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Endotoxaemia in paediatric critical illness - a pilot study

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Introduction:

The aim was to investigate the prevalence of endotoxaemia in children admitted to pediatric intensive care unit (PICU), and its association with disease severity and outcome.

Methods:

Prospective, observational cohort study of children admitted to PICU at St. Mary's Hospital, London over a 6-month period. 100 consecutive patients were recruited. Demographic and clinical data were collected. Severity of illness was assessed by the paediatric index of mortality 2 (PIM2 score). The paediatric logistic organ dysfunction score (PELOD) score was performed daily for the first 4 days. Patients were categorised according to primary reason for PICU admission.

Blood samples were taken within 24 hours of admission and endotoxaemia was measured using the endotoxin activity assay (EAA). Patients were stratified according to EAA level (high, EAA > 0.4, low, EAA < 0.4) and categorized as septic, post surgical, respiratory or other. Data were analysed using appropriate non-parametric tests.

Results:

EAA level was significantly lower in PICU controls vs other PICU admissions (P = 0.01). 55 children had endotoxaemia on admission. 41 (75%) of these were eventually diagnosed with an infectious cause of admission. 9 children without infection had elevated EAA on admission. An infectious cause of admission was significantly associated with endotoxaemia ($P < 0.005$). Of 15 children with gram-negative infection, only 9 (60%) had endotoxaemia on admission. Endotoxaemia on admission was not associated with shock or death. However there was a tendency for increased PELOD score and length of stay in endotoxaemic children.

Conclusions:

Endotoxaemia is common in children admitted to intensive care. Understanding the implications of endotoxaemia and potential anti-endotoxin strategies may have the potential to reduce severity of illness and length of PICU stay in critically ill children.

Introduction

Sepsis is a major cause of admission to Paediatric Intensive Care Units (PICUs) and causes significant morbidity and mortality in children. A recent study from the United States, estimated the incidence of paediatric severe sepsis to be 0.56 cases per 1000 population, with an overall hospital mortality rate of 10.3%, 7% of all deaths in children [1].

In 2009 there were nearly 17000 admissions to PICUs in the UK. Of these, nearly 9000 (53%) were unplanned medical admissions including sepsis, pneumonia, bronchiolitis and respiratory failure. (PICANET Annual Report 2009) [2]. The unadjusted case fatality rate for children admitted to PICUs in the UK is 4.1% and these children account for over 100,000 bed days. A recent audit of referrals of children with sepsis to PICUs in the UK, found that 17% of these children died [3].

The earliest events in the pathogenesis of sepsis are the interaction of pathogen-related antigens, (e.g. endotoxin [lipopolysaccharide (LPS) and peptidoglycan]), with cell surface pattern recognition receptors such as the Toll-like receptors 4 and 2 respectively [4].The interaction of LPS with its receptors, and the subsequent cellular responses have been well described and is a pivotal process in the inflammatory response leading to the manifestations of sepsis [5].

Measuring the concentration of LPS in human disease has been notoriously difficult. The most commonly used method, the chromogenic limulus amebocyte lysate (LAL) assay, is based on the ability of endotoxin to induce coagulation of hemolymph in the horseshoe crab, Limulus polyphemus [6]. The utility of this assay has been limited because of circulating inhibitors of the coagulation reaction. In addition the assay is not specific for endotoxin.

A novel endotoxin assay, which is simpler and more accurate than the LAL assay, has recently been described [7]. This Endotoxin Activity Assay (EAA) detects LPS in whole blood by the use of neutrophil-dependent chemiluminescence. This assay has been used in a study of critically ill adults, where an association between

endotoxaemia with infection and an increased risk of adverse outcome was demonstrated [8].

Because of the link between endotoxin and inflammation, we sought to define the prevalence of endotoxaemia in critically ill children, and determine the association of endotoxaemia with infection, severity of illness and outcome.

Materials and methods

We undertook a prospective observational cohort study of critically ill children admitted to the PICU at St. Mary's Hospital, London, over a 6-month period, January to June 2007. The unit does not admit cardiac-surgical or neurosurgical patients. Informed consent was obtained from parents/carers. The study was approved by the local research ethics committee.

Demographic and clinical data were collected on admission to PICU. Patients were categorised into their primary reason for admission: respiratory failure (i.e. acute viral bronchiolitis, croup, asthma, pneumonia); neurological failure (i.e. meningo-encephailitis, status epilepticus); sepsis; cardiac (i.e. myocarditis, arrhythmia); surgical or other (i.e. trauma, cardiac arrest).

We defined a PICU control group as children who were electively admitted to PICU for post-operative care. They were all electively ventilated, but had no other organ failure on admission.

Patients were diagnosed with sepsis and/or septic shock according to Goldstein's criteria [9]. Infection was diagnosed with standard microbiological techniques.

Severity of illness was assessed by the Paediatric Index of Mortality 2 (PIM2) score [10], and the Paediatric Logistic Organ Dysfunction Score (PELOD) [11] was performed daily for the first 4 days as 4 days was the mean length of stay.

Chemiluminescent assay for endotoxin

All patients had a single measurement of endotoxin activity (EA) in whole blood within 24 hours of PICU admission, as described previously [7].
2ml whole blood samples were collected into EDTA vacutainer tubes. Samples of 0.5mL of whole blood in duplicate were immediately incubated with saturating concentrations of a murine IgM monoclonal antibody against the lipid A of Escherichia Coli J5. This antibody is broadly cross-reactive against gram-negative bacteria; it does not cross-react with gram-positive bacteria. Any LPS present in the blood, complexes with the anti-LPS antibody. This complex primes the patient's neutrophils for an augmented response to stimulation with zymosan. The resulting respiratory burst activity is detected as light release from the lumiphor luminol, using a chemiluminometer (Autolumat LB953; E.G. & G. Berthold). By measuring basal (no antibody) and maximally stimulated (4600 pg/mL LPS) responses in the same blood sample, the endotoxin activity of the test specimen is calculated by integrating chemiluminescence over time. Levels are expressed as EA units and represent the mean of duplicate determinations from the same sample. An EA level of > 0.4 is approximately equivalent to an endotoxin concentration of 25–50 pg/mL of E. coli 055:B5 LPS.
As the EAA requires adequate numbers of patient neutrophils to provide the respiratory burst for the assay read out, we excluded patients who had an absolute neutropenia of $<0.1x10^6/cm^3$.

For the purposes of this study, as defined previously [7], a cut off level of 0.4 EA units was used to determine the presence of endotoxaemia - i.e. $\leq$ 0.4 negative, > 0.4 Endotoxin activity present).

Statistics

Data were non parametric and are presented as median $\pm$ IQR. The results were analysed using Graph Pad Prism. The Mann-Whitney U Test was used to compare groups of data and the Kruskal Wallis test was used if there were >2 groups of data. The Chi Square test was used to compare proportions.

Results

One hundred and four consecutive admissions were asked to participate in the study, of which 4 refused. There were no children excluded, therefore 100 patients were recruited for the study. Their demographics are shown in Table 1.

Ten children (10%) died and 90 were discharged alive from PICU. The mean length of stay (LOS) for survivors was 7.1 days (median 5 days, range 1 – 57).

There were nine children in the control group, 48 in the respiratory group, 18 in the sepsis group and 25 in "other". The control group consisted of two children who had tonsillectomy and adenoidectomy for obstructive sleep apnoea; three children who had a Nissen's fundoplication and gastrostomy; one child who had a gastric pull up for oesophageal atresia; one child who had an oesophageal dilatation; one child who had resection of a colonic duplication cyst; and one child who had a partial nephrectomy. None suffered periods of intraoperative hypotension, or needed prolonged post-operative ventilation.

EAA level was significantly lower in the control group (0.16, IQR 0.11-0.37) compared to the respiratory (0.48, IQR 0.34-0.74), sepsis (0.55, IQR 0.4-0.7) and other groups (0.37, IQR 0.25-0.59). (p= 0.01, Kruskal-Wallis test, Figure 1).

Fifty-five children had endotoxaemia on admission to PICU. Only two of these were in the control group (one a child who underwent an elective Nissen's fundoplication who had extensive bowel manipulation during surgery; the other a child who underwent an adenotonsillectomy). Of these 55 children, 41 (75%) were eventually diagnosed with an infection-related cause of admission (Table 2).

Seventeen children were bacteraemic on admission. Fifteen children had Gram-negative infection as the cause of admission, of which only 9 (60%) were endotoxaemic on admission. Of the 15 children with Gram-negative infection, only 5 had Gram-negative bacteraemia (all meningococcus serogroup B). Only 3 of the 5 children with meningococcal bacteraemia had endotoxaemia detected on admission.

There were 62 children who were diagnosed with an infection-related admission, of which 41 (66%) had endotoxaemia, compared to 14 of 38 (37%) children with a non-infectious cause of admission (p=0.0043, Chi square test). 75% of children with respiratory failure of any cause had endotoxaemia on admission to PICU.

All children admitted to our PICU where there is a suspicion of infection are treated with a standard regimen of antimicrobials, consisting of a third-generation cephalosporin (usually Ceftriaxone) with or without Gentamicin, if there is a suspicion of pseudomonas infection (such as patients with chronic lung disease). There was no clear correlation between antimicrobial use and endotoxaemia (data not shown).

The presence of endotoxaemia on admission to PICU was not significantly associated with shock or death. Twenty two of 34 children (64%) who had shock at PICU admission had endotoxaemia, compared with 35/66 (53%) without shock (ns, Chi square test).
Seven of the 10 (70%) children who died had endotoxaemia on admission compared to 48/90 (53%) survivors (ns, Chi square test).

Daily PELOD score, PIM2 score on admission and length of stay (LOS) in survivors was not significantly different between the groups with and without endotoxaemia on admission, although there was a tendency for increased PELOD score and LOS in the group with endotoxaemia (Table 3).

Discussion

Our study shows that endotoxaemia is common in a heterogeneous population of critically ill children. Although there was no clear correlation with severity of illness or the presence of shock, there was a tendency toward longer length of stay in patients with endotoxaemia. This is consistent with a recent study in adult surgical intensive care patients, which showed an association between endotoxaemia and length of ICU stay [12].

Despite the high prevalence of endotoxaemia found in our study, only 15 children were diagnosed with gram-negative infection, a scenario classically associated with endotoxaemia. In addition, only 9 of 15 children with documented gram-negative infection had endotoxaemia. This may reflect the timing of the assay in relation to admission and treatment. It has previously been noted that endotoxin level in blood declines rapidly following appropriate antimicrobial therapy [13]. We did not record the timing of performance of the EAA in relation to start of antimicrobial therapy, but 75% of samples were taken within 12 hours of admission to PICU.

The commonest cause of PICU admission in our study was respiratory failure usually due to viral lower respiratory tract infection. The majority (75%) of children with respiratory tract infection had endotoxaemia on admission, despite having low severity of illness scores and good outcome. Several studies have documented high levels of endotoxin in lung and blood of patients with respiratory infection, as well as those on artificial ventilation. Pulmonary to systemic translocation of LPS has been demonstrated in non-protective ventilation strategy studies in experimental animals [14], and an association between ventilator-induced lung injury, ventilator-associated pneumonia and systemic LPS and cytokine levels has been reported [15]. Although the lung has no endogenous microflora that could provide a source of LPS, It is likely that bacterial colonisation of the airway or lung may be a potential source of LPS which can translocate systemically following mechanical ventilation. The high prevalence of endotoxaemia in our patients seems to confirm this.

The gut provides a second and major potential source of systemic LPS. The indigenous flora of the gastrointestinal tract contains large amounts of endotoxin, and translocation of both endotoxin and viable bacteria from the gut has been demonstrated in animal models and in human illness associated with splanchnic hypoperfusion [16, 17]. Consistent with this, we observed that endotoxaemia was more common in critical illness compared to control children. In addition, patients who had endotoxaemia on admission appeared to be a sicker population, as reflected by longer length of stay and trend toward higher PELOD scores. Thus, the presence of endotoxaemia on admission may identify a high-risk subpopulation of critically ill children.

The chemiluminescent assay used in our study is both sensitive and specific for endotoxin and can be performed in <1 hour, which permits the rapid detection of endotoxin in fresh whole blood. However, it uses the patient's own neutrophils as a readout system and so presents inherent limitations; in particular, it is not possible to store specimens for later assay; measurements must be performed within 3 hours of obtaining the sample. Because of the requirement for neutrophils, the assay is not reliable in patients with an absolute neutrophil count of $<0.1x10^6/cm^3$.
The assay relies on neutrophil chemiluminescence in response to IgM anti LPS antibody and LPS. However, the pre-activation state is accounted for by correcting for baseline chemiluinescence in the EAA.
In addition, the assay detects exposed lipid A in the endotoxin molecule and so may not reflect endotoxin bioactivity in vivo. However, the availability of a relatively simple bedside assay for blood endotoxin level may identify a high-risk population of critically ill patients who may benefit from adjunctive therapy.

We did not correlate the EAA with the best known endotoxin assay, the Limulus Amoebocyte Lysate (LAL) assay. The LAL assay has been widely used to detect endotoxin contamination of drugs and fluids; however, its utility in biological samples has been limited, because of circulating inhibitors of the coagulation reaction. In addition, other microbial products, notably from fungi, can activate the LAL reaction, so the assay is not specific for endotoxin [18]. Studies comparing EAA and LAL show considerable variability in the prevalence of endotoxaemia or its association with Gram-negative infection, with the EAA able to detect endotoxaemia associated with Gram-negative infection from any source, a diagnosis of sepsis and an elevated white blood cell count; no such correlations being found when samples were assayed using the LAL method [19].

Antibiotics can accelerate endotoxin release and may result in falsely negative blood cultures [20]. We did not correlate endotoxaemia with type and duration of antimicrobial therapy. However, most patients would have been treated with our typical empiric antimicrobial regimen which consisted of a 3rd generation cephalosporin, with additional gentamicin in certain cases, such as those with specific risk factors for pseudomonas infection.

Whether the increased length of stay and possible increased severity of illness associated with endotoxaemia might be reduced by specific measures to neutralize endotoxin needs to be studied further, but the hypothesis remains attractive. High levels of endotoxin have been associated with increased severity of illness in meningococcal disease [13], and studies of anti-endotoxin therapy in human sepsis suggest that the greatest potential benefit occurs in patients in whom endotoxaemia is likely to be present [21].

Inferences from the present study are limited by the observational nature of the study and the relatively small number of patients in each disease category. In addition, the timing of the assay may have "missed" the peak of endotoxaemia in those patients where antimicrobial therapy had been initiated prior to PICU admission. In addition, intrinsic anti-endotoxin activity (such as endotoxin antibodies, and lipoproteins may quench endotoxin activity.

Conclusions

The demonstration of significant endotoxaemia in a high proportion of children admitted to PICU for a variety of pathologies suggests an important interaction between critical illness and inflammation induced by, or augmented by, intrinsically derived endotoxin. It is possible that this endotoxaemia contributes to severity of illness and ouotcome. The hypothesis that LPS contributes to disease severity in critical illness requires further exploration.

Our study suggests endotoxaemia in critically ill children is common and can be detected with a simple, bedside test. The use of anti-endotoxin agents potentially may have a role in the treatment of critical illness of all causes in children, and this should be the subject of future studies.

Key messages

- Children with critical illness of any cause are likely to have endotoxaemia.

- Endotoxaemia can be accurately detected by a simple bedside test.
- Endotoxaemia may be associated with increased severity of illness and length of stay in PICU.
- It is likely that the source of endotoxin in these children is from their own gut or lung.
- If the findings of this study are confirmed in larger studies, anti-endotoxin therapies may be a logical adjunct to treatment of paediatric critical illness.

Abbreviations:

EA: endotoxin activity, EAA: endotoxin activity assay; a test for endotoxin, EDTA: ethylenediaminetetraacetic acid; an anticoagulant, HHV6: human herpes virus 6, IQR: interquartile range, LAL: limulus amebocyte lysate assay; a test for endotoxin, LOS: length of stay, LPS: lipopolysaccharide; endotoxin, PCP: pneumocystis jirovecii Pneumonia, PELOD: paediatric logistic organ dysfunction score; a severity of illness score, PICU: paediatric intensive care unit, PICANET: paediatric intensive care audit network (UK), PIM2: paediatric index of mortality 2; a risk of mortality score, RSV: respiratory syncytial virus.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions:

SD recruited patients, performed the assay and wrote the first draft of the manuscript.
DI gave statistical advice and contributed to the manuscript.
HB recruited patients, performed the assay and contributed to the manuscript.
SN initiated the project, recruited patients, performed the assay and contributed to the manuscript. All authors have read and approved the article for publication.

Figures:

Figure 1: EAA level against clinical classification.

For statistical analysis see text.

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Table 1: Demographic data

	n
Total population recruited	**100**
Median Age (months)	**33.8 (range 0-180)**
Gender	**Male 63**
Diagnosis – Respiratory*	**48**
Sepsis	**18**
Other**	**25**
Control***	**9**
Shock on admission	**34**
Died before discharge	**10**

* Includes 8 with respiratory syncytial virus bronchiolitis
** Other includes 7 primary neurological failure and 4 primary cardiac failure
*** Post operative elective admission

Patients may fall into more than one category.

Table 2: Infectious causes of admission (n=62)

Viral	**n**
Adenovirus	**3**
Influenza A	**2**
HHV6	**1**
Metapneumovirus	**2**
Parainfluenza 1	**1**
RSV	**8**
Varicella	**2**
Bacterial	
Staphylococcus aureus	**8**
Group A Streptococcus	**4**
Group B Streptococcus	**2**
Haemophilus influenzae	**3**
Klebsiella spp.	**1**
Meningococcus	**6**
Pneumococcus	**8**
Pseudomonas	**4**
Salmonella spp.	**1**
Other	
PCP	**2**
Malaria	**1**
Aspergillus	**1**
Candida	**2**

HHV6; human herpes virus 6, RSV; respiratory syncytial virus, PCP; pneumocystis jirovecii pneumonia.

Table 3: Severity of illness (median and IQR shown)

	EAA </= 0.4	EAA > 0.4	Mann Whitney
PIM (%) score	5 (1.5-13)	6 (2-20)	p=0.35
PELOD score Median (Interquartile range)	1.7 (1.15-20.8)	16.2 (1.3-20.8)	p=0.31
LOS (survivors) Median (interquartile range)	4 (2-7)	5 (4-8)	p=0.05

PIM; paediatric index of mortality, PELOD; pediatric logistic organ dysfunction, LOS; length of stay (days).

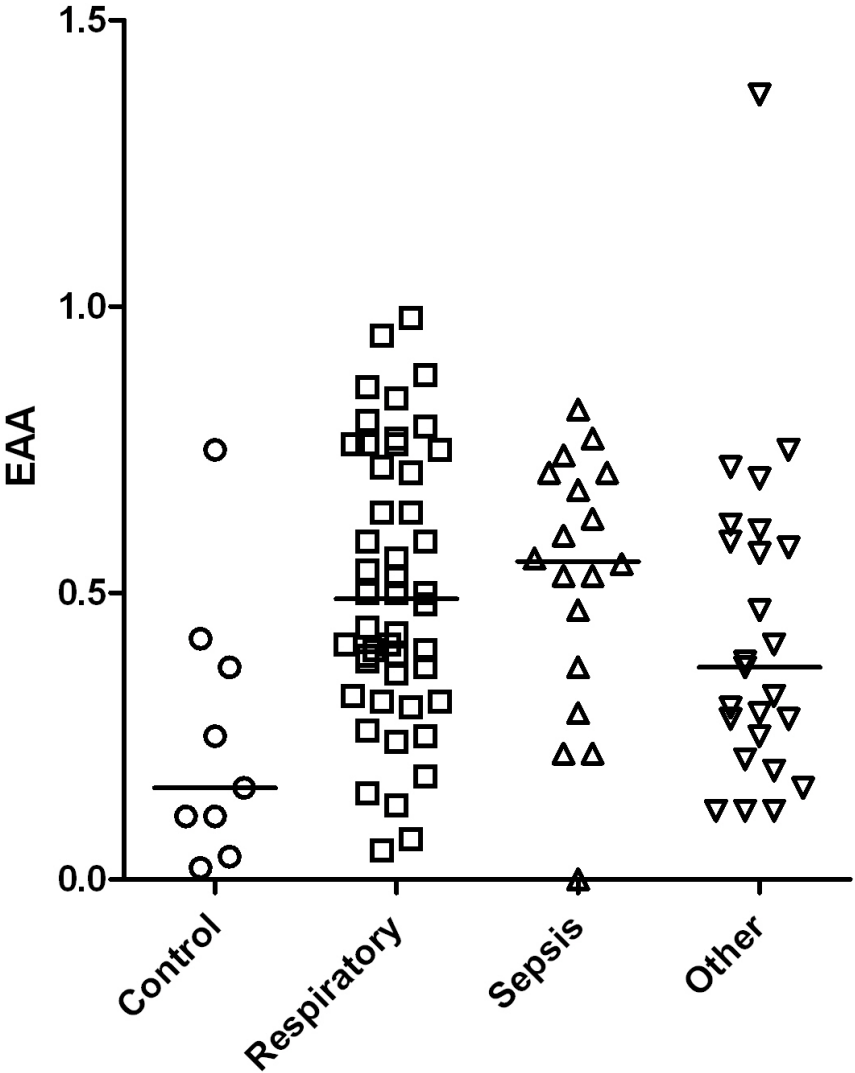

Figure 1