Antenatal inflammatory insults and preterm brain injury

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Over the past decades, improved perinatal health care has led to more infants that survive preterm birth and consequently a shift in survival to earlier gestational ages [1]. However, the chance of event-free survival is still low in extremely preterm babies. Nowadays, around 35% of preterm infants survive with neurodevelopmental impairments such as cognitive deficits and attention deficit disorder which rises up to 50-60% in extremely preterm babies (<27 weeks of gestation), and around 10% develop motoric spasticity (CP) [2]. This results in a prevalence of CP around two per 1000 live births in developed and in developing countries [3, 4]. Assuming that in the Netherlands 180.000 children are born annually, this accounts for 360 babies born with CP every year. EoP does not only affect the patient but has an enormous impact on its families and society [5, 6]. Substantially increased lifetime costs are therefore attributed to EoP. Annually, in the Netherlands the costs for all CP patients comprises € 737,5 million Euros (0,10 % of BNP) [4].

In this thesis, I addressed the time dependent changes in the preterm brain in the course of perinatal stress and identified (cell-based, cell-derived and anti-infective) therapies. For this purpose, we used a translational ovine model of preterm brain injury caused by either HI or intrauterine infection. The long gestational period of sheep enables us to study antenatal developmental processes in detail and to administer our therapies at specific time-points in during this development. Moreover, ovine brain development in utero and the cerebral disease progression following perinatal inflammatory insults (global HI and chorioamnionitis) has essential overlap with man [7].

The data presented in this thesis **(chapter 2-6)** support and extend the concept that EoP has a multifactorial and complex origin, indicating that every newborn child has its own "fingerprint" of risk factors potentially contributing to EoP development. More precisely, the timing of the peripheral and cerebral inflammation and its pathophysiological cerebral

changes were shown to be different following a sterile (HI) insult when compared to an infectious trigger **(chapter 3 & 6)**. Accordingly, we show that defined windows of opportunity emerge following exposure to multiple perinatal inflammatory triggers. These important findings highlight that a potential mismatch might be present between the optimal timing of a treatment (in relation to the nature of the insult(s)) and the current clinical initiation of a treatment. This mismatch impedes development and translation of neurological therapeutics for preterm infants. Correct timing of treatment initiation in relation to the distinct nature and stage of injury is of great clinical importance in the near future. Translation of our findings into the clinical setting demands diagnostic tools for the recognition of these perinatal risk factors in a safe and non-invasive manner. Our group is currently working on analyzing volatile organic compounds in breath condensate of pregnant women to determine the presence and/or predict the onset of intra-amniotic infections.

In addition, we performed diffusion MRI sequencing in our study to further improve the clinical use of DTI as a biomarker of individual neurodevelopment and therapeutic effect in the future. Correlation of such new crucial imaging biomarkers with microstructural histological changes will improve clinical application and subsequently result in an individualized treatment approach **(chapter 5).** Such personalized medicine in which tailor made treatments will be adjusted to individuals need is nowadays focus of research in every facet of health care, from prevention to terminal care. The overall aim is to develop new methodologies and technological applications that make it easier to predict outcomes in individual cases, align treatment with those predictions, and administer reliable tests to determine the success of that treatment. Closer cooperation and back-to-back analysis between translational/basic science and clinical data will help to achieve this aim.

Our data **(chapter 4)** suggests that eradication of Candida Albicans with anti-fungal treatment is not sufficient to prevent brain injury. Our work in **chapter 3** supports other reports that describe that cerebral inflammation that is initiated upon exposure with an infectious trigger continuous (postnatally) and might even persist into adulthood. Therefore, future treatment protocols should combine multiple therapeutics that harbor anti-microbial, immunomodulatory and regenerative capacities to protect

against neurodevelopmental disorders in later life. Potential candidates that have proven to possess anti-inflammatory or immunomodulatory capacities are erythropoietin, cell-based therapies including MSCs/MAPCs **(chapter 3 & 5)** and AnnexinA1 **(chapter 6)**. In **chapter 6**, we investigate the potential therapeutic role of AnxA1 in hypoxic-ischemic encephalopathy (HIE). We are the first group to demonstrate a strong potential for AnxA1 in the treatment of HI-induced brain injury. The intellectual property has been patented which offers new scientific and economic opportunities. Future research should focus on route, dose and timing of AnxA1 and elucidate whether AnxA1 can act synergistically with other therapies including cell-based therapies like MAPCs (Multistem®) that have been successfully tested **(chapter 5)**. The potential for MultiStem® to treat ischemic stroke is currently being evaluated in a registered trial in Japan and in a pivotal Phase 3 clinical trial conducted in North America and Europe (clinicaltrial.gov). In addition, this program received the Fast Track designation, as well as the Regenerative Medicine Advanced therapy designation from the FDA (fda.gov). These designations are designed to accelerate the development, regulatory review and subsequent commercialization of product candidates like MultiStem® cell therapy for ischemic stroke if the clinical evaluation demonstrates appropriate safety and therapeutic effectiveness. As a subsequent step, MAPCs can be evaluated as therapeutic intervention in neonatal brain injury. In conclusion, the findings in this thesis are on short term primary of value to other researchers, but in long term could be translated into commercially available products and/or services to improve the outcome of babies at risk for EoP.

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