

REVIEW

Exhaled breath measures of inflammation: are they useful in neonatal chronic lung disease?

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Neonatal chronic lung disease is a common problem for surviving infants of extreme prematurity. Although the precise pathophysiology is still not known, it is clear that inflammation provides a common link that amplifies the injury to the premature lung. Current invasive measures of pulmonary inflammation include markers in blood and airway effluent, with the cellular composition of tracheal fluid being the "gold standard". In this article available exhaled breath measures, particularly nitric oxide, carbon monoxide, volatile hydrocarbons, and exhaled breath condensate, are reviewed with particular reference to sample collection, analysis, and common pitfalls as they apply to the ventilated premature newborn at risk of chronic lung disease. Although they have great potential, all measures require thorough validation before being used clinically.

including vascular endothelium, airway epithelium, and inflammatory cells.¹⁰ Endothelial derived NO has been extensively studied in the establishment of pulmonary circulation.¹¹ Proinflammatory cytokines (tumour necrosis factor α , interleukins 1 and 6) increase the expression of inducible NO synthase¹² and are increased in bronchoalveolar lavage specimens from newborns with CLD.¹³ Bronchoalveolar lavage nitrite and nitrate, byproducts of NO metabolism, remain raised in premature newborns who develop CLD, possibly reflecting inflammatory upregulation.¹⁴ In adults, eNO has been validated against measures of inflammation in bronchoscopic specimens^{10 15} and has been measured in obstructive sleep apnoea¹⁶ and acute respiratory distress syndrome,¹⁷ and in asthma can predict both loss of control and response to corticosteroids.¹⁸

eNO has recently been measured in ventilated newborns.¹⁹ In a small group of ventilated infants, lower airway eNO fell after treatment with dexamethasone.²⁰ This is consistent with the inflammatory modulation effect of corticosteroids.²¹

Collection

In adults, eNO is collected by a single-breath technique and reflects both airway and alveolar production. Both single breath against resistance and multiple tidal breath techniques²² have been used to prevent contamination from nasal NO. In ventilated newborns, a 4 French catheter positioned adjacent to the end of the endotracheal tube²⁰ has been used for collection of lower airway specimens.²³

Analysis

An international taskforce has defined standards and procedures for measurement of eNO in adults and children.²⁴ eNO measurement by chemiluminescence has been standardised and is reproducible.²⁵

Potential pitfalls

NO is produced by the paranasal sinuses in adults and is increased in nasal samples from ventilated newborns.^{23 26} In addition, eNO concentrations increase in normal subjects after inhaled, intravenous, or digested L-arginine.^{27 28} This needs consideration in newborns receiving parenteral nutrition solutions.

Abbreviations: CLD, chronic lung disease; eNO, exhaled nitric oxide; eCO, exhaled carbon monoxide; EBC, exhaled breath condensate

Neonatal chronic lung disease (CLD) is a common pulmonary outcome for extreme preterm infants.¹ It is probably the result of a number of interacting factors.^{2 3} Although the precise pathophysiology is not known, it is clear that inflammation provides a common link that amplifies the injury to the lung. There are a number of broad strategies to ameliorate and/or prevent neonatal CLD.^{4–7} Some have serious side effects^{8 9} and are prescribed after an individualised cost-benefit analysis.

The cellular composition of tracheal effluent remains the optimal measure of pulmonary inflammation. Exhaled breath has been used to measure inflammatory markers in adults¹⁰ for over 30 years. A validated breath measure may be of benefit to ventilated newborns by providing a surrogate non-invasive outcome measure, allowing titration of anti-inflammatory treatments, and by predicting at risk infants by defining the profile of CLD.

It is the purpose of this review to outline available exhaled breath measures of inflammation, particularly exhaled nitric oxide (eNO), exhaled carbon monoxide (eCO), volatile hydrocarbons, and exhaled breath condensate (EBC), and to summarise current evidence with particular reference to sample collection, analysis, and common pitfalls.

EXHALED NITRIC OXIDE (ENO)

NO is synthesised from arginine by NO synthetase. It is produced by a number of different cells

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Summary

eNO has great potential in ventilated newborns provided that problems with collection are addressed, particularly partitioning of nasal and lower airway sampling. In addition, further validation against gold standard measures of airway inflammation is needed before eNO can be widely used in premature ventilated newborns.

EXHALED CARBON MONOXIDE (eCO)

CO is produced endogenously from the degradation of haem-containing proteins (haemoglobin) and is a recognised exogenous contaminant. Haem oxygenase is the rate limiting enzyme responsible for degradation of haem to biliverdin and CO in equimolar amounts. It exists in at least two isoforms, and one of these (HO-1), found mostly in the spleen and liver, is inducible.²⁹ CO binds to haemoglobin as carboxyhaemoglobin and is subsequently excreted in exhaled breath. Thus, eCO can be used as a measure of bilirubin production in vivo.^{30–33} After the exclusion of exogenous CO from environmental contamination and passive diffusion across the placenta, eCO has been shown to be a valid measure of haemolysis³⁴ and corrected reticulocyte count in Coombs' test positive term neonates.³⁵

Increased concentrations of eCO (fractional concentration of CO in expired gas) have been reported in patients with inflammatory airway disorders, such as asthma,³⁶ cystic fibrosis,³⁷ and bronchiectasis.³⁸ In adults with cystic fibrosis, concentrations are reduced with antibacterial treatment and corticosteroids, suggesting that it may be a useful marker of inflammation and oxidative stress.³⁹ Both oxidants and inflammatory cytokines are capable of inducing haem oxygenase, thereby increasing endogenous CO production.⁴⁰ eCO is increased in infants who later develop CLD,⁴¹ and plasma CO concentrations are raised in the paediatric sepsis syndrome.⁴²

Collection

The alveolus is the main site of diffusion, as CO production is unaffected by flow rate but increased with breath holding.^{43–44} In adults, end tidal eCO concentrations have been collected after a period of breath holding. This method is non-invasive, reproducible, and well tolerated.⁴⁵ In a method developed for non-ventilated infants (Natus CO-stat End Tidal Breath Analyser (Natus Medical, San Carlos, California, USA), a small sampling tube inserted into the nostril has been used to draw air continuously across a measuring device at a fixed flow rate.^{31–34}

Analysis

Previous researchers have developed a validated end tidal measuring device in non-ventilated infants using an electrochemical sensor.³² This device measures both endogenous and exogenous eCO.

Potential pitfalls

Passive diffusion of CO from infants of smoking mothers may exaggerate eCO in the first 24 hours of life.³⁴

Summary

eCO has already been validated in non-ventilated newborns as a measure of haemolytic disease. However, further validation against standard measures of airway inflammation is needed for concise interpretation, particularly in a population in which sepsis, hyperbilirubinaemia, and inflammatory lung disorders often co-exist.

VOLATILE HYDROCARBONS

Volatile hydrocarbons are byproducts of lipid peroxidation. The concentration in expired gas, measured by the

hydrocarbon breath test,⁴⁶ reflects both endogenous production and exogenous contamination.

Ethane and pentane are low molecular mass hydrocarbons. They are generated by the free radical peroxidation of omega 6 (linoleic) and omega 3 (linolenic) polyunsaturated fatty acids. They have been used as non-invasive markers of active inflammation in obstructive sleep apnoea,¹⁶ acute asthma (pentane),⁴⁷ colitis (animal model),⁴⁸ and rheumatoid arthritis⁴⁹ and as outcome measures in adults with adult respiratory distress syndrome⁵⁰ and chronic obstructive pulmonary disease⁵¹ and premature newborns with respiratory distress syndrome.⁵²

In an animal model of colitis, increases in exhaled breath pentane were delayed until day 7 at which time histological examination confirmed acute colonic inflammation.⁴⁸ Pentane concentrations fell during the phase of healing and fibrosis when acute inflammatory cells were no longer present. Further, in adults with rheumatoid arthritis, there is a correlation between both joint inflammation and erythrocyte sedimentation rates and exhaled pentane excretion.⁴⁹ Finally, corticosteroids have been shown to reduce exhaled ethane in adults with asthma.⁵³

Infants with CLD have increased lipid peroxidation products measurable in both plasma (thiobarbituric acid reacting substances) and tracheal aspirates (malondialdehyde).⁵⁴ This lipid peroxidation is likely to be increased in the presence of acute neutrophilic inflammation.⁵⁵ In preterm ventilated infants with hyaline membrane disease, pentane concentration peaks on days 4 and 5. This peak correlates with death or CLD, although not with inspired oxygen tension.⁵² Work by Nycyk⁵⁶ in ventilated preterm infants showed a correlation between both exhaled pentane concentration and death and pentane concentration on day 1 and CLD. It is possible that this early pentane peak reflects fetal pulmonary sequestration from placental inflammation.^{57–58} Clearly, further validation work is needed before conclusions can be drawn.

The profile of lipid peroxidation byproducts, including exhaled pentane and serum and tracheal aspirate measures, is similar to the evolution of inflammatory cells in tracheal aspirate specimens.⁵⁹ It is plausible that lipid peroxidation reflects oxidative stress that is a consequence of acute pulmonary inflammation.

This is supported by work with isolated granulocytes.⁶⁰ When incubated in pure oxygen, isolated granulocytes produce small amounts of pentane. After granulocyte activation (with the addition of phorbol 13-myristate 12-acetate), this production increased by 540%. This response was blunted, although not completely, by the addition of superoxide dismutase and catalase, both well established antioxidants.

After removal of environmental contamination, exhaled hydrocarbon concentrations reflect endogenous lipid peroxidation through a blood-breath interface. This peroxidation is likely to be increased in the presence of acute inflammation, when the activated neutrophil is the most likely metabolically active inflammatory cell.⁵⁴ Although volatile hydrocarbons have been measured in the breath of newborns, they have not been validated against standard measures of pulmonary inflammation.

Collection

In adult studies, a single-breath technique has been used, with collection into an inert gas tight bag. This allows dead space washout during exhalation.⁶¹ Previous studies in ventilated newborns have used accessory anaesthetic rebreathing circuits in order to ventilate infants with hydrocarbon-free gas. We have developed a method to capture a significant exhaled volume from a ventilated infant

without ventilator disconnection. Exhaled gas is trapped on a carrier compound that is stable for long periods, allowing specimens to be batched. Further, ambient (exogenous) hydrocarbons can also be collected, allowing measurement of the small endogenous signal.

Analysis

The endogenous signal is small (probably 1–10 ppb) and closely approximates the sensitivity of available measuring devices. Mostly, gas chromatography with mass spectroscopy has been used for analysis. Further care needs to be taken to avoid incorrect analysis of overlapping breath components.⁶²

Pitfalls

Consideration needs to be given to the relatively large concentration of hydrocarbons in ambient air. Some studies have used washout periods with hydrocarbon-free gas.⁶³

In addition, infused lipid emulsion contains significant quantities of hydrocarbons⁶⁴ which affect interpretation. After cessation of lipid infusion, exhaled concentrations fall quickly so that, in practice, expired breath can be collected within a short period.^{65 66}

It has been suggested that exhaled hydrocarbon concentrations may be affected by contamination from bacterial flora in the gastrointestinal tract. However, during the first few days of life, pentane and ethane excretion increase when the gut is not colonised.⁵²

Summary

Volatile hydrocarbons have been studied in inflammatory illnesses in different populations. The endogenous concentration is very small, highlighting the importance of a collection method that excludes exogenous hydrocarbons, both ambient and infused. Although exhaled hydrocarbons are potentially useful, they require further validation in ventilated newborns.

EXHALED BREATH CONDENSATE (EBC)

Gas collected by active exhalation after prolonged oral breathing can be cooled or frozen for analysis. This method (EBC) enables components of lung lining fluid to be studied. Evidence suggests that the condensate composition reflects biochemical changes in the extracellular fluid. However, the proportional contributions have yet to be determined.^{10 67}

Several compounds have been measured including 8-isoprostane (from arachidonic acid metabolism),^{68–70} nitrite (byproduct of NO metabolism),⁷¹ and leukotrienes.⁷² Griese *et al*⁷³ used EBC to collect hydrogen peroxide in children aged 4 weeks to 18 years. They found that gas collection by active exhalation was very difficult in children younger than about 4–6 years.

CONCLUSION

CLD remains a problem for surviving infants of extreme prematurity. Current standard measures have been used to describe the evolution of inflammation in very low birth-weight infants, although few studies report the profile beyond 2 weeks of age. The development of a non-invasive measure would facilitate the longitudinal management of extremely preterm infants both by defining the illness profile and allowing titration of anti-inflammatory treatments. Further, non-invasive measures need to be developed for infants receiving nasal continuous positive airway pressure. Table 1 summarises the aforementioned measures.

For all measures, issues relating to (a) the large bias flow from the infant ventilator, causing signal dilution, (b) the contribution of environmental contamination, (c) airway humidification, and (d) the biochemical qualities (inertness) of the collection circuit need to be addressed.

Collection methods for both eNO and exhaled hydrocarbons have already been developed for ventilated newborns; however, both systems have potential pitfalls. In non-ventilated infants, a method for collection and analysis of eCO is currently available but interpretation is problematic.

Table 1 Summary of exhaled breath measures

	Source	Use	Collection	Analysis	Pitfalls
Exhaled NO	Derived from endothelial, epithelial, and inflammatory cells	Measure of pulmonary inflammation in ventilated premature newborns and in adults with obstructive sleep apnoea, ARDS and asthma.	Airway specimen collected from endotracheal tube in ventilated newborns	Chemiluminescence	Production by paranasal sinuses/nasal passages in both newborns and adults
Exhaled CO	NO synthesised from arginine by NO synthetase	Validated measure of Coombs positive haemolysis in non-ventilated newborns	Single breath technique in adults	Electrochemical sensor	Increased after arginine infusion
	Endogenous production after degradation of haem-containing proteins by haem oxygenase		Intranasal sampling in non-ventilated newborns		Passive diffusion of CO across the placenta in infants of smoking mothers
Exhaled volatile hydrocarbons (pentane, ethane)	Exogenous contaminant	Inflammatory measure in adults with asthma, cystic fibrosis, and bronchiectasis and in children with sepsis	End tidal breath collection in adults	Gas chromatography with mass spectroscopy	High concentration in ambient air and infused lipid emulsion
	Derived from free radical peroxidation of membrane bound polyunsaturated fatty acids	Correlated with death or later chronic lung disease in premature ventilated infants	Expiratory flow diverted after period of washout in ventilated newborns		
Exhaled breath condensate	Exogenous contaminant	Measure of inflammation in obstructive sleep apnoea, asthma, and rheumatoid arthritis in adults and in an animal model of colitis	Single breath technique in adults after similar washout	Varies with each compound measured	Potential contamination from collection circuit
	Derived from varied components of lung lining fluid	Preliminary data as a measure of pulmonary inflammation in adults and children	Active exhalation with subsequent specimen freezing after prolonged oral breathing		Difficult gas collection especially in young children

Finally, EBC has great potential, but a collection method has not been developed. Although these measures offer promise particularly for clinical research, none have been used in the clinical care of ventilated newborns. They all require methodologically sound validation studies before clinical application to ventilated newborns.

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IMAGES IN NEONATAL MEDICINE.....

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Alcohol burns in extremely low birthweight infants: still occurring

Delivery of an extremely premature infant presents a number of challenging issues, and placement of central lines is an important part of the early management of these vulnerable babies. Alcohol based skin cleansers such as chlorhexidine gluconate 0.5% in 70% methanol are widely used before insertion of umbilical and percutaneous central lines and are effective at achieving skin sterilisation in neonates.¹ Despite previous reports,² we have recently seen two cases of infants born at 24 weeks gestation who sustained extensive abdominal burns from chlorhexidine/alcohol applied during the insertion of umbilical catheters before transfer to our unit.

Hypothermia, excessive water loss, sepsis, and renal failure are all recognised consequences of severe burns in the neonate.³ Additionally pain and stress may adversely affect neuronal maturation in the brain,⁴ and skin scarring and depigmentation are common.

In summary, the use of alcohol based skin cleansers in babies with immature skin (<28 weeks) is inappropriate, and we remind paediatricians who may be inserting (usually umbilical) lines that skin cleansers should be chosen that are suitable for the skin maturity. We take great care to avoid pooling of cleanser under the infant, and use saline for immature skin.

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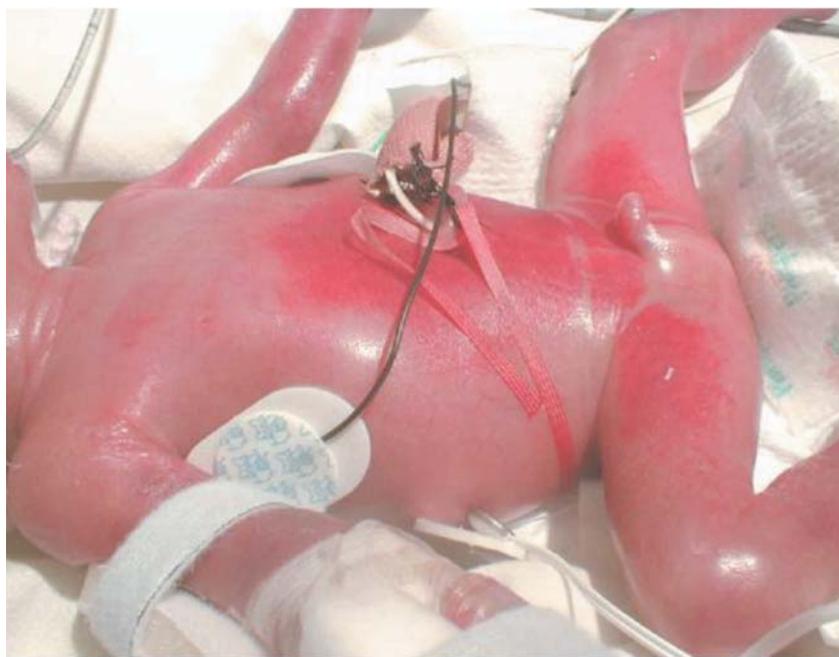


Figure 1 This 644 g baby sustained extensive burns over the abdomen and upper thighs. He became hypothermic (32.6°C), hypernatraemic, and subsequently developed systemic fungal sepsis with extensive skin breakdown. After two weeks the skin had healed without apparent cosmetic damage, but he died from renal failure 25 days later. This figure is published with parental consent.

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