

1

**How to Combine Initial Stabilisation with Cord Clamping**A. te Pas<sup>a</sup>, R. Kno<sup>b</sup>, S.B. Hooper<sup>c</sup><sup>a</sup>Department of Pediatrics, Leiden University Medical Center, Leiden, and <sup>b</sup>Department of Pediatrics, Erasmus MC – Sophia's Children Hospital, Rotterdam, The Netherlands; <sup>c</sup>The Ritchie Center, MIMR-PHI Institute of Medical Research, Melbourne, VIC, Australia

Preterm infants are most vulnerable immediately after birth, and the management in the first minutes of life can have a major impact on important morbidities associated with prematurity [1]. During the transition to life after birth, lung aeration is pivotal for the major physiological changes in respiratory and cardiovascular function that are required for survival after birth [2]. However, most preterm infants fail to aerate their immature lungs and immediate cord clamping (ICC) is then required to transfer apnoeic preterm infants to a resuscitation table to provide respiratory support. However, this cord clamping compromises cardiovascular function when it occurs in the absence of lung aeration [3]. Studies in preterm infants have shown that delayed cord clamping (DCC), as compared to ICC, reduced the risk of intraventricular/periventricular haemorrhages (I/PVH), necrotizing enterocolitis, hypotension, and need for blood transfusions [4]. While this was attributed to increased neonatal blood volume, experimental studies demonstrated that ICC reduces preload and cardiac output due to a loss of umbilical venous return [3]. These large swings in haemodynamic function increase the risk of I/PVH and associated rates of mortality and morbidity. DCC until after ventilation onset sustains preload and cardiac output and avoids the large disturbances in systemic and cerebral haemodynamics during transition [3, 5]. Preterm infants could potentially benefit from this as large swings in cardiovascular function place them at high risk of rupture of their immature vascular bed and associated rates of mortality and morbidity. Assuring lung aeration before clamping the cord greatly stabilizes the cardiovascular transition at birth and prevents compromising the cardiovascular function. In addition we have recently demonstrated that breathing has a major

impact on pulmonary and umbilical blood flows [6, 7]. This indicates that the potential benefits may be even larger in spontaneously breathing infants when subatmospheric intrathoracic pressures are produced as compared to the supra-atmospheric pressures during positive pressure ventilation. Taking into account establishing lung aeration next to placental transfusion, the moment of clamping should be based on physiology and individualized instead of using a fixed time point. The trials in preterm infants included in the meta-analysis focused on placental transfusion and used a fixed time point, with no regard for lung aeration [4]. Also in the Australian Placental Transfusion Study, the largest trial so far (1,600 infants), cord clamping is focused on transfusion and time-based (60 s) while no respiratory effort is provided (ClinicalTrials.gov NCT02606058). In recent studies where respiratory support for preterm infants was provided close to the mother, delayed cord clamping showed no differences in outcome [8, 9]. However, in these studies the moment of cord clamping was still based on time. In the ongoing CORD trial (ISRCTN21456601), cord clamping is delayed at least 2 min while respiratory support was given.

Recently, in Leiden University Medical Center cord clamping has been integrated into the stabilisation of the preterm infant. We have developed a purpose-built resuscitation table that allows infants to be kept close to their mothers, allowing the provision of standard care during stabilisation while the cord remains intact. The infant is fully monitored to ensure adequacy of ventilation and breathing effort [10]. The cord will be clamped once the infant has been stabilized and the lungs are aerated. After testing the safety and effectiveness of this approach, a large randomized trial will be planned to investigate whether this physiologically based approach of cord clamping will improve outcome.

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## 2

### How Much Oxygen to Start and Continue?

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Once extremely preterm infants begin pulmonary gas exchange, NICU staff set their oxygen targets until discharge. There are competing concerns about the risks of lower versus higher targets, uncertainty whether optimal targets are the same throughout the clinical course, and a recognition that SpO<sub>2</sub> or PO<sub>2</sub> are single elements in the multifactorial process of oxygen delivery. There are few clinical trials. Randomised trials comparing lower (<30%) versus higher (≥60%) inspired oxygen for initial stabilisation at birth report 509 infants in 8 studies who started with lower or higher FiO<sub>2</sub> but were targeted to the same achieved SpO<sub>2</sub>. Meta-analysis shows no significant difference in mortality, bronchopulmonary dysplasia, intraventricular haemorrhage, necrotising enterocolitis (NEC), or retinopathy of prematurity (ROP) [1]. The confidence intervals leave a possibility of small but important differences if larger studies are done. Worldwide collaboration in a prospective meta-analysis of 2 large trials is planned (TORPIDO2 and HiLo) aiming to study >9,000 infants. Recent ILCOR guidance [2] is that resuscitation should be initiated with 21–30% oxygen, placing value on not exposing preterm infants to high concentrations of oxygen without proven benefit. Current best practice is to begin with 21–30% oxygen and target the SpO<sub>2</sub> patterns of healthy infants or join a clinical trial. As existing trials varied FiO<sub>2</sub> in pursuit of the same SpO<sub>2</sub> targets, a further question is to compare higher or lower SpO<sub>2</sub> targets. In the neonatal unit the evidence is greater. Five masked randomised controlled trials (RCTs) in almost 5,000 infants (the Neonatal Oxygen Prospective Meta-analysis – NeOProm [3]) compared lower (85–89%) with higher (91–95%) SpO<sub>2</sub> targets from birth to 36 weeks. A meta-analysis [4] shows that lower SpO<sub>2</sub> targets increase the risk of mortality and severe NEC. Higher targets increase the risk of ROP treatment but not blindness. There was no difference in risk of severe disability, either as a composite of blindness, deafness, cerebral palsy or cognitive im-

pairment, or in the individual components. Current best practice is to avoid SpO<sub>2</sub> targets below 90% or join a trial. Guidance from the American Academy of Pediatrics [5] predated the full trial data and should be revised. The first BOOST trial [6] and the STOP-ROP trial [7] tested whether convalescent infants benefit from SpO<sub>2</sub> targets of 95–98 and 96–99%, respectively, compared with targets below 95%, showing no late advantage to these higher ranges. In the earlier weeks the question whether higher targets might show a further survival advantage remains open. There has been speculation that lower SpO<sub>2</sub> in the early weeks might diminish severe ROP without consequence but the hazard plots from the NeOProm trials warn against this, showing a mortality risk to lower SpO<sub>2</sub> emerging from around 2 weeks and presumably determined before this. Post hoc analysis of the SUPPORT trial [8] suggested a possibility that mortality risks of lower SpO<sub>2</sub> might largely be confined to small-for-gestational-age infants but this is not supported by the other trials. The increased relative risk of ROP requiring treatment associated with higher SpO<sub>2</sub> was also highest in SUPPORT, which began the intervention from birth. Infants in the other trials seldom began the intervention before 6 h, so there is insufficient data in the NeOProm trials to consider this further. Current practice is to monitor and target SpO<sub>2</sub> in the absence of a proven superior measure. Future trials should consider different targets or technologies such as servo control or near-infrared spectroscopy.

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### Caffeine: From Birth to Discharge?

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A major frustration for practising clinicians is the limited amount of valid randomized controlled trials (RCTs) available to support treatment decisions. However, compared to other disciplines, the field of neonatology is not doing badly, as there are a number of high-quality neonatal RCTs available. As for caffeine treatment, there is the international Caffeine for Apnea of Prematurity (CAP) trial that was launched to determine whether survival without neurodevelopmental disability at a corrected age of 18 months is improved if apnoea of prematurity is managed without caffeine in infants at a high risk of apnoeic attacks [1]. Fortunately the CAP trial convincingly showed that neonatal caffeine therapy reduces the risks of important short-term morbidities such as bronchopulmonary dysplasia (BPD) and also decreases the incidence of cerebral palsy and cognitive delay at 18 months [2]. Thus, if we treat our newborn patients according to the CAP trial we can be reasonably sure to do them something good while at the same time following the principles of evidence-based medicine (EBM) [3].

The median age of starting the study medication in the CAP trial was 3 days, and the median age of stopping caffeine was 34.4 weeks postmenstrual age (PMA) [1]. Understandably neonatologists now ask whether caffeine started earlier than 3 days and given longer than 35 weeks PMA also provides benefit and further improves health outcomes. Unfortunately there is less valid evidence available to answer these 2 questions, and thus at first glance practicing EBM seems to be more difficult. EBM initially proposed concepts to rank the available evidence that exclusively focused on the design of clinical studies [4]. For therapy, the hierarchy was spearheaded by RCTs and they were generally considered superior over observational studies for determining the trustworthiness of evidence related to treatment effects [5]. As the awareness of the limitations of the initial simple hierarchy of evidence grew, an alternative framework named “Grades of Recommendation, Assessment, Development, and Evaluation (GRADE)” was proposed that takes into account all elements related to the credibility of bodies of evidence: study design, risk of bias, precision, consistency, directness, publication bias, magnitude of effect, and dose-response gradients [6]. Thus, GRADE does not only allow for limitations in bodies of evidence from RCTs, but also the rating of observational studies as high-quality evidence and under certain circumstances the provision of causal evidence [5]. In conjunction with the issue of the best timing of caffeine treatment in preterm infants, the application of the GRADE criteria is helpful. A recent systematic review comparing early versus late caffeine therapy in preterm neonates identified 14 studies, including retrospective and prospective cohort studies, as well as RCTs [7]. If we apply the GRADE criteria to the key studies, keeping in mind that the level of respiratory support is the single most important predictor of BPD [8] and that approximately half of the neuroprotective effect of caffeine at 18 months corrected age can be explained by the earlier discontinuation of positive airway pressure in infants assigned to caffeine [2], a recommendation to initiate caffeine therapy in infants with very low birth weight on non-invasive respiratory support soon after

birth seems to be reasonable. Thereby we can possibly avoid mechanical ventilation, and any therapy that reduces the duration of mechanical ventilation will likely reduce the risk of BPD and contribute to a favourable neurosensory outcome. But what about extending caffeine treatment beyond 35 weeks PMA? An RCT published in 2014 in which 98 preterm infants were assigned to either an extension of caffeine treatment or to usual care showed that intermittent hypoxia (IH) can be reduced by extended caffeine treatment but did not answer the important question whether reducing the extent of IH has any long-term neurodevelopmental benefits [9]. Thus, pending results from more randomized or high-quality observational studies, the current clinical practice based on the results of the CAP trial should not be changed.

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### 4

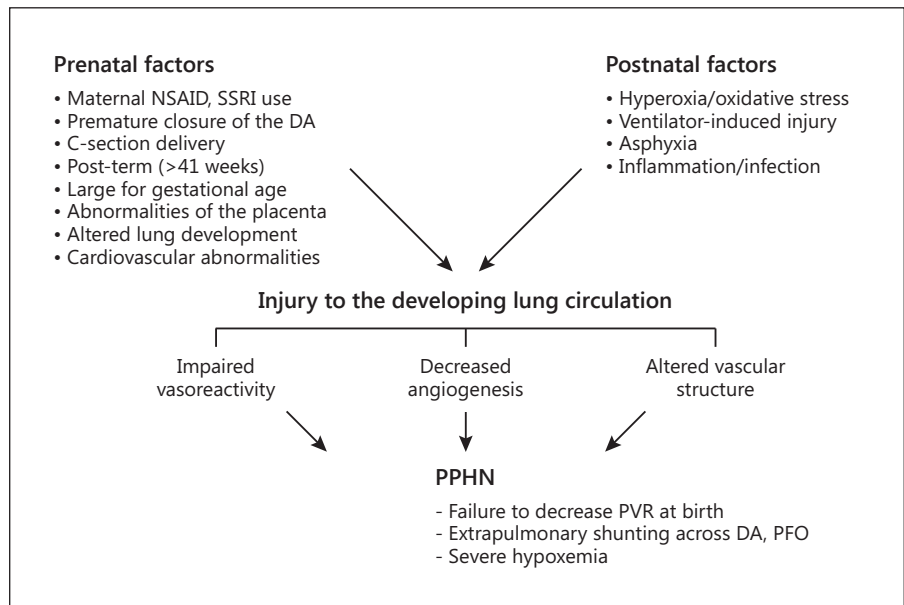
#### Challenges of Persistent Pulmonary Hypertension of the Newborn

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With the loss of the placenta at birth, the pulmonary circulation must undergo a striking vasodilation to allow the lung to assume its critical postnatal function of gas exchange. Mechanisms contributing to the normal decline in pulmonary vascular resistance (PVR) include a variety of physiological responses related to mechanical, hormonal, and local factors. Persistent pulmonary hypertension of the newborn (PPHN) represents the failure of this normal transition after birth, leading to hypoxemia due to extrapulmonary right-to-left shunting of blood across the foramen ova-

**Fig. 1.** Pathogenesis of persistent pulmonary hypertension of the newborn (PPHN). NSAID, nonsteroidal anti-inflammatory drug; SSRI, selective serotonin reuptake inhibitor; DA, ductus arteriosus; C-section, cesarean section; PFO, patent foramen ovale; PVR, pulmonary vascular resistance.



le and/or ductus arteriosus. Over the years, diverse clinical factors have been associated with the development of PPHN, which include perinatal stresses as well as postnatal injury (Fig. 1). The recognition of the importance of nitric oxide (NO)-cGMP signaling during development and the potent vasodilator effects of inhaled NO (iNO) as a therapy has lowered the need for extracorporeal membrane oxygenation (ECMO) therapy in term neonates [1, 2]. Despite these successes, however, many neonates with PPHN-related physiology remain refractory or poorly responsive to iNO therapy, highlighting the complexity of cardiopulmonary interactions during the transition. Past studies have demonstrated the critical roles of lung recruitment to directly lower PVR and improve gas exchange, the importance of sufficient cardiotoxic support to sustain right and left ventricular performance and systemic hemodynamics, and heightened awareness of the potential presence of developmental lung diseases (especially alveolar capillary dysplasia, genetic surfactant protein disorders, thyroid transcription factor-1 deficiency syndrome, *ABCA3* mutation, and other lung diseases) as important contributors to poor outcomes. PPHN associated with congenital diaphragmatic hernia (CDH) remains a significant challenge, with the severity and persistence of pulmonary hypertension contributing to high ECMO use and mortality in subgroups of CDH patients, despite the modern era of pulmonary hypertension-targeted therapies. Greater understanding of the physiological contributions of lung disease, pulmonary vascular growth and remodeling, and left heart dysfunction or underdevelopment should further improve outcomes of severe CDH patients. Finally, preterm infants, especially in the setting of oligohydramnios, prolonged premature rupture of membranes, lung hypoplasia, and severe growth restriction, are also at high risk for PPHN in the days after birth. Although controversial, several case series have highlighted the striking role of pulmonary vasoconstriction in the hypoxemic preterm infant, as reflected by responsiveness to iNO therapy in many cases. Although recent guidelines have been developed regarding the management of term and preterm infants with PPHN, there remains an ongoing need for stud-

ies to better understand the potential for early iNO therapy in preterm infants with PPHN, how to best optimize interactive cardiopulmonary strategies, and novel therapies to further enhance short- and long-term outcomes of newborns with PPHN.

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## 5

### Non-Invasive Ventilation Modes in the Delivery Suite and Beyond

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Continuous distending pressure (CDP) is a cornerstone of respiratory management in neonatology [1–4]. Positive effects of CDP include splinting of the airways, increase in functional residual capacity, improved oxygenation, and improvement of apnea-bradycardia syndrome. Since Gregory's first description of head-box continuous positive airway pressure (CPAP), a plethora of different CDP devices and nasal interfaces has become available [3]. Recently, small nasal cannulae, providing a steady flow of heated and humidified gas (0.5–8 L/min), have become popular [4]. Differently from nasal CPAP where physiological and clinical

properties from the delivery room to discharge have been studied over decades, laboratory and clinical data on nasal high-flow therapy (nHFT, or high-flow nasal cannulae) are just emerging [5, 6]. Scientific evidence from benchtop and clinical studies has helped to understand nHFT mechanisms of action. The efficacy and safety of nHFT has now been studied in over 25 controlled clinical trials. Results of these trials show that nHFT was as safe and not inferior to CPAP when used as postextubation respiratory support [4–7]. Common to all clinical studies was the consistent finding of significantly less nasal trauma with nHFT [7]. Very recently, 2 randomized clinical trials (RCTs), including a respectable number of patients, investigated the use of nHFT as primary treatment for respiratory distress syndrome with conflicting results (summarized in Wilkinson et al. [4] and Roehr et al. [6]): in a single-centre, non-inferiority randomized controlled trial (NI-RCT), Lavizzari et al. [8] compared nHFT or CPAP as primary respiratory support which showed no difference in the rates of respiratory decompensation and the need for intubation. Conversely, a larger, NI-RCT by Roberts et al. [9] compared nHFT to CPAP as primary mode of respiratory support. The authors found that the use of nHFT was associated with a greater number of infants being offered “rescue CPAP” due to perceived greater instability on nHFT. In summary nHFT has been shown to be as efficacious and safe in very low-birth-weight (VLBW) infants when used after extubation, whereas the use of nHFT as primary therapy requires further study [6].

Several alternatives to nasal CPAP or nHFT have been tested in the clinical neonatal setting. Promising data regarding the use of synchronized non-invasive positive pressure ventilation (NIPPV) and, to a lesser extent, non-synchronized NIPPV. As for NIPPV, bilevel CPAP or SiPAP systems, the absence of clinical data with regard to the optimal inspiratory pressure, the respiratory frequency and the basal CPAP when using respiratory-generated NIPPV are largely chosen empirically. Non-invasive, nasal high-frequency oscillation ventilation (HFOV), neurally adjusted ventilatory assist (NAVA) techniques, and adaptive loop feedback for oxygen control whilst receiving CDP [10] have also been trialed with beneficial effects, however, in smaller cohorts. Whilst NIPPV can be applied by means of almost all modern ventilators or pressure transducers via a patient interface (usually a small mask or prongs), synchronized NIPPV and NAVA require specific technology to detect the patient’s diaphragmatic activity and feed this back to the ventilator to synchronize. Nasal HFOV, among other claimed benefits, utilizes the oscillatory effect of the ventilator to facilitate improved CO<sub>2</sub> wash-out. However, at present, results from adequately powered RCTs are under way [Rimensberger P., pers. commun.].

To summarize, nasal CPAP remains the mainstay of non-invasive respiratory support for many neonatologists; however, an exciting spectrum of newer and more refined CDP applications and machines becomes available, showing very promising first results. Next to the ongoing studies of the above, further research needs to focus on identifying the optimal form of respiratory support for an individual patient, tailored to the particular state of disease progression of neonatal lung disease.

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## 6

### Can We Optimize Conventional Mechanical Ventilation by Oxygen Targeting?

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Most premature infants require supplemental oxygen for long periods of time. Because of their respiratory instability these infants spend considerable periods of time above or below the intended clinical target [1]. Hyperoxia and hypoxia can have significant negative effects on multiple organs such as the lung, brain and eye, and increase the risk for long-term disability [2, 3]. To avoid these problems, arterial oxygen saturation is continuously monitored by pulse oximetry (SpO<sub>2</sub>), and FiO<sub>2</sub> is manually adjusted to keep SpO<sub>2</sub> within a desired range. Maintenance of SpO<sub>2</sub> within the target range becomes more difficult as the infant ages and becomes more active but is improved by using tight SpO<sub>2</sub> alarms. Maintenance of oxygenation within the intended range is also influenced by the nurse-to-patient ratio [4]. In an effort to attenuate the frequency of hypoxemia episodes, the clinical staff often uses excessive inspired oxygen and tolerates high SpO<sub>2</sub> levels. Systems for automatic adjustment of FiO<sub>2</sub> to maintain blood oxygen within targets have been developed and are now available for clinical use in different parts of the world [5, 6]. Clinical stud-

ies have shown that automatic FiO<sub>2</sub> control can improve maintenance of SpO<sub>2</sub> within the target range [6–8]. These results show consistent reductions in exposure to hyperoxemia and hypoxemia and less exposure to supplemental oxygen with automatic control when compared to routine clinical management [9, 10]. Some studies have shown an increased frequency of brief fluctuations in SpO<sub>2</sub> below the target range during automated adjustments. However, automatic FiO<sub>2</sub> control consistently reduced the number of episodes of severe and/or prolonged hypoxemia [7, 10]. Automatic FiO<sub>2</sub> control does not prevent the occurrence of hypoxemia episodes but through a faster and more consistent response can reduce the exposure to severe and prolonged episodes of hypoxemia. Automatic FiO<sub>2</sub> control can also reduce the exposure to inspired oxygen and possibly reduce oxidative lung injury. Automated FiO<sub>2</sub> control also reduces staff workload that can be considerable when caring for infants with frequent episodes of hypoxemia. In these infants, automatic control can reduce the number of manual FiO<sub>2</sub> adjustments by as much as 90%. Automated FiO<sub>2</sub> control may have unwanted consequences, the most important being overreliance and reduced attentiveness. For this reason automatic systems must include monitoring features that alert the staff about changes in patient status, e.g. a persistently higher FiO<sub>2</sub> to maintain SpO<sub>2</sub> in range, or poor signal quality of the SpO<sub>2</sub> sensor. Caution must be exercised when selecting the SpO<sub>2</sub> target range during automatic FiO<sub>2</sub> control because specific ranges of SpO<sub>2</sub> may have undesirable clinical and physiological effects that become more relevant when targets are more tightly maintained by these systems. In summary, conventional modalities of ventilation require frequent adjustments by the staff to adapt to the changing needs of the preterm infant. Automated modalities can potentially address these limitations but larger clinical trials are needed to determine the long-term impact of extended use of this technology on ophthalmic, respiratory, and neurodevelopmental outcome in preterm infants.

*Potential conflict of interest:* The University of Miami and Drs. Bancalari and Claire have a patent on a system for automated control of inspired oxygen.

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## 7

### High-Frequency Ventilation in 2017

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Bronchopulmonary dysplasia (BPD) remains an important complication of preterm birth. Lung injury caused by inappropriate ventilation strategies is considered an important risk factor in the development of BPD. For this reason, a lung protective ventilation strategy avoiding lung overdistension (volutrauma) and atelectasis (atelectotrauma), also referred to as open lung ventilation, should be used at all times in preterm infants. High-frequency ventilation (HFV) is often used to apply such an open lung strategy, because, by design, it delivers very small tidal volumes (less volutrauma). However, animal studies have shown that reversal of atelectasis via lung recruitment is essential for optimal lung protection during HFV [1].

Despite promising animal studies, randomized controlled trials have only shown a small and inconsistent benefit of HFV on the incidence of BPD. Some have suggested that HFV might reduce BPD but mainly in patients with severe respiratory distress syndrome and if applied at every episode of respiratory failure thereafter. A recent follow-up study suggests that HFV might also reduce the risk of small airway disease, even in the absence of BPD reduction [2]. Despite this lack of convincing benefit, HFV is (increasingly) used in 15–60% of preterm infants [3, 4].

In contrast to a decade ago, almost all modern (hybrid) ventilators are capable of delivering HFV, and their performance has improved over time. However, there are still considerable differences between these ventilators, and clinicians need to be aware of the HFV characteristics and especially the limitations of the ventilator they use in their unit [5].

To reduce the risk of hypocapnia during HFV, most recent ventilators have also incorporated volume targeting during HFV. This will stabilize the tidal volume delivered during HFV by automatically adjusting the oscillation amplitude. However, there are only limited data on the efficacy of this add-on mode and no data on the possible impact on clinically relevant outcomes. In addition, clinicians need to be aware that the impact of changes of oscillation frequency on CO<sub>2</sub> clearance are opposite during HFV with and without volume targeting. Finally, clinicians need to be aware that, independently of the mode or strategy, invasive mechanical ventilation will increase the risk of BPD. Therefore, it

needs to be seen as a temporary mode of support and the aim should be to extubate patients back to noninvasive support as soon as possible.

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## 8

### Neurally Adjusted Ventilatory Assist in Neonates: A Real Improvement in Mechanical Ventilation?

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Current ventilators use a change in airway flow as the trigger to initiate a mechanical breath cycle. This mechanical cycle has a set tidal volume or  $\Delta$  pressure above PEEP, a rate and an inspiratory time defined by time or flow cycling that may or may not be in synchrony with the patient. False triggering, late triggering, and missed triggering as well as early or late cycling are common problems with this type of inspiratory and expiratory triggering. These asynchrony events become even more marked during non-invasive ventilation in the presence of large air leaks at the interface (nasal prongs or masks). Synchronized respiratory support delivery with the infant's inspiration is improving patient comfort and work of breathing and is believed to increase the efficacy of invasive and especially of non-invasive ventilation. Neurally adjusted ventilatory assist (NAVA), using the electromyographic signal from the diaphragm (EADi) for triggering and cycle termination, allows to synchronize better the spontaneous respiratory effort with mechanical ventilation [1–3]. Furthermore, NAVA offers a proportional support to the patient's effort (i.e., EADi signal intensity). With this the ventilator determines the inspiratory pressure in proportion to this electrical signal, whereas the patient, controlling his neural respiratory drive, determines the respiratory rate, tidal volume, and inspiratory and expiratory times. According to respiratory system mechanics, which change with progressing or improving lung disease, this neural control loop concept will allow the patient to best adapt  $V_t$  and respiratory rate to his actual condition allowing to optimally reduce work of breathing. In fact, infants with poor respiratory system conditions (low lung compliance) chose much smaller  $V_t$  than recommended by actual con-

cepts of lung protective ventilation, whereas when compliance is improving larger  $V_t$  than recommended can be observed [2, 4, 5]. Despite this, most of the observational crossover studies showed NAVA to provide better blood gas regulation with lower peak inspiratory pressure and oxygen requirements when compared with standard conventional ventilation. Furthermore, non-invasive NAVA improved patient-ventilator synchrony and diaphragmatic unloading in preterm infants even in the presence of large air leaks [3]. These observations and the closed loop neural control concept would make NAVA an ideal ventilatory mode for assisting spontaneous breathing not only for invasive, but certainly also for non-invasive support in the presence of large air leaks. However, especially in the premature infant, backup settings including apnoea ventilation have to be set carefully. Despite these theoretical advantages of NAVA and its potential to improve and somewhat simplify mechanical ventilation, it has hardly been studied in the clinical setting, and no appropriately powered outcome studies are available yet. Many studies could show NAVA to be safe and superior to conventional modes of mechanical ventilation in terms of delivering assistance in synchrony with patient efforts; however, it still has not been shown to improve short- or long-term outcomes in the neonatal unit. Therefore, well-designed appropriately powered studies are urgently needed before one could recommend NAVA or any similar concept of neurally controlled proportional or non-proportional assist, invasive or non-invasive ventilation for preterm infants as a primary ventilator mode or to prevent extubation failure [6]. However, with the advent of NAVA emerging in neonatal units, we learned a lot for the better understanding of the infant's breathing control and its variables in the presence or absence of acute or chronic lung disease.

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## Bronchopulmonary Dysplasia and Pulmonary Hypertension

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Advances in vascular biology have provided new insights into basic mechanisms of lung development during embryonic, fetal, and postnatal life. Investigation of growth and development of the pulmonary circulation is critically important for understanding clinical disorders that are associated with pulmonary hypertension (PH), such as bronchopulmonary dysplasia (BPD) [1, 2]. Past studies have shown that persistent echocardiographic evidence of PH beyond the first few months of life is associated with up to 40% mortality in infants with BPD [3, 4]. The association of PH with poor survival in BPD has continued into the recent era of the “new BPD,” especially in infants with severe disease who require prolonged support with mechanical ventilation. Thus, developing insights into the pathogenesis and pathobiology of PH and related pulmonary vascular disease (PVD) in BPD continue as an important challenge and may help to improve early and late cardiopulmonary outcomes after preterm birth [5]. Mechanisms that coordinate normal vascular growth and alveolarization during development or cause abnormal lung growth in BPD are poorly understood. Disruption of key signals between airway epithelium and endothelial cells can alter vascular and alveolar growth, resulting in decreased arterial and airspace structure [6, 7]. For example, hyperoxic lung injury in newborn animals decreases expression of the critical proangiogenic and endothelial cell survival factor, vascular endothelial growth factor (VEGF) [8]. Early impairment of VEGF production inhibits vascular growth and impairs endothelial function, which leads to PH [6, 9]. In addition, disruption of angiogenesis due to adverse antenatal factors, such as chorioamnionitis, preeclampsia or maternal smoking, and postnatal events after premature birth, can cause vascular injury that not only lead to PH, but can also impair distal lung growth [6, 10–12]. Abnormalities of the pulmonary circulation in severe BPD include altered tone and reactivity, structure and growth, which can cause right heart failure, impaired gas exchange, pulmonary edema, decreased exercise capacity and other clinical problems [5]. Physiological abnormalities of the pulmonary circulation in BPD include elevated pulmonary vascular resistance (PVR) and abnormal vasoactivity, as evidenced by the marked vasoconstrictor response to acute hypoxia and by impaired gas exchange due to abnormal distribution of lung blood flow [13]. Abnormal pulmonary vascular structure also contributes to high PVR due to increased smooth muscle cell hyperplasia and altered vascular compliance caused by increased production of an abnormal extracellular matrix. Growth of the distal lung circulation is abnormal in infants with severe BPD [14], and decreased arterial growth (angiogenesis) reduces vascular surface area that further impairs gas exchange and increases the risk for the development of PH and impaired exercise capacity in older children. Clinical studies have recently shown that early echocardiographic findings of PVD after preterm birth are strongly associated with the development and severity of BPD and PH at 36 weeks corrected age [15]. Interestingly, these findings

were not only associated with a worse respiratory course during the initial hospitalization, but also late respiratory outcomes, including respiratory exacerbations, hospitalizations, and the need for asthma medications. Ongoing studies are exploring the impact of PH-specific drug therapies, such as sildenafil and other agents, on PH and related complications. Most importantly, the primary approach to PH therapies in BPD is rigorous evaluation and management of the underlying lung disease [16]. Recent studies have revealed the magnitude of PH in preterm infants, but many aspects of PVD remain understudied, and ongoing investigations continue to explore risk factors, mechanisms of disease, and long-term outcomes. Prospective studies are needed to definitively establish standardized clinical criteria for PVD and PH in BPD, and to determine the best methods for early diagnosis, risk stratification, and disease monitoring.

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## Postnatal Corticosteroids and BPD: Inhaled or Mixed with Surfactant?

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A prerequisite for the determination of the efficacy and effectiveness of interventions aimed at the prevention or treatment of bronchopulmonary dysplasia (BPD) is a clear definition of the target disease. In a scoping review Hines et al. [1] found that the incidence of BPD varied between 6 and 57%, depending on the definition chosen. Furthermore studies that investigated correlations with long-term pulmonary and/or neurosensory outcomes only reported moderate-to-low predictive values [1]. Not only because BPD is often used as a primary outcome measure in randomized clinical trials (RCTs), a contemporary definition of BPD that better correlates with morbidity in childhood is needed.

Multiple surveys indicate that a large number of preterm infants receive inhaled corticosteroids for the prevention and/or treatment of BPD in routine clinical care despite the lack of data supporting this practice [2, 3]. This discrepancy prompted the Neonatal European Study of Inhaled Steroids (NEUROSIS) that investigated the role of inhaled budesonide for the prevention of BPD [4] and showed a significant reduction in the incidence of BPD [5]. However, the primary outcome (a composite of death or BPD at 36 weeks postmenstrual age, PMA) was only of borderline significance as a result of a non-significant trend to increased mortality in the budesonide group [5, 6].

Systematic reviews from the Cochrane Collaboration attempted to separate studies of prevention [7] and treatment [8] of BPD while another recent systematic review did not distinguish between “early” and “late” administration of inhaled corticosteroids [9]. This review pooled all studies in which inhaled corticosteroids were compared to placebo for the prevention or treatment of BPD in preterm infants in various meta-analyses [9]. There is now increasing evidence, both from RCTs and systematic reviews, that the early administration of inhaled corticosteroids to preterm infants is effective in reducing the incidence of death or BPD at 36 weeks’ PMA among either all randomized infants or among survivors [5, 7]; if administered late, there seems to be no effect on this composite outcome [8]. Nevertheless, the NEUROSIS trial and the systematic reviews questioned the clinical significance of this positive finding and concluded that more information about the long-term effects of inhaled corticosteroids for the prevention of BPD is needed before firm clinical recommendations can be made. More information from NEUROSIS about the effects of inhaled corticosteroids on neurodevelopmental disability is expected to be published later in 2017. The mixing of surfactant with budesonide before tracheal instillation results in a rapid and uniform distribution of the corticosteroid to the distal airspaces [10], a result that is hardly achieved if budesonide is given by inhalation. Further advantages of using budesonide locally in a mixture together with surfactant are prolonged local anti-inflammatory effects, a decreased risk of systemic adverse effects and a better calculability of the actually delivered dose. Unfortunately to this day the clinical evidence supporting this mode of administration is still limited. The largest published randomized study compared an initial sur-

factant treatment that included budesonide with surfactant alone for preterm infants with severe respiratory distress syndrome to decrease BPD [10]. The mixture of surfactant and budesonide decreased BPD by 21% with a significant effect only seen for severe BPD when using the NICHD definition [10]. No acute adverse effects and no effect on neurodevelopmental outcomes at 2–3 years were reported [10]. These results are promising but need to be replicated in large RCTs performed in different clinical settings. In summary, neither mode of administration of corticosteroids for the prevention of BPD, early inhalation or instillation, is currently sufficiently studied respectively reported to be introduced into clinical practice. For early inhalation, data on the long-term outcomes from the NEUROSIS trial, and for instillation, multinational confirmatory RCTs are needed.

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## Mesenchymal Stem Cells: A Breakthrough in the Treatment of BPD?

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Bronchopulmonary dysplasia (BPD) celebrates its 50th anniversary and remains the most common complication of extreme prematurity. This is because past advances in perinatal care have allowed the survival of ever more premature infants, rendering pre-

vention of the ever more immature lung more challenging as we approach the biological limit of viability. BPD today is a multifactorial disease interrupting the normal development of the extreme preterm lung. Therapies targeting multiple pathways to prevent lung injury/induce lung repair while promoting lung growth are required to significantly decrease the incidence/severity of BPD. Recent insight into stem cell biology has created excitement about the repair potential of these cells. Mesenchymal stromal cells (MSCs) [1] have received particular attention because of their ease of isolation, expansion, their pleiotropic effects, and apparent safety. Studies in experimental neonatal lung injury have demonstrated the lung protective effects of bone marrow and umbilical cord-derived MSCs [2]. MSCs do not engraft, but exert their therapeutic benefit through the release of bioactive molecules, likely responsible for the multifactorial effects of these cells [3]. In addition, MSCs release numerous growth factors important during normal lung development. These promising findings in the laboratory have led to early phase clinical trials in preterm infants at risk of developing BPD demonstrating the feasibility and safety of allogeneic cord blood-derived MSCs in a few patients [4]. Over the coming decade, it will be important to deepen our knowledge about MSCs (and other potential repair cells), understand the mechanism of action of these cells, develop reliable potency assays to optimize the manufacturing of high-quality, safe, and efficient clinical-grade MSCs, and conduct well-designed clinical trials based on robust rationales. It is only through this methodical and rigorous approach in both the preclinical and clinical setting that safety and efficacy of cell-based therapies can be determined with the ultimate hope of not having to celebrate BPD's 60th anniversary.

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## 12

### Current Approach to the Patent Ductus Arteriosus in Extremely Low-Birth-Weight Infants

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The management of the patent ductus arteriosus (PDA) in extremely low-birth-weight (ELBW) infants continues to be one of the most contested topics in neonatal medicine with large variations in the approach to treatment [1]. Many ELBW infants fail to close their ductus spontaneously increasing the risk of pulmonary and hemodynamic adverse effects due to the shunting of blood from the systemic to the pulmonary circulation. Despite multiple experimental and observational studies linking PDA to lung in-

jury and other neonatal morbidities, multiple randomized controlled trials comparing early or prophylactic PDA closure with delayed closure of symptomatic PDA have not shown to significantly affect long-term outcomes [2, 3]. This lack of clear evidence of benefits of treatment, in addition to the limited efficacy and possible side effects of the available treatment options, has resulted in a more selective treatment of PDA limiting its closure to cases that become hemodynamically and/or clinically significant [4]. This has resulted in an increased number of preterm infants that are exposed to PDA for longer periods of time. The effects of this change in practice on clinical outcomes have been inconsistent but 1 recent single-center cohort study reports an increased incidence of hypotension and bronchopulmonary dysplasia (BPD) in infants born during the epoch with delayed closure of the PDA when compared to the previous epoch of prophylactic closure with indomethacin [5]. This is in contrast to large observational studies that failed to show a correlation between prophylactic or more aggressive use of nonsteroidal anti-inflammatory drugs for PDA closure on the incidence of BPD [4, 6]. Possible explanations for these contrasting results may include differences in patient population or in duration of exposure to PDA between studies. Because only a fraction of infants with PDA develop hemodynamic and respiratory compromise due to the left-to-right shunting, there have been many attempts to identify these infants for early targeted treatment. There are multiple scoring systems including echocardiographic, laboratory, and clinical markers which have been evaluated for the diagnosis of a significant PDA but there is limited evidence for their impact on neonatal outcomes [7]. Due to the potential side effects of NSAIDs on renal and gut function, acetaminophen has recently been evaluated as a treatment option with similar efficacy and less potential adverse effects. The impact of its use on neonatal outcomes and safety profile in preterm infants remains to be fully investigated [8]. The surgical closure of the PDA is usually restricted for infants in whom pharmacological treatment fails or is contraindicated. In addition to risks associated with anesthesia exposure, possible clinical deterioration soon after surgery, and postsurgical complications, observational studies have associated ligation with increased BPD, retinopathy of prematurity and neurodevelopmental impairment. Even though there is a renewed debate whether the increase in adverse long-term outcomes associated with PDA ligation is just reflective of underlying comorbidities associated with a hemodynamically significant PDA rather than secondary to the ligation per se, limiting surgical closure of the PDA seems prudent [9]. In conclusion, despite limited evidence of its impact on outcomes, the approach to PDA management in premature infants has shifted towards a more conservative and selective treatment of only hemodynamically significant PDAs. There is a pressing need for well-designed placebo-controlled trials evaluating the different PDA management strategies on long-term outcomes in these infants. Until then the optimal management of PDA will continue to be an open question.

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### 13

#### Family-Centered Care: More than a Good Feeling?

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Since 1952 when Bowlby [1] first described severe depressive symptoms in children separated from their parents during hospital stay, changes have been made in pediatric wards allowing parents to stay with their children 24/7 and participating in their care. However, this development has lagged behind in neonatal wards, where even today most infants are separated from their parents the majority of hours of the day. Rooming-in is still very uncommon, and staff is reluctant to let parents participate in the care of their own newborn infant. Common assumptions behind this practice are “lack of controlled scientific studies showing the impact of early separation,” “lack of space and appropriate localities,” and “risk of infections transferred from parents to their child.” But obviously this is no longer the case for children in pediatric wards, since we no longer accept a routine separation for 2- or 8-year-olds admitted to hospital. Parents can provide something an incubator or hospital staff never can: unique sensory input, unique microbiota, and the closeness/love such a delicate and developing brain needs. There is actually overwhelmingly scientific data on the importance of physical closeness and empathetic parental care in the neonatal period. Emergent findings from both human and animal model studies and the nature of parental-infant interaction in early post-natal life have a profound role in mediating variations in offspring phenotype, including emotional and cognitive development with long-term health consequences [2]. Prolonged and/or repeated physical separation between parent and newborn and, especially, the preterm infant alters brain development, impairs the ongoing bonding/attachment process, and has long-lasting effects on e.g.

emotional programming [3]. Premature children need to be protected from stress induced by pain and/or unpleasant proprioceptive stimulation, disturbed sleep/wake cycling, exposure to unpleasant odors/taste, large feeding volumes, from high noise levels and constant positioning in the horizontal position, bright light and the potentially pathogenic microbiota of the NICU environment [4]. Physical closeness between parent and child has crucial regulatory aspects for both parent and child and reverses epigenetic change caused by perinatal stress in human infants [5]. Closeness versus separation in the neonatal period affects the infant's temperature, blood glucose levels, and breathing patterns [6]. Besides profound physiological effects, closeness instead of routine separation affects maternal depression rates, the length of the hospital stay for the child, and gives the family a possibility of activity and control, factors important in order to protect and regulate potentially traumatic stress [7]. A body of literature demonstrates the beneficial effects of neonatal care that actively involves parents in the care of their infants. Prenatal presence on the units promotes sensitive and consistent parent-infant interaction [8]. Hence, providing emotional support and practical help in a way that facilitates closeness between parent and child strengthens innate resilience in both parent and child. But in spite of good evidence recent studies show a large variation in parent-infant closeness and parental participation between units and countries. Some units with single rooms and rooming-in provided as little as 0.3 h closeness a day, and units with limited space provided as much as 22 h. This indicates that despite the widespread assumption on the importance of single rooms in order to provide for parent-infant closeness, factors such as culture, policy making, and leadership may be more central to a modernized approach, rather than localities alone [9]. Introducing changes towards a family-centered care where the infant's family is actually present, the majority of the day is challenging and often dependent on barriers in organizational context, leadership, and resistance from care culture. In a busy NICU in Uppsala, Sweden, we have, by small and successive steps, been able to gain experience in having parents present together with their critically ill and/or extremely preterm born infant in an adult bed skin to skin most hours of the day [10].

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## 14

### The Importance of Nonobtrusive Monitoring in the NICU

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During the last few decades, mortality decreased substantially especially in preterm infants, born after a gestation below 27 weeks [1]. This result was achieved due to the new therapeutic approaches that have been developed. However, in follow-up studies it was noted that ex-preterm infants show more developmental impairments when they grow up. These include cognitive disorders, concentration, and behavioral disorders [2]. Evidence is accumulating that apart from medical conditions, pain and stress experienced during their stay at the NICU in combination with the separation from their mother are responsible for these developmental impairments [3]. Volumes of brain areas of ex-preterm infants are reported to be significantly smaller at the age of 9 years, compared to their full-term-born peers [4], and a significant correlation was found between the number of skin-breaking procedures and the developmental index in the second year of life [5]. In parallel with the exposure to noxious stimuli, preterm infants experience maternal separation that also affects brain development [6, 7]. Development of preterm infants can be improved by avoiding pain and stress as much as possible and to compensate for maternal separation. Kangaroo mother care is advocated as an excellent way to promote mother-child bonding and to improve growth and development. Adhesive electrodes for cardiorespiratory monitoring are causing pain when infants pull at the wires inadvertently and on removal. Therefore we worked on solutions for nonobtrusive monitoring: a smart jacket that features textile electrodes [8], a mattress with incorporated capacitive electrodes [9], and video photoplethysmography [10]. In summary, more research should be directed to find solutions to minimize pain and stress for preterm infants in the NICU, in order to improve their developmental outcomes.

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## 15

### Lower Limits of Viability – A Resume in 2017

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Controversies inherent in the care of infants born near the limits of viability (22–25 weeks gestational age [GA]) continue to generate profuse research activity. This review will focus on recent topics of interest. **Limits of Progress:** The downward progress of the GA-defined limit of viability has historically been driven by consistent improvement in morbidity and mortality in ever smaller infants. However, recent studies suggest that nature may have finally set a limit to progress, with either no significant change (Norway) or only a modest increase in survival or survival without neurodevelopmental impairment (USA) in the last decade [1, 2]. The most recent large-scale US data found survival without neurodevelopmental impairment in 1, 13, and 32% at 22, 23, and 24 weeks, respectively. **Lack of Consensus:** Despite extensive study of outcomes, consensus eludes neonatologists. Studies of approaches to delivery room (DR) care in case scenarios show marked individual differences among senior practitioners [3]. Published professional guidelines similarly vary regarding initiation of care based on GA and the inclusion or exclusion of additional confounding variables such as sex, estimated fetal weight, exposure to antenatal steroids, and plurality [4, 5]. Despite the recognition of the critical importance of such clinical factors, together with the limitations of GA assessment by combined obstetric-neonatal methods, the vast majority of published guidelines continue to rely solely on GA for DR decision-making. The extent of variation in approach particularly at 22 and 23 weeks GA is remarkable. A recent study from the NICHD network found that the proportion of infants offered active intensive care in the DR at 22 weeks varied between 0 and 100% in academic medical centers [6]. Clearly, this degree of variance cannot be explained simply by varying parental preferences but may well reflect directive guidance offered by med-

ical caregivers based on personal values, cultural norms, religious beliefs, and other factors. **Guidelines and Outcomes:** Changes in guidelines based on poor outcome data may lead to active intensive care being offered to more infants at the designated limits, and this may lead to improved outcomes. Documented progress has been seen to generate further changes in recommendations within relatively short periods of time [7]. However, other professional bodies may interpret the same data as proof of concept of the boundaries of nature and/or the limitations of medical resources and justify perpetuating restrictive guidelines that maintain the achieved outcomes without change. **Treatment Limitation Discussions:** Withholding active intensive care at birth may not be considered ethically equivalent to later withdrawal of care that is intended to reduce pain and suffering in the short term or to avoid a poor future quality of life. Most NICU deaths today occur following a treatment limitation discussion (TLD) concerning a very sick infant. However, a recent study found that in only 55% of cases did physicians claim absolute certainty regarding the prognosis, suggesting that neonatologists may make recommendations of profound individual significance even in the presence of a certain ambiguity regarding prognosis [8]. In addition, other studies show that in TLDs, neonatologists and parents do not necessarily agree and that some infants survive after their parents have refused to limit intensive care [9, 10]. Outcomes in this setting continue to highlight the limitations of our prognostic abilities.

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## Early Onset Sepsis: Unnecessary Investigations and Excessive Use of Antibiotics

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Culture-proven early onset neonatal sepsis (EONS) is confirmed in fewer than 1% of admissions to neonatal units, yet accounts for 10% of all neonatal mortality and contributes to significant morbidity. Culture-negative sepsis occurs more frequently and represents a challenge for neonatologists. Early diagnosis of EONS is challenging because clinical characteristics are non-specific and difficult to differentiate from those of non-infectious aetiologies. The repertoire of ancillary laboratory tests is also limited and not always reliable [1, 2]. Fear of infection results in excessive use of antibiotics, with more than 95% of babies being investigated and treated for suspected infection, ultimately having no evidence of infection [3]. The hazards of antibiotics are beyond the fact that antibiotic therapy drives antibiotic resistance. Because gut flora drives the nascent immune system, peripartum antibiotic exposure is increasingly recognized as a major driver of immune dysregulation, resulting in an increasing incidence of atopy and asthma in childhood [4]. Neonatologists are constrained by the availability of insufficiently sensitive and specific microbiological diagnostic tools, which would allow more judicious antibiotic prescribing. Risk factors contribute to a clinical diagnosis but are insufficiently robust to be reliable in making a diagnosis of EONS. A number of risk factor scores have been devised for asymptomatic neonates at risk of EONS [5], none of which has been shown to be sufficiently robust for widespread use. More recently pulse oximetry has gained momentum as a screening tool to detect illnesses including EONS in addition to congenital heart disease, which the “PulseOx study” was originally designed to investigate [6]. The “gold standard” for diagnosing infection is historically a positive blood or CSF culture with a minimum reporting delay of 36–48 h. Microbial cultures suffer from low sensitivity and specificity: a negative blood culture result is almost inevitable for a large proportion of blood cultures because of the submission of inadequate volumes of blood and only 1 culture being drawn [7]. False-positive culture of coagulase-negative staphylococci is common, making diagnosis of EONS using the historical gold standard a challenge. Although real-time PCR and broad-range conventional PCR amplification methods have higher sensitivity (0.9; 95% CI 0.78–0.95) and specificity (0.96; 95% CI 0.94–0.97) than other assays, molecular assays still do not have sufficient sensitivity to replace microbial cultures in the diagnosis of neonatal sepsis but may perform well as “add-on” tests [1]. The ideal diagnostic test would be rapid, 100% sensitive, 100% specific and not affected by maternal antibiotic therapy. Reliable diagnostic techniques may better inform antibiotic management of neonates, and negative results enable clinicians to have confidence in prescribing antibiotics for a short period of time or not at all [1]. **Antimicrobial Stewardship:** Antibiotic therapy drives antibiotic resistance. Antimicrobial stewardship (AMS) refers to the coordinated interventions to prescribe and measure the most appropriate, pathogen-specific, narrow-spectrum drug regimen, dose, duration of therapy, and route of administration [8]. Ele-

ments of an AMS should also include ward-focused antimicrobial teams and evidence-based antimicrobial prescribing guidelines [8]. Neonatal units should also focus on infection prevention [9].

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## 17

### The Danger of Multiresistant Microorganisms and Fungal Infections: Is There a Solution?

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The long-term solution to the problems of both multiresistant bacteria and fungal infection in neonatal units has to be the better use of existing antibiotics. This means using narrow-spectrum antibiotics in preference to broad-spectrum antibiotics, and stopping antibiotics as soon as possible if cultures are negative, preferably within 2–3 days and certainly within 5 days whenever possible. Several studies have shown that it is safe to stop antibiotics after 2–3 days [1–4]. Continuing antibiotics longer than 5 days for very low-birth-weight babies in the face of negative systemic cultures is associated with a 50% increase in mortality and a 42% increase in necrotizing enterocolitis [5]. The incidence of neonatal fungal infections increases when broad-spectrum antibiotics, notably third-generation cephalosporins, are used for prolonged periods. The incidence of neonatal fungal infections can be reduced using prophylactic antifungals [6, 7]. Cochrane systematic reviews show the incidence of invasive fungal infection can be reduced by systemic antifungals like fluconazole by 57% and by non-absorbed oral antifungals such as nystatin by 80%, but in trials with methodological weaknesses.

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## 18

### Cellular Growth Factors, Immunoglobulins and Immunomodulators: Do They Protect the Very Immature Preterm Infant from Developing Sepsis?

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The preterm infant is born with insufficient immunoglobulins, neutrophils that are functionally immature, and lymphocytes that are skewed away from producing the pro-inflammatory cytokines that drive antibacterial immunity. The first approach to address these deficiencies and reduce blood stream sepsis was to test intravenous immunoglobulin (IVIg) given as sepsis prophylaxis. Randomised trials achieved a small reduction in sepsis incidence but did not reduce mortality [1]. Other studies administering IVIg at the time of sepsis to reduce mortality were more encouraging, leading to the large International Immunotherapy Study (INIS) [2]. INIS recruited almost 3,500 infants with proven or suspected serious infection to receive 2 doses of IVIg or placebo. Two-year mortality was almost identical, 18% for infants who received either active IVIg or placebo. The next approach was to target the neutrophil, using the newly available recombinant cytokines, G-CSF (granulocyte colony-stimulating factor) and GM-CSF (granulocyte-macrophage CSF), which stimulate neutrophil (and monocyte) production and, to some extent, function. It was recognised that sepsis in preterm infants often precipitates a fall in neutrophil count, a consequence of reduced neutrophil production and marrow reserves. Randomised trials, giving G- or GM-CSF to infants with suspected late onset sepsis, proved difficult to recruit into, and the small number of studies completed gave inconclusive results [3]. Two large randomised prophylactic studies were reported in 2009: one commenced GM-CSF in small-for-gestation preterm infants soon after birth irrespective of neutrophil count and the oth-

er G-CSF in infants developing postnatal neutropenia [4, 5]. Neither led to reduced sepsis or all-cause mortality. A secondary analysis in the GM-CSF (PROGRAMS) trial revealed that, contrary to popular belief, neutropenia during the first postnatal week does not increase the risk of subsequent bacterial sepsis; only gestational age and birth weight were significant predictors. A recent large retrospective survey of clinical use of G-CSF in the USA compared outcomes of infants given G-CSF as treatment for neutropenia ( $\leq 1.5 \times 10^9/L$  for  $\geq 24$  h) compared to those not given G-CSF. Treated infants had faster neutrophil recovery but higher incidence of subsequent sepsis and/or death. This may reflect the use of G-CSF in more immature, sicker infants rather than an adverse effect of G-CSF itself [6]. While there has been no definitive study testing whether administration of G-CSF to septic neutropenic infants reduces mortality, infants in the PROGRAMS trial who developed sepsis while receiving GM-CSF did not have better immediate or long-term outcomes [4, 7]. Recent attention has focused on probiotics to reduce the incidence of sepsis and/or necrotizing enterocolitis (NEC). With many studies reported, a picture is emerging that probiotic administration to preterm infants may reduce the incidence of NEC but not blood culture-positive sepsis. The 2 largest studies, PIPS in the UK and ProPrams in Australia, each recruiting more than 1,000 preterm infants, found no difference in late onset sepsis or all-cause mortality between the treated and placebo groups [8, 9]. While PIPS found no difference in the incidence of NEC, ProPrams did report a modest reduction ( $p = 0.03$ , number needed to treat 45). Easier to administer, with some physiological basis and emerging evidence of benefit, is lactoferrin. The landmark trial of Manzoni et al. [10] found early administration of oral lactoferrin to very low-birth-weight infants led to a significant reduction in late onset sepsis, particularly if given together with a probiotic. Lactoferrin, present in high concentrations in human colostrum, seems to have a beneficial effect on the development of newborn gut immune competence. It is most effective in reducing infection if administered soon after birth and in the most preterm infants. Whether it has benefit over and above human breast milk remains uncertain. At this moment lactoferrin seems the most interesting intervention to watch [11].

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## 19

### Therapeutic Drug Monitoring of Antibacterial Agents in the NICU

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Therapeutic drug monitoring (TDM) of antibacterial agents in the NICU has traditionally been used to tailor both the dose and dosing interval of these drugs in a sick neonate with suspected or proven bacterial infection with the ultimate goal to maximize therapeutic benefits of the prescribed medication while minimizing drug-related toxicity [1]. Classically, this approach has been performed by measuring drug concentrations in plasma or serum, applying pharmacokinetic and pharmacodynamic principles, and then adjusting the dose to keep the drug concentration in the desired therapeutic range. By its very nature, TDM is primarily applicable for drugs that have a narrow therapeutic window (i.e., the drug concentration required for a therapeutic effect is close to a concentration that will result in toxicity) and for drugs that show a useful correlation between serum concentrations and pharmacological effect. Neonates are in a unique and very dynamic pharmacokinetic state, where they undergo relatively rapid maturational changes in drug absorption, distribution, metabolism, and excretion [2]. These pharmacokinetic variables are strongly influenced by both gestational and postnatal age. The body composition of the newborn has a large impact on the distribution of these drugs in the neonatal body. Term but especially preterm neonates have a relatively high proportion of total body water and a low proportion of fat resulting in a higher volume of distribution for water-soluble compounds and a lower volume of distribution for fat-soluble compounds. In addition, because the brain-to-body mass ratio is relatively high and the body fat content low, lipid-soluble drugs tend to concentrate in the CNS. Protein binding in neonates is lower as compared to older kids and adults resulting in higher free drug concentrations even if the total serum drug concentrations are the same as in older patients. The clearance of most drugs is prolonged, caused by both immature drug-metabolizing capacity as well as decreased renal clearance, ultimately resulting in high concentrations of both parent drug and its metabolite(s) [3]. Currently, TDM is routinely used in neonates to tailor the use of ami-

noglycosides and vancomycin whereas TDM is not routinely used for optimizing the use of many other antibacterial agents such as penicillins, cephalosporins, carbapenems, etc. Aminoglycosides are small hydrophilic molecules with a volume of distribution similar to the extracellular fluid volume and clearance directly proportional to the glomerular filtration rate [4, 5]. The bactericidal effect of aminoglycosides (gentamicin, tobramycin, amikacin) is strongly associated with reaching appropriate peak concentrations in relation to the minimal inhibitory concentration of the pathogen, the so-called concentration-dependent killing. As a consequence, a relatively large dose is needed to reach a potentially effective peak concentration whereas the low renal clearing capacity of the neonate will necessitate increasing the dosing interval from once a day to once every other day or even once every 72 h. Clearly TDM seems indicated in this situation to assure optimal efficacy (peak concentrations) and minimal toxicity (through concentrations). Vancomycin is a glycopeptide used to treat infections caused by *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus*. Unlike aminoglycosides, vancomycin does not need a high peak concentration for optimal effect but it is much more important to be exposed to vancomycin as long as feasible during the entire dosing period to be effective, due to the so-called time-dependent killing. The use of TDM to tailor the use of vancomycin is quite variable across the globe. In general, monitoring of peak concentrations is not recommended because these concentrations do not correlate with efficacy or toxicity. For adults with a normal renal function a trough concentration of 15–20 mg/L reliably predicts a sufficient exposure throughout the entire dosing period. However, in neonates we lack that kind of very useful information. Moreover, for toxicity reasons, it is unclear whether the currently accepted pharmacokinetic targets in adults will be associated with nephro- and/or ototoxicity in neonates [6]. Finally, safe and effective use of medicines in critically ill neonates is very challenging because of the limited knowledge concerning the effect of both the underlying disease state and ongoing developmental changes on both the pharmacokinetics and pharmacodynamics of frequently used antibacterial agents. In this critically ill neonatal population, changes in drug distribution and organ function responsible for drug handling can be substantial, both as a consequence of the primary underlying pathophysiology and in response to clinical interventions provided in the neonatal intensive care setting. In the adult intensive care and even in the pediatric intensive care setting, an additional complicating factor for optimizing drug therapy has emerged called augmented renal clearance (ARC) [7, 8]. At this stage it is still unclear what the clinical relevance of this finding is but it might be useful to realize that even in our neonates ARC might happen, and it is perhaps the right moment to start designing the appropriate studies to investigate this in our vulnerable patients.

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## 20

### Neonatal Encephalopathy: Are We Improving Outcomes in All Settings?

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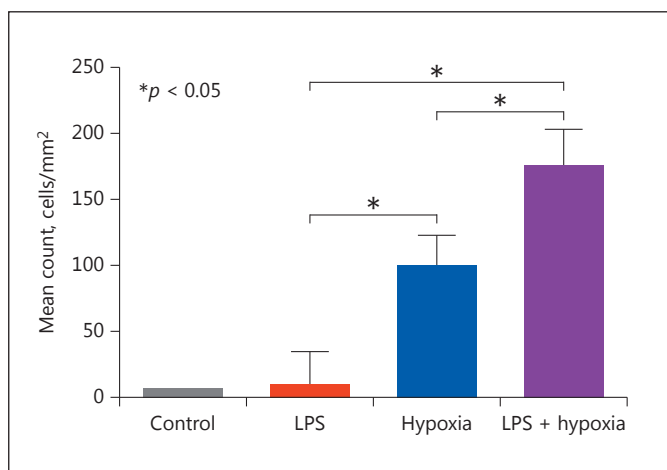
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#### Neonatal Encephalopathy in High, Mid, and Low Settings:

Neonatal encephalopathy (NE) continues to represent a significant global health burden with an estimated 650,000 deaths worldwide; in 2010, NE was the second leading cause of neonatal mortality [1]. Where babies are born in the world impacts outcome significantly [2]. In high-resource settings, therapeutic hypothermia with intensive care support has improved outcomes (mortality and neurodevelopmental disability) at 18 months (typical RR 0.75, 95% CI 0.68–0.83) [3]; this improvement persists into childhood [4].

Studies of low-tech therapeutic hypothermia in low- and mid-income (LMI) countries in contrast have not shown a significant reduction in mortality (RR 0.74, 95% CI 0.44–1.25) [5]. The wide CI suggests that a clinically important benefit or harm cannot be excluded and may be due to small studies and inclusion of mild NE. However, in these settings, NE rates are high, hypoxia may have started many hours before delivery, resuscitation may not be optimal, and there may be other comorbidities such as sepsis. LMI countries therefore have insufficient evidence that cooling (using current protocols) is safe and protective in their setting. Resource limitations are severe in some *low-income* countries, and at present cooling should not be considered standard care. In contrast, collaboration between *high- and mid-income* countries may be beneficial for speeding up therapies while understanding the different cultural and medical backgrounds [6]. For example, some mid-income units facilitate “endogenous cooling” over 16 h [7]. One of the challenges is the high seizure burden and difficulty recognizing and thus treating seizures without EEG/aEEG. **Infection Sensitization:** Another challenge across the world is the multifactorial aetiology of NE, with the combination of antenatal infection and a potentially asphyxiating condition at birth dramatically increasing the risk of cerebral palsy. In a large animal model of birth asphyxia (the newborn piglet), we have recently shown the effect of 4-h exposure to lipopolysaccharides (LPS) prior to hypoxia. LPS is found in the wall of gram-negative bacteria, such as *Escherichia coli*, and is a potent endotoxin, stimulating a Toll-like receptor 4 (TLR-4) response. We observed increased mortality (60 vs. 0%)





**Fig. 1.** Mean cell death ( $n/mm^2$ , mean TUNEL count across all brain regions  $\pm$  SEM) with LPS, hypoxia, and LPS + hypoxia combined. Synergy was seen with LPS + hypoxia.

and worse brain injury, predominantly in the white matter compared to hypoxia alone. Most notably, the number of dead cells (TUNEL count) for the LPS+ hypoxia group was significantly greater than both hypoxia alone and LPS alone groups, with the total being more than the cumulative total of each, demonstrating synergistic injury to the brain. The estimated mean cells per square millimetre for LPS+ hypoxia are shown in Figure 1. The mean cell death per square millimetre across all 8 brain regions for LPS+ hypoxia was 176 (95% CI 117–234) compared to 100 (53–148) for hypoxia alone ( $p < 0.05$ ) versus 10 (–41 to 62 for LPS alone ( $p < 0.05$ ). Possible mechanisms for this increased vulnerability to injury with LPS exposure for 4 h include: (i) suppression of mitochondrial respiration leading to a cumulative larger ATP depletion during hypoxia; (ii) TLR-4 activation in microglia and other nerve cells triggering increased NF- $\kappa$ B, IL-1 $\beta$ , and TNF- $\alpha$  expression which leads to apoptosis, necrosis, and excess pro-inflammatory cytokine production. **Melatonin as a Potential Treatment for Inflammation-Sensitized Hypoxia:** Melatonin is a versatile molecule known for its antioxidant and free radical scavenger actions. Pharmacological levels of melatonin augment the hypothermic protection after hypoxia in the piglet [8]. Melatonin is also an important regulator of the immune response; a recent study shows that melatonin administration at the same time as LPS in pregnant rats completely reversed the inflammatory state produced by LPS and sensitization to injury [9]. Melatonin may be an effective therapy in infection-sensitized NE. **Conclusions:** Global health research in NE needs to be culturally appropriate, evidence based, and economically feasible. Knowledge of the inflammatory status of a newborn with NE will be important in optimizing brain protection and choosing the most effective therapies.

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## 21

### Mesenchymal Stem Cells: Magic Cure for Severe Intraventricular Hemorrhage?

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Severe (grade  $\geq 3$ ) intraventricular hemorrhage (IVH) that increased the risk of developing posthemorrhagic hydrocephalus (PHH) remains a major cause of mortality and long-term neurological morbidities in premature infants. However, despite recent advances in neonatal intensive care, no effective treatment is currently available to prevent the development of PHH to attenuate brain injury after severe IVH in preterm infants. Although the pathogenesis of brain injury and progression to PHH after severe IVH is complex and has not yet been clearly elucidated, inflammatory responses caused by blood products in the subarachnoid space are known as some of their main mechanisms [1]. Several studies reported the beneficial effects of mesenchymal stem cell (MSC) transplantation, such as antiapoptotic, anti-inflammatory, and antioxidative paracrine effects in various neonatal animal disease models [2, 3]. Therefore, MSC transplantation can be regarded as a promising new therapeutic modality of severe IVH in preterm infants. Recently, we have shown that intraventricular administration of MSCs significantly attenuates brain injury and PHH after severe IVH in immunocompetent newborn rats [4]. Owing to not only their easy obtainability and lower immunogenicity [5], but also higher proliferation capacity [6] and paracrine potency [7], MSCs obtained from birth-related tissues, such as umbilical cord blood (UCB) or Wharton's jelly, may be optimal sources for clinical use for severe IVH in premature infants. We have performed several preclinical studies regarding the optimal route [8] and timing [9] of MSC transplantation in a severe IVH model in neonatal

rat pups. The benefits of MSC transplantation are primarily mediated by a paracrine effect, and the brain-derived neurotrophic factor secreted by transplanted MSCs is at least one of the critical paracrine factors that play cardinal roles in attenuating brain injury and PHH after severe IVH [10]. We have shown that either the systemic or intraventricular local transplantation of MSCs was equally effective to attenuate brain injury and PHH. However, the MSC dose for systemic intravenous injection was 5 times higher compared to the local intraventricular injection [8]. Moreover, since the newborn infant has an open anterior fontanelle, local transplantation of MSCs via a bedside ventricular tap is clinically feasible in premature infants with severe IVH. We have also shown that early timing is better than late timing for intraventricular MSC transplantation in severe IVH of neonatal rat pups [9]. Based on these promising pieces of evidence of neuroprotective effects of MSC transplantation in a newborn animal model of severe IVH, we designed and conducted a phase I clinical study of human UCB-derived MSC transplantation in preterm infants with severe IVH (NCT02274428). This study was an open-label, single-center, and dose-escalating clinical trial to assess the safety and feasibility of intraventricular transplantation of a single dose of allogenic human UCB-derived MSCs within 7 days of detection of severe (grade  $\geq 3$ ) IVH in preterm infants. The primary outcomes were death or anaphylactic shock within 6 h of MSC transplantation, and the secondary outcomes were death or hydrocephalus requiring shunt surgery. In total 9 patients were enrolled and the MSCs were transplanted with a low dose of  $5 \times 10^6$  cells/kg (in 1 mL/kg saline) in 3 patients and a high dose of  $1 \times 10^7$  cells/kg (in 2 mL/kg saline) in 6 patients. The MSC treatment was well tolerated, and no patients showed serious adverse effects related with transplantation. We are planning to conduct long-term follow-up studies of these enrolled infants and subsequent phase II clinical trials to assess the efficacy of MSC transplantation for severe IVH in premature infants.

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## 22

### Outcome Prediction in Preterm Infants by Advanced MRI at Term

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Very preterm infants exhibit impaired brain growth and delayed maturation compared with full-term infants, which is likely the result of a combination of primary injury and secondary maturational disturbances. Both white and gray matter structures, expressed in neuronal networks or cortical folding, are affected at term-equivalent age. Preterm infants have been reported to display volume changes in cortical gray matter, basal ganglia and thalami, cerebellum, cerebral white matter, and, in particular, in corpus callosum size, compared with term-born controls. In children born before term, the association between smaller brain volumes and cognitive impairments, such as lower overall intelligence, memory deficits, and impairments in executive functioning has been well established. Total brain tissue volume accounted for 20–40% of the variance in cognitive and educational performance. Brain volume alterations in the neonatal period seem to be associated with neurodevelopment across a wide range of functional outcomes (cognition, behavior, as well as neuromotor performance). Advances in neuroimaging techniques seem to pave a way towards a comprehensive understanding of the maturational pathways of brain network development and of how premature birth may affect these trajectories and neurodevelopment outcome. Recent insights into brain network development, such as white matter connectivity matrices and cortical reconstructions, are fundamental for our further understanding of cognitive and educational performance, for early identification of infants at risk, as well as for future neuroprotective strategies.

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## 23

### Preventive Strategies and Up-to-Date Treatment of Severe Retinopathy of Prematurity

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Severe retinopathy of prematurity (ROP) is a 2-phase disease. In the first phase, starting at birth, oxygen exposure increases abruptly, and the nutrient supply becomes insufficient. Angiogenesis, which is stimulated by relative hypoxia in the retina and other

organs, slows down, and already formed vessels are lost. The second phase is characterized by catch-up growth and retinal hypoxia due to impaired vascularization. Uncontrolled neovascularization may lead to retinal detachment and blindness. Current treatment targets proliferative ROP but since any ROP is associated with smaller brain size and impaired neurodevelopmental outcome, prevention of all ROP is desirable. **Prevention of the First Phase of ROP:** *Oxygen:* After birth, promotion of normal neurovascular development may prevent ROP by avoidance of hyperoxia and oxygen fluctuations. The application of an oxygen saturation SpO<sub>2</sub> target of 90–95% has led to increased ROP rates in many settings. It is well known that alarm levels are often set to high, that SpO<sub>2</sub> above target is common [1], and that SpO<sub>2</sub> >93% is associated with hyperoxia (PaO<sub>2</sub> >80 mm Hg) [2]. Education, strict oxygen control with the alarm limit close to 95%, and titration algorithms preventing large oxygen fluctuations can reduce ROP [1]. *Nutrition:* Most infants at risk for ROP have extrauterine growth retardation, low serum IGF-1 and a need for parenteral nutrition. Currently provided solutions lack many of the ingredients that are normally transferred from the mother during the third trimester and during breastfeeding. Early human milk feedings decrease the ROP risk, and promotion of breastfeeding may prevent ROP [3]. Experimental and human studies indicate that fish oil containing the ω-3 long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (DHA) reduces severe ROP [4]. Most lipid solutions for intravenous use lack DHA which is normally selectively transferred from mother to fetus during the third trimester and which is important for retina and brain development as well as for metabolism. Supplementation with ω-3 LCPUFA may suppress the ω-6 LCPUFA arachidonic acid (AA) which plays important roles in the vasculature. A study comparing Clinoleic with SMOFlipid in the prevention of ROP has recently been finalized (www.clinicaltrials.gov No. NCT02760472) and formed the basis for a new study with supplementation with both DHA and AA to prevent ROP and other morbidities in extremely preterm infants. **Prevention of Progression in the Second Phase of ROP:** *Oxygen:* There is evidence that, in the second hypoxic phase, higher oxygen saturation reduces the progression to proliferative ROP [5]. *β-Receptor Blocker:* Systemic propranolol has the potential to decrease progression to proliferative ROP but with serious adverse effects [6]. **Treatment of Sight-Threatening ROP:** *Laser:* Diode laser photocoagulation of sight-threatening ROP is the gold standard treatment. It destroys the avascular retina and induces regression of neovascularization in most cases. Treatment may need to be repeated in the neonatal period. There is a life-long increased risk of retinal detachment and other ocular complications. *Anti-VEGF:* In recent years off-label intravitreal injections of bevacizumab, an anti-vascular endothelial growth factor (VEGF) antibody, have increased in popularity despite a lack of studies on dosage, pharmacokinetics, and safety. Bevacizumab causes rapid regression of neovascularization. However, recurrences are common and may occur after several years. Most importantly, bevacizumab escapes the eye and suppresses serum VEGF for months at a stage of intense neurovascular development with unknown consequences for the development of the brain and other organs [7]. At the age of 2 years, which is too early to assess effects on cognition, both no difference in neurodevelopmental outcome [8] and a threefold increased incidence of severe neurodevelopmental disability [9] after bevacizumab versus laser have been reported. Ranibizumab is an alternative with a shorter half-life. Studies on both substances are under way.

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## 24

### Implementing Best Practice in Preterm Enteral Care

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The number of preterm deliveries is growing with current estimates indicating a contribution worldwide ranging from 7 to 15% of all births. Progress is being made in defining optimal practice in enteral care but there are many continuing areas of uncertainty. There are strong theoretical grounds for advocating the immediate or early administration of fresh colostrum to preterm babies but objective evidence of benefit is lacking. Randomised controlled trials such as Speed of Increasing Milk Feeds (unpublished) and others [1] show that it is safe to commence enteral feeds within 48 h of birth and to advance as tolerated, at volumes of up to around 30 mL/kg/day in even growth-restricted and very preterm babies. Quasi-randomised and observational studies indicate that milk from a baby's own mother reduces sepsis and necrotising enterocolitis [2], and confers neurodevelopmental advantage. Evidence for a positive impact on metabolic and cardiovascular health remains limited, and a reduction in risk of later obesity remains uncertain. A cardinal uncertainty is the optimal pattern of postnatal growth [3]. The majority of preterm babies have experienced some degree of intrauterine growth restriction, hence aiming to achieve a weight by term equal to that of a full-term baby requires a period of accelerated postnatal growth, which may be harmful. Optimal macronutrient requirements, especially in relation to protein in-

take and multicomponent fortification of human milk, are unknown. Recommendations on theoretical grounds have been made on the basis of mimicking intrauterine growth velocity but to-date the impact of such an approach on functional outcomes has not been evaluated. Optimum protein intake is likely to lie within an as yet undefined range. Safety is a key consideration with reports of adverse outcomes in relation to both too high as well as too low protein intakes. As the protein content of human milk is very variable, and the optimum protein intake is unknown, so-called individualised fortification has little merit. Human milk banks exist in many parts of the world but evidence for the efficacy and effectiveness of pasteurised human donor milk is scant [4]. Pasteurisation destroys or reduces many non-nutrient bioactive components of human milk, and the profile of, for example, hormones and human milk oligosaccharides varies between mothers [5, 6]. Pasteurised human donor milk cannot therefore be considered identical in biological effectiveness as fresh milk from a baby's own mother. The rise in commercialisation of human milk is another area of potential concern. Optimal micronutrient, short-chain polyunsaturated fatty acid, nucleotide content of preterm feeds, and other components are also areas of continuing uncertainty [7]. A reasonable conclusion must be that preterm enteral nutrition remains largely experimental. This argues for approaches that embed incremental reduction in uncertainties in everyday care into routine clinical practice as the optimal way forward [8, 9].

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### Parenteral Nutrition in Very Immature Infants: Advances and Hidden Risks

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Intravenous nutrition is necessary in preterm infants until full enteral feeding is achieved, but it is not without risks. Increased use of intravenous nutrition, for example, has markedly increased the incidence of hyperglycemia. The principal cause is a higher than needed rate of intravenous dextrose infusion [1], which is compounded by other common risks of hyperglycemia in preterm infants, including increased stress-induced or infused catecholamine, glucagon, and cortisol concentrations, reduced insulin secretion, lack of enteral feeding, and gut production of “incretins” that promote insulin secretion, and persistent glucose production that does not decrease in response to higher glucose and insulin concentrations, as happens later in life [2]. Adverse effects of hyperglycemia in preterm infants have likely been underestimated. Many studies in animal models and adults have documented hyperglycemia-induced production of reactive oxygen species in many cells throughout the body and increased cell, organ, and systemic inflammation [3]. Serious adverse effects of hyperglycemia on neurodevelopment have also been documented in animal models, including decreased neuronal dendritic spine and synapse formation [4], reduced neuronal density, and reduced antioxidant and increased oxidant injury status [5]. While the principal approach to treating hyperglycemia during intravenous nutrition is to reduce dextrose infusion rates, many treat hyperglycemia with intravenous insulin. Unfortunately, such insulin treatment can augment excess cellular glucose carbon uptake leading to metabolic acidosis, cellular and systemic oxidant injury, and local and systemic inflammation [3]. Furthermore, insulin treatment to prevent or treat hyperglycemia leads to an increased incidence of hypoglycemia [6]. Therefore, a safer and more successful approach to prevent and treat hyperglycemia is the use of higher amino acid infusion rates (3.5–4.0 g/kg/day), which enhance insulin secretion [7] and reduce time-averaged glucose concentrations and the number of documented episodes of hyperglycemia [8]. Starting enteral nutrition sooner and advancing it faster will also help limit the incidence, severity, and duration of hyperglycemia [9]. The most consistent evidence supporting the use of intravenous nutrition in preterm infants is the positive effect on nitrogen/protein balance. Unfortunately, current standard intravenous nutrition and feeding regimens do not support normal fetal rates of brain growth in infants born very preterm [10]. Furthermore, because the mix of amino acids in the intravenous amino acid solutions was not designed to meet the unique metabolic needs of physiologically unstable, very preterm infants during the first postnatal days, simply giving higher rates of infusion is not likely to produce positive benefits. There is reason, therefore, to develop more optimal intravenous amino acid solutions with relative higher concentrations of essential amino acids, especially leucine, that might specifically promote a positive protein balance in infants who have severe catabolic conditions such as intermittent hypoxia, sepsis with endotoxemia [11], and stressful conditions such as complex abdominal surgery [12].

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## 26

### Update on the Pathophysiology of Necrotizing Enterocolitis

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Necrotizing enterocolitis (NEC) remains a common and feared disorder of newborn infants. Although much has been discovered about mechanisms that may promote inflammation, there are as yet no models that may explain all elements of this disorder. Perhaps not surprisingly, given the vast numbers of bacteria separated by a single cell layer from the majority of the infant’s immune cells, epithelial integrity appears critical. When NEC first emerged about 60 years ago, most reports were in term infants who had suffered peri- or postnatal asphyxial stress [1]. With time, as smaller and more preterm infants were successfully treated, the disorder has become much more common and most frequently seen in extremely preterm very low-birth-weight infants. Other important predisposing factors have emerged, notably the intestinal microbi-

ome, antibiotics, and cow’s milk-derived formula feeds [1–4]. Various pathogens have been implicated in individual cases or outbreaks of NEC, although without any clear common theme, most cases developing without clear evidence of a causative pathogen [5]. Gut microbial populations are abnormal in NEC. Even before the onset of disease there develops a “proteobacterial bloom” characterized by both an increased abundance of Proteobacteria and a decreased relative abundance of Firmicutes and Bacteroidetes [3]. Recognition of the driving role of the microbiome has led to trials of various probiotics in infants at risk of NEC, and this stratagem has been broadly successful in that a reduced incidence of NEC and death has been reported in many, although not all, trials [6]. Subgroup analysis has identified that *Bifidobacteria* and *Lactobacillus* species appear most effective and that the groups of infants most likely to benefit are those who have been breastfed and have not been exposed to antenatal corticosteroids [6]. The pro-inflammatory drive for NEC may potentially be initiated by an enhanced epithelial NF- $\kappa$ B response to gut bacteria, mediated by signalling through the enterocyte Toll-like receptor 4 [7]. Within the mucosal lamina propria, there emerges a skewed T helper response in which Th17 cytokines dominate and T regulatory responses are attenuated [7]. The impact of this unopposed Th17 response upon epithelial integrity includes reduced proliferation and apoptosis of stem cells within the crypts. Numerous secondary pro-inflammatory mediators are released [2, 7]. The reduced incidence of NEC afforded by exclusive breastfeeding [8] suggests a possible protective role of breastmilk components, which include bacteria from the mother’s intestine, gut-derived immune cells, and cytokines as well as immunomodulatory microvesicles and multipotent stem cells that are incorporated into the tissues of the infant [9]. An alternative explanation may be a direct immunostimulatory effect of cow’s milk protein in infant formulae: lymphocytes from infants with NEC show an enhanced pro-inflammatory response in vitro to cow’s milk-derived protein [4]. Allergic dysmotility is a well-recognized component of non-IgE-mediated allergy to cow’s milk, and it is notable that many affected infants show dysmotility for some time prior to the onset of disease. Whether some infants diagnosed as having NEC are in fact suffering from food protein-induced enterocolitis syndrome is an intriguing concept that warrants further study. Finally it should be noted that the NEC response is highly unusual, with sudden onset in many cases and a remarkable incidence of perforation or necrosis. Animal models of disruption of myenteric plexus glial cells are characterized by a similarly high incidence of perforation and death, and a number of studies have identified significant disruption of enteric glia by focal immune responses [10].

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27

### Discovery of Novel Biomarkers for Necrotising Enterocolitis

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Necrotising enterocolitis (NEC) is the most common surgical emergency for premature infants and carries a high morbidity and mortality. Advances in neonatal intensive care have witnessed significant improvement in the prevention and/or treatment of respiratory distress syndrome, bronchopulmonary dysplasia (BPD), parenteral nutrition-associated cholestasis, intracerebral pathologies and mortality in past decades. Yet, the occurrence of NEC and its associated complications have not decreased. One of the major difficulties is detecting the disease at its early stage. To date, there has been no surveillance or early diagnostic biomarkers to alert frontline neonatologists at the initial process of intestinal injury. In the past 2 decades, researchers have focused on utilising known mediators (e.g., acute phase proteins, cell surface antigens, cytokines, chemokines, etc.) of the inflammatory signalling pathways, coupled usually with commercially available laboratory measurement kits for evaluating their diagnostic utilities (i.e., sensitivity, specificity, positive predictive values [PPV] and negative predictive values [NPV]). This “conventional” approach has greatly limited the scope of discovering novel biomarkers for specific organ injury or disease. With recent advances in medical and omics technologies, we have currently applied a “hypothesis-free” approach to identify novel and specific biomarkers for intestinal injury and NEC. We adopted a 3-phase sequence for discovery of new biomarkers. First, we employed global gene (or nucleic acids) expression profiling of relevant specimen samples (e.g., tissues, blood, or other relevant body fluids) on definitive cases of NEC versus conditions we wish to differentiate (i.e., neonatal sepsis without NEC and non-infection cases such as exacerbation of BPD, apnoea of prematurity, severe anaemia, etc.). Differential comparison of microarray data enables researchers to identify potentially promising targets specific to NEC. Secondly, selected targets are subjected to a case-control study in order to assess their suitability, including diagnostic characteristics, absolute concentrations at an early stage of NEC, instrumentation and turnaround time, etc. as biomarkers. This process permits us to calculate the sensitivity, specificity, and areas under the receiver-operating characteristic curves for early

identification of NEC cases from non-NEC conditions. Those with favourable diagnostic characteristics are further evaluated through a prospective cohort study to delineate the optimal cut-off values for each or combination of biomarkers and full diagnostic utilities, including sensitivity, specificity, PPV and NPV, positive and negative likelihood ratios, and diagnostic odds ratios. We have recently completed such a comprehensive study and managed to identify 2 microRNAs which could specifically differentiate both medical (mild) and surgical (severe) NEC from neonatal sepsis and other non-NEC neonatal disorders with sensitivity >0.80 and specificity >0.85. We anticipated that this “hypothesis-free” approach could be applied to other tissue/organ injuries and diseases, and assists frontline neonatologists in discovering novel biomarkers for early disease identification. Thus, neonatologists and paediatric surgeons would be more confident in prescribing specific treatment at a very early stage for preventing disease progression and minimising potential complications, thereby permitting more efficient and effective management in future.

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28

### Recent Advances in Diagnosing Neonatal Nonimmune Hemolytic Anemias

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Hemolysis is a pathological shortening of the red blood cell (RBC) life span and differs from the natural removal of senescent RBCs at the end of their normal life span. Immune-mediated hemolysis in a neonate is recognized when the direct antiglobulin test (or Coombs test) is positive, indicating antibody (typically of maternal origin) on the RBC surface [1]. Neonatal hemolysis that is *not* immune-mediated can be complex and challenging to diagnose. Nonimmune hemolysis can be acute or chronic, and can be genetically based or acquired. The initial steps in evaluating nonimmune hemolysis involve examining the complete blood count and blood smear. Even if these do not produce a definitive diagnosis, they often exclude some causes of hemolysis and provide clues about which tests should be ordered next [2]. Table 1 lists blood smear features that can help identify the underlying condition. Hemolysis can be established by an elevated end-tidal CO. Also, the level of CO can serve to quantify the hemolytic rate [3]. If end-tidal CO measurements are not available, hemolysis can be established by the combination of hyperbilirubinemia, a falling hemoglobin, hemoglobinuria, and/or undetectable serum haptoglobin (Table 2). In many cases the exact diagnosis will be obvious from the clinical presentation, family history, and initial testing. Validation of the common RBC enzymopathies such as glucose-6-phosphate dehydrogenase deficiency or pyruvate kinase deficiency can be accomplished by analysis of the enzyme activity per gram hemoglobin. When the cause of the condition remains obscure, 2 new tests can be helpful: eosin-5-maleimide (EMA) flow (also called band 3 reduction), when hereditary spherocytosis (HS) is suspected in the

**Table 1.** Blood smear features that classify neonatal hemolytic conditions

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<i>Blister and bite cells:</i> especially when accompanied by schistocytes, suggest an acute hemolytic crisis due to glucose-6-phosphate dehydrogenase deficiency or an unstable hemoglobin species
<i>Dense distorted erythrocytes:</i> particularly with irregular projections and polychromasia (when associated with anemia, elevated aspartate transaminase, and absence of other etiologies) suggest infantile pyknocytosis
<i>Echinocytes:</i> with polychromasia, jaundice, and anemia, these cells suggest pyruvate kinase deficiency
<i>Elliptocytes:</i> if no other morphological abnormalities are seen, except for polychromasia, elliptocytes suggest hereditary elliptocytosis
<i>Marked poikilocytosis:</i> the combination of microspherocytes, RBC membrane budding, elliptocytes, polychromasia, and anemia with a low mean corpuscular volume suggests hereditary pyropoikilocytosis
<i>Schistocytes:</i> several fragmented RBCs per field, with thrombocytopenia, suggest macroangiopathic hemolytic anemia; hypochromic microcytic Heinz body-positive schistocytic anemia suggests HbH disease
<i>Spherocytes:</i> several per field, with polychromasia and no other morphological abnormalities, suggest hereditary spherocytosis
<i>Stomatocytes:</i> accompanying polychromasia suggests hereditary stomatocytosis; hereditary xerocytosis is suspected when dehydrated stomatocytes are seen

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**Table 2.** Laboratory tests to detect, quantify, and identify the cause of hemolysis

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<i>Elevated carbon monoxide:</i> an elevated end-tidal (breath) concentration of CO, or an elevated level of carboxyhemoglobin in the blood, indicates an elevated hemolytic rate
<i>Elevated indirect bilirubin:</i> particularly when severe hyperbilirubinemia is apparent on the day of birth
<i>Hemoglobinuria:</i> a urine analysis supports the diagnosis of hemolysis if hemoglobin is present in the absence of intact red blood cells
<i>Undetectable (or low) serum haptoglobin:</i> if hemolysis is significant, free hemoglobin will bind to haptoglobin, and serum haptoglobin will fall to undetectable levels
<i>Eosin-5-maleimide flow (band 3 reduction):</i> rapid and precise detection of hereditary spherocytosis, hereditary elliptocytosis, or hereditary pyropoikilocytosis
<i>Next-generation sequencing panel:</i> time-consuming (3 weeks) and expensive (> USD 1,000), but can identify well-known and novel mutations involved in hemolysis or bilirubin metabolism

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absence of a family history, and a next-generation sequencing panel when the cause remains unclear (Table 2). EMA is a rapid automated flow cytometry-based test that measures the fluorescent EMA uptake by RBC band 3. Decreased binding can confirm membrane defects such as HS, hereditary elliptocytosis, or hereditary pyropoikilocytosis [4]. Next-generation sequencing of a panel of genes involved in hemolysis and bilirubin metabolism is available [5–7]. This can identify responsible mutations when other tests fail to do so and can disclose well-described or novel mutations.

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## Neonatal Jaundice and Phototherapy: Are We Color-Blind and Worried Too Much about Light?

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**Neonatal Jaundice:** Neonatal jaundice is one of the most frequent diagnoses made in neonates. The bilirubin production rate in the newborn is between 6 and 10 mg/kg/day. For each gram of hemoglobin metabolized, 34 mg of bilirubin is produced. Unconjugated bilirubin that is not bound to albumin is neurotoxic. Basal ganglia and various brain stem nuclei are more susceptible to bilirubin toxicity. Significant hyperbilirubinemia is defined as total serum bilirubin (TSB)  $\geq 17$  mg/dL ( $>95$ th percentile for age), severe hyperbilirubinemia when TSB  $\geq 20$  mg/dL ( $>98$ th percentile for age), extreme hyperbilirubinemia when TSB  $\geq 25$  mg/dL ( $>99.9$ th percentile for age), and hazardous hyperbilirubinemia when TSB  $\geq 30$  mg/dL ( $>99.99$ th percentile for age). Among late preterm and term infants, TSB  $>95$ th percentile occurs in 81/1,000 live births, and TSB  $>99.9$ th percentile occurs in 1/1,000 live births. Severe hyperbilirubinemia in high-income countries is 32/100,000 live births. However, in low- and middle-income countries, the incidence is very high, ranging from 4 to 49% [1]. There are many causes for jaundice: hemolytic disease of the newborn due to ABO/Rh minor blood group incompatibilities, RBC enzyme deficiencies, such as glucose-6-phosphate dehydrogenase, pyruvate kinase or hexokinase, structural defects of RBCs, like hereditary spherocytosis or elliptocytosis, hemoglobinopathies, like  $\alpha$ -thalassemia, disorders of hepatic uptake, like Crigler-Najjar syndrome, disorders of enterohepatic circulation, such as breastfeeding jaundice, breast milk jaundice, and metabolic causes, like hypothyroidism or hypopituitarism, and infections. The risk for kernicterus is about 1 in 16 when TSB  $>25$  mg/dL and 1 in 5.5 when TSB  $>30$  mg/dL. Elevated TSB from any cause, including breast milk jaundice, can cause kernicterus. Many infants with kernicterus do not have hemolytic disease of the newborn. The best way to assess jaundice in newborns is to get a TSB or transcutaneous bilirubin (TcB) assessment. Plotting TSB or TcB values in the nomogram helps with follow-up management of jaundice [2]. The most important way to monitor for the development of pathological jaundice is to measure the rate of rise of bilirubin, rather than relying on a single measurement. A rate of rise of bilirubin  $>5$  mg/dL per day is abnormal. Inexpensive and simple devices to measure TSB and effective treatment of severe jaundice are essential to reduce acute bilirubin encephalopathy, and improve outcomes for thousands of neonates around the world [1]. **Phototherapy (Light Therapy):** Major reasons to treat pathological jaundice are to avoid acute and chronic bilirubin-induced encephalopathy. Even moderate hyperbilirubinemia has been shown to be associated with adverse neurodevelopmental outcomes. Lack of concern regarding jaundice, delay in measuring bilirubin despite marked jaundice and not initiating phototherapy are the root causes for the re-emergence of kernicterus. Guidelines for initiating phototherapy recommend using high-intensity phototherapy [3]. In resource-limited settings, phototherapy with filtered sunlight in a canopy made of air blue-80 film or Gila titanium film has been shown to be very effective

[4]. Recent retrospective studies have raised some concerns about phototherapy and risk of infantile cancer. In a retrospective study of 5,144,849 neonates born at  $\geq 35$  weeks' gestational age, cancer in the first year was 32.6/100,000 among phototherapy-exposed newborns compared to 21/100,000 among infants unexposed to phototherapy (relative risk 1.6, 95% CI 1.2–2.0,  $p = 0.002$ ) [5]. Number needed to harm (NNH) was 10,638, and NNH in neonates with Down syndrome was 1,285. The study authors concluded that phototherapy might slightly increase the risk of cancer in infancy, although the absolute risk increase is small. This risk should be considered when making decisions regarding phototherapy, especially for infants with bilirubin levels below treatment guidelines [5]. Most of the guidelines published regarding phototherapy are for infants  $\geq 35$  weeks' gestational age [2]. Kernicterus has been reported with bilirubin levels  $<10$  mg/dL among extremely low-birth-weight infants [6]. Maisels et al. [7] have suggested guidelines for phototherapy and exchange transfusion in infants born at  $<35$  weeks' gestational age. In summary, bilirubin is neurotoxic and almost all cases of kernicterus are preventable. Phototherapy is very effective in decreasing the risk for kernicterus; however, concerns regarding light therapy and potential association with cancer need to be considered when using light therapy. Exchange transfusion is used more frequently in low- and middle-income countries as compared to high-income countries, mostly due to lack of guidelines to monitor for severe hyperbilirubinemia and initiating phototherapy at appropriate TSB levels. A tool incorporating bilirubin nomogram, assessment for the presence of neurotoxicity risk factors and clinical signs of acute bilirubin encephalopathy, and providing effective phototherapy will decrease unnecessary exchange transfusion in low- and middle-income countries [8].

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## Acute Interventions in Life-Threatening Metabolic Diseases of the Newborn

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Early recognition and treatment of metabolic emergencies depends on a suspicion of such a disorder with a strategy that combines diagnostic studies with immediate intervention. Intake of potentially toxic dietary precursors is interrupted by stopping all oral intake and providing intravenous glucose. The classic presentation of inborn errors of metabolism is after uneventful pregnancy and delivery with a period of apparent health for hours or days, but then followed by overwhelming life-threatening disease [1]. Metabolic decompensation is most common in the immediate postnatal period, often complicated by acute infection and its attendant catabolism. Initial laboratory evaluation needs only the routine values to establish acidosis or alkalosis, hyperammonemia, ketosis, hypoglycemia, or lactic acidemia, and to detect acute organ dysfunction (e.g., acute renal failure, acute liver failure, cardiomyopathy, rhabdomyolysis, or pancreatitis). The ultimate success is dependent on rapid and appropriate intervention and specific diagnostics. Already hours of delay may lead to irreversible neurological damage or death. Fortunately, there is only a limited repertoire of pathophysiological sequences in the response of an infant to metabolic decompensation and, consequently, a limited number of therapeutic measures to be taken. Three major groups of disorders at risk for acute metabolic decompensation that require specific therapeutic approaches in emergency situations were outlined by Prietsch et al. [2]:

- Disorders with reduced fasting tolerance
- Disorders of cerebral/neurotransmitter metabolism
- Disorders of intermediary metabolism that cause acute intoxication through the accumulation of toxic molecules

A preliminary differentiation of these 3 groups is possible with the help of basic investigations, available in every hospital setting, namely the determination of acid-base balance, glucose, lactate, ammonia, and ketones. With this information, appropriate therapy can be initiated even before a precise diagnosis is known. Measures must be quick, precise and not halfhearted, and every effort must be undertaken to obtain the definitive diagnostic information within 24 h or sooner, i.e., results of acylcarnitines in dried blood spots or plasma, amino acids in plasma, and organic acids in urine. Primary genetic investigations, e.g. "neonatal disease panels," are far too slow. Disorders with reduced fasting tolerance, such as defects of fatty acid oxidation and gluconeogenesis, require vigorous administration of glucose in amounts sufficient to restore and maintain euglycemia. Comatose patients without hyperammonemia or acidosis most commonly have nonketotic hyperglycinemia. The identical presentation can be seen in babies suffering from the well-treatable disorders of pyridoxine metabolism or methylene tetrahydrofolate reductase deficiency. If intractable seizures dominate the clinical picture, a therapeutic trial of pyridoxine and pyridoxal phosphate is pursued and, if negative, followed by the administration of folinic acid. In diseases, in which symptoms develop because of "acute intoxication," rapid reduction of toxic molecules is essential. In disorders of amino acid catabolism,

such as maple syrup urine disease, classical organic acidurias, or urea cycle defects, the toxic compounds may be derived from exogenous as well as endogenous sources. In addition to stopping the intake of natural protein, but for no longer than 12–48 h, turnaround of catabolism by promotion of anabolism reverses the breakdown of endogenous protein. Large amounts of energy are needed to achieve anabolism, e.g., in neonates >100 kcal/kg body weight/day. In a sick baby, this can only be accomplished by hyperosmolar infusions of glucose together with fat through a central venous line. Insulin should be started early, especially in the presence of significant ketosis or in maple syrup urine disease, to enhance anabolism. The administration of lipids intravenously can often be increased to 3 g/kg, if serum levels of triglycerides are monitored. The pharmacological approach to the detoxification of ammonia in urea cycle defects, and also in those organic acidurias that present with hyperammonemia, is the provision of alternative methods of waste nitrogen excretion. Sodium benzoate is effectively conjugated with glycine to form hippurate, which is then excreted in the urine. Similarly, sodium phenylacetate is conjugated with glutamine to form phenylacetylglutamine, which is efficiently excreted. In a metabolic emergency with an as yet unknown diagnosis and a documented hyperammonemia  $\geq 200$   $\mu\text{mol/L}$ , benzoate and phenylacetate should be given intravenously, along with arginine. The ultimate goal of therapy is to prevent irreversible damage to the patient's brain. The diseases leading to acute intoxication carry the greatest risk of major sequelae. Additional supportive therapeutic measures are used informally in some centers to enhance this goal. These include mannitol for the treatment of cerebral edema, which may enhance detoxification through increased diuresis. Increased intracranial pressure may be monitored neurosurgically. New trends include the use of hypothermia in neonatal hyperammonemia due to organic acidurias or urea cycle disorders [3]. Mild systemic hypothermia is used with the rationale of lowering the ammonia production. Overall supportive care is critical in patients in intensive care units, with special vigilance for the detection and prompt treatment of infection.

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