Quantification of acute phase proteins in 134 neonatal dried blood samples from CELSPAC birth cohort study

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Introduction: Acute phase proteins (APPs) are generally not transported across the placenta and thus provide a measure of the activity of the innate immune system in the neonate (1). Circulating plasma concentrations of APPs changes by at least 25 % in response to inflammation (2). Protein concentrations are typically determined by immunoanalytical methods, such as immunoturbidimetry or immunonephelometry. However these methods require blood sample collection via venipuncture, un-suitable for newborns and babies. On the other hand, collection of dried blood samples (DBS) is less invasive and requires less sample processing and handling. Neonatal DBS are collected from a heel prick and they serve as conventional material for newborn screening for endocrine and metabolic disorders (3).

Methods: Proteins from DBS were extracted with 50mM ammonium bicarbonate buffer (pH 7.8) internally standardized using stable isotopically labeled peptides with a trypsin cleavable tag (SIL-TCT). The extracts were enzymatically digested overnight at 37 °C using trypsin and digests were desalted applying solid phase extraction (SPE). The tryptic peptides were analyzed by ultra-high performance liquid chromatography (UHPLC) and selected reaction monitoring (SRM) mass spectrometry (MS) in positive ion mode. Previously developed multiplex assay was applied to 134 neonatal DBS for absolute quantification of alpha-1-antitrypsin (A1AT), alpha-1-acid glycoprotein 1 (A1AG1), alpha-1-acid glycoprotein 2 (A1AG2), C-reactive protein (CRP), serum amyloid A1 (SAA1), serum amyloid A2 (SAA2), serum amyloid A4 (SAA4) and immunoglobulin A (IgA) (4).

Results: Blood levels of A1AT, A1AG1 and A1AG2 may increase several fold in response to inflammation (5, 6). In our study, median of blood concentrations of A1AT, A1AG1 and A1AG2 were 189.8 mg/l; 105 mg/l and 23.2 mg/l respectively. Median of blood concentration of constitutive protein SAA4 was 7.7 mg/l. Circulating levels of SAA1, SAA2 a CRP are low in healthy individuals, but they increase between 10- to 1000-fold during acute phase of inflammation. Only a single neonatal DBS revealed CRP level above limit of quantification (7.2 mg/l), still within the normal range for CRP (7). However, SAA is arguably a more reliable marker of inflammation compared to CRP as SAA levels rise earlier, more rapidly and with higher amplitude (8). In our study, higher than normal levels of SAA1 (> 10 mg/l) were observed in 13 newborns, presumably indicating an inflammatory condition.

Conclusion: In this study we have quantified inflammatory markers in neonatal DBS. Levels of 7 proteins (A1AT, A1AG1, A1AG2, CRP, SAA1, SAA2, SAA4) were determined in 134 newborns. Increased levels of SAA1 in 13 newborns is potential indicator of inflammatory response.

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