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ETIOLOGY AND TREATMENT OF PEDIATRIC PLEURAL EMPYEMA.

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Parapneumonic effusions (PE) complicate pediatric community-acquired pneumonia (CAP) in 40% of cases. It is estimated that empyema forms in more than half of such cases but evidence-based data regarding appropriate treatment is limited. This study evaluates treatment modalities as they relate to duration of hospitalization and cost of care in such cases. A quality improvement based retrospective chart review was undertaken to evaluate outcomes for empyema that were diagnosed between 12/00-3/04 at Children's Mercy Hospital in Kansas City, MO. Identification of empyema was based on strict criteria that was independently confirmed by 2 evaluating physicians. Cases were included if ultrasound and/or CT showed pleural fluid loculation and septation OR pleural fluid was grossly purulent OR bacteria was identified on pleural fluid culture. Data abstracted included demographic data, radiographic imaging studies, pleural fluid analysis, treatment modality, length of hospital stay (LOS), and cost of care. 96 cases of CAP with PE were reviewed; 57 cases were classified as pneumonia, uncomplicated PE, 5 cases as necrotizing pneumonia/abscess but w/o empyema and 34 cases of empyema. Children with empyema ranged in age from 17 months-16 years (mean 5 years) and 20 were boys (59%). In 27 empyema cases, pleural fluid evaluation was performed. Empyema patients more often had neutrophil >90% and glucose <20 mg/dl. Those with LDH >10,000 were more likely to be bacteriologically confirmed (7/11). Bacteriologic diagnosis was confirmed in 38% of cases; gram-positive pathogens predominated and *S. pneumoniae* was most commonly identified. Ten cases occurred annually and *S. pneumoniae* remained consistent despite widespread implementation of PCV in our community. Treatment included 4 modalities: Group 1 (n=2): antibiotics (A) only; Group 2 (n=14): A + thoracostomy tube; Group 3 (n=10): A + tube + alteplase fibrinolysis; and Group 4a/b (n=3/5): VATS (early ≤7 d sxs; late >7 days sxs). Patients in Group 2 had the greatest failure rate and longest stay (11.5 days) as well as cost of care. Patients in Group 3 and 4a had the shortest LOS and cost of care (7 days and median cost \$21,062). Empyema was identified in 35% of cases of CAP with PE and the annual incidence remained stable over the last 3 years. Cases caused by *S. pneumoniae* persist despite PCV and may represent non vaccine strains (type 1 and 3). Among invasive interventions, tube thoracostomy alone had longer LOS and more failures. Early VATS and intrapleural fibrinolysis have shorter stays and cost. More evidence based investigation is necessary to confirm these results; utilization of a strict definition of empyema would facilitate such work.

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A COMPARISON OF AUTOMATED AND MANUAL LEUKOCYTE DIFFERENTIAL COUNTS AND THE DETECTION OF LYMPHOID BLAST CELLS FOR DIAGNOSIS AND TREATMENT DECISIONS IN PEDIATRIC ONCOLOGY.

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The absolute granulocyte count (AGC) is one of the most useful laboratory values used in pediatric oncology. AGCs can be derived from manual or automated leukocyte differential (LD) counts. We wondered whether a primarily automated system could reliably be used for making decisions regarding treatment in pediatric oncology and how this might affect diagnoses of acute leukemia in childhood. We retrospectively collected all pediatric oncology complete blood cell counts at our institution with matched automated and manual LD counts over a one-month period (n = 439) and conducted correlation analyses. Following an initial analysis of automated compared to manual AGCs, correlation analyses of three sub-groups based on treatment protocols were conducted: AGCs of <0.5 × 10⁹/L (n = 110); >0.5-1.0 × 10⁹/L (n = 55); and >1.0 × 10⁹/L (n = 274). In addition, we analyzed the sensitivity and specificity of the machine to detect blast cells. We subsequently collected all available matched automated and manual LD counts at the time of diagnosis of acute leukemia (ALL and AML) over a two-year period (n = 33) and analyzed the sensitivity and specificity of the machine to detect blast cells in the peripheral blood. There was a highly positive correlation between automated and manual AGCs (R² = 0.879). Sub-group analyses revealed that automated and manual AGCs of 0.00-0.5 × 10⁹/L were not correlated (R² = 0.565) and of >0.5-1.0 × 10⁹/L were not correlated (R² = 0.268). There was a strong positive correlation between automated and manual AGCs of >1.0 × 10⁹/L (R² = 0.826). The sensitivity of the machine to detect blast cells was 37% and the specificity was 74.3%. The sensitivity of the machine to detect blast cells at the time of diagnosis of ALL or AML was 41.4% and the specificity was 66.7%. Overall, automated AGCs are predictive of manual AGCs in pediatric oncology patients. Automated AGCs of <1.0 × 10⁹/L, however, are not satisfactory for decisions regarding treatment. Further, automated detection of blast cells is poor, even at the time of diagnosis of acute leukemia. Therefore, we recommend that both automated and manual LD counts be completed on pediatric oncology patients with automated AGCs of <1.0 × 10⁹/L. Automated counts for AGCs >1.0 × 10⁹/L are sufficient on their own, however, it is recommended that these samples be manually scanned by a technician for abnormal cellular morphology.

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COMPARISON OF MULTIDRUG RESISTANCE PROTEIN-1 (MRP-1) AND P-GLYCOPROTEIN (PGP) EXPRESSION IN THE DEVELOPING HUMAN CENTRAL NERVOUS SYSTEM: CELLULAR AND TISSUE LOCALIZATION.

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Background: MRP-1 and Pgp are multidrug efflux pumps that share substantial overlap in substrate specificity including their possible transport of unconjugated bilirubin. Although Pgp is reportedly expressed on endothelial cells and perivascular astrocytes of the blood-brain barrier (BBB) in human newborns, the pattern of MRP-1 expression in the CNS of human neonates has not been characterized. **Objective:** To test the hypothesis that MRP-1 is expressed in a regionally specific, developmentally modulated fashion in human CNS and compare the pattern of MRP-1 cellular and tissue localization with that of Pgp. **Design/Methods:** Paraffin embedded postmortem brain tissue sections from infants born at 23-42 weeks gestation were subjected to antigen retrieval in 10 mM sodium citrate at 97°C and immunostained for MRP-1 and Pgp using the monoclonal antibodies MRPr1 (Kamiya) and C219 (Signet) respectively. Immunostaining as a function of age, cell type and brain region was semiquantified. **Results:** MRP-1 was expressed in choroids plexus epithelium, ependymal cells of the lateral ventricles, oligo-dendroglial cells, neurons in selected brainstem nuclei, and large pyramidal cells of the cerebellum. MRP-1 immunostaining did not change between 23 and 42 weeks gestation. MRP-1 was not observed in capillary endothelial cells or astrocytes. In contrast, Pgp immunostaining was prominent in capillary endothelial cells and perivascular astrocytes of the BBB and observed in choroids plexus epithelium and large pyramidal cells of the cerebellum; Pgp immunostaining increased with gestational age from 23-42 weeks. **Conclusions:** We conclude that MRP-1 and Pgp are expressed in a regional and cell specific fashion in the human CNS. Pgp is primarily expressed in endothelial cells and perivascular astrocytes of the BBB; cells that do not express MRP-1. MRP-1 is expressed in choroids plexus epithelium and ependymal cells of the ventricles, elements of the blood-CSF barrier. Both Pgp and MRP-1 are expressed in selected parenchymal neurons most notably, large pyramidal cells of the cerebellum. We speculate that the complementary pattern of MRP-1 (blood-CSF barrier) and Pgp (BBB) expression may serve together to limit CNS bilirubin levels during neonatal hyperbilirubinemia. **Disclosure:** Funded by NINDS (038993), 25 Club of Magee-Womens Hospital, and Mario Lemieux Centers for Patient Care and Research.

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BILIRUBIN EFFLUX BY BRAIN CAPILLARY ENDOTHELIAL CELL MONOLAYERS IN VITRO: ROLE OF P-GLYCOPROTEIN.

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Background: The passage of bilirubin across the blood-brain barrier into the CNS is central to the development of kernicterus. Indirect in vivo evidence suggests that P-glycoprotein (Pgp), a multidrug transporter expressed on brain capillary endothelial cells, may limit the influx and CNS retention of unconjugated bilirubin (Pediatr Res 44:763-766, 1998). This phenomenon has not been studied in brain capillary endothelial cell monolayers *in vitro*. **Objective:** To test the hypothesis that Pgp mediates bilirubin transport across brain capillary endothelial cell monolayers *in vitro*. **Design/Methods:** Bovine brain capillary endothelial cells (Cell Systems Corp.) were grown in confluent monolayers at a density of 5 × 10⁴ cells/insert in 1 cm² Transwell dishes. [³H]-bilirubin (100nM) transport (pmol/cm²/min) was tested in both the apical to basolateral [A→B] and basolateral to apical [B→A] directions in the presence and absence of a Pgp inhibitor (cyclosporin A[5uM]). Involvement of a Pgp mediated efflux mechanism is suggested by a B→A/A→B ratio of greater than 1.5 (Pharm Res 16:1206, 1999). Brain capillary endothelial cell Pgp expression was confirmed by Western immunoblotting techniques. **Results:** Brain capillary endothelial cells express Pgp as seen on Western immunoblots. Bilirubin transport in the B→A direction (0.67±0.05 pmol/cm²/min) was 6.4 fold higher than the rate for A→B direction (0.11±0.02) suggesting active efflux of bilirubin across brain capillary endothelial cell monolayers. B→A bilirubin transport decreased (0.60±0.07) and A→B bilirubin transport was enhanced (0.17±0.03) in the presence of the Pgp inhibitor, with an overall decrease in bilirubin efflux of 27% suggesting that bilirubin transport by brain capillary endothelial cells is mediated in part by Pgp. **Conclusions:** We conclude that i) bilirubin is transported by brain capillary endothelial cell monolayers *in vitro* in a net B→A direction (i.e. active efflux); ii) Pgp plays an active role in this barrier function, and iii) unconjugated bilirubin is a substrate for Pgp. We speculate that i) brain capillary endothelial Pgp limits the CNS passage and retention of unconjugated bilirubin and ii) that low brain capillary endothelial cell Pgp expression, as reported in premature neonates, may enhance brain bilirubin levels during hyperbilirubinemia. **Disclosure:** Supported by NINDS (038993), the 25 Club of Magee-Womens Hospital and the Mario Lemieux Centers for Patient Care and Research.

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DEVELOPMENTAL IRON DEFICIENCY IMPAIRS RAT WATERMAZE PERFORMANCE.

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Background: Iron deficiency (ID), a common nutritional disorder during development, has been associated with cognitive and behavioral impairments. Previous studies suggest developmental ID in rats affects hippocampal dendritic morphology and alters performance in the Morris Water Maze (MWM). **Objective:** To explore the nature of MWM deficits in rats that had ID during development. **Design/Methods:** 8-week old dams were randomized to iron sufficient (IS) or iron deficient (ID) groups. IS or ID diets were given during gestation and lactation. All pups received the IS diet after postnatal day (P)20. At P35, 8 to 9 IS and ID group males began one of two MWM assessments. 1st: Place learning (standard MWM) and memory (platform removed at 24 hours - probe) were assessed. Rats continued with trials to enhance learning to search the target quadrant then had a second probe trial. 2nd: IS and ID rats had thigmotaxis training before standard MWM assessment and probe. Data (days to criteria, latency, quadrant preference and thigmotaxis) were analyzed using one-way and repeated measures ANOVA (RPM). **Results:** 1st: ID group rats had longer latencies on place learning (RPM p<0.001). Two ID rats reached criteria (ID+) but six did not (ID-). ID- rats had significantly more thigmotaxis in place learning trials than IS rats (RPM p<0.002) but ID+ and IS rats did not differ. In the probe, IS rats had greater quadrant preference (30.94 ± 9.03) than ID- rats (-19.62 ± 8.84) and less thigmotaxis. After additional trials, latency was similar for IS and all ID rats. However, IS vs ID- remained different on probe quadrant preference (25.49 ± 5.83 vs -20.46 ± 10.32, p<0.05). 2nd: After thigmotaxis training, IS rats still had shorter latencies than ID rats in the standard MWM (RPM, p<0.001) and greater quadrant preference than ID- rats on the probe (10.29 ± 11.31 vs -33.30 ± 0.01). **Conclusions:** Previously ID rats demonstrate poorer MWM performance that appears related to persistent thigmotaxis behavior. Although learning improved for ID rats with additional training, memory for platform location did not. Thigmotaxis training did not impair learning measures for IS rats. The results suggest that developmental ID is associated with poorer ability to leave thigmotaxis and switch-strategies in spatial learning and memory tasks.