresponding author. Tel: +44-1482-875875 ext. 2267; Fax: +44-1482-622492; E-mail: gavin.barlow@hey.nhs.uk

Sepsis is a leading cause of mortality in critically ill patients. Delay in diagnosis and initiation of antibiotics have been shown to increase mortality in this cohort. However, differentiating sepsis from non-infectious triggers of the systemic inflammatory response syndrome (SIRS) is difficult, especially in critically ill patients who may have SIRS for other reasons. It is this conundrum that predominantly drives broad-spectrum antimicrobial use and the associated evolution of antibiotic resistance in critical care environments. It is perhaps unsurprising, therefore, that the search for a highly accurate biomarker of sepsis has become one of the holy grails of medicine. Procalcitonin (PCT) has emerged as the most studied and promising sepsis biomarker. For diagnostic and prognostic purposes in critical care, PCT is an advance on C-reactive protein and other traditional markers of sepsis, but is not accurate enough for clinicians to dispense with clinical judgement. There is stronger evidence, however, that measurement of PCT has a role in reducing the antibiotic exposure of critical care patients. For units intending to incorporate PCT assays into routine clinical practice, the cost-effectiveness of this is likely to depend on the pre-implementation length of an average antibiotic course and the subsequent impact of implementation on emerging antibiotic resistance. In most of the trials to date, the average baseline duration of the antibiotic course was longer than is currently standard practice in many UK critical care units. Many other biomarkers are currently being investigated. To be highly useful in clinical practice, it may be necessary to combine these with other novel biomarkers and/or traditional markers of sepsis.

Keywords: procalcitonin, intensive care, antibiotic stewardship

Introduction

Bacterial infections and sepsis are common problems in critically ill patients, both as a cause of admission to critical care units and healthcare-associated infection following admission. It is now widely accepted that starting effective antibiotic therapy early in the course of an infection decreases morbidity and mortality in this cohort of patients.[1] Balanced against this is the need for antibiotic stewardship in order to combat escalating rates of antibiotic resistance. Given that a high proportion of critically ill patients have the systemic inflammatory response syndrome (SIRS), the ability to accurately distinguish between SIRS and sepsis (sepsis is defined as SIRS as a result of bacterial infection) has become one of the holy grails of medicine. It is therefore unsurprising that there has been considerable interest, debate and, sometimes, argument over the last two decades regarding the use of biomarkers to achieve this goal. Proposed sepsis biomarkers have included procalcitonin (PCT),[2] various interleukins (ILs),[2] eosinophil count,[3] adrenomedullin (ADM) and pro-ADM,[4] atrial natriuretic peptide (ANP) and pro-ANP,[5] pro-vasopressin (copeptin),[4] interferon-γ (IFN-γ),[6] triggering receptor expressed on myeloid cells 1 (TREM-1),[6] and resistin.[7] Of these and others, PCT has been the most studied and, in some countries, is now being included in routine clinical practice and guideline recommendations.[8] Consequently, this review will concentrate primarily on the potential uses of PCT in critical care, but will also briefly discuss other candidate biomarkers. The interpretation of some studies is difficult due to variations in PCT assays and predictive cut-off points used. In the UK, PCT has not been widely employed to date, but we are aware of the increasing use of the newly available, highly sensitive assays, including in our own hospital.

To be clinically useful, a sepsis biomarker needs to provide information additional to that already available from established clinical assessments (e.g. history and examination) and investigations [e.g. C-reactive protein (CRP) and white cell count (WCC)]. To do this, it needs to be able to differentiate accurately bacterial infection from non-infective and viral causes of SIRS, and be available in a timely and cost-effective manner. The utility of a biomarker is potentially further enhanced if it can indicate the severity of infection and is able to act as a guide to the effectiveness of therapy. PCT has been studied in critical care patients both as a diagnostic and prognostic test, and for its ability to aid antibiotic stewardship by safely shortening antibiotic course length. Although there is some overlap between studies, the literature for each of these areas will be reviewed separately. Experimental study designs that randomize critically ill patients to biomarker-guided clinical management or 'usual

care' are clearly the gold-standard research method for the investigation of biomarkers. However, such studies present considerable methodological challenges in this cohort of patients (e.g. in the initial design and subsequent recruitment of patients). Much of the evidence to date is, therefore, observational in nature, although some randomized trials have been performed and are ongoing.

This article does not apply to paediatric, pregnant or breast-feeding, immunosuppressed, or end-stage cancer patients, or to those patients with a 'do not resuscitate' order; most of the studies discussed below excluded these patients. Additionally, the article does not apply to patients with diagnoses for which it is well-accepted practice to give prolonged courses of antibiotics (e.g. infective endocarditis, chronic osteomyelitis, tuberculosis etc.), although PCT and/or other biomarkers may be shown to have a role in some of these infections in the future.

Data sources

A literature search was conducted using PubMed. The search strategy used combinations of the following terms: procalcitonin; critically; critical; intensive; biomarkers; and sepsis. The reference lists of identified articles were searched to identify additional publications. We have predominantly focused on articles published between 2000 and 2010.

What is procalcitonin?

PCT is a precursor of the hormone calcitonin and is synthesized physiologically by thyroid C cells. In normal physiological conditions, PCT levels in the serum are low (<0.1 ng/mL). However, in bacterial infection PCT is synthesized in various extrathyroidal neuroendocrine tissues. Systemic PCT secretion is a component of the inflammatory response that appears to be relatively specific to systemic bacterial infections. Bacteraemic infections appear to cause the highest rises in PCT with lower or negligible rises in localized, viral and intracellular bacterial (e.g. *Mycoplasma pneumoniae*) infections.[9,10] There is evidence that Gram-negative bacteraemias cause higher PCT rises than Gram-positive bacteraemias.[11] Importantly, PCT levels in response to sepsis do not appear to be significantly affected by the use of steroids,[12] although at least one study has shown elevated PCT levels at 24 h in volunteers given ibuprofen at the time of endotoxin challenge compared with control volunteers given endotoxin and placebo.[13]

To be a useful diagnostic biomarker in bacterial sepsis, the substance being measured must rise above normal levels early in the course of the infectious process. In bacterial infections, serum PCT levels start to rise at 4 h after the onset of systemic infection, and peak at between 8 and 24 h. In contrast, CRP, which with the exception of the WCC is the most commonly used biomarker of infection in the UK, rises slowly and peaks 36 h after an endotoxin challenge.[10,14] PCT can also be elevated in renal impairment in the absence of infection.[15] In surgical patients the picture is less clear, as PCT can increase after trauma or surgery, particularly major abdominal surgery, and in pancreatitis. However, some authors have found that PCT levels only transiently increase for 12–24 h after surgery before, in the absence of infection, falling back to normal levels.[16] Again, this is in contrast to both CRP and WCC, which can stay elevated for a number of days after surgery without there being underlying infection. PCT has a half-life of ~24 h, so a sample can be collected and sent to the laboratory as with other routine biochemical blood tests.[10] Laboratory turnaround times will vary depending on local circumstances and the PCT testing kit used, but an average turnaround time appears to be ~3 h, although Uzzan *et al.*[17] quoted half an hour for their laboratory.

PCT as a diagnostic biomarker of bacterial sepsis

Using PCT to diagnose sepsis in critically ill patients

There have been a number of studies looking at the diagnostic ability of PCT in critically ill patients and, more specifically, its ability to differentiate between SIRS and bacterial sepsis. These studies are generally small (<200 patients), are methodologically heterogeneous and use different PCT cut-off points to define normal. The results are conflicting, making it difficult to draw any firm conclusions. There have been two meta-analyses performed within the last 5 years, however, that have attempted to clarify the situation.[17,18] The first by Uzzan *et al.*,[17] published in 2006, reviewed and analysed 25 studies with a total of 2966 patients. A subanalysis was performed on 15 studies to compare the diagnostic ability of PCT versus CRP. In the 25 studies that looked at PCT, the sensitivities ranged from 42% to 100% and the specificities from 48% to 100%. The sensitivities and specificities for CRP ranged from 35% to 100% and from 18% to 84%, respectively. The results of their meta-analysis found a global diagnostic accuracy odds ratio for PCT of 15.7 [95% confidence interval (CI) 9.1–27.1] and of 5.4 (95% CI 3.2–9.2) for CRP. The Q^* value, a measure of performance that is less affected by study heterogeneity than other measures, was significantly higher for PCT than CRP: 0.78 versus 0.71, $P=0.02$. Similar results were found by Tang *et al.* in their meta-analysis.[18] They reviewed 18 studies, some but not all of which overlapped with the review by Uzzan *et al.*[17] They found mean values of 71% (95% CI 67%–76%) for both sensitivity and specificity, and an area under the receiver operating curve (AUROC) of 0.78 (95% CI 0.73–0.83); the Q^* value for PCT was 0.72. Interestingly, the larger studies included in these reviews tended to find lower estimates of PCT sensitivity and specificity than smaller studies. Despite these limitations, Uzzan *et al.*[17] concluded that 'PCT represents a good biological diagnostic marker for sepsis, severe sepsis and septic shock' and 'should be included in diagnostic guidelines for sepsis and in clinical practice in intensive care units'. Tang *et al.*[18] were more measured, concluding that the diagnostic performance of PCT was low and that it cannot reliably differentiate sepsis from non-infectious causes of SIRS in critically ill adult patients.

Subsequently, Ruiz-Alvarez *et al.*[19] found that PCT (AUROC=0.81) and the sequential organ failure assessment (SOFA) score (AUROC=0.82), but not CRP, were the only independent predictors of infection in 103 intensive care patients with suspected sepsis. Rau *et al.*[20] also found PCT to be useful, and better than CRP, in predicting infections and multiorgan dysfunction syndrome in 104 patients with acute pancreatitis. Based on these studies, PCT appears to be a genuine advance on CRP in the diagnosis of sepsis, but given that CRP is widely regarded by experienced clinicians to be a relatively non-specific marker

of inflammation, the difference in performance between the two is not impressive enough for clinicians to rely on PCT as a sole diagnostic tool in the initial care of critically ill patients; clinical judgement will remain the mainstay of diagnostic clinical decision-making. Reflecting this, the American College of Critical Care Medicine and the Infectious Diseases Society of America have recently recommended, graded as Level 2 evidence ('Reasonably justifiable by available scientific evidence and strongly supported by expert critical care opinion'), that serum PCT can be used as 'an adjunctive diagnostic tool for discriminating infection as the cause for fever or sepsis presentations' in their guidelines for the evaluation of new fever in critically ill adults.[10]

Given that PCT can be elevated in certain non-infective conditions,[15] it is probably better used to rule out than rule in systemic bacterial infection. However, false-negative results can occur if samples are taken too early in the course of infection and few physicians will be persuaded not to prescribe antibiotics on the basis of a single low PCT value performed on or shortly after admission to hospital for a critically ill patient without a clear diagnosis; a repeat test should be performed at 6–12 h.[10,21] However, if all microbiological cultures are negative and a clear source of infection has not declared itself by 24 h, a repeat low PCT, combined with clinical judgement, provides a strong argument for discontinuing antimicrobial therapy and searching for an alternative diagnosis. Such an approach is likely to avoid ≥3–4 days of broad-spectrum antibiotic therapy per patient in the UK. However, PCT tests are currently more expensive than CRP (£20.00 versus £1.05 in our hospital) and although this approach is attractive, it is yet to be subjected to robust cost-effectiveness analyses.

Using PCT to diagnose healthcare-associated infection in critically ill patients

PCT is also potentially useful diagnostically in critically ill patients who deteriorate during their admission when intercurrent bacterial infection is in the differential diagnosis as the cause for the deterioration. Ventilator-associated pneumonia (VAP) is one of the more common problems, the diagnosis of which is particularly challenging because clinical signs are often non-specific and there is no gold-standard diagnostic test. In addition, microbiological tests, often endotracheal aspirates in the UK, can be difficult to interpret because of microbial colonization of the respiratory tract. This uncertainty can lead to the delayed diagnosis and treatment of VAP and poorer clinical outcomes, but it is also widely acknowledged that VAP is over-diagnosed and, even when a clear diagnosis can be made, patients are often treated for too long. A diagnostic test that indicates the early presence of bacterial respiratory tract infection with some certainty in ventilated patients would clearly represent an important clinical advance.

Most of the studies performed in this area have been small and observational in nature. The results of two recently published studies, however, provide useful insights. The first, by Charles *et al.*,[22] looked at consecutive patients with either suspected VAP or confirmed bacteraemia. Seventy patients were included, 37 with 'proven' VAP, 10 with bacteraemia and 23 controls with suspected, but unproven VAP. PCT results in the three groups of patients were compared on the first day of fever (day 0) as well as the difference between PCT levels taken 1, 2 or 3 days before day 0. The day 0 PCT levels were significantly higher in cases of proven infection than in those without (5.5 versus 0.7 ng/mL, $P=0.018$). The absolute difference between the day 0 result and that on any of the preceding days was also significantly different between cases and controls (+5.8 versus −0.5, $P=0.035$ for day 0 to day 1). The AUROC for PCT on day 0 was 0.80 (95% CI 0.68–0.91). The sensitivity and specificity for the ability of PCT to diagnose VAP on day 0 (cut-off=0.44 ng/mL) were 65.2% and 83%, respectively. Luyt *et al.*[23] also measured PCT before and on day 1 in 73 patients with suspected VAP. In contrast, they found PCT on day 1 (cut-off=0.5 ng/mL) to perform less impressively with a sensitivity and specificity of 72% and 24%, respectively. A PCT rise (compared with the before level) had a sensitivity of 41%, specificity of 85%, positive predictive value of 68% and negative predictive value of 65%.

Tsangaris *et al.*[24] also looked at the diagnostic ability of PCT compared with CRP and WCC in 27 patients who had been in an intensive care unit for >10 days and had developed proven infection (bacteraemia, respiratory or abdominal) compared with 23 patients without infection. The AUROC for PCT was 0.85 (95% CI 0.71–0.93), for CRP it was 0.65 (95% CI 0.46–0.78) and for WCC it was 0.68 (95% CI 0.49–0.81). The sensitivity and specificity for a PCT cut-off of 1.0 ng/mL were 70% and 91%, respectively. Importantly, they also found that the difference in the PCT values from day 0 and any of the values for the 10 days before day 0 was significantly different in both cases and controls. The sequential measurement of PCT in identifying healthcare-associated infection is undoubtedly attractive, and there is some evidence that PCT measured twice or thrice weekly and on the day infection is suspected for the first time might be sufficient and clinically useful. PCT used in this way may also reduce unnecessary antibiotic prescribing in patients who deteriorate for non-infection reasons, but it will also add to critical care admission costs. The cost-effectiveness of such an approach needs to be evaluated, including against strategies that aim to prevent healthcare-associated infections in the first place. The results of The Procalcitonin and Survival Study (PASS), a randomized trial of a PCT-guided treatment strategy in 1200 critically ill patients (NCT00271752), may provide important insights and is therefore eagerly awaited.[25]

Procalcitonin as a prognostic biomarker

Serum PCT levels have also been noted to increase with increasing severity of sepsis and organ dysfunction. This was demonstrated by Giamarellos-Bourboulis *et al.*[26] (see Table 1). This has led to interest in using PCT as a prognostic indicator in critical care patients and a number of studies have now been performed.

One of the largest studies was performed by Jensen *et al.*[27] This study prospectively looked at daily PCT measurements in 472 critical care patients and correlated the results with all-cause mortality in a 90 day study period. They found that a high maximum PCT and an increase of PCT value following the first reading >1.0 ng/mL were both independent predictors of 90 day mortality. The relative risk for mortality increased with every day the PCT value continued to rise after the first reading >1.0 ng/mL: 1.8 (95% CI 1.4–2.4) for 1 day; 2.2

Table 1. PCT levels by organ dysfunction status (adapted from Table 2 in Giamarellos-Bourboulis *et al.*[26])

Organ dysfunction status	PCT (mean ± SE; ng/mL)
Patients not progressing to MODS	4.47 ± 1.22
ARDS	10.48 ± 4.77
ARDS plus ARF	8.08 ± 2.25
ARDS plus ARF plus DIC	32.72 ± 13.41
ARDS plus ARF plus DIC plus HF	43.35 ± 20.98

MODS, multiple organ dysfunction syndrome; ARDS, acute respiratory distress syndrome; ARF, acute renal failure; DIC, disseminated intravascular coagulation; HF, hepatic failure.

(95% CI 1.6–3.0) for 2 days; and 2.8 (95% CI 2.0–3.8) for 3 days. In contrast, levels of CRP and WCC were not found to be predictive of mortality. In a smaller study by Pettila *et al.* ($n=61$),[28] there was a significant difference in PCT values between survivors and non-survivors on day 1 and day 2 after admission to intensive care. However, there were similar statistically significant differences for IL-6 levels, and Acute Physiology and Chronic Health Evaluation (APACHE) II and SOFA scores; only the APACHE II score and male gender were found to be independent predictors of death in multivariate analyses. In the study by Ruiz-Alvarez *et al.*[19] ($n=103$) discussed earlier, PCT did not predict mortality, although CRP, SOFA score, age and gender did.

The evidence for using PCT to predict mortality in patients after surgery is more complicated to interpret, not least because studies have found markedly different cut-off points. Schneider *et al.*[29] found an optimum cut-off point of 1.44 ng/mL in their study of 220 unselected post-surgery patients requiring post-operative critical care. Using this cut-off for a serum PCT measured on the day after surgery, the ROC curve for combined mortality and morbidity was 0.75 (95% CI 0.66–0.85) and for the APACHE II score was 0.69 (95% CI 0.59–0.78). In contrast, in a study of post-elective coronary bypass patients, Fritz *et al.*[30] found a cut-off for mortality prediction at 2.5 ng/mL, whereas Rau *et al.*[31] found a cut-off of 16 ng/mL in patients who had undergone surgery for peritonitis. The requirement to use markedly different cut-off points for subgroups of surgical patients would certainly complicate clinical decision-making and reduce the clinical usefulness of PCT for prognostic assessment in critical care.

So, based on the above, does PCT add anything to the already established clinical methods of prognostic assessment in critical care? APACHE II and SOFA scores have been validated for mortality risk stratification, but are clinically unwieldy and tend to be used more for audit and research than clinical decision-making. A rapidly available biochemical test that provides similar prognostic information could therefore be useful, e.g. to help discussions about prognosis with patients' relatives and decisions regarding earlier interventions. It seems doubtful that such a test, unless highly prognostic, will heavily influence day-to-day clinical decision-making for the latter; although, as suggested by Giamarellos-Bourboulis *et al.*,[26] a rising PCT level might be used as an indicator that an infectious process is not under control and that better source control is required. The danger of this is that any intervention triggered by a rising PCT (e.g. further debridement surgery in a physiologically unstable patient) might result in poorer clinical outcomes than would have occurred without intervention. Although attractive, before being widely adopted in critical care clinical practice, this approach therefore also requires further validation.

The role of PCT in antibiotic stewardship

The use of PCT as an antimicrobial stewardship tool is extremely attractive in the current climate of increasingly antibiotic-resistant microbes. The theory is that with daily or serial PCT measurements, antibiotics can be safely stopped once the PCT level declines below a certain cut-off point or reduces to a certain percentage of its initial value. The use of PCT in the avoidance of antibiotic initiation and in reducing antibiotic course length has been extensively studied outside of the critical care environment. Several large, high-quality randomized controlled trials have demonstrated significant decreases in antibiotic use without any apparent increase in harm in lower respiratory tract infection,[32,33] exacerbations of chronic obstructive pulmonary disease[34] and community-acquired pneumonia.[35]

There have now been a number of studies using the same principles in critically ill patients. Nobre *et al.*[36] performed a small randomized open-label study comparing PCT-guided antibiotic duration (39 patients) against usual care (40 patients). All patients had severe sepsis or septic shock on enrolment. The results showed a significant reduction in antibiotic duration in patients who were strictly treated by PCT guidance [6 days (range 4–16) versus 12.5 days (range 8–16)], but a non-significant difference in the intention-to-treat analysis. There was no difference in mortality and infection recurrence in the two groups, but intensive care unit stay was shortened in the PCT cohort. Hochreiter *et al.*[37] ($n=110$) found that PCT-guided patients also received a significantly shorter duration of antibiotic therapy (5.9 ± 1.7 days versus 7.9 ± 0.5 days) in their randomized controlled trial of surgical intensive care patients with confirmed or highly suspected infections. In patients with VAP ($n=101$), Stolz *et al.*[38] found that PCT use significantly increased the number of antibiotic-free days alive [13 (range 2–21) days versus 9.5 (range 1.5–17) days] with an overall reduction in antibiotic exposure of 27%.

The PRORATA trial is the largest randomized PCT trial to date and is therefore worthy of more detailed discussion.[21] The study was performed in eight French intensive care units, was open-label and compared PCT-guided antibiotic therapy (307 patients) to usual care (314 patients) for predominantly non-surgical patients (10% were surgical) with suspected bacterial sepsis on either entry to intensive care or during their admission; see Figure 1 for the algorithm used. It is worth noting that 1315 patients were assessed for eligibility and 685 were excluded for various reasons, including that they were considered to have a poor chance of survival or were expected to remain in intensive care for <3 days. Of the 307 patients randomized to the PCT group, recommendations were not followed in 219 patients (71%); of these, the algorithm was overruled at inclusion or during follow-up in 57 patients (19%). On admission, a high and similar proportion of patients in both cohorts were prescribed antibiotics, but, overall, PCT patients had significantly more days without antibiotic exposure than control patients [14.3 ± 9.1 days (mean ± SD) versus 11.6 ± 8.2 days] and received significantly fewer days of antibiotics [10.3 ± 7.7 days

Guidelines for starting antibiotics[a]			
If the blood sample taken for procalcitonin level was taken at the early stage of the episode, obtain a second procalcitonin level at 6–12 h			
[a]Excludes situations requiring immediate antibiotic treatment (e.g. septic shock, purulent meningitis)			
Concentration <0.25 μg/L	Concentration ≥0.25 to <0.5 μg/L	Concentration ≥0.5 to <1 μg/L	Concentration ≥1 μg/L
⇓	⇓	⇓	⇓
Antibiotics strongly discouraged	Antibiotics discouraged	Antibiotics encouraged	Antibiotics strongly encouraged
Guidelines for continuing or stopping of antibiotics			
Concentration <0.25 μg/L	Concentration decrease by ≥80% from peak OR ≥0.25 to <0.5 μg/L	Concentration decrease by <80% from peak AND ≥0.5 μg/L	Concentration increase compared with peak AND ≥0.5 μg/L
⇓	⇓	⇓	⇓
Stopping of antibiotics strongly encouraged	Stopping of antibiotics encouraged	Continuing antibiotics encouraged	Changing antibiotics strongly encouraged

Figure 1. Recommendations for starting/stopping antibiotics based on the PRORATA study.[21] Adapted from Figure 1 in Bouadma *et al.*[21]

(mean ± SD) versus 13.3 ± 7.6 days]. There was no difference in the proportion of patients with emerging multidrug-resistant bacteria; a cost-effectiveness analysis was not performed.

In contrast, but in a smaller observational study ($n = 75$), Venkatesh *et al.*[39] found that in critically ill patients with culture-positive sepsis, the mean PCT level remained elevated throughout the course of antibiotics, only falling to <1.0 ng/mL on day 10 and to <0.5 ng/mL on day 14. The mean PCT levels of patients in the culture-negative sepsis group were generally lower, and went to <1.0 ng/mL by day 7 and to <0.5 ng/mL by day 10. On the basis of these results, the authors concluded that 'due to significant overlap in PCT levels it was not possible to define a cut-off point for PCT under which it was safe to discontinue antibiotics'. They suggested a number of potential reasons for their results, including that PCT may have been raised in the presence of organ dysfunction, irrespective of its aetiology, and that improvement in PCT levels may reflect improvement in the underlying inflammatory response rather than eradication of infection.

However, the key question is not whether PCT can be used safely to guide antibiotic duration in patients—the answer would appear to be 'yes' based on studies within and outside the critical care environment—but is it necessary to use it at all? The studies that have demonstrated a significant reduction in antibiotic duration are mostly studies in which the length of a course of 'usual care' antibiotic therapy appears to be ~10–14 days. However, Chastre *et al.*[40] demonstrated, without the aid of PCT, that 8 days of antibiotics was as effective as 15 days for critically ill patients with VAP, without any difference in mortality or duration of critical care. There was a higher recurrence rate in patients with *Pseudomonas* spp. infections in the 8 day group, although the emergence of multiresistant bacteria occurred less often. In keeping with this, and as a result of the strong recent drive for antibiotic stewardship in the UK to control healthcare-associated methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia and *Clostridium difficile*-associated diarrhoea, the 'usual care' duration of antibiotic courses in most UK critical care units, without the aid of PCT, has now decreased to 5–7 days. In our own hospital, in the absence of clinical evidence of ongoing infection, an infection that requires prolonged therapy (e.g. endocarditis) or *Pseudomonas* spp. infection, 5 days is often used. Overall, antibiotic use within our own hospital has decreased considerably over the last 5 years, and the monthly number of MRSA bacteraemia and *C. difficile*-associated diarrhoea cases is now approaching zero; many acute UK hospitals have had similar success. Therefore, while the use of PCT has had a significant impact within clinical trials, its impact in real life is likely to be dependent on the baseline length of a 'usual care' antibiotic course within the intended environment of use. This is known to vary considerably from country to country and between hospitals within countries. In units that usually use antibiotic courses in the region of 10–14 days or more for infections such as VAP, PCT may well be a highly cost-effective strategy to provide the necessary confidence to clinicians in stopping antibiotics earlier. Unfortunately, the Procalcitonin Level to Discontinue Antibiotics on ICU Patients with no Obvious Site of Infection study (NCT00407147) was recently terminated due to slow patient recruitment. A key question for future research is: how short can the length of a 'standard' antibiotic course safely be in critical care patients and what is the role of PCT in achieving this?

Studies of other potential diagnostic and prognostic biomarkers of sepsis in critical care

Eosinopenia is an attractive potential biomarker in sepsis, as the eosinophil count is already serially measured in routine clinical

practice and the additional costs would therefore be minimal. Abidi *et al.*[41] found an AUROC of 0.89 (95% CI 0.83–0.94) for an eosinophil count cut-off of <50 cells/mm^3, performed on admission, in differentiating between non-infected and infected patients ($n=177$) in a medical intensive care unit in Morocco. Although this is promising, Ho and Towler[3] ($n=66$) found that CRP was a better marker of bloodstream infection in critically ill patients in Australia, and Shaaban *et al.*[42] ($n=68$) found that CRP (cut-off = 70 mg/L) and PCT (cut-off = 1.5 ng/mL) outperformed eosinopenia as a marker of sepsis in a North American critical care unit (negative predictive value = 94%, 87% and 80%, respectively). In a small study of 29 patients, Group II phospholipase A2 and PCT were both found to be superior to CRP as an early marker of bacteraemia in patients with infections.[43]

Changes to the biomarkers of coagulation have also been demonstrated in severe sepsis, with activated partial thromboplastin time waveform analysis appearing to have notable clinical potential.[44,45] For example, in a study of 331 patients, a biphasic waveform had a specificity of between 92% and 98% for the diagnosis of sepsis, depending on the threshold value used, although sensitivity was lower at between 22% and 55% for a diagnosis of sepsis on admission and between 48% and 74% for a diagnosis during admission. Chopin *et al.*,[46] in a study of 187 patients, showed a biphasic waveform to be more useful than PCT or CRP in distinguishing severe sepsis and septic shock, being significantly higher during days 1–3 in those who died of sepsis compared with those who did not or those who died of non-sepsis causes, and to have the highest specificity (91%) and negative predictive value (98%) for sepsis-related mortality on day 3. Zakariah *et al.*[47] subsequently showed that combining a biphasic waveform with PCT improved sensitivity (96%) for the diagnosis of sepsis in intensive care patients in Belgium.

In a study of 99 patients with septic shock, Guignant *et al.*[4] recently found that pro-ADM and pro-vasopressin (copeptin), when measured in the first week after the onset of shock, were both significantly elevated in those who died and significantly associated with mortality in multivariate analyses. Predictive ability was improved when both biomarkers were combined. This is interesting as these biomarkers have competing biological properties, and the results support the hypothesis that measurement of more than one biomarker is the way forward in high accuracy diagnostic and prognostic assessment in sepsis. Similarly, Scheutz *et al.*[48] demonstrated that the ratio of two counteracting peptides, pro-ADM and the precursor of endothelin-1, had higher prognostic accuracy than CRP and was comparable to the APACHE II score. Pro-ADM, measured on admission, was also found to significantly predict mortality in a study of 51 critically ill patients in China (AUROC = 0.87).[49] In the same study, pro-ANP also significantly predicted mortality (AUROC = 0.89), and both biomarkers performed similarly when compared with the APACHE II score and PCT. Pro-ANP was also found to predict mortality, when measured on days 0 and 4, in patients with VAP ($n=71$).[5] Pro-ANP was the only independent predictor of outcome in a multivariate model that included age, gender, APACHE II score and serum creatinine. Plasma brain natriuretic peptide level measured on day 2 has also been shown to have potentially useful prognostic value in septic shock.[50]

Hoffmann *et al.*[51] investigated the role of matrix metalloproteinases (MMP) and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMP), in a study of 37 patients with severe sepsis and 37 healthy controls. MMP-9, TIMP-1, TIMP-2 and IL-6 were significantly higher in ill patients; TIMP-1 was also found to be significantly higher in those who died compared with survivors. Harbarth *et al.*[2] previously compared the diagnostic value of IL-6, IL-8 and procalcitonin performed within 12 h of admission in 78 patients with SIRS. Although, IL-6 and IL-8 were both significantly predictive of sepsis (AUROC = 0.75 and 0.71, respectively), only procalcitonin (AUROC = 0.92) significantly added diagnostic value when combined with traditional markers of sepsis. Wu *et al.*[6] also investigated IL-6 together with IFN-γ, IL-10, IL-12 and plasma transforming growth factor-β1 (TGF-β1) in 63 patients admitted to an intensive care unit with severe community-acquired pneumonia. IL-6, IL-10, TGF-β1 and APACHE II score all significantly predicted mortality, but TGF-β1 was the only independent predictor of mortality in multivariate analyses. Resistin has also been shown to be significantly elevated during the first 2 weeks following admission in severe sepsis and septic shock patients ($n=95$) compared with healthy controls. Resistin correlated well with APACHE II and SOFA scores.[52] In a recent study of 170 patients, however, Koch *et al.*[7] confirmed some of these findings, but found that resistin, measured on admission, only predicted mortality in critically ill patients without sepsis.

The above studies, whilst not representing an exhaustive review of non-PCT biomarkers in sepsis, demonstrate the wide range of potentially clinically useful biomarkers currently being investigated. As has already been demonstrated to some extent in a few studies, to be highly predictive of sepsis and/or clinical outcome, it is likely that a combined panel of novel biomarkers, with or without traditional markers of sepsis, will be required. The challenge will be to identify the most cost-effective markers and how to incorporate such a panel into daily clinical practice.

Conclusions

In the diagnosis and prognosis of sepsis in critically ill patients, PCT is an improvement on CRP and other traditional markers, but, based on current evidence, it lacks the necessary accuracy to be used without clinical judgement, which should retain a pivotal role in clinical decision-making. This is particularly important in patients who present early in the course of illness or have focal rather than systemic infection and in surgical patients in whom various cut-off points have been identified for different diagnoses. PCT may be better employed to rule out rather than rule in systemic sepsis in the critical care environment, particularly if repeated measures are used.

There is stronger evidence for its use as a tool to reduce antibiotic course length and it is perhaps in this role that it will prove most useful. However, the cost-effectiveness of PCT as an antibiotic stewardship tool is likely to depend on baseline antibiotic course length and its, as yet unknown, impact on antibiotic resistance. Critical care units intending to use PCT should consider these issues pre-implementation. In the future, to improve the accuracy of the diagnosis and prognosis of sepsis, the use of a combined panel of novel biomarkers and traditional markers of sepsis, reflecting different aspects of the human

body's response to infection, is an attractive proposition and is worthy of further investigation.

Transparency declaration

This article is part of a Supplement sponsored by the BSAC. The authors received an honorarium from the BSAC for writing this article.

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