99

ANTI MULLERIAN HORMONE (AMH/MIS) ELISA: A DIAGNOSTIC TOOL IN NEONATOLOGY AND PAEDIATRY

J Guibaudenche¹, N Lucidarme², M Noël¹, A Artus³, D Porquet¹ ¹Hôpital Robert Debré, Biochemistry, Paris, France; ²Hôpital Robert Debré, endocrinology, Paris, France; ³Beckmann Coulter, Immunotech, Marseille, France

Background: Dimorphic expression of the Anti-Müllerian hormone (AMH), also called Müllerian inhibiting substance (MIS), in ovary and testis is crucial for normal differentiation of reproductive structures. Its absence in the female embryo allows Müllerian ducts to form the uterus, oviducts and upper vagina. AMH assay in girls is restricted to granulosa cancer and evaluation of ovarian follicular status. In males, AMH is produced by the testes reflecting Sertoli cell maturation. AMH induces Müllerian duct regression and is involved in testicular differentiation and function. Clinical applications of AMH assay include external abnormal genitalia, precocious puberty and hypogonadotropic hypogonadism. However, AMH measurement belongs to specialised laboratories which performed home made assays. Recently, Immunotech® has developed an assay available in all clinical laboratories. We established usual AMH values for this assay and we improved its interest.

Methods: AMH measurement required only 25 µl of plasma and 3 hours (detection range: 0–200 ng/ml; sensitivity: 0.3 ng/ml; within-assay and between-assays coefficients of variation: below 6% and 9% respectively). Usual values were established on blood from, 48 eutrophic fetuses (18 to 37 weeks of gestation), 101 full-term healthy newborns, and 425 healthy infants aged from one day to 10 years. 88 children with external gonadal abnormalities (14 clitoral hypertrophy; 50 micropallus; 43 hypospadias; 25 cryptorchidism; 9 non palpable testis) were evaluated. Clinical overlap (ambiguous genitalia) was observed in 42 cases.

Results: In female, AMH was undetectable before birth and then weakly produced (< 6 ng/ml). In males, AMH increases from fetal life, picks up between 1 and 12 months of life (99.7 ng/ml) with wide interindividual variations and then decrease with no overlap with female values. In clitoral hypertrophy, isolated micropallus and isolated hypospadias, AMH concentrations were within the usual range in 90% of cases. AMH was decreased in 73% of isolated cryptorchidism cases and was undetectable in anorchia. In ambiguous genitalia, AMH concentrations were increased, decreased or normal.

Conclusions: At birth, AMH levels seems useful when investigating ambiguous genitalia antenatally suspected. In children with isolated micropallus or hypospadias, normal AMH values exclude testis dysfunction. A single AMH measurement distinguishes between anorchia and cryptorchidism when testis palpation is abnormal. In ambiguous genitalia, AMH measurement must be combined with other investigations.

100

EFFECTS OF ANTENATAL MAGNESIUM SULFATE ADMINISTRATION ON NEUTROPHIL ACTIVATION IN THE UMBILICAL CORD BLOOD OF PRETERM NEWBORNS

E M A Gulkzynska¹, M Banasik², A Zjawiona¹, B Cyranowicz², J Gadzinowski¹ ¹Research Institute of Polish Mother's Memorial Hospital, Department of Neonatology, Lodz, Poland; ²Research Institute of Polish Mother's Memorial Hospital, Department of Clinical Immunology, Lodz, Poland

Background: The results of epidemiological studies concerning neurological outcome of preterm newborns revealed beneficial effects of maternal magnesium sulphate administration on reduction of intracranial hemorrhage and periventricular leukomalacia and consequently the incidence of cerebral palsy. One hypothesis for this fact is modulation of inflammatory response by elevated magnesium (Mg²⁺) concentration.

Aim: The purpose of this study was to investigate alterations in the expression of adhesion molecules CD11b, CD11b, CD16 on neutrophils in very low birth weight babies prenatally exposed on magnesium sulfate in comparison to the control group. **Material and methods:** The prospective analysis involved 29 newborns with birth weight ≤1500g delivered at Research Institute of Polish Mother's Memorial Hospital in 2003. 9 neonates were born from mothers receiving magnesium sulfate before delivery, remaining 20 preterms consisted control group without magnesium therapy. The cord blood samples were collected from all patients immediately after birth to evaluate magnesium level and to identify integrins. The expression of adhesive particles (CD11b, CD11b, CD16) – was assumed with flow-cytometer (Becton-Dickinson Co) and monoclonal antibodies. The number of cells with molecular expression and mean intensity of fluorescence given in arbitrary units (MFI) was estimated.

Results: The mean magnesium level (Mg²⁺) in study and control group was respectively 1.4 vs 0.79 mmol/l. The number of neutrophils with CD11b, CD11b, CD16 expression was increased in the magnesium sulfate treated group (95.6 vs 88.3; 93.9 vs 85; 87.9 vs 80.2). The CD11b and CD11b values were statistically significant (p<0.05), whereas the difference in CD16 number almost reached statistical significance (p=0.07). The essential variations of mean fluorescent intensity (MFI) were not observed. This difference cannot be explained by fetal acidosis (pH 7.2 vs 7.22 in the control group).

Conclusions: In the group of preemies with high blood magnesium ions (Mg²⁺) concentration due to magnesium sulfate administration to pre-eclamptic women, the significant increase in expression of adhesion molecules CD11b, CD11b, CD16 on neutrophils was observed. Supported by the grants No. 5P05E 09224 from the State Committee for Scientific Research, Poland.

101

WATER PERMEABILITY IN ASTROCYTES AND THE EFFECT OF LEAD INTOXICATION

E Gunnarsson¹, G Baturina², S Zelenin¹, M Zelenina¹, A Aperia¹ ¹Karolinska Institutet, Department of Woman and Child Health, Pediatric Unit, Stockholm, Sweden; ²Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Laboratory of Physiological Genetics, Novosibirsk, Russian Federation

Background: The water channel aquaporin 4 (AQP4) is abundantly expressed in astrocytes. There is now compelling evidence that AQP4 may contribute to an unfavorable course in brain edema. Acute lead intoxication is a condition that causes brain damage preceded by brain edema. Here we have investigated the effect of lead on AQP4 water permeability in astrocytes and AQP4 mRNA expression in brain.

Methods: An astrocyte cell line that did not express AQP4 was transiently transfected with AQP4 tagged with green fluorescent protein (GFP). Using confocal laser scanning microscopy we measured water permeability (P_w) in these cells and in AQP4-negative cells located on the same plate. P_w in astroglial cells in primary culture was also measured after lead exposure. The effect of lead on AQP4 mRNA distribution in the brain was studied by *in situ* hybridization.

Results: AQP4-expressing astrocytes had more than 3-fold higher water permeability than astrocytes not expressing AQP4. Lead exposure induced a significant, 30%, increase in P_w in astrocytes expressing AQP4, but had no effect on P_w in astrocytes not expressing AQP4. The increase in water permeability persisted after lead washout. Lead exposure also increased P_w in astroglial cells in primary culture, which express endogenous AQP4. Lead had no effect on P_w in astrocytes transfected with another aquaporin, AQP3. *In situ* hybridization studies on adult rat brain showed no change in distribution of AQP4 mRNA after oral lead intake for three days.

Conclusion: We here show that lead specifically increases water permeability in astrocytes expressing the water channel AQP4. Lead is so far the only heavy metal shown to modulate the effect of AQP4. It is suggested that lead-triggered stimulation of water transport in AQP4-expressing astrocytes may be a contributing factor to brain edema in acute lead intoxication.

102

POST-HAEMORRHAGIC HYDROCEPHALUS (PHH) WITH VENTRICULO-PERITONEAL SHUNT (VP) IN INFANTS < 30 WEEKS: A POPULATION STUDY

R Hagan¹, N P French¹, S Sinhal² ¹University of Western Australia, School of Women's and Infants' Health, Perth, Australia; ²Women's & Children's Health Service, Neonatal Paediatrics, Perth, Australia

Background: Population based trends in infantile hydrocephalus in preterm infants have been suggested as a useful clinical indicator.

Aims: To describe the incidence, clinical features, outcome and trends over time of infants with PHH and a shunt in Western Australia from 1986–2003 inclusive. **Design:** Population-based retrospective cohort study.

Methods: Cases were identified from three independent databases; the two WA tertiary neonatal units; the neonatal follow-up program. Total livebirths 23–29 weeks were obtained from the Midwives Notification System for all births in WA, cerebral palsy status was confirmed with the statewide Cerebral Palsy register. Outcome data were obtained from the follow-up program and additional data extracted from the case notes. Data were analysed in three 6 year time periods; 1986–1991; 1992–1997; 1998–2003.

Results: Eighteen cases were identified in 2,970 livebirths who were <30w at birth (6.1/1000 livebirths <30w) with a median (iqr) gestation of 26 (24,27) weeks and birthweight 785 (685,870) g. There was an increase in the prevalence over time (table). There were 6 early infected shunts in 4 children. Five children had ventricular taps or reservoirs pre shunt. Disabilities were common with 10 children (56%) having cerebral palsy; eight children (44%) had more than one disability; 4 (22%) were severely disabled. Six children (33%) had no disability. All 5 children with ventricular taps or reservoirs had CP as did 2 of the 4 who had early infected shunts. The median (iqr) GQ/DQ of the 12/16 (75%) of eligible children tested were 86(74,92).

	1986–1991	1992–1997	1998–2003	Total
Livebirths<30w	961	1039	970	2970
survival (%)	69.7	81.8	83.4	78.4
VP shunt (n)	1	8	9	18
VP rate/1000lb	1.0	7.7	9.3	6.1
rate/1000 surv	1.5	9.4	11.1	7.7
Disabilities				
CP (n)	0	5	5	10
seizures	0	3	2	5
visual/hearing	0	5	3	8

Conclusion: PHH requiring VP shunting is uncommon in this geographical population of infants of 23–29 weeks gestational age. This would not be a useful clinical indicator of neonatal care in this highly centralised perinatal care system.

103

ADDITIVE PROTECTION OF THE NMDA-RECEPTOR BLOCKER MK-801 AND PAN-CASPASE INHIBITORS IN BILIRUBIN-INDUCED APOPTOSIS IN HUMAN NT2-N NEURONS

E Hanks¹, T W R Hansen², R Almaas³, J K Lindstad¹, T Rootwelt¹ ¹Rikshospitalet, Department of Pediatric Research, Oslo, Norway; ²Rikshospitalet, Department of Pediatrics, Oslo, Norway; ³Sykehuset Oestfold i Fredrikstad, Department of Pediatrics, Fredrikstad, Norway

Background: We recently showed that unconjugated bilirubin (UCB) in low and moderate doses (≤ 10 µM) induced cell death predominantly as apoptosis in cultured human NT2-N neurons. The execution of apoptotic cell death often requires the action of cysteine proteases, known as caspases. In this study we investigated the protective properties of the NMDA-receptor blocker MK-801 and two caspase-inhibitors in UCB-induced apoptosis.

Methods: Pretreatment was given with either MK-801 (10 µM) alone, the caspase-3 inhibitor DEVD-FMK (20 and 100 µM), the pancaspase inhibitor zVAD-FMK (20 and 100 µM), or the combination of MK-801 and DEVD-FMK (20 µM) or zVAD-FMK (100 µM). Thereafter the cell cultures were exposed to UCB (2–25 µM) and bovine serum albumin at a 1.5 molar ratio or 2 µM staurosporine (positive apoptosis control) for up to 48 h. MTT reduction, LDH release, classification of nuclear appearance after staining with Hoechst 33342 and ethidium homodimer, detection of caspase-3 activity, and DNA-electrophoresis were used to evaluate cell death.

Results: Both caspase inhibitors dose-dependently attenuated apoptosis in staurosporine-treated neurons. 5 µM UCB, but not 25 µM UCB, induced modest caspase-3 activation, however, caspase-3 inhibition did not attenuate apoptosis. The general caspase inhibitor zVAD-FMK (20 and 100 µM) almost completely abolished nuclear fragmentation and DNA-laddering as shown with DNA-electrophoresis. However, protection against UCB-induced cell death was only achieved with 100 µM zVAD-FMK in neurons treated with 2 µM UCB. MK-801 almost completely abolished nuclear condensation and attenuated cell death as measured with nuclear morphology and MTT reduction. Combined with MK-801, pancaspase inhibition caused additive protection against apoptosis in neurons treated with 5 and 25 µM UCB as measured with all apoptotic markers.

Conclusion: While caspase inhibition effectively attenuated staurosporine-induced apoptosis in human NT2-N neurons, the anti-apoptotic effect was limited in UCB-induced apoptosis. Caspase-3 inhibition did not protect from UCB-induced apoptosis in human NT2-N neurons. Pancaspase inhibition protected from apoptotic morphology in neurons treated with low UCB concentrations (2 µM), and caused additive protection in combination with MK-801 in neurons treated with moderate UCB concentrations (5 and 25 µM).

104

DOES COMMON VARIATION IN THE CYCLOOXYGENASE-2 GENE AFFECT NEUROCOGNITIVE OUTCOME AFTER PREMATURE BIRTH?

D Harding¹, H E Montgomery², S E Humphries², N Marlow³, A Whitelaw⁴ ¹St. Michael's Hospital, Peter Dunn NICU, Bristol, United Kingdom; ²University College London, Cardiovascular genetics, London, United Kingdom; ³University of Nottingham, School for Human Development, Nottingham, United Kingdom; ⁴University of Bristol, Neonatal Medicine, Bristol, United Kingdom

Introduction: Development of the preterm brain is adversely affected by inflammation. Depending upon the organ, the insult and the temporal relationship between injury and assay cyclooxygenase (COX; prostaglandin H synthase) may have both anti-inflammatory and pro-inflammatory actions. The gene encoding the inducible form of cyclooxygenase, COX-2, is polymorphic for a G/C substitution within the promoter region of the gene (at position -765). In vitro the G allele increases COX-2 activity relative to the C allele.

Aim: To determine if the COX-2 -765 G/C polymorphism influences neuro-developmental progress after preterm birth. **Patients/Methods:** 209 surviving Caucasian preterm infants of median gestation 30 weeks (range 22–32 weeks) had COX-2 genotype determined. Neuro-cognitive outcomes were: any disability and Griffiths Developmental Quotient (DQ) at 2 yrs and General Cognitive ability (GCA) at 5 ½ yrs (British Ability Scales-11).

Results: Genotypes were: GG 139 (66%), GC (32%), CC (1%). When comparing COX-2 G allele (GG+GC) with COX-2 CC genotype, there were no associations with neuro-developmental outcomes. However, COX-2 C allele (GG+GC) was associated with worse DQ and GCA when compared to COX-2 GG genotype; COX-2 C allele (GC+CC) was not associated with disability.

	COX-2 C allele	COX-2 GG	probability
Gestation (weeks)	31 (28–32)	31 (29–32)	NS
Severe IVH	3 (5%)	10 (8%)	NS
PVL/Ventriculomegaly	7 (10%)	16 (12%)	NS
Any Disability at 2 years	10 (15%)	22 (16%)	NS
DQ (n=182)	92.1(1.7)	97.2(1.6)	0.042
GCA (n=142)	94.3(2.2)	100.7(1.7)	0.033
Verbal subscale	93.8(3.3)	103.7(1.5)	0.002
Non-verbal subscale	95.4(2.4)	99.7(1.7)	0.16
Spatial subscale	93.5(2.3)	98.9(1.8)	0.08

Pearson Chi Square, Mann Whitney U or Student T Test: n (%), median (interquartile range), mean (SE) as appropriate. NS is not significant. There were no differences in family/social variables across genotypes.

Conclusion: The presence of a C allele at position -765 of the COX-2 gene, encoding relatively low COX-2 activity in vitro, is associated with worse neuro-cognitive performance at 2 and 5 ½ years of age in children born at <33 weeks gestation. The use of COX-2 inhibitors as anti-inflammatory agents in the preterm may be deleterious.