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Brief Report

Plasma secretory phospholipase A2 as an early marker for late-onset sepsis in preterm infants - a pilot study

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Very preterm infants (≤ 32 weeks gestational age; GA) are at high risk of developing late-onset sepsis (LOS; onset after 72 hours of age), which is associated with increased mortality and short- and long-term morbidity.[1] The early symptoms of suspected LOS are non-specific and are managed with empiric broad-spectrum antibiotics, with well-documented adverse effects, including mortality.[2] Microbial blood culture, with adjunctive diagnostic biomarker(s) (e.g. C-reactive protein (CRP) and interleukin (IL)-6), are commonly used for LOS diagnosis, but have sub-optimal sensitivity and specificity, and median time to positivity of 12 - 36 hours for blood culture.[3] Thus, there is an urgent and unmet need for accurate and more rapid adjunctive diagnostics to optimise and minimise antibiotic use in this vulnerable population.

Increased activity of secretory phospholipase A2 group IIA (sPLA2-IIA), a key enzyme in the synthesis of inflammatory prostaglandins and leukotrienes, is reported in preterm and term infants with sepsis during the first week of life.[4] We aimed to evaluate the diagnostic performance of sPLA2-IIA as an early indicator of LOS in preterm infants born < 30 weeks GA. We hypothesised that the positive and negative predictive values (PPV; NPV) of sPLA2-IIA at the time of blood culture sampling for suspected LOS would identify infants without LOS and allow cessation of antibiotics earlier than possible with current diagnostics.

Infants from two independent prospective cohort studies with common sampling protocols for suspected LOS were included. The first study investigated the innate immune responses in infants born < 30 weeks GA (n=129) with the primary blood sample collection at weekly intervals for the first month of life and secondary opportunistic collection at the time of evaluation for suspected LOS. The second study evaluated transcriptomic pathways in infants born < 42 weeks GA investigated for suspected sepsis (n=57). For cohort homogeneity, preterm infants born < 30 weeks GA with suspected LOS and a blood sample collected when blood culture was performed and antibiotic therapy commenced were included in the analysis (study 1 n=35, study 2 n=21). Both studies were approved by the institutional Ethics Committee at King Edward Memorial Hospital, Perth (1627EW and 2014091EW), and written, informed consent was obtained from parents.

Plasma was collected within 2 hours of blood culture for suspected LOS (median, IQR: 0.0, 0.0-1.9 hours) and stored at -80 °C until analysis. Plasma concentrations of sPLA2-IIA and

IL-6 were measured by enzyme immunoassay (Cayman Chemicals, MI, USA) and bead-based immunoassay kit (ProcartaPlex Biosystems eBioscience, CA, USA), respectively, according to manufacturer's instructions. CRP measurements were collected pragmatically as part of routine care.

Suspected septic episodes with a positive blood culture and/or a CRP >20 mg/L within 72 hours of blood culture sampling were defined as 'LOS' (n=28), whereas a negative blood culture and 2-4 serial CRP <20 mg/L were defined as 'no LOS' (n=21). Blood cultures positive for coagulase-negative staphylococci (n=5), *Enterococcus faecalis* (n=1) or *Klebsiella pneumoniae* (n=1), in infants with 2-4 serial CRP <20 mg/L, and no sustained clinical features of LOS were excluded. Logistic regression models assessed the association of log-transformed sPLA2-II2 and IL-6 plasma concentrations with LOS (Walds test). Mean area under the receiver operating characteristic (AUROC) models, generated by 2-fold cross validation (repeated 250 times), assessed the diagnostic performance of sPLA2-IIA and IL-6 in predicting LOS (fit with R v4.0.2 and ROCR). Youden's index determined the optimal concentration cut-off of sPLA2-IIA and IL-6, which was then used to calculate PPV and NPV (cutpointr).

The demographic and clinical details between the LOS (n=28) and no LOS (n=21) infants were similar (Table S1), as were the first and second (n=30 and 19) study cohorts, and the LOS infants with positive and negative blood cultures (n=16 and 12, respectively), except for a higher CRP in infants with blood culture positive LOS (median (IQR) mg/L: 57 (32-91) and 31 (27-53); p=0.046).

sPLA2-IIA plasma concentrations were elevated in infants with LOS compared to uninfected infants (Fig. 1A). sPLA2-IIA from blood samples obtained on average within 2 hours of blood culture sampling for suspected LOS had a mean AUROC of 0.88 for predicting LOS (Fig. 1B). Using a cutoff of ≥6,330 pg/mL, the PPV of sPLA2-IIA was 83% (95% CI 66.4-92.7%) for infants with LOS and the NPV was 84% (95% CI 62.4-94.5%) for infants who were subsequently categorised as no LOS after negative blood culture and serial CRP measurements (both of which are robust indicators that bacterial infection is unlikely).[5] Prior to being categorised as no LOS, these infants received antibiotic therapy on average for 30 hours (median (IQR) 30.2 (19.9-43.6)).

IL-6 was also elevated in infants with LOS compared to the no LOS group at the time of blood culture sampling (Fig. 1A). IL-6 had a mean AUROC of 0.81 for predicting LOS (Fig. 1B) and at an optimal cutoff ≥41 pg/mL the PPV was 84.6% (95% CI 66.5-93.8%) and NPV

was 73.9% (95% CI 53.5-87.5%). sPLA2-IIA had superior overall performance for identifying LOS than IL-6, and sooner than reported diagnostic utilities of CRP and procalcitonin.[3]

This pilot study has limitations, including being a single centre study with a small sample size, limiting generalisability. Analysis was limited to sPLA2-IIA and IL-6 due to limited blood volume. We could not comment on the association between sPLA2-IIA levels and respiratory distress syndrome (RDS), as previously reported, since RDS has typically resolved by the median onset of LOS (14 days) in our cohort.[4]

In summary, we found that sPLA2-IIA may be a clinically useful marker for the earlier diagnosis of LOS in preterm infants and could support clinical management decisions and reduce unnecessary antibiotic exposure in conjunction with blood culture and other adjunct inflammatory markers. Larger prospective studies should confirm these preliminary findings and further explore the role of sPLA2-IIA in LOS.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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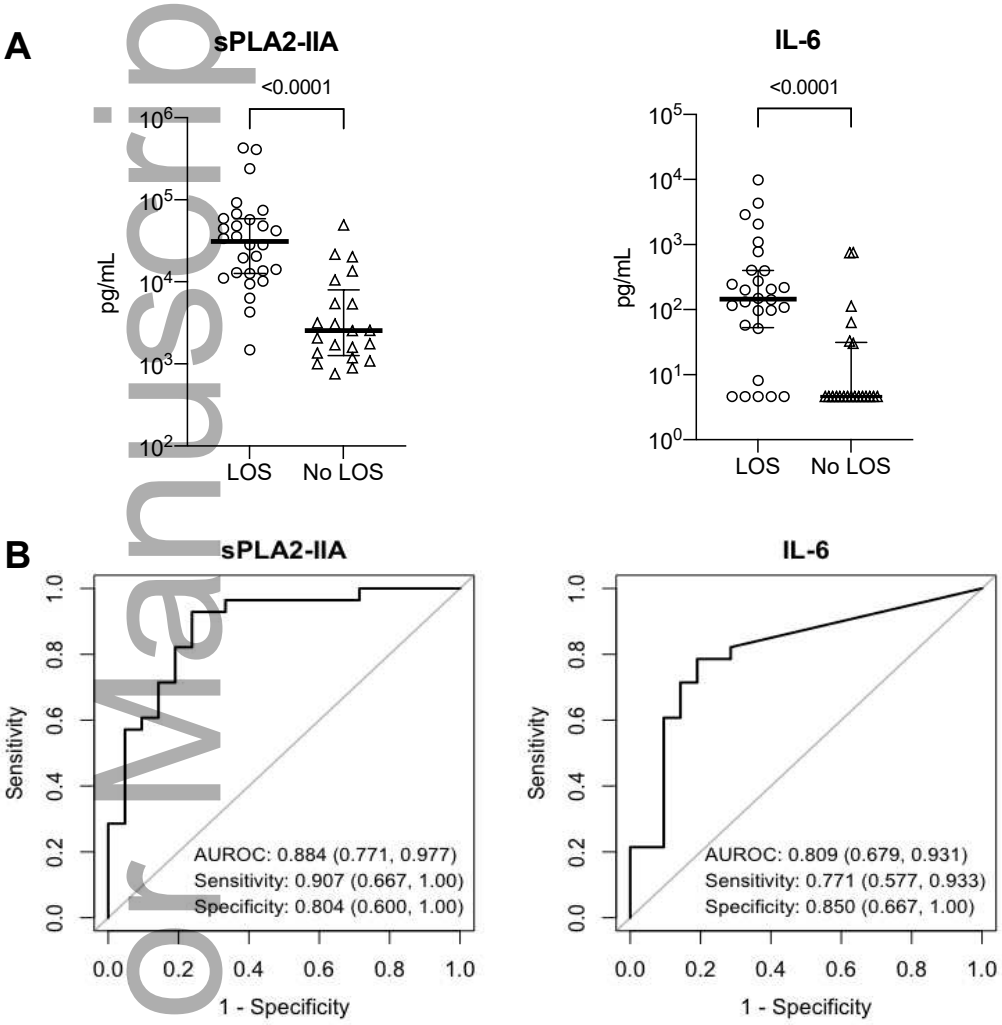

Fig. 1. In plasma from very preterm infants with (n=28) and without (n=21) LOS: **A)** Median (IQR) concentration of sPLA2-IIA (30,970 (12,677-58,710) and 2,534 (1,269-7,991)) and IL-6 (145 (53-400) and 4.6 (4.6-32)), and **B)** Mean AUROC, sensitivity and specificity (95% CI) of sPLA2-IIA and IL-6 for the diagnosis of LOS.