ORIGINAL RESEARCH

Severe respiratory viral infection induces procalcitonin in the absence of bacterial pneumonia

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ABSTRACT

Introduction Procalcitonin expression is thought to be stimulated by bacteria and suppressed by viruses via interferon signalling. Consequently, during respiratory viral illness, clinicians often interpret elevated procalcitonin as evidence of bacterial coinfection, prompting antibiotic administration. We sought to evaluate the validity of this practice and the underlying assumption that viral infection inhibits procalcitonin synthesis.

Methods We conducted a retrospective cohort study of patients hospitalised with pure viral infection (n=2075) versus bacterial coinfection (n=179). The ability of procalcitonin to distinguish these groups was assessed. In addition, procalcitonin and interferon gene expression were evaluated in murine and cellular models of influenza infection.

Results Patients with bacterial coinfection had higher procalcitonin than those with pure viral infection, but also more severe disease and higher mortality (p<0.001). After matching for severity, the specificity of procalcitonin for bacterial coinfection dropped substantially, from 72% to 61%. In fact, receiver operating characteristic curve analysis showed that procalcitonin was a better indicator of multiple indices of severity (eg, organ failures and mortality) than of coinfection. Accordingly, patients with severe viral infection had elevated procalcitonin. In murine and cellular models of influenza infection, procalcitonin was also elevated despite bacteriologic sterility and correlated with markers of severity. Interferon signalling did not abrogate procalcitonin synthesis.

Discussion These studies reveal that procalcitonin rises during pure viral infection in proportion to disease severity and is not suppressed by interferon signalling, in contrast to prior models of procalcitonin regulation. Applied clinically, our data suggest that procalcitonin represents a better indicator of disease severity than bacterial coinfection during viral respiratory infection.

Key messages

What is the key question?
► Should procalcitonin be used as a marker of bacterial coinfection during respiratory viral illness?

What is the bottom line?
► Procalcitonin rises during pure viral respiratory infection and is a better marker of disease severity than bacterial coinfection.

Why read on?
► We use a novel approach that combines clinical, murine and cellular models to gain basic mechanistic insights into procalcitonin regulation, which in turn inform the clinical interpretation of procalcitonin during respiratory viral infection.

INTRODUCTION

Pneumonia is a highly prevalent and deadly disease, claiming nearly four million lives per year worldwide.[1] Early empiric antibiotics reduce mortality and are therefore a cornerstone of therapy.[2] However, antibiotic overuse for respiratory infection is now recognised as a major driver of multidrug resistance—an epidemic considered one of the 10 greatest threats to human health by the WHO.[3] Indeed, by WHO estimates, drug-resistant bacteria will take more than ten million lives annually by 2050 if left unchecked.[4] Therefore, it is imperative to develop effective diagnostic tools to guide antibiotic therapy for respiratory infection.

A principal challenge is to differentiate patients with bacterial and viral infection in order to withhold antibiotics from the latter. This problem is of growing importance, as the primary aetiology of pneumonia has shifted from bacterial to viral in recent years, increasing the risk that empiric therapy will result in unnecessary antibiosis.[5–7] The reasons for this epidemiological shift are not fully known, though decreased prevalence of pneumococcal pneumonia due to widespread vaccination and increased recognition of viral pathogens due to improved testing have been implicated.[8]

Numerous methods for distinguishing bacterial from viral infection have been proposed, ranging from serum biomarkers to next-generation-sequencing techniques. These aid in diagnosis via direct detection of pathogens or identification of pathogen-specific host immune responses.[9] Among them, the immune biomarker procalcitonin (PCT) has emerged as the most intensely studied and widely used ancillary test for bacterial infection.[10] Its use is based on two key suppositions: (1) that bacteria stimulate PCT expression, both directly via lipopolysaccharide and indirectly through induction of proinflammatory cytokines such as interleukin-1β (IL-1β), IL-6 and tumour necrosis factor

α (TNF-α)[11–18]; and (2) that viruses indirectly suppress PCT production by inducing inhibitory interferons (principally, interferon-γ (IFN-γ)).[16 17 19 20]

Accordingly, during respiratory viral infection, clinicians often interpret elevated PCT as evidence of concurrent bacterial pneumonia, and thus justification for antibiotic therapy. Some studies support this strategy, particularly during influenza,[21–24] while others provide contradictory evidence.[25] The recently updated guidelines for managing community-acquired pneumonia recognise this equipoise, calling for further studies to clarify the role of PCT in diagnosing viral–bacterial coinfection.[26]

Here, we sought to address this question through a multidisciplinary approach—first using a clinical cohort of patients with respiratory viral infection to assess the ability of PCT to diagnose bacterial coinfection, and then a set of biological models to test the basic assumption that viral infection suppresses PCT production.

METHODS

Clinical study design

We performed a retrospective cohort study of patients≥18 years of age who were admitted between 27 September 2016 and 1 March 2018 to Yale-New Haven Hospital, a 1541-bed tertiary care centre in Connecticut, USA (see online supplementary figure E1 for details of study design). Inclusion criteria were: (1) positive testing for respiratory viruses based on nasopharyngeal swab or bronchoscopic sampling and (2) at least one PCT measurement during admission. We excluded patients with any evidence of extrapulmonary infection. A diagnosis of bacterial coinfection required both: (1) microbiological evidence of bacterial infection (defined by positive respiratory culture and/or positive urine antigen for *Legionella pneumophila* or *Streptococcus pneumoniae*) and (2) clinical evidence of pneumonia (defined by imaging consistent with pneumonia or documented diagnosis and treatment for pneumonia by an attending physician). Respiratory viruses were detected using a panel developed at Yale-New Haven Hospital consisting of real-time TaqMan PCR for influenza viruses A and B, respiratory syncytial viruses A and B, human metapneumovirus, parainfluenza virus types 1–4, adenoviruses, rhinoviruses and multiple coronaviruses (2259E, OC43, NL63, HKU1). A rapid PCR for influenza viruses A and B (GeneXpert, Cepheid, Sunnyvale, USA) was also used. PCR and culture were used to detect varicella-zoster virus, cytomegalovirus and herpes simplex virus. Clinical data were obtained by the Joint Data Analytics Team at Yale-New Haven Hospital and subsequent medical record review. Clinical data included demographics, comorbidities, laboratory and imaging data, medication exposure, diagnoses (by International Classification of Diseases-10 (ICD-10) codes and discharge diagnoses) and critical care interventions (eg, mechanical ventilation). Comorbidities were defined via ICD-10 codes as follows: respiratory diseases (J00–J99); cardiovascular diseases (I00–I99); neurological diseases (G00–G99); diabetes (E08–E13). Immunocompromising conditions were defined as in the online supplement. Informed consent was not required due to the non-interventional study design. In the manual chart review of patients with positive viral testing and highly elevated PCT (>20 ng/mL), pure viral infection was considered to be confirmed if the patient met at least one of the following prespecified criteria: (1) imaging was inconsistent with bacterial pneumonia as assessed by an independent radiologist; (2) lower respiratory cultures were negative; (3) antibiotics were withheld and (4) final diagnosis at discharge was pure viral infection.

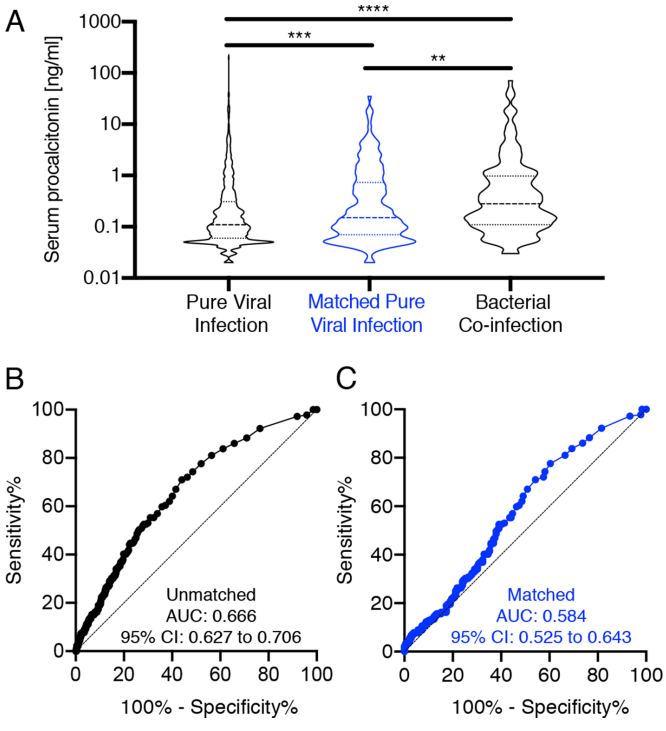

Figure 1 Serum procalcitonin in patients with respiratory viral infection and its utility in diagnosing bacterial coinfection. (A) Procalcitonin levels for three groups of patients are represented using violin plots: 2075 with pure viral infection; 179 with bacterial coinfection; and 179 with pure viral infection matched to the bacterial coinfection group in terms of severity (shown in blue). Median and IQRs are indicated. (B) Receiver operating characteristic curve (ROC) analysis applied to the unmatched pure viral group. (C) ROC analysis applied to the matched pure viral group. The AUC decreased significantly after matching for severity (p=0.023 by DeLong's test). Statistical significance in A was calculated by the Mann-Whitney U test. **P<0.01; ***P<0.001; ****P<0.0001.

Statistical analysis of clinical data

Two primary study groups were identified: pure viral infection and bacterial coinfection. An additional subgroup was assembled by matching patients in the pure viral group to patients in the coinfection group without replacement in a 1:1 ratio based on gender, intensive care unit (ICU) admission, mechanical ventilation and in-hospital mortality. The number of matching variables were constrained by the ability to populate a 1:1 match among patients with available data (see online supplementary for full methodological details). Differences in demographic and clinical variables were assessed and p values were calculated using either the X^2 or Mann-Whitney U test, with or without Bonferroni correction as indicated. Patients with absent demographic or clinical data were omitted from analysis related to that characteristic, as described in the online supplementary material. To evaluate the test characteristics of PCT, receiver operating characteristic curves (ROCs) were plotted and area under the curve (AUC) analyses were performed. To evaluate the relative diagnostic accuracy of PCT for bacterial coinfection versus other clinical conditions we compared AUCs, using DeLong's test for ROCs to detect statistical differences (see online supplementary material for further details).[27] On average, patients underwent testing for PCT 1.28 times during admission; our analysis focused on the maximum PCT recorded. This occurred early after admission

(median 9.84 hours, IQR 4.32–21.84 hours), consistent with the decision point for diagnosing bacterial coinfection. Data analysis was conducted using the R software environment (V.3.6.0, Vienna, Austria). Statistical significance throughout was defined at a level of $p<0.05$.

Murine and cellular studies

Wild-type male and female C57BL/6 mice (Jackson Laboratories, Bar Harbor, USA) were bred at Yale University in specific pathogen-free conditions. All animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee of the Yale School of Medicine. For infection experiments, 96 total 8–12 weeks old and gender-matched mice were used, with a group size of 5–6 to achieve sufficient power for statistical analysis. Mice were anaesthetised with 100 mg/kg and 10 mg/kg of ketamine and xylazine, respectively, and different inocula of H1N1 A/PR8/34 influenza were administered via nasal aspiration in 50 µL of phosphate-buffered saline (PBS); control mice received PBS alone. Weights were measured daily thereafter. The inoculum used for lethal infection was 200 plaque-forming units (pfu); 10 pfu were used for sublethal infection. Additional techniques, including in vitro assays, were performed as described in the online supplement.

Statistical analysis of murine and cellular data

As appropriate, groups were compared using a two-tailed Student's t-test, Mann-Whitney U test or Kruskal-Wallis test with Dunn's multiple comparison test. Data shown are representative of multiple independent repetitions of the experiment. Values are expressed as mean±SE of the mean unless otherwise indicated. Analysis was performed using Prism V.8 (GraphPad Software, San Diego, USA). Statistical significance was defined throughout at a level of $p<0.05$.

RESULTS

Overall, 11 721 patients in our retrospective cohort underwent testing for both PCT and respiratory viruses (online supplementary figure E1). Of the 2811 patients testing positive for respiratory viruses, 557 were excluded due to the presence of extrapulmonary infection. Of the remaining 2254 patients, 2075 (92%) had pure viral infection and 179 (8%) had bacterial coinfection.

As shown in figure 1A, PCT was significantly higher in coinfection than in pure viral infection ($p<0.0001$). However, there were also significant differences in demographic and clinical characteristics between the two groups, especially relating to disease severity (table 1). To evaluate the potential confounding effect of disease severity, we used a matching strategy (see online supplementary material for details). In this approach, we selected a subgroup of 179 patients with pure viral infection that matched the 179 with coinfection in terms of four correlates of severity: gender, ICU admission, mechanical ventilation and mortality (microbiological data in table 2 and online supplementary table E1). As shown in figure 1A, the severity-matched subgroup (in blue) had significantly higher PCT than the original group with pure viral infection ($p<0.001$), although still lower than the group with bacterial coinfection ($p=0.007$). Matching against several other combinations of severity correlates gave nearly identical results. These data suggested that elevated PCT in patients with viral infection may relate to disease severity.

Table 1 Demographic, clinical and laboratory characteristics of patients with respiratory viral infection

Demographic	Pure viral infection (n=2075)	Matched pure viral infection (n=179)	Bacterial coinfection (n=179)	P value: pure viral versus coinfection	P value: matched pure viral versus coinfection
Age, median (IQR)	69.0 (56.0–82.0)	68.0 (57.0–79.0)	69.0 (59.0–80.5)	0.414	0.291
Male, n (%)	902 (43.5)	**102 (57.0)**	**102 (57.0)**	<0.001	**1.000 (matched)**
ICU admission, n (%)	390 (18.8)	**93 (52.0)**	**93 (52.0)**	<0.001	**1.000 (matched)**
BMI, median (IQR)	27.9 (23.7–33.5)	27.4 (22.8–31.8)	25.4 (21.6–30.6)	<0.001	0.213
Peak temperature (°C), median (IQR)	37.7 (37.2–38.6)	38.0(37.4–38.8)	38.3 (37.6–39.1)	<0.001	0.032
Mechanical ventilation, n (%)	86 (4.1)	**52 (29.1)**	**52 (29.1)**	<0.001	**1.000 (matched)**
Length of stay (days), median (IQR)	3.0 (2.0–6.0)	5.0 (2.5–9.5)	9.0 (4.0–13.5)	<0.001	<0.001
Death during admission, n (%)	34 (1.6)	**13 (7.3)**	**13 (7.3)**	<0.001	**1.000 (matched)**
Laboratory values					
Maximum PCT (ng/mL), median (IQR)	0.11 (0.06–0.31)	0.16 (0.07–0.67)	0.28 (0.11–0.95)	<0.001	0.007
WCC (x10∧9/L), median (IQR)	8.0 (5.8–11.3)	9.1 (6.5–12.9)	10.1 (7.1–13.9)	<0.001	0.170
Creatinine (mg/dL), median (IQR)	0.9 (0.7–1.3)	1.0 (0.7–1.4)	1.0 (0.7–1.4)	0.664	0.925
Comorbidities					
Respiratory diseases, n (%)	1544 (75.6)	134 (76.6)	145 (83.3)	0.022	0.149
Cardiovascular diseases, n (%)	1393 (68.3)	122 (69.7)	128 (73.6)	0.147	0.497
Neurological diseases, n (%)	676 (33.1)	61 (34.5)	53 (30.5)	0.473	0.492
Diabetes, n (%)	287 (14.1)	23 (13.3)	29 (16.7)	0.346	0.466
Immunocompromise, n (%)	311 (15.2)	27 (15.4)	30 (17.2)	0.482	0.754
Weighted Charlson Comorbidity Score, mean (SD)	2.26 (2.36)	2.28 (2.33)	2.68 (2.88)	0.177	0.453

P values were calculated via either the X^2 Test or the Mann-Whitney U test, as appropriate.
Bold: Variable was used as a matching criterion.
BMI, body mass index; ICU, intensive care unit; PCT, procalcitonin; WCC, white cell count.

Table 2 Microbiologic characteristics of patients with respiratory viral infections

Viral aetiology	Pure viral (n=2075) (%)	PCT (median)	PCT (min, max)	PCT (IQR)
Influenza	1524 (49.0)	0.13	0.02–200.00	0.07–0.29
Rhinovirus	735 (23.6)	0.09	0.02–38.66	0.05–0.27
Respiratory syncytial virus	510 (16.4)	0.14	0.02–12.94	0.07–0.35
Human metapneumovirus	158 (5.1)	0.14	0.02–234.12	0.07–0.40
Parainfluenza	140 (4.5)	0.11	0.03–9.70	0.06–0.31
Adenovirus	35 (1.1)	0.09	0.02–7.04	0.05–0.25
Cytomegalovirus	5 (0.2)	0.50	0.17–1.49	0.27–0.88
Viral aetiology	**Matched pure viral (n=179) (%)**	**PCT (median)**	**PCT (min, max)**	**PCT (IQR)**
Influenza	135 (50.0)	0.15	0.02–35.37	0.08–0.63
Rhinovirus	66 (24.4)	0.13	0.02–18.23	0.05–0.53
Respiratory syncytial virus	41 (15.2)	0.14	0.04–4.98	0.09–0.83
Human metapneumovirus	14 (5.2)	0.36	0.06–6.25	0.14–1.52
Parainfluenza	8 (3.0)	0.50	0.04–3.98	0.19–2.25
Adenovirus	3 (1.1)	0.06	0.02–0.09	0.04–0.08
Bacterial aetiology	**Coinfection (n=179) (%)**	**PCT (median)**	**PCT (min, max)**	**PCT (IQR)**
Staphylococcus aureus	78 (37.7)	0.44	0.03–37.36	0.15–1.17
Pseudomonas aeruginosa	28 (13.5)	0.15	0.05–6.69	0.08–0.30
Streptococcus pneumoniae	23 (11.1)	0.37	0.03–70.97	0.15–2.73
Haemophilus influenzae	23 (11.1)	0.17	0.05–7.12	0.08–0.71
Non-tuberculous mycobacterial spp.	10 (4.8)	0.69	0.04–47.53	0.26–3.23
Beta-Hemolytic Streptococcal spp.	10 (4.8)	0.11	0.03–1.96	0.07–0.18
Klebsiella pneumoniae	7 (3.4)	0.91	0.10–1.32	0.26–1.20
Escherichia coli	6 (2.9)	1.12	0.22–5.03	0.56–1.54
Moraxella catarrhalis	5 (2.4)	0.15	0.10–0.46	0.15–0.19
Haemophilus parainfluenzae	3 (1.4)	0.13	0.10–0.19	0.12–0.16

Organisms identified in ≤2 tests not shown (4 total for viruses (2 in the matched group and unmatched groups each) and 14 total for bacteria).
PCT, procalcitonin.

Next, we performed ROC analysis to evaluate the efficacy of PCT for diagnosing bacterial coinfection. Based on the AUC, PCT had fair test characteristics for distinguishing coinfection from pure viral infection in the unmatched group (figure 1B, AUC 0.67). However, its performance dropped substantially when applied to the matched subgroup (figure 1C, AUC 0.58)—a statistically significant difference by Delong's test (p=0.023). At a threshold of 0.25 ng/mL, the specificity of PCT for coinfection

Table 3 Test performance of PCT in identifying bacterial coinfection versus other clinical conditions

Clinical condition	Median PCT in ng/mL (IQR)		P value*	AUC†	Specificity	Sensitivity
	Absence of condition	Presence of condition	(absence vs presence)		(%, PCT ≥0.25)	(%, PCT <0.25)
Bacterial coinfection	0.11 (0.06–0.30)	0.45 (0.11–1.65)	9.84E-13	0.666	71.7	52.5
Renal insufficiency (Cr >1.5)	0.10 (0.06–0.24)	0.24 (0.09–1.09)	3.92E-74	**0.786‡**	70.9	**80.9**
Death	0.11 (0.06–0.32)	0.16 (0.07–0.56)	1.01E-09	**0.773‡**	**77.8**	**65.0**
Renal failure requiring dialysis	0.11 (0.06–0.28)	0.21 (0.09–0.91)	4.45E-17	**0.745‡**	**72.0**	**73.4**
Cardiovascular shock	0.11 (0.06–0.30)	0.17 (0.07– 0.45)	8.56E-16	**0.713**	**71.8**	**61.9**
Respiratory failure requiring PPV	0.11 (0.06–0.31)	0.28 (0.11–0.95)	9.03E-15	**0.703**	**71.9**	**62.3**
Fever (temperature >37.9 °C)	0.10 (0.05–0.24)	0.16 (0.07–0.50)	3.81E-37	0.658	**78.3**	41.7
ICU admission	0.09 (0.05–0.20)	0.34 (0.15–1.09)	1.02E-23	0.652	**75.2**	49.9

Patients are from the unmatched pure viral group and the bacterial coinfection group (n=2254).
Bold: AUC, specificity, or sensitivity value is superior to that of bacterial coinfection.
*P values were calculated by the Mann-Whitney U test and adjusted via Bonferroni correction.
†AUC is for the distinction of subjects with and without the specified condition.
‡Statistically significant improvement in AUC compared with bacterial coinfection by De Long's test.
AUC, area under the curve; Cr, creatinine; ICU, intensive care unit; PCT, procalcitonin; PPV, positive pressure ventilation.

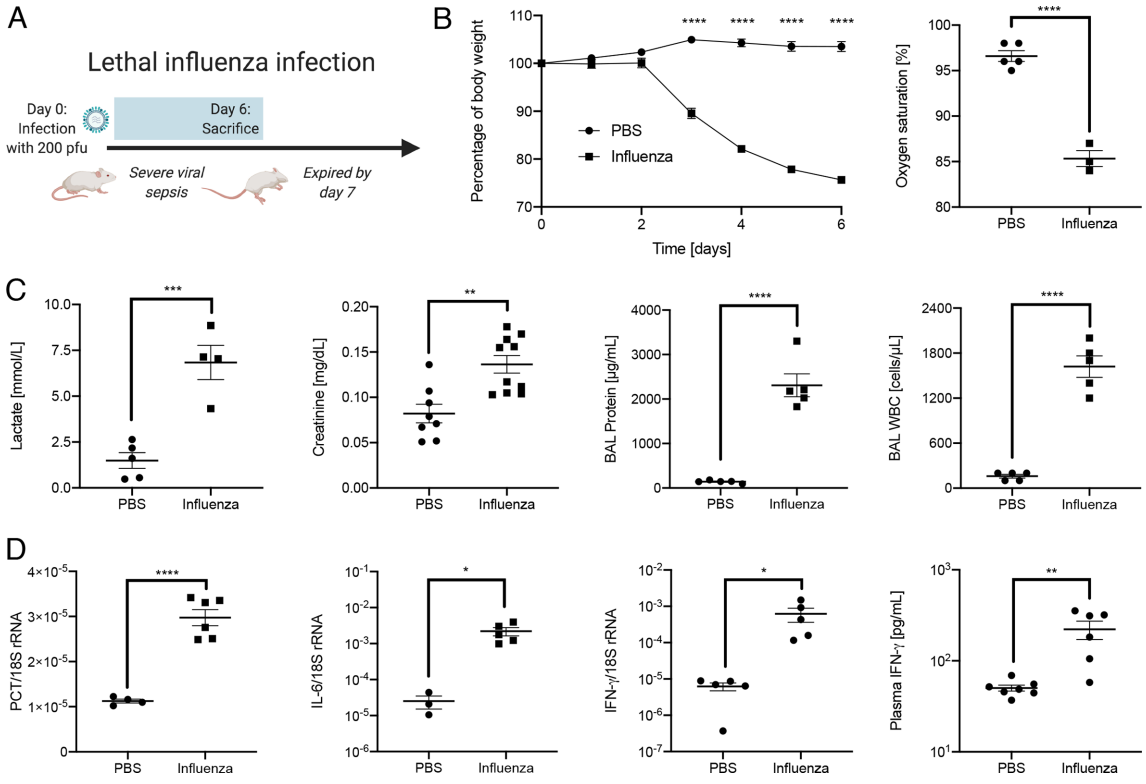

Figure 2 Procalcitonin (PCT) expression in lethal murine influenza. (A) Schematic of the lethal influenza model. (B) Weight loss and arterial oxygen saturation. (C) Plasma lactate, plasma creatinine, BAL protein content, BAL WBC count. (D) Lung expression of PCT, IL-6, IFN-γ and plasma levels of IFN-γ. Statistical significance for B–D was calculated using a two-tailed Student's t-test or Mann-Whitney U test (where n<6). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. BAL, bronchoalveolar lavage; IFN-γ, interferon-γ; IL-6, interleukin-6; WBC, white blood cell count.

dropped from 72% to 61% after matching for severity (online supplementary tables E2 and E3). Of note, we also evaluated the diagnostic potential of PCT in a subset of patients with mild or moderate disease. To this end, we identified 86 patients with coinfection admitted to non-ICU floors, and selected a corresponding subgroup of patients with pure viral infection matched for severity. As above, PCT proved a relatively poor biomarker for bacterial coinfection in this non-ICU population, with an AUC of only 0.63.

All together, these results suggested that PCT may better reflect disease severity than bacterial coinfection. To test this hypothesis, we compared the AUC of PCT for identifying bacterial coinfection to the AUCs of PCT for identifying clinical indices of severity. As shown in table 3 and online supplementary table E2, both ROC analysis and specificity values showed that PCT is a superior indicator of multiple severity indices—including respiratory failure, shock, renal failure and mortality—than of coinfection.

Returning to the distribution of PCT in pure viral infection (figure 1A), we noted a subset of patients with highly elevated PCT values. This observation was significant because it showed that PCT levels are not necessarily low in pure viral infection, as previously suggested.[16 17 19 20] It was, therefore, essential to rule out occult bacterial pneumonia in these patients and confirm pure viral infection, to the best of our ability. To do so, we undertook a detailed chart review of patients with pure viral infection and PCT >20 ng/mL, most of whom had severe influenza with multiorgan dysfunction (online supplementary table E4). Within this group of 20 patients, pure viral infection was confirmed in 17 individuals, while three remained indeterminate, thus indicating that the large majority of patients in the pure viral infection group were correctly assigned.

Nevertheless, we acknowledged the difficulty of definitively excluding bacterial infection as an occult cause for elevated PCT in the clinical setting. Therefore, to further evaluate whether pure viral infection can elicit PCT expression, we turned to a murine model of influenza, in which bacteriological sterility could be confirmed. Based on our clinical evidence that PCT correlates with disease severity, we selected a lethal model of influenza to maximise severity and therefore induction of PCT (figure 2A).

After lethal inoculation with influenza, morbidity was pronounced, with rapid weight loss from day 3 onwards (figure 2B). When sacrificed on day 6, mice were in septic shock with multiorgan failure, as evidenced by hypoxemia, elevated plasma lactate and renal insufficiency (figure 2B, C). Marked inflammation and lung injury was present, as indicated by elevated white blood cells and protein in the bronchoalveolar lavage (BAL) fluid (figure 2C). No bacterial growth was observed in BAL fluid plated on sheep's blood agar plates, a highly permissive growth media, thus confirming the absence of bacterial coinfection.

In this highly controlled model of severe, bacteriologically sterile viral infection, we observed significant upregulation of PCT transcription in lung tissue (figure 2D), thus confirming that pure viral infection can elicit PCT synthesis. This was associated with increased expression of IL-6, a marker of inflammation and a known trigger of PCT synthesis.[28] We also observed upregulation of IFN-γ in both lung tissue and blood (figure 2D), challenging the conventional theory that IFN signalling abolishes PCT expression.

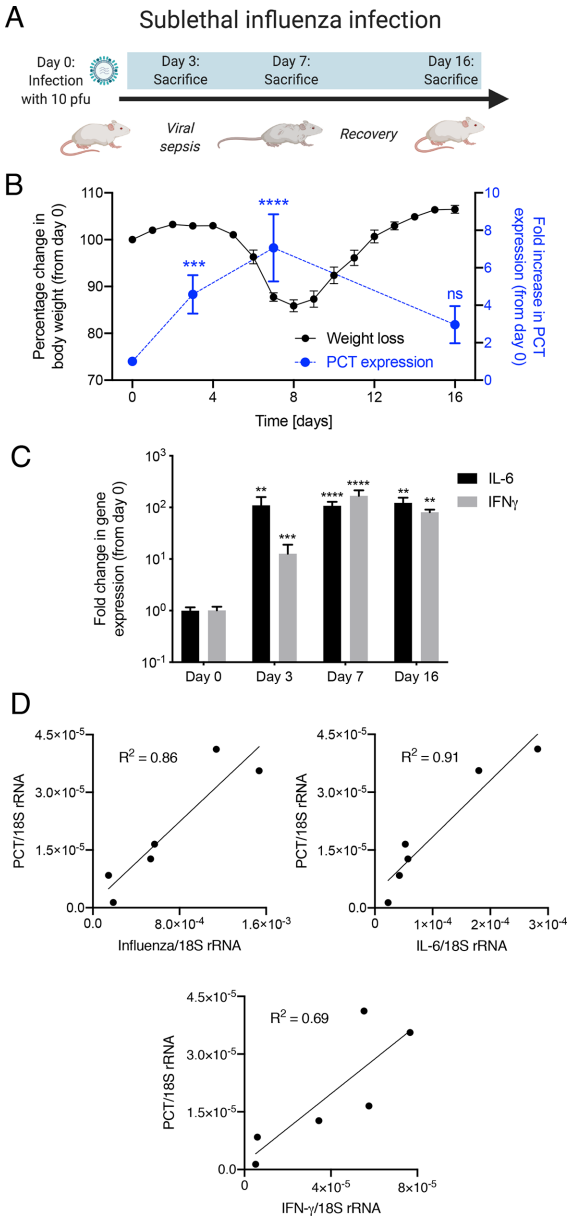

Figure 3 Procalcitonin (PCT) expression in sublethal murine influenza. (A) Schematic of the sublethal influenza model. (B) Weight loss and PCT transcription in lung. (C) IFN-γ and IL-6 transcription in lung. (D) Correlations between PCT and influenza viral load, IL-6 and IFN-γ, respectively. Statistical significance in B, C was calculated using the Kruskal-Wallis test with Dunn's multiple comparison test comparing gene expression at indicated time points to baseline expression at day 0. In (D) linear regression was performed and coefficients of determination are given. *P<0.05; **P<0.01; ****P<0.0001. IFN-γ, interferon-γ; IL-6, interleukin-6.

Next, we sought to test if PCT would correlate with severity of viral infection in mice as it had in patients. For this purpose, we employed a sublethal model of influenza infection in which mice develop severe morbidity and then recover fully (figure 3A), a course reflected in their pattern of weight loss (data in black, figure 3B). As shown by the blue curve in figure 3B, PCT expression tracked closely with morbidity—rising slightly at day 3, peaking at day 7 around the nadir of weight loss, and then returning to near baseline by day 16. Furthermore, PCT expression correlated with severity of both infection and inflammation, as indicated by influenza viral load and IL-6 synthesis, respectively (figure 3D). Importantly, we found that PCT expression not only persisted despite IFN signalling, it correlated positively with IFN-γ—arguing strongly against the premise that IFNs preclude PCT synthesis (figure 3D).

Finally, we assessed whether viral infection could elicit PCT synthesis at the cellular level. To do so, we used the human lung epithelial cell line A549, a common in vitro model for lung infection. Unlike other cell lines used to study viral infection, A549 has an intact IFN system, enabling interrogation of the putative suppressive effects of IFN-γ signalling on PCT expression. As shown in figure 4A, influenza infection significantly upregulated PCT despite active IFN synthesis, consistent with our murine investigations. Also in line with our in vivo data, PCT expression correlated positively with viral load, IL-6 and IFN-γ (figure 4B), providing further evidence that PCT corresponds with severity and persists despite IFN signalling.

DISCUSSION

Taken together, these clinical, murine and cellular studies provide two principal insights. First, respiratory viral infection can stimulate PCT production in the absence of bacterial pneumonia. This observation challenges the premise that PCT expression remains low during viral infection due to inhibitory IFN signalling (online supplementary figure E2A).[16 17] It also contradicts the corollary—that elevated PCT during viral illness indicates bacterial coinfection (online supplementary figure E2B). As such, our data argue against the use of PCT as a positive predictive test for coinfection, a conclusion supported by multiple recent reports.[2 25 29]

Second, our data suggest that PCT represents a better biomarker of clinical severity than bacterial coinfection during viral illness (table 3). An association between PCT and severity has been described in the context of pneumonia,[30–32] sepsis[33] and trauma.[34 35] However, the present study is the first to demonstrate the superior diagnostic accuracy for disease severity compared with bacterial infection. This distinction has important clinical implications, as it challenges the validity of using high PCT to diagnose bacterial infection in critically ill patients, since PCT will be elevated in this population per se. Conversely, it indicates that low PCT may be better interpreted as evidence of mild disease rather than absence of bacterial infection, as suggested by Bergin and Tsalik.[20] Further studies will be needed to parse this question fully.

In light of these findings, we favour a model of PCT as a biomarker not of bacterial infection but of a nonspecific systemic inflammatory response syndrome (SIRS), which in turn drives disease severity (organ failures and mortality) (online supplementary figure E2C). Although bacterial infection represents a common inflammatory trigger for SIRS, there are several others as well, including trauma, burns and surgery[19]; the present study helps to add viral infections to this list.[29] Indeed, there is a growing literature to suggest pure viral infections can elicit sepsis.[7 36–38] Cytokines such as IL-1β, IL-6 and TNF-α may play an important mechanistic role in viral sepsis, driving both PCT synthesis and organ dysfunction.[11–18 28 39] In this vein, it is worth noting that expression of IL-6 correlated tightly with PCT in our murine and cellular models of influenza infection (figures 3D and 4B). However, viruses may stimulate PCT expression by cytokine-independent mechanisms as well (online supplementary figure E2C); additional studies are required to elucidate these pathways.

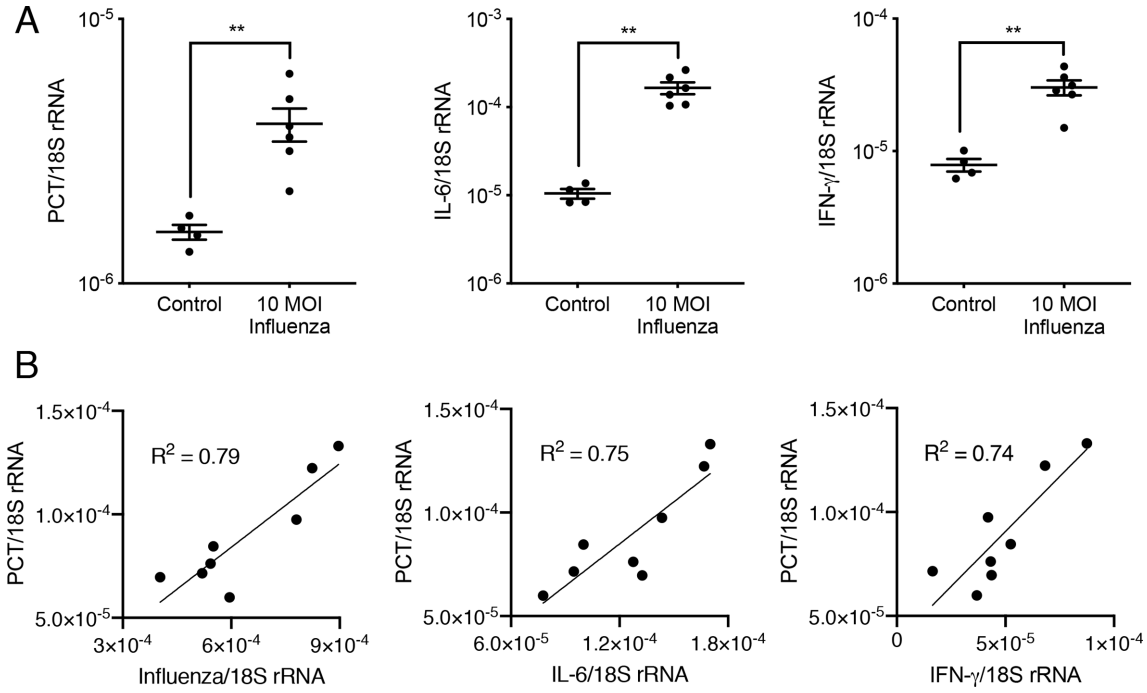

Figure 4 Procalcitonin (PCT) expression in human lung epithelial cells. (A) A549 cells were infected with influenza at a multiplicity of infection (MOI) of 10 and collected after 48 hours; transcription of PCT, IL-6 and IFN-γ are presented. (B) A549 cells were infected with influenza at an MOI of 25 and collected after 24 hours; correlations between PCT and influenza viral load, IL-6 and IFN-γ, respectively, are presented. In (A) statistical significance was calculated using a Mann-Whitney U test (n<6): In (B) linear regression was performed and coefficients of determination are given. **P<0.01. IFN-γ, interferon-γ; IL-6, interleukin-6.

It is important to note that PCT remained higher in patients with bacterial coinfection than in those with pure viral infection even after adjusting for clinical severity. This difference may be explained by a more robust stimulation of PCT by bacterial pathogen-associated molecular patterns (PAMPs) compared with viral PAMPs, a partial inhibitory effect of virus-induced IFN or other mechanisms. Despite this difference, our data argue against the use of PCT as a diagnostic for ruling in coinfection, based on the poor AUCs and specificities described in table 3 and online supplementary table E2, respectively. A sudden rise in PCT in patients hospitalised with respiratory viral illness may represent a useful biomarker for the development of postviral bacterial pneumonia, but PCT sampling in our cohort was too sparse to answer this question. Our study was also not designed to evaluate the ability of PCT to rule out bacterial coinfection, though we did observe moderate sensitivity, particularly at low PCT levels (<0.1 ng/mL). Stronger evidence comes from large clinical trials, which support the use of PCT in excluding bacterial pneumonia and safely reducing antibiotic use (reviewed in reference 40).

The present work has certain additional limitations that require mention. First, the external validity of the clinical studies is limited due to performance at a single centre. Second, due to sample size, we were unable to match simultaneously for all factors that differed between the pure viral and coinfection groups in table 1. However, sequential matching for each factor (see online supplementary material) was performed to support the conclusion that severity is a primary driver of PCT expression, not a confounding variable. Third, the post hoc nature of our analysis may have resulted in underestimation of bacterial coinfection. Indeed, mandatory lower respiratory cultures in a prospective study design may have identified additional patients with occult bacterial infection, possibly altering the test characteristics of PCT for identifying coinfection. However, as shown by the analysis in online supplementary table E4, these cases would have represented a small fraction, unlikely to influence the data substantially. Furthermore, our murine and cellular data help to confirm the essential biological finding that severe viral infection upregulates PCT in the absence of bacterial pneumonia.

Regarding the basic science studies, a lack of effective commercially available reagents precluded evaluation of PCT protein levels in the murine influenza model, for example, by serum ELISA. Additionally, the murine and cellular studies were highly focused and leave some pertinent questions unanswered. For instance, although IFN-γ was not sufficient to fully suppress PCT synthesis in our murine and cellular models, it may yet exert some inhibitory effect as indicated by Linscheid *et al*, as may other types of IFN (eg, IFN-α and IFN-β).[16 17] Indeed, partial inhibition of PCT expression by IFN may help to explain the lower PCT levels observed in patients with viral vs bacterial infection, even after matching for severity (figure 1A).

Mechanistic investigations into such questions are necessary to elucidate the regulation of PCT. A better understanding of PCT biology, in turn, should improve implementation of this biomarker in practice and inform the design of clinical trials. If applied correctly, PCT may well serve as a valuable aid in the diagnosis of respiratory infection, especially to exclude bacterial pneumonia.[40] Further biological and clinical studies are needed to clarify these questions, which have broad implications for antibiotic stewardship and the management of respiratory infections.

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