Diagnostic importance of pulmonary interleukin-1 beta and interleukin-8 in ventilator-associated pneumonia

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ABSTRACT

Background

Ventilator-associated pneumonia (VAP) is the most commonly fatal nosocomial infection. Clinical diagnosis of VAP remains notoriously inaccurate. We tested the hypothesis that significantly augmented inflammatory markers distinguish VAP from conditions closely mimicking VAP.

Methods

A prospective, observational cohort study in two university hospital intensive care units recruiting 73 patients with clinically suspected VAP, and a semi-urban primary care practice recruiting a reference group of 21 age- and sex-matched volunteers.
Growth of pathogens at $>10^4$ colony forming units/ml bronchoalveolar lavage fluid (BALF) distinguished VAP from 'non-VAP'. Inflammatory mediators were quantified in BALF and serum. Mediators showing significant differences between patients with and without VAP were analysed for diagnostic utility by receiver operator characteristic (ROC) curves.

Results.

Seventy-two patients had recoverable lavage - 24% had VAP. BALF interleukin (IL)-1β, IL-8, granulocyte-colony stimulating factor and macrophage inflammatory protein 1α were significantly higher in the VAP group (all P<0·005). Using a cut off of 10 pg/ml, BALF IL-1β generated negative likelihood ratios for VAP of 0·09. In patients with BALF IL-1β <10 pg/ml the post-test probability of VAP was 2·8%. Using a cut off value for IL-8 of 2 ng/ml, positive likelihood ratio was 5.03. There was no difference in cytokine levels between patients with sterile BALF and those with growth of $<10^4$ CFU/ml.

Conclusions

BALF IL-1β and IL-8 are amongst the strongest markers yet identified for accurately demarcating VAP within the larger population of patients with suspected VAP. These findings have potential implications for reduction in unnecessary antibiotic use but require further validation in larger populations.

Introduction

Ventilator-associated pneumonia (VAP) occurs in up to 20% of patients mechanically ventilated for more than 48 hours and is typically associated with mortality rates of around 30%.[1,2] VAP therefore has a higher mortality than any other hospital-acquired infection, and exerts a large financial burden on health services.[3] Although estimates of the attributable mortality vary, most studies describe a significant associated mortality and morbidity.[1,3,4]

The optimal methods and criteria required to make a diagnosis of VAP remain contentious.[1] However it is generally accepted that only approximately one quarter to one third of patients with clinically suspected VAP satisfy pre-defined microbiological criteria for pneumonia.[5,6] The clinical diagnosis of VAP is made relatively frequently in the intensive care unit (ICU). This can result in empirical antibiotic treatment, with the inherent risk of over-prescribing. [7-9] Therefore in recent years attempts have been made to identify biological markers that can distinguish VAP accurately, rapidly and practically.[10-12]

Given the key roles played by inflammatory cytokines and neutrophils in the natural history of pneumonia, it is plausible that discriminatory markers of infection may be found in the innate immune system. We hypothesised that in critically ill patients pneumonia would drive a further increase in pulmonary inflammation with inflammatory mediators distinguishing VAP from conditions that mimic VAP.

Materials and Methods

Patients

This prospective, observational, cohort study was performed in two university hospital general ICUs admitting all patients except those undergoing cardiothoracic surgery. Patients were screened daily for possible VAP, and were assessed for enrolment as soon as the clinical suspicion was raised. Where enrolment occurred bronchoscopy took place within 6 hours of clinical diagnosis. Demographic details, co-morbidities and prescribed medications were recorded. The severity of presenting illness was assessed by an acute physiology and chronic health evaluation II (APACHE II) score calculated within 24 hours of ICU admission. Patients were eligible if they fulfilled recognised criteria for *clinically suspected VAP* i.e. mechanical ventilation for at least 48 hours, new and persisting infiltrates on a chest radiograph and at least two of the following – purulent tracheal secretions, temperature >38°C, or white cell count $>11x10^9$ per litre, based on a modification of previously published clinical criteria.[1] Exclusion criteria comprised PaO_2<8kPa on FiO_2>0·7, positive end-expiratory pressure >15cmH_2O, active bronchospasm,

myocardial infarction within the last 3 months, unstable arrhythmia, mean arterial pressure <65mmHg on vasopressor therapy, bleeding diathesis (including platelet count $<20x10^9$ per litre) and initiation or modification of antibiotics in the preceding 3 days.[1] Patients who had had no change in prescribed antibiotics for more than three days were included [1,13].

Patients had fibreoptic bronchoscopy and bronchoalveolar lavage (BAL) performed by a single experienced operator using a pre-defined, standardised technique.[14,15] Briefly, where focal infiltrates were present, the bronchoscope was wedged in a subsegment corresponding to the area of radiological involvement. In the case of diffuse radiographic change the bronchoscope was wedged in a subsegment producing visible purulent secretions or (in the absence of purulent secretions) in the posterior segment of the right lower lobe. Twenty ml of sterile saline was instilled and the aspirate (representing a 'bronchiolar' sample) discarded, then 200ml of sterile saline was instilled in aliquots and the aspirate (representing an alveolar sample) retained. Whole blood was collected into 0·38% sodium citrate (final concentration).

Volunteer reference group

After recruitment of 40 patients the (anonymised) age and sex of each patient was communicated to a local primary care practice, where staff unconnected with the study randomly identified matching individuals and sent out invitations to participate. The first twenty-one respondents were enrolled to form a reference group. Exclusion criteria comprised hypoxia (SaO_2 <92% on air), bleeding diathesis, anticoagulant therapy, insulin-dependent diabetes mellitus, arrhythmia, bronchospasm not responding to nebulised β2 agonist, or clinical evidence of respiratory tract infection. Eligible volunteers provided blood and had fibreoptic bronchoscopy and BAL performed by the same investigator as above.

Processing of BAL fluid (BALF) and whole blood

An aliquot of BALF was sent to the National Health Service (NHS) Clinical Microbiology laboratory for culture, whilst simultaneous cultures were undertaken in our research laboratory. Samples were processed using a standard operating procedure (SOP) in accordance with the SOP for the processing of BAL issued by the United Kingdom Health Protection Agency (HPA).[16] Analyses were limited to those routinely performed on BALF in our NHS laboratory (i.e. detailed analysis for viruses was not included), with the exception that we additionally performed anaerobic cultures. BALF from healthy volunteers was only cultured in the research lab.

Growth of $>10^4$ colony forming units (cfu) per ml of lavage fluid *confirmed VAP*.[1] This definition is used by several infection control and critical care organisations including the Hospitals in Europe Link for Infection Control through Surveillance (HELICS) program. [17] Patients whose BALF grew $<10^4$ cfu/ml formed a 'non-VAP' category. For further subgroup analysis 'non-VAP' was subdivided into 'sub-VAP growth' and 'sterile' groups. One ml of BALF was collected for culture, and the remainder centrifuged at 700g for 10 minutes. Supernatant was immediately frozen at -80^oC until further analysis. The cellular pellet was resuspended in warmed Iscove's Modified Dulbecco's Medium (IMDM, Invitrogen, CA, USA) and cytospins produced. Cytospins were stained with Diff-Quik (Reagena, Toivala, Finland) and differential cell counts established.

Thirty ml of citrated whole blood was separated into cellular and plasma components by centrifugation.[18] Serum was prepared by adding 1M calcium chloride to plasma.

Quantification of cytokines and inflammatory mediators

Concentrations of tumour necrosis factor alpha (TNFα), interleukin (IL)-1β, IL-6, IL-8, IL-10, granulocyte colony stimulating factor (G-CSF), and macrophage inflammatory protein 1 alpha (MIP-1α) in serum and BALF were estimated using cytometric bead array (CBA) kits (BD Bioscience, NJ, USA). Concentration of type 1 soluble triggering receptor expressed on myeloid cells (sTREM-1) and monocyte chemoattractant peptide 1 (MCP-1) were measured by enzyme-linked immunosorbent assay (ELISA; R&D Systems, MN, USA). Samples measured by CBA and ELISA were diluted in an assay-dependent manner to ensure they lay within the limits of the calibration curves. The dilution required ranged from neat to 1:100 for the highest values. Urea was measured by a colorimetric method (QuantiChrom, Bioassay Systems, CA, USA) and specifically used as a recognised means of correcting for dilutional effects in BALF.[19]

Consent and ethical approval

Informed, witnessed assent was obtained from a relative or main carer for all patients. Informed, written consent was obtained from all volunteers. The study was approved by the relevant Research Ethics Committees.

Statistical analysis

Statistical analysis was conducted using Prism (Graphpad Software, CA, USA). Non-normally distributed data were analysed using the Mann-Whitney U-test for two variables and the Kruskal-Wallis test for greater than two, using Dunn's method for post-hoc analysis. Normally distributed data were analysed using Student's t-test or ANOVA with Bonferroni's method for post-hoc analysis. Preliminary identification of candidate biomarkers was undertaken by noting those with significant differences between the VAP and 'non-VAP' median values. The diagnostic utility of these variables was assessed using area under the receiver operator characteristic (ROC) curves. For those with area under curve values of $\geq$ 0·5 optimal cut-offs and likelihood ratios were determined by the value with the maximum Youden index[20]; a likelihood ratio is a likelihood that a person with a positive (or negative) test has the disease in question. For the two most promising candidates discriminating VAP from 'non-VAP' multi-level likelihood ratios were calculated to illustrate diagnostic potential. Combinations of measures were assessed for enhanced diagnostic potential by statistical modeling via logistic regression and classification tree methods.

Results

There were seventy-four eligible patients, seventy-three were enrolled with one excluded due to lack of a relative's informed assent.. Seventy-two patients had recoverable BALF and so entered the analysis. Seventeen (24%) grew organisms at $>10^4$ cfu per ml of BALF ('VAP group') (growing a median of $5.7x10^4$ cfu/ml IQR $3x10^4$ – $6x10^4$ cfu/ml). Seven grew a Gram positive organism, six a Gram negative organism, and four patients grew fungi including 3 yeasts (Table 1). One patient growing a gram positive organism also grew anaerobes. The remaining 55 patients formed the 'non-VAP' group, of whom 22 were categorised as 'sub-VAP' (i.e.

organisms cultured but at $<10^4$ cfu/ml of BALF, growing a median $2.1x10^2$ cfu/ml (IQR 7.5 to $4x10^2$ cfu/ml)) and 33 as 'sterile'. Comparisons between cultures conducted in the NHS laboratory and our laboratory revealed strong agreement, with all patients identified as having VAP by the research laboratory being similarly identified by the NHS laboratory. There were no discordant cultures in this group. Amongst the non-VAP group two patients grew bacteria ($<10^2$ cfu/ml) which were not reported by the NHS lab, and one sample grew bacteria ($<10^2$ cfu/ml) in the NHS lab but not the research lab. Twenty-one volunteers were recruited to form the reference group. Twelve samples grew mixed oral commensal flora but always at $<10^2$ cfu/ml.

Table 1: Organisms grown in patients with and without VAP
In the VAP group 18 species were grown from 17 patients (i.e. 2 organisms were grown from 1 patient); by definition organisms were grown at $>10^4$ cfu/ml BALF. In the non-VAP group 27 species were grown from 22 patients (i.e. 2 organisms were grown from 5 patients); by definition organisms were grown at $<10^4$ cfu/ml BALF.

	ORGANISMS	VAP	NON-VAP
GRAM-POSITIVE	*Streptococcus pneumoniae*	0	1
	Methicillin-sensitive *Staphylococcus aureus*	2	2
	Methicillin-resistant *S. aureus*	3	6
	Coagulase negative Staph spp.	2	1
GRAM-NEGATIVE	*Haemophilus influenzae*	1	2
	Escherichia coli	3	1
	Citrobacter freundii	1	0
	Citrobacter koseri	0	1
	Enterobacter cloacae	1	2
	Acinetobacter baumannii	0	1
	Pseudomonas aeruginosa	0	2
	Serratia marcescens	0	1
	Klebsiella pneumoniae	0	4
FUNGI	*Candida albicans*	3	2
	Aspergillus fumigatus	1	1
OTHER	*Anaerobes*	1	0

The three groups studied were closely matched with respect to age (Table 2). The VAP and non-VAP groups were similar with regard to duration of mechanical ventilation, severity of illness and co-morbidities (Table 2). Although there was a greater likelihood of patients in the VAP group being male, having a surgical reason for admission to ICU and having less acute lung injury (ALI)/adult respiratory distress syndrome (ARDS), these differences did not reach statistical significance (Table 2).

Table 2: Demographic and clinical details of patients and age/sex-matched volunteers.
95% CI, 95% confidence interval; IQR, interquartile range; NA, not applicable.

	VAP (n=17)	*Non-VAP(n=55)*	*Matched Volunteers (n=21)*
Mean age (range)	57 (31-83)	58 (25-87)	59 (24-84)
%male	76%	55%	79%
Mean (95% CI) APACHE II score	23 (20-26)	21 (20-23)	NA
Median (IQR) days of ventilation before enrolment	8 (6-9)	8 (5-10)	NA
ICU mortality	35%	36%	NA
% with surgical diagnosis on admission	65%	47%	NA
% with ≥ 1 co-morbidity	59%	56%	NA
% receiving immunosuppressant drugs (including corticosteroids)	12%	11%	NA
% receiving antibiotics on day of diagnosis	29%	60%	NA
% with acute lung injury/acute respiratory distress syndrome	17%	35%	NA
% with systemic inflammatory response syndrome (SIRS)	88%	81%	NA

All measured cytokines and inflammatory mediators in serum showed similar concentrations in the VAP and non-VAP groups (Supplemental Table S1). No serum marker appeared to have potential value for discriminating VAP from non-VAP, though a trend in this direction was observed for sTREM-1. In general, serum markers were higher in both patient groups than in volunteers with the exceptions of IL-1β and TNFα, which were broadly similar in all three groups.

In contrast, the VAP group had significantly higher concentrations of IL-1β, IL-8, G-CSF and MIP-1α in BALF than the non-VAP group (Table 3). Trends in the same direction were observed for IL-6 and sTREM-1 (Table 3).

Table 3: Inflammatory profile of BALF.

Data expressed as median and inter-quartile range. BALF values are corrected for dilution against concentrations of urea.[19] Analysis by Kruskall-Wallis ANOVA allows detection of any difference between the 3 groups (*<0.05. **<0.01, ***<0.001 shown in left hand column). Post-hoc comparison between VAP and non-VAP groups was undertaken by Dunn's post-hoc test with p value displayed in the right hand column.

	VAP (n=17)	*Non-VAP(n=55)*	*Matched Volunteers (n=21)*	*P value (Dunn's post-hoc test between VAP and non-VAP)*
Neutrophils (10^5/ml)***	2.8 (0.6–21)	3.2 (0.3–12)	0(0–0.03)	NS
Macrophages (10^5/ml)*	2.3 (0.4-3.9)	1.6 (0.2-3.9)	2.9 (1.4-3.9)	NS
IL-1β (pg/ml)***	103 (27-755)	3 (0-48)	0 (0-1.6)	<0.001
IL-8 (pg/ml)***	6773 (2633-11762)	230 (68-1072)	69 (26-374)	<0.001
TNF-α (pg/ml)	1 (0-18)	0 (0-4)	0 (0-0)	NS
IL-6 (pg/ml)***	266 (105-503)	99 (10-465)	0 (0-6)	NS
G-CSF (pg/ml)**	107 (38-383)	17 (5-89)	6 (0-25)	<0.05
IL-10 (pg/ml)*	0 (0-9)	1 (0-16)	0 (0-0)	NS
sTREM-1 (pg/ml)	13 (0-530)	0 (0-7)	0 (0-93)	NS
MIP-1α (pg/ml)***	51 (14-269)	3 (0-27)	2 (0-13)	<0.01
MCP-1 (pg/ml)**	293 (94-554)	173 (49-1097)	21(0-54)	NS

On the basis of these findings the capacity for IL-1β, IL-8, G-CSF, MIP-1α, IL-6 and sTREM-1 to distinguish VAP among the population with clinically suspected VAP was tested (Figure 1). For the ROC curves plotted in Figure 1, area under the curve correlates with the discriminatory value of the marker being analysed. In this context IL-1β and IL-8 appeared to delineate VAP most accurately. When optimal cut off values were derived, concentrations of BALF IL-1β below 10 pg/ml appeared to be particularly powerful for the exclusion of VAP. Specifically, where BALF IL-1β was < 10 pg/ml, the negative likelihood ratio of 0.09 gives a post-test probability of having VAP was calculated at 2·8% (95% confidence interval 0·1% to 15·9%). IL-1β proved less useful as a positive discriminator of VAP. In contrast IL-8, whilst being less powerful for the exclusion of VAP, had a broader diagnostic value. Thus, relative to the identified cut off value in Figure 1 of 2000 pg/ml, a high BALF IL-8 concentration increased the post-test probability of VAP being present to 61·4% (95% CI 36·2%-76·8%), derived from the positive likelihood ratio of 5.03 . These data are presented as scatter plots in Figure 2, illustrating that IL-1β has utility in excluding VAP, but yields a number of false positives. In contrast, IL-8 has a broader utility, but demonstrates a number of false positive and false negative values. Multi-level likelihood ratios demonstrated that increasing levels of BALF IL-8 lead to increased confidence for 'ruling in' VAP, with the highest level examined (4000 ng/ml) producing a post-test probability of 75%. However at this level patient numbers are small and there is a marked reduction in sensitivity (details are presented in Supplemental Table S2). A similar relationship was not demonstrated for IL1-β (see Table S2). G-CSF, MIP-1α, IL-6 and sTREM-1 had markedly less discriminatory value than IL-1β or IL-8. Statistical modeling using combinations of mediators (via logistic regression and classification tree methods) failed to add discriminatory value to that achieved by either IL-1β or IL-8 alone. We did not find any difference in cytokine levels between VAPs caused by different classes of organism (Gram +ve, Gram-ve or fungi). Interestingly all cases associated with *Candida albicans* had cytokine levels above the optimum cut-off point for diagnosis for IL-1β, IL-8, G-CSF and MIP-1α.

To examine the specificity of our findings, we analysed the cytokine levels in the non-VAP group subdivided into those with no growth ('sterile') and those with growth below the 10^4 cfu/ml cut off ('sub-VAP growth'). Cytokine concentrations did not differ significantly between the sub-VAP and sterile groups (Figure 3). No correlation was found between bacterial growth and cytokine concentration when examining those patients with growth below the 10^4 cfu/ml cut off (r=0.14 p=0.31 for IL-8, r=0.06 p=0.65 for IL-1β by Spearman's Rho).

Discussion

These data have implications for both the diagnosis of VAP and for understanding the biology of the disease. The practical implications are that, in our hands, a patient with a BALF IL-1β concentration <10 pg/ml has approximately a 3% probability of having VAP. In contrast, the probability of VAP being present increases as BALF IL-8 concentration increases, a level >2 ng/ml corresponding to a 61% probability of VAP being present.

VAP remains common and associated with a high mortality.[1,4] It is difficult to diagnose accurately on clinical grounds alone,[5,7,8] with a tendency to over-

diagnosis leading to over-prescription of antibiotics.[1,7,8] Diagnostic confirmation remains largely reliant on standard microbiological culture techniques which generally take 24-48 hours to yield results.[15] In this regard the capacity of IL-8 to increase the likelihood of a correct diagnosis 5-fold and/or the capacity of low BALF IL-1β to reduce the likelihood of VAP 10-fold is an important development. The assays used here can yield a result within four hours. These tests could therefore have a significant impact on clinical decision-making.

These results also provide an interesting perspective on the biology of VAP. For the majority of markers both patient groups had higher levels than volunteers, implying a pro-inflammatory state in critically ill patients as might be expected. However it is intriguing that acute inflammatory cytokines such as IL-1β and MIP-1α were so similar when comparing the non-VAP group with volunteers. Although non-significant, there was a trend towards more cases of ARDS/ALI in the non-VAP group. One might have anticipated that these (ARDS/ALI) patients would also demonstrate elevated cytokine levels [21], however we found no difference between IL-8 and IL-1β levels within the non-VAP group when sub-divided into those with ARDS/ALI and those without. We believe this relates to the time point of sampling, with our patients median length of stay prior to being recruited being 8 days.

In contrast, the VAP group appeared to have a brisk inflammatory response confined to the lung. We have no data relating to the source of the relevant cytokines, but diffusion from the serum seems unlikely, suggesting a prominent role for alveolar epithelium and/or lung macrophages. It is tempting to speculate that sufficient pathogens in the alveolar space drive a renewed and compartmentalised inflammatory response. However this study was not designed to address this specific question and we cannot exclude the possibility that VAP arises in those patients who first develop an up-regulation of inflammation in the lung.

A number of potential biomarkers for VAP have been proposed, including sTREM-1, procalcitonin, copeptin and adrenomedullin.[10-12,22,23] However only sTREM-1 has been found to have stronger negative predictive value (or area under ROC curve) for VAP than the findings presented here [10], and recent studies have found less impressive predictive capabilities for sTREM-1 in BALF [24,25] in line with our observations. It is interesting to consider why IL-1β and IL-8 have not been identified as potential discriminatory markers in previous studies. The compartmentalised nature of pro-inflammatory cytokines in VAP has been noted previously,[26] but the diagnostic capability observed in our study was not seen. We believe this discrepancy relates to the timing of investigation and reliance on rigorously collected alveolar samples in our study.

We recommend caution when considering the general applicability of our data. Firstly, our results were generated using bronchoscopy and lavage which may be contra-indicated in some patients, and remains relatively labour intensive and operator-dependent.[27] An ideal diagnostic marker for VAP would involve a highly discriminatory blood test. However this remains elusive as reflected in Supplemental Table S1. Debate continues as to the optimal method for diagnosing VAP.[28] The ‘gold standard’ for the diagnosis of pneumonia remains histology,[29] however this is neither desirable nor practicable in critically ill patients. Therefore uncertainty over the optimal method of diagnosis affects all studies concerning VAP, especially those

focusing on diagnostic markers. A strength of our study lies in the fact that we only recruited patients with pre-defined clinically suspected VAP using established criteria [1,17], and employed a rigorously standardised, visually directed BAL procedure that was conducted by a single experienced operator and relied on quantitative cultures to confirm or refute VAP. To our knowledge these specific, strict criteria have not been simultaneously applied in previous studies seeking diagnostic markers for VAP. Our incidence of confirmed VAP (24%) is at the lower end of the reported incidence, but is consistent with previous reports [30] and the 95% confidence interval (14-34%) for the true population incidence overlaps with the estimate from several other reports [31,32]. The precise timing of sampling may also influence the diagnosis of VAP. Our data relate specifically to the time of first clinical suspicion of VAP, and are based on the use of a particular assay system. Therefore, while IL-1β and IL-8 are promising diagnostic markers, their diagnostic usefulness (and the derived cut-off values) should be validated in wider populations of ICU patients.

Secondly there was a non-significant trend towards more 'non-VAP' patients being on antibiotics at the time of bronchoscopy. Antibiotics could have suppressed microbial growth to some degree in these patients, although previous studies suggest that after 3 days of unchanged antimicrobial therapy false negative cultures are unusual. [1,13] Nevertheless this does not deflect the fact that marked rises in IL-1β and IL-8 only occurred in patients with $>10^4$ cfu/ml (Figure 3). This suggests that low levels of bacterial growth may be tolerated in the alveolar space and support the concept of clinical VAP emerging above a bacterial threshold [1,15].

Third, the use of non-ventilated volunteers in this study can be questioned. We would stress that this group was recruited as a reference group only. The important question in clinical practice is whether patients with a high clinical suspicion of VAP have the condition or not, hence our critical comparison was between the VAP and non-VAP groups.

A fourth caveat relates to the variable case-mix and microbiological epidemiology of ICUs.[33] We included a wide variety of surgical and medical cases but no cardiothoracic cases were involved. The range of bacteria isolated in this study broadly reflects those described elsewhere, but is notable for the absence of *P. aeruginosa*. It may also be potentially relevant that we did not culture for respiratory viruses. Finally, the inclusion of fungi, especially Candida species, as causative organisms in VAP remains controversial [1,34,35]. However we analysed our data strictly in accordance with our pre-defined diagnostic definitions which were based on the concentration of organisms (without specifying the type). Interestingly we did not find any difference in cytokine levels between fungal and bacterial VAPs, with both demonstrating a profound and characteristic pulmonary inflammation. In our patients with VAP associated with fungal growth, all four cytokines described in Figure 1 were above the optimal cut off levels defined by the ROC curves. While we acknowledge that the small numbers involved mean that statistical non-significance could reflect a type two error, the data suggest that fungal infection was associated with the same pulmonary inflammation as the bacterial pathogens more typically associated with VAP. Furthermore, exclusion of *C. albicans* and/or coagulase negative Staphylococci (sometimes considered 'non-pathogenic' organisms) had no significant effect on the diagnostic usefulness of the cytokines as assessed by area under ROC curve (data not shown).

In conclusion, VAP is associated with increased pulmonary IL-1β and IL-8 Further studies are warranted to validate IL-1β and IL-8 as diagnostic markers able to influence important clinical end-points such antibiotic prescribing in ICUs.

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Conflict of Interest statement

Andrew Conway Morris has received an academic prize (travel and accommodation to attend an international conference) funded by Eli Lily.

A John Simpson has received expenses from Astra Zeneca and Glaxo Smith Kline (for travel and accommodation) to attend international educational conferences

Ian F Laurenson has received expenses from Astra Zeneca (for travel and accommodation) to attend international educational conferences.

Timothy S Walsh is the recipient of an unrestricted educational grant from Wyeth pharmaceuticals for work concerning epidemiology of ICU-acquired infection.

All other authors have no conflicts of interest to declare

Figure Legends

Figure 1: ROC curves and optimal sensitivity, specificity, positive predictive values and negative predictive values for BALF cytokines (n=72, 55 non-VAP and 17 VAP).

Data are derived from the patients with clinically suspected VAP. Broken line shows identity. PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

Figure 2: Scatter plots of pulmonary cytokine levels (n=72, 55 non-VAP and 17 VAP).

Each dot represents a single observation. The solid lines mark the median values, the hashed line marks the optimal diagnostic cut-off. Log scale used due to skewed nature of cytokine levels.

Figure 3: Comparison of pulmonary cytokine levels between patients with VAP (n=17), patients with growth of pathogens in BALF below the diagnostic 10^4 cfu/ml cut off (n=22), and patients with no growth in BALF (n=33).

Panel A: IL-8 levels. Data presented as median and inter-quartile ranges, $p<0.0001$ by Kruskal-Wallis, NS $P>0.05$, $^{**}p<0.01$,$^{***}p<0.0001$ by Dunn's post-hoc test.

Panel B: IL-1β levels. Data presented as median and inter-quartile ranges, $p=0.0006$ by Kruskal-Wallis, NS $P>0.05$, $^{**}p<0.01$,$^{***}p<0.0001$ by Dunn's post-hoc test.

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Table S1: Inflammatory profile of serum.
Data expressed as median and inter-quartile range. BALF values are corrected for dilution against concentrations of urea.[16] Analysis by Kruskall-Wallis ANOVA (*<0.05. **<0.01, ***<0.001 shown in left hand column), comparison between VAP and non-VAP undertaken by Dunn's post-hoc test with p value displayed in the right hand column.

	VAP (n=17)	*Non-VAP(n=55)*	*Matched Volunteers (n=21)*	*P value(Dunn's post hoc test between VAP and non-VAP)*
IL-1β (pg/ml)	4 (1.3-6.7)	5 (3-6)	2 (0-5)	NS
IL-8 (pg/ml)***	80 (62-324)	77 (35-257)	3 (0-12)	NS
TNF-α (pg/ml)	3 (1-5)	3 (0-4)	2 (0-5)	NS
IL-6 (pg/ml) ***	106 (55-174)	112 (32-358)	3 (0-4)	NS
G-CSF (pg/ml) **	29 (7-69)	15 (6-35)	4 (0-19)	NS
IL-10 (pg/ml)***	8 (5-15)	9 (5-21)	0 (0-3)	NS
sTREM1 (pg/ml)	30 (0-127)	0 (0-185)	0 (0-2)	NS
MIP-1α (pg/ml)***	5 (0-19)	5 (3-11)	2 (0-3)	NS
MCP-1 (pg/ml)	343 (136-868)	276 (88-502)	62 (44-552)	NS

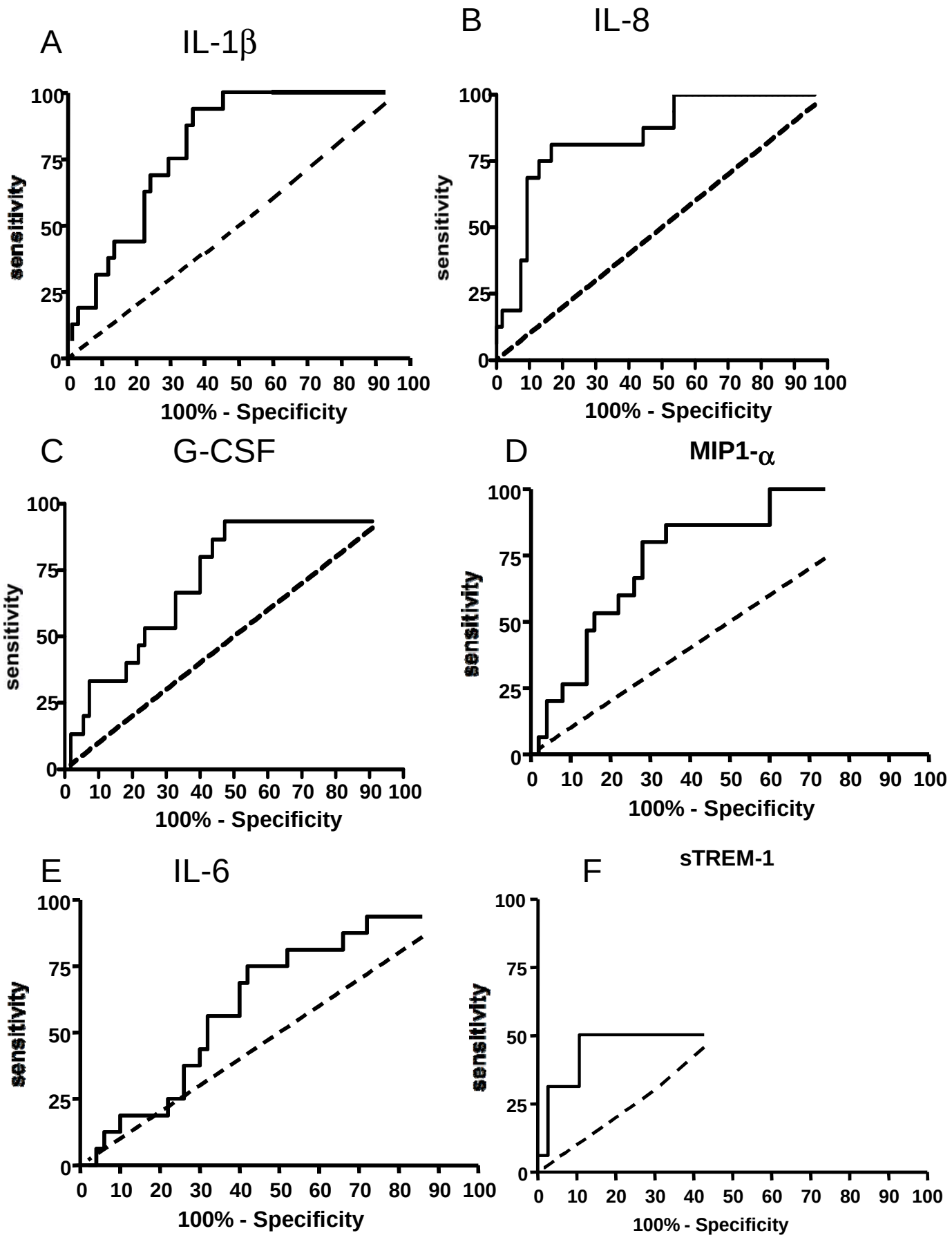
Table S2: Multi-level likelihood ratios with 95% confidence intervals for various cut off values of IL-8 and IL-1β.
+LR, positive Likelihood ratio; -LR, negative likelihood ratio; numbers refer to the number of patients who fall above or below the specified cut-off values.

IL-8

Cutoff (pg/ml)	*VAP (above/below)*	*Non-VAP (above/below)*	*+LR (95% CI)*	*-LR (95% CI)*
200	15/2	25/30	1.6 (0.9-2.2)	0.25 (0.04-1.2)
2000	14/3	9/46	5.03 (1.8-10.5)	0.2 (0.04-0.6)
4000	11/6	7/48	5.08 (1.68-16.9)	0.4 (0.12-0.77)
40000	3/14	1/54	9.7 (0.4-913)	0.83 (0.54-1.1)

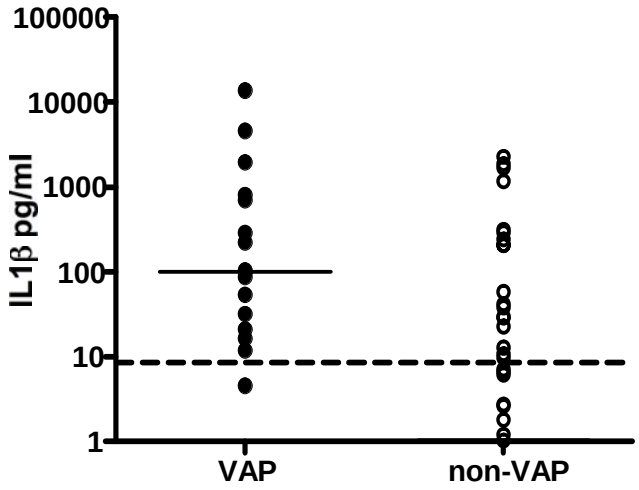
IL-1β

Cutoff (pg/ml)	*VAP (above/below)*	*Non-VAP (above/below)*	*+LR (95% CI)*	*-LR (95% CI)*
5	16/1	25/30	2.04 (1.3-3.1)	0.1 (0-0.5)
10	16/1	21/34	2.46 (1.4-3.9)	0.09 (0-0.6)
50	12/5	13/42	2.9 (1.1-6.7)	0.38(0.12-0.9)
100	9/8	12/43	2.4 (0.66-6.3)	0.60 (0.3-1.2)
1000	3/14	4/51	2.4 (0.22-22.5)	0.88(0.55-1.2)

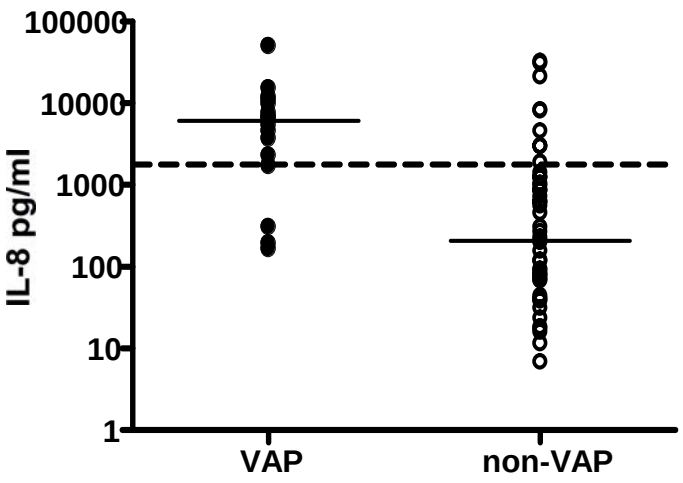


Cytokine	*Area under Curve (95% CI)*	*Optimal cut-off (pg/ml)*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*+LR*	*-LR*
IL-1§	0.81 (0.71-0.91)	10	94%	64%	43%	97%	2.46	0.09
IL-8	0.83 (0.74-0.95)	2000	81%	83%	59%	94%	5.03	0.20
G-CSF	0.73 (0.58-0.86)	37	80%	60%	35%	92%	2.0	0.33
MIP-1α	0.773 (0.66-0.9)	14	80%	72%	46%	92%	2.9	0.27
IL-6	0.63 (0.48-0.78)	Area under ROC curve does not differ significantly from identity						
sTREM-1	0.66 (0.48-0.83)	Area under ROC curve does not differ significantly from identity						

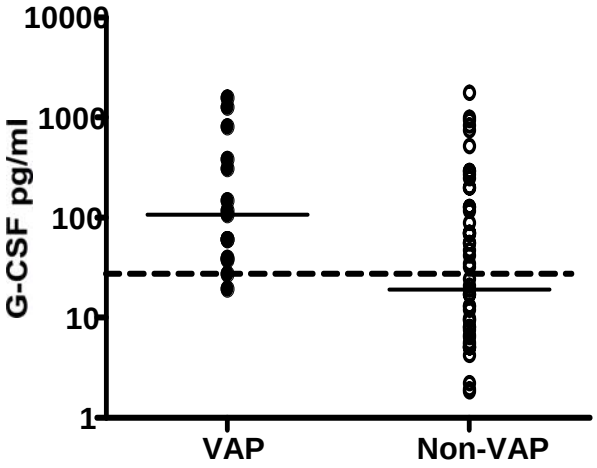
A IL1-β



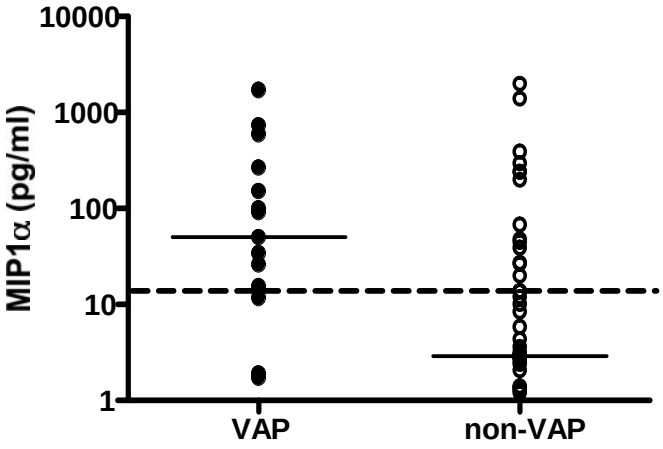
B IL-8



C G-CSF



D MIP1-α



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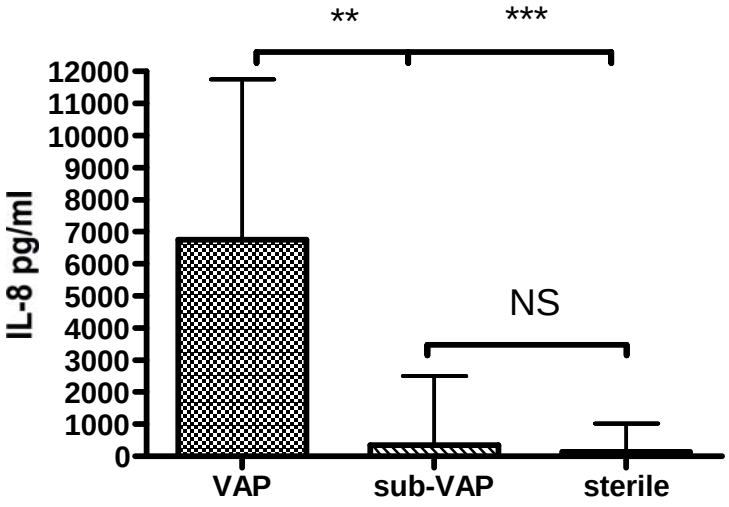

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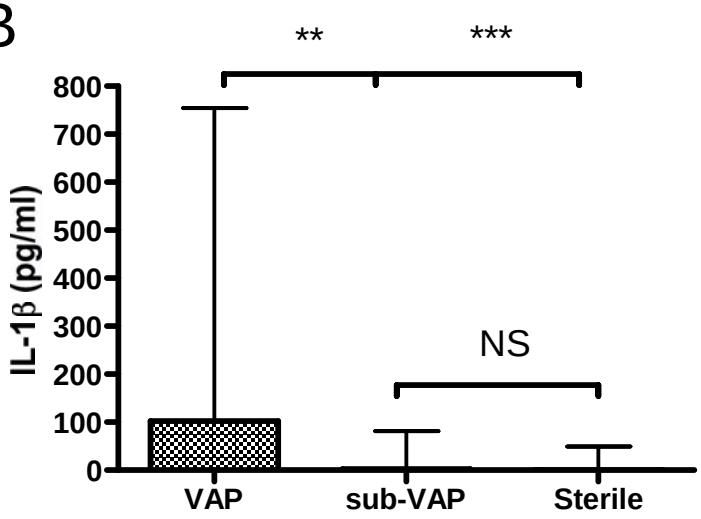

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Corrections

doi:10.1136/thx.2009.122291corr1

Conway Morris A, Kefala K, Wilkinson TS, *et al*. Diagnostic importance of pulmonary interleukin-1b and interleukin-8 in ventilator-associated pneumonia. *Thorax* 2010;**65**:201–7. This article should have included the note that Dr Kefala was joint first author.

doi:10.1136/thx.2009.124776corr1

Polverino E, Dambrava P, Cilloniz C, *et al*. Nursing home-acquired pneumonia: a 10 year single-centre experience. *Thorax* 2010;**65**:354–59. The correct affiliation for affiliation 1 should have read "Respiratory Department, Hospital Clinic-IDIBAPS, Barcelona-Spain, Centro de Investigación Biomedica En Red-Enfermedades Respiratorias (CibeRes, CB06/06/0028, el Ciberes es una iniciativa del ISCIII) – 2009SGRQ - http://www.idibapsrespiratoryresearch.org."

doi:10.1136/thx.2009.133108corr1

Millett C, Glantz SA. Assigning an '18' rating to movies with tobacco imagery is essential to reduce youth smoking. *Thorax* 2010;**65**:377–8. The authors referred to a paper by McNeil *et al*; this should have been Lyons *et al (* Lyons A, McNeill A, Chen Y, *et al*).

doi:10.1136/thx.2009.130716corr1

Lyons A, McNeill A, Chen Y, *et al*. Tobacco and tobacco branding in films most popular in the UK from 1989 to 2008. *Thorax* 2010;**65**:417–22. There is an error in figure legend 2 which currently reads "Trends in all tobacco intervals and tobacco use intervals per hour per **day** by British Board of Film Classification (BBFC) category (all figures expressed as means)." It should have read: " Trends in all tobacco intervals and tobacco use intervals per hour per **year** by British Board of Film Classification (BBFC) category (all figures expressed as means)."

doi:10.1136/thx.2009.127274corr1

Kemp SV, El Batrawy SH, Harrison RN, *et al*. Learning curves for endobronchial ultrasound using cusum analysis. *Thorax* 2010;**65**:534–8. The author name A Roselli should have read A Rosell.