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European Society for Developmental, Perinatal and Paediatric Pharmacology, 8th Congress

The 8th Congress was held in Liège, Belgium on October 25–28, 2002. It was the largest Congress of the Society. The president for the meeting was Jean-Paul Langhendries. The Congress covered research in the European Community, use of antibiotics, immunosuppressive agents in paediatric transplantation, aspects of pain, the effects of the environment on the developing fetus and infant and immune mechanisms in children.

There were 15 oral free communications (O), 11 mini oral presentations (M) and 65 poster presentations (P). These are shown below.

O1
Multicenter Ibuprofen Prophylaxis Study (Mips) In Preterm Infants

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Background: Indomethacin has been used to reduce both the incidence of PDA and intraventricular hemorrhage (IVH) in preterm infants. Ibuprofen may induce closure of patent ductus arteriosus (PDA) and has different effects on cerebral circulation. We aimed to evaluate the effect of ibuprofen prophylaxis on IVH and PDA in preterm infants.

Methods: In seven centers 358 preterm infants < 30 weeks of gestation were randomized to receive double-blinded either saline (1 – 0.5 – 0.5 ml/kg; n = 186) or ibuprofen-lysine (10 – 5 – 5 mg/kg; 10 mg/ml; n = 172) intravenously at 24 hour intervals with the first dose < 6 h after birth. Cerebral and cardiac ultrasound were performed before and after treatment. Perinatal characteristics and possible side effects were registered.

Results: IVH grade 3-4 occurred in 18/186 (10%) in the saline and in 17/172 (10%) in the ibuprofen-lysine group. Ductal closure on day 3 was significantly lower (108 /186 (58%) *vs* 140 /172 (81%), $P < 0.001$), urine production higher on day 1 (2.3 ± 1.4 *vs* 1.5 ± 1.2 ml/kg.h, $P < 0.001$) and serumcreatinine lower on day 3 (1.01 ± 0.22 *vs* 1.14 ± 0.29 mg/dl; $P < 0.001$) in the saline versus the ibuprofen-lysine group. There were no significant differences for antenatal medication, gestational age, Apgar scores, birthweight, respiratory evolution, occurrence of necrotizing enterocolitis and death.

Conclusions: Ibuprofen prophylaxis reduced significantly PDA and had no influence on the incidence of grade 3–4 IVH. Ibuprofen caused minimal renal effects without other side effects.

O2
Fluoroquinolones Safety in Paediatric Patients: A Prospective Multicenter Controlled Cohort Study in France

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Background: Fluoroquinolones (FQ) off-label use is widespread in paediatric patients despite their cartilage toxicity in juvenile animal models. Their safety in this population has never been evaluated in a large prospective controlled study.

Methods: A multicenter observational comparative cohort study was performed between 1998 and 2000 in French paediatric and cystic fibrosis (CF) wards. All consecutive paediatric patients receiving systemic FQ were included and matched to controls receiving other antibiotics. Potential adverse events (PAE) were prospectively collected in both groups up to day 15 after the end of the treatment. PAE had to be followed up until recovery. The rate of PAE in each group was compared using uni- and multi-variate analysis.

Results: Seventy three centers included 276 patients exposed to FQ and 249 controls. Among patients exposed to FQ, 23% were less than 2 years old and 33% had CF. In the FQ group, 52 PAE occurred, leading to stop 11 FQ courses. The odds-ratio for PAE in the FQ group was 3.7 (95% confidence interval: 1.9–7.5; $P < 0.0001$). No significant interaction with age or underlying condition (CF or not) was found and similar results were obtained after adjustment on these 2 potential confounding factors. Joint PAE were also more frequent in the FQ group (P =0.02). They occurred in 10 patients (including 2 with CF), all older than 6 years, receiving "usual" dose regimens of FQ. The main joints involved were knees. All joint PAE were of limited intensity and transient. Rechallenge was performed in three patients and was positive in 1.

Conclusion: The overall rate of PAE and the rate of joint PAE were more frequent with FQ than with other antibiotics. The rate of joint PAE was much higher than those reported in adult patients (0.01–0.2%). No serious joint PAE was observed. The status quo proposed by the American Academy of Paediatrics, justifying but restricting the off-label use of FQ in paediatric patients to a second line treatment in a limited number of situations, is supported by our findings.

O3
Surveillance for Fatal Suspected Adverse Drug Reactions in the UK

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Background: To determine the nature and number of suspected adverse drug reactions (ADRs) associated with fatal outcomes in children reported through the yellow card scheme.

Methods: All yellow card reports of suspected ADRs with a fatal outcome in children received by the Committee on Safety of Medicines over a 36 year period from 1964 until December 2000 were reviewed. Reports associated with vaccines and overdose were excluded. The medicine, date of the report, diagnosis, ADR and the age of the child were analysed. A formal causality assessment was not performed.

Results: There were 331 deaths with 390 suspected medicines reported for children aged 16 years or less where the outcome has been fatal since 1964. Medicines most frequently prescribed in the reports of fatalities were anticonvulsants (65 deaths), cytotoxics (34 deaths), antibiotics (29 deaths) and anaesthetic agents (30 deaths). The individual drug most frequently prescribed was sodium valproate (31 deaths). The nature of suspected ADRs associated with fatalities were diverse and hepatic failure was the most frequent.

*Conclusion*s: A wide range of suspected ADRs are associated with fatalities in children. Anticonvulsants were associated with the greatest number of reports of fatalities and hepatotoxicity in particular.

O4
A Concept for Early and Detailed Detection of Adverse Drug Reaction on Paediatric Wards: A Computerized Monitoring System in Process of Development

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Background: Adverse drug reactions (ADRs) are common in paediatric patients but not well documented, particular in spontaneous reporting systems. Manual detection systems are more sensitive but very expensive. Computerized monitoring systems (CMSs) appear promising in adult patients but have not yet been evaluated in paediatric patients. We tested the practicability of a CMS before implementing costly adaptions, and secondly adapted CMS in parallel to manual chart review.

Methods: In a pilot project a 8 months prospective study was conducted on a paediatric ward. Charts were reviewed once weekly by a pharmaco-epidemiological team. Clinical signs as well as laboratory changes were documented. Algorithms were used to assess the probability and severity of each detected event. In a second trial a similar experimental approach was used over a period of 6 months, in parallel with CMS that automatically generating signals caused by defined laboratory parameters. The results of the two methods were compared.

Results: In the pilot project 214 patients were enrolled. A total of 68 ADRs were detected in 46 of 214 patients by the pharmacoepidemiological team. 34 ADRs (50%) were detected by the staff physician and 27 (40%) primarily by analyzing laboratory parameters. The overlap was only three ADRs between these two groups. 510 patients were enrolled in the second study. 42 ADRs were detected by the manual reporting system. Of 16 ADRs based on laboratory findings, all 16 ADRs were also detected as a signal by the CMS.

Conclusion: The number of ADRs varied between the two studies because of the different patient populations studied. The pilot study showed that the rate of ADRs would be almost doubled by CMS analyzing laboratory data. In the follow up project a high sensitivity rate was achieved tolerating a low specifity. In a second step the data have to be reviewed again to increase the specifity by modifying the algorithm of the CMS. CMSs appear promising in detecting ADRs in children.

O5
Pharmacokinetic and Pharmacodynamic Analysis of Unlicensed and Off-Label Drug Use in Paediatric Patients

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Background: There is a poor evidence base for the selection of drugs and dosages in paediatric patients and indeed a series of studies have shown that approximately half of the drugs used in children within the hospital setting are either unlicensed or off-label. The aim of the present research programme is to use sparse data analysis methodology to gather pharmacokinetic and pharmacodynamic data for unlicensed and off-label drug use in children, thereby overcoming the ethical issues associated with traditional dose ranging studies in children.

Methods: The research programme has received ethical approval and is ongoing in two centres (Belfast and Liverpool). Having obtained written informed consent, blood samples are collected from children (usually at a time when blood is being drawn for another purpose *e.g.* electrolyte analysis) who have been prescribed drugs outside their license in the study site hospitals. At the time of sample collection the child is examined by a trained nurse to evaluate beneficial and unwanted drug effects. The blood samples are assayed for drug content using custom designed HPLC microanalyses. The drugs under investigation are: ranitidine, cisapride, diclofenac, enalapril, codeine, paracetamol, spironolactone, midazolam and omeprazole which are unlicensed in the indications or specific age groups of children under investigation. Data collected are subjected to sparse data analysis to allow pharmacokinetic/pharmacodynamic profiles of the drugs to be developed.

Results: To date 831 blood samples have been collected from a total of 387 infants/children. A total of 160 samples per drug are required to allow generalisable results to be obtained. This number has been reached for two drugs *i.e.* diclofenac and ranitidine. Preliminary results for ranitidine, for example, have indicated a bioavailability of 23%, a clearance of 0.68L/h/kg, a Vd of 3.31 l/kg and a half-life of 3.4 hours. Both clearance and Vd were linearly related to child weight.

*Conclusion*s: The methodology described offers an ethical alternative to traditional pharmacokinetic dose ranging studies and can produce much needed information on safety and efficacy profiles for unlicensed and off-label drug use in children.

Acknowledgement: Action Research (*UK*) funded the present study.

O6
Glutathione, Glutathione-Dependent Enzymes and Antioxidant Status in Erythrocytes of Children Treated with High Dose Paracetamol

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Paracetamol is the most frequently used over the counter medication in children. Repeated doses of paracetamol given for therapeutic reasons have been reported to cause hepatotoxicity in adults and children.

Aim: To investigate glutathione and antioxidant status changes in the erythrocytes of febrile children receiving repeated supratherapeutic paracetamol doses.

Methods: 51 children aged from 2 months to 10 years participated in the study. Three groups of children were studied: Group 1 (n=24) included afebrile children who did not receive paracetamol. Groups 2 (n=13) and 3 (n=14) included children who had fever above 38.5°C for more than 72 hours. Patients in group 2 received paracetamol 50±15 (30–75) mg/kg/day and those in group 3 received paracetamol above the recommended therapeutic dose –107±28 (80–180) mg/kg/day. A blood sample was taken for liver transaminases, gammaglutamil transferase (GGT), reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione S-transferase (GST), superoxide dismutase (SOD) and antioxidant status.

Results: AST level in group 3 was higher than in the other groups (P=0.027). GSH, SOD and antioxidant status were significantly lower in group 3 as compared with groups 1 and 2. GR activity was significantly lower in groups 3 and 2 in comparison with group 1. Using multiple regression analysis, paracetamol dose was found to be the only independent variable affecting GR, GST and SOD levels (P = 0.007, 0.003 and 0.008, respectively).

*Conclusion*s: In febrile children, treatment with repeated supratherapeutic doses of paracetamol is associated with reduced antioxidant status and erythrocyte glutathione levels. These significant changes may indicate an increased risk for hepatotoxicity and liver damage.

O7
Minimal Effective Dose (Med) of Propofol for Successful Tracheal Intubation without Muscle Relaxant in Children Aged 2 to 6 Years

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Background: Tracheal intubation without muscle relaxant has been used for many years in paediatric anaesthesia. In a recent study we found a relatively high incidence of unsuccessful tracheal intubation when propofol was used alone (*Paed Anesth* 2002; 12:36–42). The purpose of our study was to determine the minimal effective dose of propofol for successful tracheal intubation in children aged from 2 to 6 years.

Methods: A prospective double-blind study was conducted by sequential reassessment bayesian method. MED was defined as the propofol dose allowing successful tracheal intubation in 90% of children. After review by the local ethics committee of our university hospital, 25 ASA I children were included. Six propofol doses were tested (5 to 10 mg/kg). One hour after premedication (hydroxyzine, 1 mg.kg^{-1}), we injected at T0 a predetermined dose of propofol associated with lidocaïne 0.5 mg/kg and at T120 sec alfentanil 20 µg/kg. At T180 sec tracheal intubation was performed and the intubation conditions were rated using 5 criteria ranged from 1 to 4 (success = global score ≤ 2). Changes in heart rate and blood pressure were compared to baseline.

Results: 25 children 49.3 ± 14 months, weighting 17.2 ± 3.5 kg were included. Estimated success probability was 90.3% (95% credibility interval: 65.7–99.1%) for propofol 8 mg/kg, which was the estimated MED. The mean absolute changes in heart rate, systolic and diastolic arterial pressures were 16.2%, 18.1%, and 22.2% respectively (n=25).

*Conclusion*s: A high propofol dose (8 mg/kg) is necessary to successfully intubate children without muscle relaxant in more than 90% of cases. Despite such high propofol doses, haemodynamic side effects were comparable to those reported in other studies.

Acknowledgements: We would like to thank Pr S. CHEVRET and Mrs S. ZOHAR (Biostatistical Departement of Saint Louis Hospital, Paris, France) for their active participation and biostatistical advises concerning the sequential reassessment bayesian method.

O8
Does Rectal Acetaminophen as Adjuvant to Intravenous Morphine Decrease Morphine Consumption after Major Surgery in Children 0–1 Year of Age?

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Introduction: The administration of acetaminophen (APAP) as adjuvant to continuous morphine infusion (CMI) has become more and more popular, although the efficacy of this combination has not been studied in young infants. Therefore we conducted a study assessing the additional value of APAP as adjuvant to morphine on postoperative pain relief in children 0–1 year of age undergoing major non-cardiac thoracic or abdominal surgery.

Methods: A RCT was performed in 54 patients receiving either APAP (90–100 mg/kg/day) or placebo rectally. Postoperatively, after a loading dose of 100 µg/kg, children of < 45 weeks gestational age (GA) received 5 µg/kg/h CMI and children ≥ 45 weeks GA received 10 µg/kg/h. Analgesic efficacy was assessed 2–3 hourly using validated pain scores (VAS and COMFORT) during the first 48 hours. Extra morphine was administered or CMI was increased when VAS ≥ 4 cm. The infusion rate was decreased in the second 24 hours when VAS < 4 cm. Blood samples for APAP, morphine, MG3 and MG6 plasma concentrations analysis were collected. Urine was collected for APAP glucuronide/sulphate ratio. Data were analysed using Mann Whitney U test and multivariate logistic regression.

Results: Median (25–75th percentile) age was 0 (0–2) months. APAP was administered to 29 patients, 25 received placebo. The APAP and placebo group did not differ in total morphine consumption. However, irrespective of APAP or placebo group, children < 45 weeks GA, receiving 5 µg/kg/h, experienced more painful periods resulting in a higher incidence of extra morphine and increases in CMI compared to children ≥ 45 weeks GA, receiving 10 µg/kg/h; odds ratio (95% CI): 29 (5–171) and 9 (2–44), respectively. Mean APAP plasma concentrations ranged from 9.5 to 27.6 mg/l.

Conclusion: APAP as adjuvant to CMI appears not to have an additional analgesic effect and should not be considered as standard care.

O9
Busulfan Pharmacokinetics in Paediatric Bone Marrow Transplant Patients: Predictive Value of Predose Pharmacokinetics for Individual Dose Adjustment

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Background: Busulfan (BU), a bi-functional alkylating agent is a major component of conditioning regimens for patients undergoing bone marrow transplantation. BU is administered at the standard dose of 1mg/kg orally every 6 hours over 4 days and followed by cyclophosphamide at the daily dose of 40 mg/m^2 intravenously for 2 days. BU plasma concentrations following oral administration vary widely from patient to patient and high BU concentrations have been correlated with the incidence of hepatic toxicity (primarily veno-occlusive disease) and low concentrations with allograft rejection and relapse. Our aims were to evaluate the predictive value of a predose pharmacokinetics on initial (D1) and steady-state (D13) BU pharmacokinetics.

Methods: The protocol was approved by the institutional review board and informed consent was obtained from the parents of our patients. A test-dose (0.5 mg/kg) was administered four days before starting the preparative regimen. Seven samples were collected after the test-dose, the first (D1) and the thirteen (D13) doses and BU pharmacokinetics were determined. Bu concentrations were determined by HPLC-MS. Pharmacokinetic analysis used the USC*PACK software.

Results: 19 patients aged 6 ± 4 years, were enrolled in the present prospective study. Expected AUC by the test dose was significantly correlated with the AUC measured after D1 and D13 (r=0.80 and 0.78 respectively). The predictability of the test dose was 91.1 ± 18%. In our series, five patients experienced a veno-occlusive disease (26%) and 8 (42%) had a stomatitis.

Conclusion: Our results demonstrate that the test dose could be used for individual dosage adjustment in order to reduce averse effects associated with BU administration.

O10
Developmental Regulation of Prostaglandin (PG)E2 Synthases in the Pig Ductus Arteriosus

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Background: PGE_2 is the most important vasodilator regulating ductus arteriosus (DA) tone.

Objective: We investigated the ontogenic regulation of PGE_2 synthases (microsomal (mPGES), cytosolic (cPGES)), inducible cyclooxygenase (COX-2) and cytosolic PLA2 (cPLA2) by Western immunoblots and densitometric quantification. PGE_2 levels were measured by radioimmunoassays.

Results: There was no difference in DA protein expression of $cPLA_2$, or cPGES between pig fetus (F: ~50–75% gestation) and the newborn (NB, first hrs). In contrast, mPGES protein expression in NB DA was 60% greater than in fetal DA, and COX-2 was increased by 20% in NB DA. These changes were associated with a 6-fold increase in PGE_2 production in the NB DA. Many cytokines like PAF are increased in the perinatal period; we investigated role of PAF in the developmental regulation of COX-2 and mPGES. Treatment of NB pigs with PAF receptor antagonists, BN52021 and THG315, for 8 hrs decreased expression of mPGES by ~50% and COX-2 by ~35% compared to vehicle-treated pigs; PAF antagonists did not affect cPGES or $cPLA_2$. PAF antagonists also reduced local and plasma PGE_2 levels. Consistent with these findings, treatment of NB DA with PAF (0.1 μM, 6 h) induced mPGES and COX-2 expression, and increased by 7-fold local PGE_2 production; cPGES and $cPLA_2$ were minimally affected. Correspondingly, DA tone was augmented 6 h after exposure to PAF-receptor antagonists and conversely reduced by PAF; mPGES inhibitor, MK886, blocked effects of PAF.

Conclusions: Increased PGE_2 production in the NB DA is associated with increased expression of mPGES and COX-2. These developmental changes contribute to DA tone and appear to be regulated, in part, by the increased production of PAF in the perinatal period. mPGES may be a novel target for control of DA tone.

O11
Erythromycin Breath Test in Neonates

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Background: The erythromycin breath test (EBT) as used in adults to study the major drug metabolising enzyme CYP3A4 is not appropriate for infants. However an oral stable isotope-labelled tracer N, N-dimethyl-$^{13}C_2$ erythromycin (ERT) may be a suitable alternative. We hypothesised that as CYP3A4 exclusively catalyses N-demethylation of ERT, the C atom in the resulting formaldehyde should appear in the breath as labelled CO_2.

Methods: Neonates weaning from mechanical ventilation and tolerating at least 0.5 ml/hr of enteral feeds were eligible for inclusion. ERT was given via a nasogastric tube at 8mg/kg (t = 0). 10ml aliquots of breath in exhalation were sampled from the endotracheal tube at t =-20,-10, 0 and at 20 minute intervals for 6 hours after. ^{13}C enrichment of breath CO_2 was determined by continous flow isotope ratio mass spectrometry.

Results: Seven neonates were recruited (mean gestation at test of 30+4 weeks). The mean%CO_2 in breath was 3.22 (SD=0.40). Changes in parts per million of ^{13}C from baseline are shown in the Figure. The maximum tracer recovery was low, approximately 6% of the dose.

Conclusion: We reliably obtained sufficient %CO_2 in breath for accurate ^{13}C measurement. No consistent change in ^{13}C signal from the baseline was seen within 6 hours. A variety of factors may influence the background ^{13}C levels. This makes interpreting oral ^{13}C EBT in preterm neonates difficult and prevents drawing firm conclusions about the CYP3A4 system. However we have demonstrated this approach to studying drug metabolism is feasible. Applying this technique may be possible in the more mature neonate.

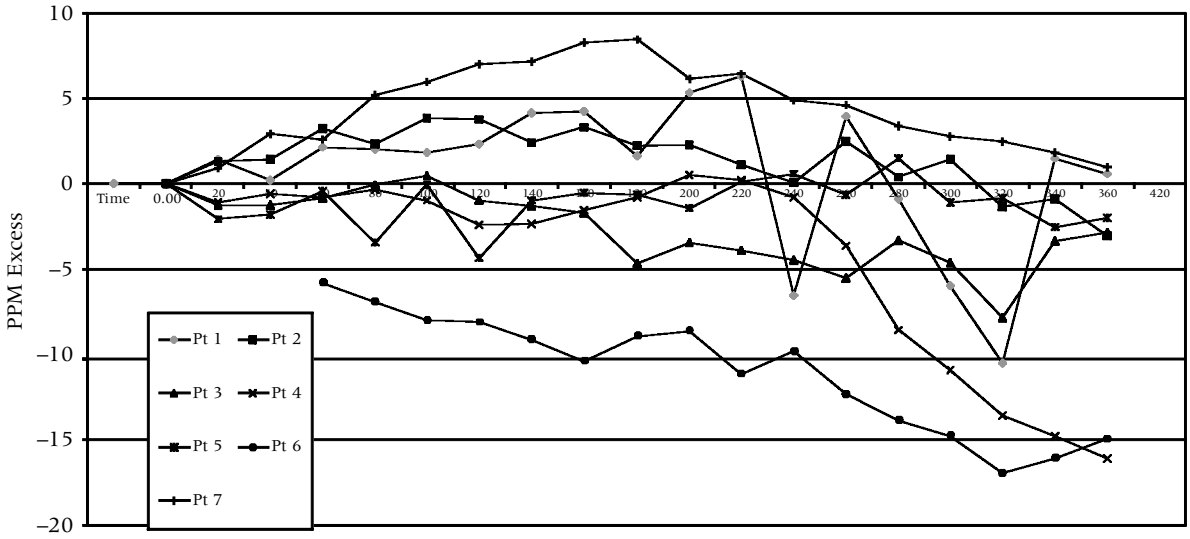

Figure O11.

O12
Maternal-Fetal Transfer and Amniotic Fluid Accumulation of Antiretroviral Drugs in HIV Infected Pregnant Women

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Background: An increasing number of antiretroviral drugs are now being used in HIV-infected pregnant women either for the prevention of vertical transmission or as therapy for the mother. However, little information is available on the use of antiretroviral drugs in pregnancy. Our objective was to investigate placental transfer and amniotic fluid concentrations of antiretroviral drugs when given to HIV infected pregnant women.

Methods: The mothers were receiving antiretroviral therapy in a clinical setting. Maternal, cord blood and amniotic fluid samples were obtained at the time of delivery in 102 mother-infant pairs.

Results: The most frequent combinations of antiretroviral drugs used in our population of pregnant women were zidovudine, lamivudine, nelfinavir (*n*=20), zidovudine, lamivudine, nevirapine (*n*=13), zidovudine, lamivudine (*n*=8), didanosine, stavudine, nelfinavir (*n*=7). A significant relationship was evidenced between maternal and cord plasma concentrations for zidovudine, lamuvidine, stavudine, didanosine, nelfinavir and nevirapine. Cord/maternal plasma ratio was high for zidovudine (R=1.22), stavudine (R=1.32), lamuvidine (R=0.93) and nevirapine (R=0.88) and low for didanosine (R=0.38) and nelfinavir (R=0.24). Concentrations of lamuvidine in amniotic fluid were higher than in maternal and cord plasma (median 0.45; 0.41 and 1.68 mg/l respectively).

Conclusion: Our findings are consistent with previous studies concerning zidovudine and could have important implications for the choice of the drugs during pregancy.

O13
Stiripentol in Childhood Partial Epilepsy: A Randomized Placebo-Controlled Trial with an Enrichment and Withdrawal Design

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Purpose: Stiripentol (STP), a new antiepileptic drug which inhibits several cytochromes P450 and increases plasma concentrations of concomitant antiepileptic drugs, has shown some efficacy when combined to carbamazepine (CBZ) in an open trial in children with epilepsy. The aim of the present trial was to study STP as an add-on therapy to CBZ in refractory partial epilepsy in children.

Methods: An enrichment and withdrawal design was used. After a 1 month single-blind placebo baseline followed by a 3 month open add-on STP, 32 out of 67 patients were responders. They were double blindly randomized for 2 months either to STP (*n*=17) or placebo (*n*=15.) If seizures increased by at least 50% or became more severe after randomisation compared to baseline, patients dropped out the study (primary endpoint).

Results: During double blind, six patients on STP (35%) and eight on placebo (53%) dropped out (difference not significant.) However, seizure frequency (secondary endpoint) decreased significantly more on STP (–75%) than on placebo (–22%). During double blind, STP dose was 81 $\pm$ 15 mg/kg/d (C_{min}:11,0 $\pm$ 5.5 mg/l) and CBZ dose was 12.6 $\pm$ 5.6 mg/kg/d (C_{min}: 12.8 $\pm$ 2.6 mg/l) in the STP group while CBZ dose was 17.0 $\pm$ 7.0 mg/kg/d (C_{min}: 7.1 $\pm$ 1.6 mg/l) in the placebo group. Twelve patients experienced at least one adverse event on STP (71%) compared to 4 on placebo (27%).

Conclusion: It is possible to take into account specific paediatric recruitment limitations and ethical issues in choosing the suitable design for trials devoted to children, even for drugs with interactions. Stiripentol proved to reduce seizure frequency when combined with CBZ in children with refractory partial epilepsy, although it failed to show a significant impact according to the escape criteria selected as primary endpoint in the present study.

O14
Expression of Duodenal CYP3A and P-GP in Children: An Immunohistological Study

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Background: Cytochromes P4503A (CYP3A) are the most abundant CYP in adult liver and gut and contribute to the first-pass metabolism of many orally administered drugs. P-glycoprotein (P-gP) is an integral membrane protein, whose function is the energy-dependent export of substances from the inside to the outside of the cells. In the enterocytes, CYP3A and P-gP form a coordinated barrier to the absorption of orally taken drugs by limiting their uptake.The ontogeny of CYP3A in the liver showed an increase in CYP3A4, the adult form of CYP450, from birth to adulthood together with a corresponding decrease in CYP3A7, the main fetal form. The ontogeny of the CYP3A enzymes in human enterocytes remains unknown.

Methods: We analysed the localisation and expression of CYP3A and P-gP in normal duodenal biopsies by immunoblot technic using an rabbit anti human polyclonal CYP3A antibody (Pr Beaune, Paris) and a mouse anti human monoclonal P-gP antibody (C494, Dako, Denmark) on formalin-embedded tissue sections.

Results: CYP3A and P-gP were expressed in almost all biopsies whatever the child'age. CYP3A expression increased from 50% in biopsies from children under 6 months of age to 100% in biopsies from older patients. The P-gP expression, restrained to the apical and basolateral side of the enterocytes was heterogenous, with variations between enterocytes and villi. It varied with age and was low before 6 months of age, increases to high levels between 3 and 6 months and decreased in a very heterogeneous way in biopsies from older children.

Conclusion: The present study of CYP3A and P-gP expression by immunohistochemical technics will be completed by a real time qantitative RT-PCR of mRNA expression. These findings will allow better understanding of the intestinal metabolism and absorption of orally taken drugs in new-borns, infants and children and have practical applications for oral drug administration in high risk paediatric populations.

O15
Lack of an Association Between the Frequency of Off-Label Prescribing and the Risk of Adverse Drug Reactions in Paediatric Outpatients

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Background: Many studies throughout Europe have shown that a substantial number of children receive off-label prescribed drugs which lack marketing authorisation for paediatric use. This might contribute to a loss of drug safety and efficacy in these 'therapeutic orphans'. Though a few hospital-based studies have suggested an increased incidence of adverse drug reactions (ADRs) due to off-label prescribed drugs, this issue has not yet been addressed for the outpatient setting. Thus, this primary care based study aimed to compare the incidence of reported ADRs and off-label drug prescribing among different therapeutic groups using the Swedish ADR register and a computerised prescription database.

Methods: We ranked all drugs which were prescribed for children younger than 16 years of age in the Stockholm County in the year 2000, by the number of prescription items and restricted the retrospective, descriptive analysis to drugs that accounted for 90% of total prescribing (DU90%). The proportion of off-label drug prescribing was calculated for different therapeutic groups with respect to age, formulation, and route of administration using the *"Swedish Physician's Desk Reference"* as the primary reference source. We also determined the number and characteristics of ADR reports for these therapeutic groups in Sweden from 1988–2000.

Results: The average off-label proportion amounted to 20.7% but was found to be widely different comparing therapeutic groups being much higher for topically than for systemically used drugs. However, vaccines caused by far the highest number of reported ADRs followed by antibiotics, antiasthmatics, antiepileptics, and antihistamines for systemic use. Interestingly, less than 0.5% of reported ADRs were related to topical drugs. We did not determine any correlation between the proportion of off-label prescribing and the incidence of ADRs in different therapeutic groups.

Conclusion: This study suggests the absence of an effect of off-label prescribing on the incidence of ADRs in the paediatric outpatient setting and argues for a more administrative rather than clinical problem of this prescribing pattern in the primary health care system.

M1
Adverse Effects of ACE Inhibitors and AT1 Receptor Blockers During Pregnancy

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Backgound: Angiotensin II converting enzyme (ACE) inhibitors have been implicated in the occurrence of fetal toxic effects when administered during pregnancy and their use is contra-indicated during the second and third trimester of pregnancy. Angiotensine II receptor blockers (ARB) which selectively compete at the angiotensine II type 1 (AT1) receptor became more recently available for the treatment of hypertension. Animal studies have shown that intrauterine exposure to ARB may cause fetal death, decreased fetal body weight and renal dysfunction with histological changes of the kidneys.

Methods and results: Between 1995 and 2001, we prospectively collected maternal data on 23 pregnant women exposed top an ACE or ARB for hypertension. 12 pregnant women, treated with an ACE had their treatment modified during the first trimester of pregnancy and no drug-related side-effect occurred during pregnancy or in neonates. 11 pregnant women were exposed to an ARB. Outcome was normal in nine cases in which treatment was modified during the first trimester. In two cases, treatment was maintained until 32 and 34 week's pregnancy. An ultrasound scan showed oligohydramnios, hyperechogenic and dysplasic kidneys and craniofacial abnormalities. Termination of pregnancy was performed in one case and in the other one, the baby was born with acute renal failure.

Discussion: ACE inhibitors are known to cause severe fetal and neonatal renal dysfunction (oligohydramnios, neonatal anuria), skull ossification defects, reduced foetal growth, stillbirths and dysmorphic features. Our observations show that, similarly, the use of ARB may induce foetal toxicity and are contra-indicated during pregnancy. Women of reproductive age should be advised of the possible hazards of these drugs and should have them stopped or changed if pregnancy is planned and as early as possible when pregnancy is documented.

M2
Evolution of Oxygenation after Ibuprofen–Lysine Prophylaxis in Preterm Infants

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Background: Ibuprofen-lysine is increasingly used for treatment of patent ductus arteriosus in preterm infants. Severe hypoxemia has been recently reported in three infants after the infusion of ibuprofen-THAM solution in France (The Lancet 2002;659:1486–1488). Concerns about the occurrence and etiology of this adverse event and the possible role of ibuprofen, motivated us to investigate whether a similar hypoxemic event could have been occurred after the administration of ibuprofen–lysine during our Multicenter Ibuprofen Prophylaxis Study (MIPS) (to be published).

Methods: Data of ventilatory parameters and bloodgases were collected from the source documents of 178 infants (BW: 1030 ± 320 g; GA 27.8 ± 1.7 wks) that participated in MIPS and were double-blind allocated to receive either ibuprofen-lysine (10 – 5 – 5 mg/kg; 10 mg/ml; q 24 h) or saline (1– 0.5 – 0,5 ml/kg; q 24h) i.v. with the 1st infusion <6 h of life. Data were collected before and 1, 6, 12, and 24 h after the infusion.

Results: In none of the infants FiO_2 increased to 100% and no hypoxemic event was observed after infusion of any treatment. Evolution of respiratory support and the oxygenation index was comparable between ibuprofen-lysine and saline treated infants. Baseline echocardio-Doppler evalution demonstrated left-to-right ductal shunting and absence of pulmonary hypertension in all infants.

Conclusion: No hypoxemia could be demonstrated after prophylactic treatment with ibuprofen-lysine in preterm infants.

M3
Closure of Patent Ductus Arteriosus with Oral Ibuprofen Suspension in Premature Newborns

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Patent ductus arteriosus is a common problem among premature infants, for which indomethacin is the conventional treatment. Recently, intravenous ibuprofen has been shown to be as effective as intravenous indomethacin in preterm infants with respiratory distress syndrome and also to cause less adverse reactions. There are no published studies on oral ibuprofen for closure of patent ductus arteriosus (PDA). In this prospective study, we aimed to evaluate the efficacy and safety of oral ibuprofen for the early treatment of PDA in preterm infants. We compared our results with those published in a recent study where ibuprofen was administered intravenously.

Methods: Twenty-two preterm newborns with a gestational age of 27.5±1.75(23.9-31) weeks, weighing 979±266 (380-1500) grams were prospectively studied. All suffered from respiratory distress syndrome and PDA as confirmed by echocardiography. They were treated with enteral ibuprofen suspension, 10 mg/kilogram body weight for the first dose, followed at 24–hour intervals by a further two doses of 5 mg/kg per dose, if needed, starting on the second day of life. Echocardiography was performed 24 hours after each dose. Brain ultrasonography before and after each ibuprofen dose was performed in every child. The rate of ductal closure, the need for additional treatment, side effects, complications, and the infants' clinical courses were recorded.

Results: Ductal closure occurred in all newborns except for one (95.5%) in whom only minor ductal shunting remained without clinical significance, as compared to 70% in the intravenous study (P=0.015). No surgical treatment was needed in our patients. Serum creatinine was similar before and after ibuprofen treatment.

*Conclusion*s: Our data may indicate that oral ibuprofen suspension is effective for closure of patent ductus arteriosus in premature infants. However, larger comparative studies are needed to investigate its efficacy and safety.

M4
Systematic Evaluation of Pain in Neonates Effect on Number of Intravenous Analgesics and Sedatives Prescribed

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Background: Pain and stress have important impact on short- and longterm outcome in neonates. Systematic evaluation by pain scale may improve awareness of the staff to optimalize comfort in these infants. Trends in consumption of analgesics and sedatives are used in this study as an objective marker of this increased awareness after introduction of systematic evaluation of pain and discomfort.

Methods: Retrospective analysis of the number of prescribed vials of intravenous analgesics or sedatives in the NICU during a 6.5-year period (96–5/02) to document trends in prescriptive behaviour before (96-99) and after (00–5/02) the introduction of a pain scale. Search for potential clinical co-variables involved (number of admissions, number of surgical interventions, days on full parenteral nutrition, days on ventilation). Results are expressed on a yearly basis. Student's *t*- test is used to compare both periods and all variables were entered in a multiple regression model (MedCalc).

Results: There is a significant increase in the number of vials prescribed on a yearly basis before (mean 3848, SD 745) and after (6678, SD 711) the pain scale was introduced (P<0.006). There is no significant increase in number of admissions (mean 532, SD 6 *vs* 595, SD 46). There is a significant increase in number of surgical procedures performed (128, 14 *vs* 149, 6)(P<0.05), in days on total parenteral nutrition (2384, 170 *vs* 2834, 154)(P<0.02) but not in days on ventilation (3073, 594 *vs* 3548, SD 406). In a logistic regression model, the increase in number of prescribed vials still remained significant after correction for all other co-variables mentioned.

*Conclusion*s: We could document a significant increase in number of vials prescribed after systematic evaluation by pain scale was introduced in the unit. This increase could not be explained by other clinical co-variables. Therefore, this trend is very likely a reflection of an increased awareness to prevent and treat pain in these infants. We do want to stress that non-pharmacological strategies are equally important in the prevention of pain and stress and that effective pain management in neonates is will always be based on prevention whenever possible and on step-up/step-down pharmacological therapy when appropriated.

M5
Urinary Excretion of Midazolam and Metabolites in Preterm Infants

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Background: Both metabolism of midazolam and renal function are impaired in preterm infants. We therefore speculated that midazolam and metabolite renal excretion will be different in preterm infants as compared to adults. The aim of our study was to determine the urinary excretion of midazolam and its metabolites, after a single IV midazolam dose in preterm infants.

Methods: The urinary excretion of midazolam (M), 1-OH-midazolam (OHM) and 1-OH-midazolam-glucuronide (OHMG) were determined in 15 preterm infants (weight 0.9–1.35 kg, PNA 3–11 days, GA 26.3–33.6 wks) after a 30-minute IV midazolam infusion (0.1 mg/kg). Urine samples were collected from start of the infusion for up to 6 hours during two-hour intervals (=approximately 1 M plasma elimination half-life in preterm infants). Midazolam and metabolites were determined in urine using GC-MS. To calculate the percentage of M dose excreted in the urine as metabolites, urinary OHM and OHMG concentrations were corrected for molecular weight.

Results: The percentage of midazolam dose excreted in the urine during the 6 hours interval was for M median 0.44% (range 0.02–1.35%), for OHM 0.04% (0.01–0.14%) and for OHMG 1.1% (0.24–4.9)%. The largest proportion of drug and metabolites were excreted during the last urine collection interval (4–6 hours postdose). The percentage of M excreted unchanged, as OHM or as OHMG was for OHMG positively correlated with postconceptual age, but not for M and OHM

Conclusion: The urinary excretion of M, OHM and OHMG is higher (albeit still low) in preterm infants than in adults. These data show that in preterm infants the glucuronidation of OHM to OHMG is almost complete. Finally, the percentage urinary excretion of OHMG increases with postconceptual age, most probably consequent to maturation of renal function.

M6
Pharmacodynamics of Midazolam in Paediatric Intensive Care Patients

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Background: Although midazolam is frequently used for sedation in paediatric intensive care patients, few data are available on sedative effect of midazolam and its metabolites given as a continuous infusion in this patient population. The aim of this study was to determine the pharmacodynamics of midazolam in paediatric intensive care patients using the COMFORT® scale as validated sedation scale.

Methods: The pharmacodynamics of midazolam and its metabolites were determined in 21 paediatric intensive care patients with ages between 2 days and 17 years who received a continuous infusion of midazolam (0.05–0.4 mg/kg/h) for 3.8 hours to 25 days for conscious sedation. The rate of midazolam infusion was titrated according to sedation level, using the COMFORT® scale as a validated tool for the assessment of sedation. COMFORT® scale 'cut-off values', which were previously validated to reflect adequate sedation, were used for the decision to change midazolam infusion. Blood samples were taken at different time points during and after midazolam infusion for determination of midazolam, 1-OH-midazolam and 1-OH-midazolam-glucuronide with HPLC-UV assay.

Results: In 20 out of the 21 patients the rate of midazolam infusion could be effectively titrated to the desired level of sedation. However, the COMFORT® scale "cut-off values" could not be applied to all patients as in specific disease states a deeper level of sedation was clinically indicated. A concentration-effect relationship scale could not be detected.

Conclusion: Desired levels of sedation could be reached with midazolam in almost all paediatric intensive care patients. Based on our findings that there is no relationship between pharmacokinetic parameters and pharmacodynamic outcome, we recommend that midazolam dosing should be titrated according to the desired clinical effect with the use of the COMFORT® scale.

M7
Nasal Sumatriptan Effectively Relieves Migraine Attacks in Children

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Background: Migraine is a common disease among children and adolescents. Although all attacks cannot be adequately controlled with simple analgesics, none of the triptans is yet available for children. Objective of the study was to investigate the efficacy of nasal sumatriptan in migraine attacks of children and adolescents.

Methods: A double-blind placebo-controlled two-way crossover trial in hospital outpatient clinics. Patients were 8–17 years old children or adolescents, body weight over 20 kg with at least two migraine attacks (IHS 1988) per months with a minimum duration of four hours. The study treatment was a single dose of sumatriptan nasal spray (Imigran®, GSK) or a matching placebo administered at home at the onset of an attack. Sumatriptan dose was 10 mg for children with a body weight of 20 to 39 kg, and 20 mg for those over 40 kg. The primary efficacy endpoint was headache reduction by at least two grades on a five-grade face scale at two hours or falling asleep at that time.

Results: Totally 129 children were recruited. 94 patients, (mean age 12.4 yr) used at least one treatment and were included in the study. 83 of them used both treatments and 11 only the first one; altogether 90 received sumatriptan and 87 placebo. The mean sumatriptan dose was 0.36 mg/kg (range: 0.20–0.50). The 83 patients, who took both treatments, reached the primary efficacy endpoint at two hours twice as often after sumatriptan (n=53; 64%) as after placebo (n=32; 39%; P<0.01). However, at two hours, only 30% (n=25) after sumatriptan and 19% (n=16) after placebo became pain-free (P=NS). Already at one hour, primary endpoint was reached more often after sumatriptan (n=42; 51%) than after placebo (n=24; 29%; P=0.01). The results were even more obvious in a subgroup of adolescents receiving the 20mg dose and were also similar in intention-to-treat analyses (all the patients who used at least one treatment were included). Subjectively, 57% (n=47) preferred sumatriptan and 34% (n=28) preferred placebo (P<0.05), while 8 were undecided. Rescue medication was used by 35% (n=29) after sumatriptan and 51% (n=42) after placebo (P=NS). No serious adverse events were observed, 29% (n=26/90) reported a bad taste after sumatriptan and 3% (n=3/87) after placebo (P<0.01).

Conclusions: Nasal sumatriptan is an effective treatment for migraine attacks in children and adolescents; however, only 30% of the patients achieved complete resolution of headache at two hours.

M8
Pharmacokinetics (PK) and Pharmacodynamics (PD) of Nizatidine (NIZ) in Neonates and Young Infants

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Background: Gastroesophageal reflux (GER) disease is a frequent problem of neonates and young infants. Acid modifying agents (e.g. H_2-receptor antagonists) are used when non-pharmacologic interventions fail to ameliorate symptoms. We report the results of a study designed to evaluate the PK and PD profiles of NIZ in infants.

Methods: Children 7d to 12 mos with GER were enrolled and randomized to receive NIZ (2 or 4 mg/kg) by IV infusion. NIZ and N-desmethylnizatidine were quantitated by HPLC/MS from serial post-dose blood samples collected over a 12 hour period. Intragastric pH was measured concurrently over the same time frame.

Results: In total, 29 infants (postnatal age: 23-341 d, postconceptional age: 272–607 d) were enrolled. Evaluable NIZ PK data were available in seven subjects, metabolite PK data in 21 subjects and PD data in 24 subjects. Total body exposure (AUC) for NIZ (2999.6 ± 604.5 ng h/ml *vs* 4593.4 ± 179.2 ng h/ml) and metabolite (386.5 ± 138.5 ng h/ml *vs* 572.3 ± 168.0 ng*h/ml) increased proportionally with doses between 2 and 4 mg/kg. Both Lz and apparent Vd were constant with dose and averaged 0.634 ± 0.069 1/h and 1.2 ± 0.3 l/kg, respectively. No discernible age-dependence in NIZ PK were apparent given the limited number of evaluable datasets. PCA did appear to influence metabolite elimination rate [Lz= 0.120 + (0.0007444 * PCA), r^2=0.352, *P*=0.005], a finding consistent with the dependence of metabolite excretion on renal function. Mean pH values "on-drug" were significantly greater than observed during "off-drug" interval (3.9 ± 1.2 *vs* 2.6 ± 1.0, *P*<0.001) and the fraction of time that "on-drug" interval remained above target pH values exceeded the corresponding "off-drug" time nearly 2-fold for target pH values of 3 (0.60 ± 0.28 *vs* 0.33 ± 0.23, *P*<0.001) and 4 (0.48 ± 0.30 *vs* 0.24 ± 0.18, *P*<0.001). A sigmoid Emax PK-PD link model could be described in five subjects yielding estimates for Emax, EC_{50} and ke0 of 5.89 ± 0.08 delta pH units, 549.2 ± 124.6 ng/ml and 0.730 ± 0.287 1/h, respectively. Compared with historical adult data, E_{max} values were nearly 2-fold higher for the infants. As a result, the effect at the EC_{50} in infants was comparable to the effect at EC_{90} in adults.

*Conclusion*s: The PK profile and subsequent PD estimates in infants suggest that a dose of either 2 mg/kg or a 4 mg/kg administered to infants between the PNA of 23-341 days is sufficient to generate a PD response equal to or greater in magnitude than that observed in adults.

M9
Optimizing Cyclosporine Monitoring in Paediatric Renal Transplanted Patients

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Background: After oral administration Interindividual variabilities make oral dosing management of cyclosporine (CsA) unpredictable. Monitoring is mandatory avoid reduced efficacy or toxicity. The area under the concentration-time curve (AUC) is a better tool for CsA monitoring than predose concentration but a limited sampling strategy is required, to estimate AUC using a small number of samples collected at specific times. In order to optimize CsA monitoring, 32 renal transplanted children (10±4 years) were studied. Post-transplant therapy included antilymphocyte globulines or basiliximab (18/14), corticoids, CsA, azathioprine or mycophenolate mofetil (22/10). A pharmacokinetic study (PK1: eight samples) was performed 0.8±0.3 months after transplantation and poor profiles (C0, C2, C4, C12) were obtained 3–4 months (PK2) and 9-12 months (PK3) after transplantation. Equations were defined giving the AUC as a function of one or several concentrations. The mean prediction error, the percentage prediction error (pe%), the proportion of AUC estimated within 15% prediction error range were calculated to compare measured and predicted AUCs.

Results: AUC-PK1 were significantly correlated with postdose partial AUC ($AUC_{0\text{-}12}$, r=0.94) and with C2, C3 or C4 (r>0.8). Significant prediction was obtained with C3 (pe:27.6% *vs* 41.9% with C2 and 54.8% with CO), CO+C3 (17.2%), C0+C2+C3 (20.7%). Similar significant correlations were obtained between AUC -PK2 or AUC-PK3 and C2, C3 or C4 (r>O;75). When equations derived from PK-1 were used to predict AUC-PK2 and AUC-PK3, prediction error were >15% in all cases.

Conclusion: C2 is accepted as the best time-point predictor of AUC in CsA treated adult patients. Although validation is required, our study demonstrates that, as in adults, C0 does not correlate with AUC in paediatric renal transplanted patients. C2 or C3 or C2 and C3 should be considered to monitor CsA in paediatric renal transplanted patients.

M10
Limited Sampling Models for Monitoring of Cyclosporine in Paediatric Patients

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Background: Therapeutic drug monitoring of cyclosporine is commonly used to estimate the systemic exposure, since exposure of cyclosporine is related to nephrotoxicity and graft rejection. It has been suggested that the area under the curve (AUC) is a better marker for exposure than trough levels, but estimating the AUC is expensive and cumbersome due to the number of blood samples required. The aim of this study was to develop a simple sampling model for estimating the AUC of cyclosporine in paediatric renal transplant recipients.

Methods: Eleven patients (age 5 – 16 years) were studied. Blood samples were drawn at 0, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 4 h after administration of cyclosporine (Neoral®) in a dose based on trough levels. A 2-compartment model (MW/Pharm) was used to calculate the AUC. The AUC based on 1 sampling time (C_0, C_1, C_2, C_3 or C_4), 2 sampling times (C_0 and C_2 / C_0 and C_4 / C_2 and C_4), 3 sampling times (C_0, C_2 and C_4) and 5 sampling times (C_0, C_1, C_2, C_3 and C_4) was compared with the AUC based on all 11 sampling times.

Results: The AUC using 5 sampling times (C_0, C_1, C_2, C_3 and C_4) showed an excellent correlation with the total AUC (R^2 = 0.99). Reduction of the number of sampling times to one resulted in a correlation of 0.8 (C_3) or lower (C_0, C_1, C_2 or C_4).

Conclusion: The model with 5 sampling times (C_0, C_1, C_2, C_3 and C_4) estimates the AUC of cyclosporine accurately in paediatric patients. However, for practical application a model with one sampling time is preferred. The model with sampling time C_3 provides the most accurate estimate.

M11
Information and Education in Sanitary Setting

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Background: A directive of the European Community Council obliges pharmaceutical market companies to include leaflets addressed to users and written in clear way in boxes of a drug for human use. Nevertheless many approved documents are very difficult to understand for the patients. The aim of the study was to evaluate whether concise information and use of graphic symbols and colours could help hypothetical patients in comprehending the material.

Methods: As a collaborative project between the Mario Negri Institute and the I.T.C.S. Erasmo da Rottedarm secondary school, three groups of students were asked to create an ideal leaflet, based on the information contained in the official one, for a paediatric syrup containing ibuprofen: Nureflex. The challenge for the students was to render the information written in the official leaflet about uses of the drug, contraindications, cautions/adverse effects and dosage more comprehensible. The ideal leaflets were addressed to parents of children between 6 months and 12 years old. To analyse the level of interpretation, 40 mothers whose children were within this age range, were randomly divided into four groups and either the official leaflet or one of the three ideal leaflets developed by the students. The mothers were asked to read the document pretending to have to give this drug to their own feverish child under the paediatrician advice. The mothers were interviewed using a previously validated questionnaire concerning the uses, contraindications, side effects and dosage of Nureflex. During the interview, the definition of 3 medical terms, as well as the meaning of NSAID were asked. The end points considered were the correctness and completeness of the answers, the reading time and personal judgement on comprehension.

Results: The population consisted of the 40 mothers, whose average age was 35 years. Ninety per cent of the women had a secondary school education, whereas 10% had a primary school one. Seventeen mothers gave a correct definition for all three medical terms, and 14 for only one. One third of the population knew the meaning of NSAID. The average reading time was 4 minutes for the three leaflets, less than the time spent for the official one. In order to compare the different leaflets, items for correctness and completeness of the answers were assigned. Two of the three ideal leaflets resulted as being clearer and much more comprehensible than the others. The level of comprehension of the mothers who received any of the three ideal leaflets was higher than that of the mothers who received the original.

Conclusion: In an homogenous population of mothers, the three graphic proposals developed by the students were understood better, faster and in a more appropriate way than the official document. Even though two of the created leaflets resulted as the best, both need further modifications. The feasibility of the study was shown supporting its amplification and generalisation after minor modification.

P1
A Pilot Case Control Follow-Up Study on Hearing in Children Treated with Tobramycin in the Newborn Period

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Objective: To assess the occurrence of hearing loss in children due to neonatal exposure to long courses of tobramycin and/or high tobramycin serum concentrations.

Methods: This was a pilot case-control study in 3–4 year old children. Data on tobramycin administration were abstracted from the patient files of an earlier study. Patients exposed in the neonatal period to either long courses (>7days) or high serum concentrations of tobramycin constituted the study group. The control group consisted of patients without tobramycin exposure. Patients were matched for other risk factor according to criteria of the Joint Committee on Infant Hearing. All patients underwent the following investigations: otoscopy and pneumatic otoscopy, followed by impedance audiometry, to exclude middle ear effusion. Click-evoked oto-acoustic emissions (ce-OAE) as well as distortion product oto-acoustic emissions (dp-OAE), tested at f^2 frequencies ranging from 1 to 10 kHz, were measured to assess hearing. All patients with abnormal ce-OAE results underwent brainstem electric response audiometry (BERA) as well. Since aminoglycoside ototoxicity is usually bilateral, results were compared per patient and not per ear.

Results: A total of 29 patients were tested. Eleven patients were excluded due to middle ear effusion. Data for 18 patients were analyzed. In the tobramycin treated group (n=9) both ce-OAE and dp-OAE (at all tested frequencies) were not detectable in six ears of three patients. All other patients had normal ce-OAE's as well as normal dp-OAE's in this frequency range. Difference between the tobramycin treated and control group for OAE as well as dp-OAE showed a trend (P=0.08). In all three patients with undetectable emissions BERA confirmed a cochlear loss of 60–70 dB at 3 kHz in both ears. These three patients had the longest total exposure to tobramycin: 20–24 days and 84–92 mg/kg, respectively. No relation to either peak or trough serum concentrations could be detected.

Conclusion: There was no statistical relation between hearing loss and tobramycin exposure, probably due to sample size. Our results do indicate a need for a case-control follow-up study of hearing in neonates exposed to long courses of aminoglycosides.

P2
Effects of Treatment of Cerebrotendinous Xanthomatosis (CTX): the Experience in Three Patients

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CTX is an autosomal recessive defect in bile acid biosynthesis with accumulation of cholestanol and cholesterol in most tissues and body fluids. The enzyme 27-hydroxilase is deficient and mutations in the gene on chrom 2 have been recently described. The clinical picture of juvenile cataract, tuberous and tendon xanthomas and a progressive neurologic disorder is, although age dependent, very suggestive. Most cases are diagnosed after the age of 20 years. Our first patient was diagnosed at the age of 32 years having the complete clinical picture. Two brothers were diagnosed at the age of 12 and 14 years with cataract, a cerebellar ataxia, a peripheral neuropathy, absence of xanthomas, and a chronic diarrhea from early childhood. These two patients manifested a steatorrhea and marked cholestasis [serum total bile acid concentration: 32.7–41.5 µmol/l (nl <10)]. Cholestasis has, to our knowledge, never been described before in CTX patients. All patients had a low IQ (range 60–66), speach disturbances and normal CAT scan and MRI investigations of the brain. Molecular studies established the diagnosis of CTX in all three patients.

The older patient was treated with a combination of chenodeoxycholic acid and a HMG-coA reductase inhibitor. The younger patients first received only chenodeoxycholic acid which resulted in an improvement of well being, strength, growth and speach and a spectacular disappearance of the steatorrhea. The amount of bile alcohols excretion slowly diminished under substitution with bile acids. When a HMG-coA reductase inhibitor was added to the treatment, the bile alcohols excretion in urine practically normalized. One year follow-up under the combined therapy showed an improvement of several cognitive functions although the IQ remained low. The HMG-coA reductase inhibitor atorvastatine (and cerivastastine) has a higher therapeutic value in these patients in comparison to simvastatine and pravastatine, most probable because it has a more potent inhibitor activity on cholesterol synthesis de novo.

Conclusion: chenodeoxycholic acid and a HMG- coA reductase inhibitor should be combined in the treatment of CTX patients.

P3
Hereditary Generalized Resistance to 1α,25(OH)D2: A Rare Disorder with Severe Effects on Bone Metabolism

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We describe a Moroccan boy, second child of consanguineous parents, who was transferred to our hospital because of a severe rickets that didn't respond to vitamin D supplementation. He showed an incomplete alopecia and there was a history of postnatal growth retardation, failure to thrive and psychomotor developmental retardation (from the age of 2–3 months). Serum calcium was in the low normal range (4.38 mEq/L) with a low serum phosphate (1.24 mEq/L)and a highly elevated serum alkaline phosphatase of 1801 U/L (ref. values 140–320). Further investigations revealed an elevated PTH (240 pg/ml (ref values 13–54) and calcitonine; 25-OH-D was low normal whereas the serum concentration of 1,25-diOH-D was highly elevated: 301 pg/ml (ref. values 16.4–42.4). The urinary calcium and phosphate excretion was undetectable low. There were no additional deficiencies. Radiologic examination showed a severe rickets with osteopenia and a bone maturation compatible with that of a newborn infant at an age of 14 months. Molecular genetic investigations revealed a missense mutation in exon 1 of the Vit.D-receptor gene.

The treatment consists of oral supplementation with 1α-OH vitamin D 12 µg/d, disodiumphosphate 6 250 mg/day; intravenous Pamidronate 1 mg/kg body weight for 3 consecutive days every 3 months and subcutaneous growth hormone treatment. Under this therapy the rickets is improving with an amelioration in hair growth, psychomotor development and muscle strength. He receives gastric tube feeding while his caloric intake was insufficient. He starts to grow at the age of 32 months.

He suffered severe respiratory infections and showed an evolution towards a respiratory failure based on both an obstructive (asthma) and restrictive (narrow, deformed thorax with weak respiratory musculature) component. At the age of 3 years he receives ventilatory support during the night.

Conclusion: This case presentation proves the important role of vitamin D in calcium homeostasis, bone formation and growth, and describes the effects of specific therapeutic measurements on the evolution of this severe disease.

P4
The Effect of Atropine on Cardiovascular Autonomic Regulation in Preterm Infants

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Background: Low frequency fluctuations in heart rate (HR) are associated with the baroreceptor reflex (sympathetic *and* vagal activity) and high frequency fluctuations with respiration (solely vagal activity). The relative contribution between low and high frequency fluctuations can be considered as the sympatho-vagal balance of the autonomic nervous system. The aim of the study was to evaluate the effect of atropine, a parasympaticolyticum, on heart rate variability (HRV) and sympatho-vagal balance in preterm infants.

Methods: 10 circulatory stable preterm infants (GA 28.5 ± 2.3 wks and BW 1216 ± 555 g) received preceding non-acute intubation [apnea (*n*=5), hyaline membrane disease (*n*=3) and infection (*n*=2)] atropine (0.01 mg/kg). The median age of intubation was 27 h after birth. Heart rate (HR) and HRV was evaluated for two periods: a period of 5 min before and the period 5–10 min after atropine. A wavelet technique was used to compute spectral power (unit: s^2) in the total frequency band (TF 0.04–1.5 Hz), low frequency band (LF: 0.04–0.15 Hz) and high frequency band (HF: 0.4–1.5 Hz). LF and HF were expressed as absolute values and normalized units (*e.g.* LF/TF 100%). LF/HF ratio was considered an estimate for sympatho-vagal balance. Results presented as mean ± standard error of measurements (SEM). Comparisons made by paired Student's *t*- test.

***Results*:** HR increased from 140 ± 4 to 154 ± 6 bpm ($P<0.001$). The total power (TF) decreased from 1.52 ± 1.46 10^{-4} to 3.33 ± 6.91 10^{-5} s^2 ($P<0.05$). LF decreased from 1.25 ± 1.30 10^{-4} to 2.68 ± 6.62 10^{-5} s^2 ($P<0.05$). HF decreased from 1.41 ± 1.36 10^{-5} to 4.65 ± 2.11 10^{-6} s^2 (ns). The normalized LF decreased from 78 ± 11 to 49 ± 14% ($P<0.01$) and normalized HF increased from 16 ± 8 to 45 ± 13% ($P<0.005$). The LF/HF ratio decreased from 14 ± 15 to 5 ± 6 ($P=0.05$).

*Conclusion*s: Atropine (0.01 mg/kg) is pharmacological effective in changing cardiovascular autonomic function in preterm infants, increasing HR and decreasing overall HRV. In contrast to what is known in adults, atropine *decreases* (normalized) LF power and *decreases* LF/HF ratio. Probably the baroreceptor reflex in preterm infants is more under vagal control than in adults.

P5
Fetal Toxic Effects of Angiotensin-2-Receptor Antagonists

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Background: Angiotensin-2-receptor antagonists are a newer group of antihypertensive drugs which are more selective than ACE-inhibitors. Nevertheless, studies on rats and sheep show that they have similar fetal toxic effects as ACE-inhibitors.

Methods: We report a new case of a live-born baby and we present a review of the literature.

Results: Animal studies on rats demonstrate lower pup weights and irreversible histopathological renal changes due to intrauterine exposure to angiotensin-2-receptor antagonists compared with non-exposed rats. These findings are dose-dependent and function of time of exposure during pregnancy. These fetal toxic effects have been attributed to:

- a direct action of angiotensin-2-receptor antagonists on the fetal rennin–angiotensin system
- an ischaemia due to maternal hypotension and subsequent reduction in fetoplacental blood flow.

Both effects are mediated by inhibition of activation of the angiotensin2 AT1 receptor. There is also some recent evidence of a role for the AT2 receptor, which has been shown to play a significant role in cell growth and differentiation of murine proximal tubule cells in vitro. In human beings, we found 9 cases, which have been reported: three of them were stillborn, two died neonatally and four children survived the neonatal period. We notice that in 3 of 4 of these last cases, no congenital abnormalities or evidence of renal dysfunction had been found. In these three cases, maternal treatment with an angiotensin-2-receptor antagonist was stopped at a mean gestation of 12 weeks. The other casereports however always described oligo-hydramnion, renal tubular dysgenesis and/or pulmonary hypoplasia. In those cases, maternal treatment had been continued beyond at least 20 weeks of gestation.

Conclusion: Because of the fetal toxic effects, angiotensin-2-receptor antagonists should be avoided in pregnancy, especially during the second and third trimester.

P6
Monitoring Antimicrobial Prescribing in a Children's Hospital

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Antimicrobial agents are the commonest drug group prescribed for children. It is important that antimicrobials are used appropriately, in order to avoid unnecessary expenditure and to prevent the emergence and spread of antibiotic resistance. We undertook a prospective non-interventional study of ciprofloxacin and liposomal amphotericin (AmBisome) prescribing during an eight week period, commencing 16 January 2002. Ciprofloxacin has an important role in multiply antibiotic-resistant Gram-negative infections, but its use must take account of the limited paediatric licence. Favourable pharmacokinetics, and a ten-fold price difference between oral and intravanous preparations make ciprofloxacin an ideal candidate for switch therapy. Fungal infections are difficult to diagnose, and anti-fungal therapy is therefore usually commenced empirically. Despite their greater cost, lipid-based preparations are increasingly being used in preference to conventional amphotericin, because of their lower toxicity. In our hospital, the cost of liposomal amphotericin (AmBisome) prescriptions exceeds that of any other drug.

In 18/19 patients treated, ciprofloxacin was prescribed as a second or third line agent. Only six patients had confirmed bacterial infections, three of which proved to be with ciprofloxacin-resistant bacteria. A Microbiologist was involved in the decision to commence ciprofloxacin in only seven cases. four patients treated with iv ciprofloxacin were candidates for oral therapy from the outset, and another patient was a candidate for early switch therapy. We estimate that the cost of ciprofloxacin prescriptions could be reduced by 33.3% by elimination of inappropriate prescribing, equating to an annual saving of at least £8000 (12.800 euros).

Only 2/27 patients treated with AmBisome had proven fungal infections (respiratory aspergillosis; cerebral aspergillosis). One patient died during treatment with AmBisome, and in another patient treatment was discontinued after the first dose because of a serious reaction. Of the remaining patients, 11 (44%) patients were treated for £4 days, and only six (24%) for ≥10 days. The brevity of the majority of courses of treatment suggests that, by applying stricter criteria for commencing treatment, the amount of AmBisome used in our hospital could be reduced by at least 28.9% without detriment to patients. This amounts to an annual saving in drug costs alone of at least £20.265 (32.400 euros).

Our study shows that a ward-based clinical pharmacy service, supported by Microbiologists, can potentially have a significant impact on antimicrobial usage in hospitals.

P7
Lamprey-GnRH-III Stimulated LH and FSH Release of Newborn and Adult Rat Pituitary Cells in the Superfusion System

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Background: LH and FSH secretion by the pituitary gland is under the control of gonadotrop hormone-releasing hormone (GnRH) and the putative follicle stimulating hormone-releasing factor (FSHRF). Recently McCann et al. suggested that the newly discovered lamprey-GnRH-III (l-GnRH-III) or a closely related, yet unknown peptide could be the FSHRF that has been long sought for. We compared the LH and FSH releasing potency of the l-GnRH-III to that of the mammalian GnRH (mGnRH) in male and female rat pituitary cells. Since l-GnRH-I and -III according to the phylogenetic tree of GnRHs is closer to the ancestral GnRH molecule than the mGnRH we tested its effect also on pituitary cells of newborn rats.

Methods: The collagenase dispersed pituitary cells, mixed with Sephadex G-10, of 4–6 days old and 2-month-old male or female rats were placed into 1 ml columns and perfused with Medium 199 (flow rate 1 ml/3 min). After 3 hours the cells were stimulated with 1 nM mGnRH for 3 min followed 30 minutes later by a 15-min stimulation with 1 nM mGnRH or 330 nM l-GnRH-III. After 60 min the cells were rechallenged with 1 nM mGnRH for 3 min, and half-an hour later for 15 min with 3 nM mGnRH or 1000 nM l-GnRH-III. Three-min samples were collected and assayed for LH and FSH content by RIA kits obtained from NIDDK/NHPP.

Results: We found that the LH and FSH releasing potency of the l-GnRH-III was 0.35–0.46% and 0.1-0.22% of that of the mGnRH. In all examined groups the FSH releasing activity was slightly lower than the LH releasing activity. Age and sex of the animals did not cause any remarkable difference in the efficacy of l-GnRH-III on LH or FSH liberation.

Conclusion: Our findings using the *in vitro* superfusion method do not support the view that the l-GnRH-III could function as FSHRF since its LH and FSH releasing potency is significantly and similarly lower than that of the mGnRH in all models investigated.

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P8
Fetal Intramuscular Betamethasone Treatment to Prevent Respiratory Distress Syndrome

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Background: Maternal corticosteroid administration is the first choice of treatment to prevent the development or decrease the severity of respiratory distress syndrome (RDS) in preterm infants. However, maternal steroid administration is only effective if delivery occurs at least 24-48 hours after the first dose was given. Moreover, maternal corticosteroid therapy may be contraindicated under certain circumstances.

Methods: Ultrasonography-guided single direct fetal intramuscular injection of betamethasone (0.5 mg/kg estimated fetal weight) for the prophylaxis of RDS was developed at our department. In order to investigate the effectiveness of the single dose of fetal betamethasone therapy we evaluated the perinatal outcome of 87 pregnancies when preterm delivery was expected to occur within 24 hours after admission and were complicated with preterm premature rupture of membranes (PPROM), preterm labor (PTL), preeclampsia (PE), intrauterine growth restriction (IUGR), diabetes mellitus and Rh incompatibility. The clinical characteristics of preterm infants treated in utero whose gestational age was <32 weeks at delivery and were born between 1997-1999 (gest. age: 29.5±2.4 weeks, birth weight:1298±494 g, *P*=33) were compared to those who were born during the same period without any prevention (gest. age: 28.9±2.6 weeks, birth weight: 1278±470 g, *P*=32).

Results: In the PPROM/PTL group (*P*=22) and PE/IUGR group (*P*=44) severe RDS developed only in 5 and 9 cases, the survival rates in these groups were 90.9 and 93.2%, respectively. If the gestational age was <32 weeks at delivery the mortality rate was significantly lower in the treated group (3/33 *vs* 9/32, *P*<0.05) compared to those without any prevention. Severe RDS, bronchopulmonary dysplasia and intraventricular hemorrhage were less frequent in infants receiving in utero therapy (24 *vs* 31%, 6.4 *vs* 18.7% and 12 *vs* 18.7%, respectively).

Conclusions: The direct fetal route of steroid administration may provide further advantages and improve neonatal survival rate. We believe that ultrasonography-guided fetal intramuscular steroid treatment has great potential for becoming a clinically relevant approach to prevent RDS in selected cases of high risk pregnancies.

P9
Bartter Syndrome in a Neonate on Prostaglandin Infusion for Ductus-Dependent Congenital Cardiopathy

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Background: Prostaglandin infusion (PG) is a well-established medical treatment in the care for neonates with a ductus-dependent congenital cardiopathy. Well-known side effects of this treatment are apnoea, fever, hypotension, bradycardia, seizures, diarrhoea, hyperostosis and oedema. We describe a preterm neonate in whom PG-infusion caused marked polyuria and renal sodium loss, suggestive for Bartter syndrome.

Case report: A male infant, born at the postmenstrual age of 32 weeks (birth weight 1740g) needed ventilation the first 24 hours of life because of respiratory distress syndrome. He was referred on day 6 because of complex cardiopathy (double outlet right ventricle, transposition of the great arteries and infundibular pulmonalis stenosis with hypoplastic pulmonary arteries). To ameliorate lung perfusion, PG-infusion was administered (starting dose 0.1 _g/kg/min) on day 9. A few hours after the infusion was started, the infant was intubated because of apnoea. Whitin 24 hours after initiation of therapy, the child displayed severe hyponatraemia (115 mmol/l), high natriuresis (103.3 mmol/l) and polyuria (up to 12 ml/kg/h). Natraemia normalised under NaCl supplementation (up to 70mmol/day). Only modest hypokalaemia with slight increased fractional excretion of potassium were observed. Marked dose/effect relation with higher sodium losses when higher doses of PG's were administered was documented. Because of the renal side effects of PG's, a therapeutic catheterisation on day 20 to dilate the pulmonary valve and ameliorate pulmonary flow was performed. After discontinuation of PG's therapy on day 27, diuresis and fractional excretion of sodium returned to normal.

Discussion: This patient showed evidence of iatrogenic Bartter-syndrome, caused by administration of PG's in a neonate with ductus-dependent cardiopathy. This is a rare complication which has been described only once. It is well known that primary Barrter disease itself is associated with endogenous increased production of PG's. Therefore, we postulate that immaturity, genetic background and dose administered were factors involved in the phenotypic presentation of iatrogenic Bartter syndrome in this preterm infant.

P10
Prospective Evaluation of Methohexital for Procedural Pain Relief in Neonates During Removal of a Chest Tube

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Introduction: Adequate pain relief in neonates still remains a major challenge while during neonatal stay different types of pain- or stressful procedures are needed. Although systemic opioids are very potent analgesics, these medications are long acting and may cause hypoventilation for a longer period. We therefore investigated the use of methohexital during procedural pain relief. Methohexital is a short acting barbiturate, providing full anaesthesia for a short period of time. It might enable progressive weaning in ventilated infant and avoid intubation in a non-ventilated infant while adequate pain relief is still secured.

Methods: A single dose of methohexital, 2,6 mg/kg dissolved in an equivalent volume of intralipid was administered when a chest tube was removed. To document the effect, vital signs (blood pressure, heart rate), grade of relaxation (1–4), sedation (1–4) and intensity of pain (0–14) pain scale were recorded before, during and after the procedure (from 10 minutes before until 10 minutes after administration).

Results: The effect of methohexital was recorded in 18 procedures in 18 infants. Mean postmenstrual age was 36.3 (SD 8.8) weeks and mean postnatal age was 17 (SD 25). Mean weight was 2770g (SD 1255). infants were still ventilated. Mean blood pressure (BP) and mean heart rate (HR) 1,3,5 and 10 minutes after administration were not significantly different when compared with mean blood pressure and heart rate before the intervention (mean BP: 54, SD 27 and 54, SD 28) (HR: 141, SD 52 vs 159, SD 37). Sedation and relaxation normalised after 5 to 10 minutes in all infants. Myoclonus and hiccups were noticed in 3 infants each. Mean pain score before intervention was 1.6 (SD 2.3) and during manipulation it was 2.0 (SD 2.7). Adequate pain relief (i.e. <5) during the whole period was documented in 13/18 infants. Ventilation could be decreased at the same intensity after this procedure when compared with the 24 hours before.

Conclusions: Methohexital seems to be a useful drug for short-acting procedural pain relief and sedation. Further studies are needed to compare these findings with other therapeutic strategies to prevent procedural pain in neonates.

P11
Pharmacokinetics of Single Dose Propacetamol in Neonates: Effect of Postmenstrual Age

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Background: Document pharmacokinetics in term and preterm infants after single intravenous administration of propacetamol on the first day of life.

Methods: A single dose of propacetamol (either 20 or 40 mg/kg) was administered over a period of 15 minutes in the first 24 hours of life in infants undergoing minor but painful procedures or as additional therapy in infants receiving opioids. Arterial blood samples were collected at 30,60,90,120, 180, 240, 360 and 600 minutes and plasma samples were stored at –20°C after centrifugation. Paracetamol levels were determined by fluorescence polarisation immunoassay (Adx system, Abott Laboratories, North Chicago, Ill). A linear, one-compartment model with instantaneous input and first-order output was used to calculate distribution volume, elimination half-time and total body clearance.

Results: 213 samples from 30 infants were analysed. 15 infants received the 20mg/kg dose, 15 received the 40mg/kg dose. 19 infants were ≤ 34 weeks PMA on admission (See Table P11).

Conclusions: In addition to differences in pharmacokinetics of propacetamol already documented between infants and neonates[(1)], we documented the effect of postmenstrual age on the pharmacokinetics of this drug on the first day of life. There is no significant difference in distribution volume between infant less or over 34 weeks of gestation. However, elimination half-time is significantly longer and clearance significantly lower in infants ≤ 34 weeks when compared to infants of a higher PMA. Therefore, loading dose should not be adjusted for prematurity, but either the interval or the dose during multiple dose administration should be adjusted.

[1] Autret E. *et al*. Dev Pharmacol Ther 1993; 20: 129-134.

Table P11.				
	Overall	**≤ 34 weeks**	**> 34 weeks**	
Birth weight (g)	2111 (1094)	1290 (587)	2958 (753)	P<0.01
PMA	33.8 (3.9)	30.3 (1.9)	37 (2.2)	P<0.01
Distribution volume (l)	1.94 (1.1)	0.83 (0.34)	1.93 (1.1)	P<0.01
Relative DV (l/kg)	0.61 (0.21)	0.65 (0.16)	0.61 (0.21)	NS
C 20 mg (t=0)	17.8 (4.3)	18.0 (4.9)	17.6 (3.1)	NS
C 40 mg (t=0)	35.4 (12)	36.8 (7.3)	35.6 (21.5)	NS
T $^1/_2$ (min)	240 (128)	280 (151)	180 (57)	P<0.01
Clearance (l/kg/h)	0.132 (0.078)	0.113 (0.085)	0.152 (0.06)	P<0.05

P12
Unapproved Prescribing Practices in Primary Paediatric Clinics in Israel

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Background: Many medications have not been approved for use in children because lack of data to assure acceptable standards for safety, efficacy and optimal dose. The objective of this study was to determine the extent of unlicensed and off label medications use in primary paediatric clinics in Israel.

Methods: Prospective analysis medications prescribed to children during consecutive visits to clinics corresponding to different ethnic communities and different regions of the country, during representative months of the year. The setting was clinic practices of six board- certified paediatricians with a list size of 9,300 children. Participating physicians filled a form including patient and medication data. Medication prescriptions given to children up to age 18 years were compared with their product license for age, dose, indication and route of administration.

Results: A total of 1,925 prescriptions of 160 different medications were given to 1,802 children. One prescription was for an unlicensed medication, and 297 (or 15.4%) were given in an off-label manner to 280 children (15.5%). Antibiotics and antiasthmatics constituted the most frequently prescribed off label medication categories. Statistical analysis did not revel significant ethnic or seasonal differences in off label medication prescribing practices.

*Conclusion*s: A large number of children in primary clinics in Israel as well as in European countries receive a myriad of unapproved medication prescriptions. Serious discrepancies exist between the product license and recommendations by the paediatric literature.

P13
How Parents Use Medications to Treat Their Children's Fever

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Background: "Fever phobia" is widespread among parents and medical personnel. It could lead to inappropriate use of antipyretics in children. Severe liver damage may occur as result of paracetamol overdoses with therapeutic intent. How parents use antipyretics for their children's fever is unclear.

Methods: This was a cross- sectional study. Parents (n = 201) attending a Paediatric Emergency Room with their children up to 5 years of age with fever were interviewed. Doses of antipyretics used by parents, based on body weight, were calculated and compared with current recommendations.

Results: Mean age of children (± SD) was 20 ± 17 months. The source of parental knowledge regarding treatment of fever was medical personnel (57%), manufacturers' recommendations (16%) or other (27%). Sixty-five percent of parents gave antipyretics for temperatures ≤ 38°C. Two parents did not give medications for fever. Medications used were paracetamol (89.5%), ibuprofen (2%), alternating paracetamol and ibuprofen (8%), and other (2%), with or without cooling measures. When oral liquid formulations were used, 96% of parents used a standardized measuring device (cup or syringe). Fifty-three percent of parents gave individual paracetamol doses within ± 10% of the recommended range (10–15 mg/ kg); 11.5% gave <9 mg/ kg, 35% and 25.4% gave >10 and >20% of the maximally recommended doses respectively. Twenty-one percent of parents gave paracetamol at more frequent than recommended time intervals (less than every 4 hours).

*Conclusion*s: Children are frequently exposed to excessive paracetamol doses with therapeutic intent. This may be a manifestation of "fever phobia". There is a need of interventional studies to deal with misconceptions about children's fever. Parents must receive precise instructions on how to treat their children's fever.

P14
Medications Prescribed to Children in Primary Clinics in Israel

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Background: Most medications for children are prescribed in primary clinics. The objective of this study was to obtain information regarding the use of medications by children in the community in Israel.

Methods: Prescriptions given to children age 0–15 years, insured at the Clalit Health Services were analyzed. A computerized database on medications prescribed in primary clinics was used. Two administrative sections of the District were chosen; they reflected socioeconomic and ethnic characteristics of the population, and types of communities. Data were obtained for the months of February, May, August and November 1997 and 1998.

Results: A total of 159,426 prescriptions given to 94,997 children were analyzed. As average, each child received 2.52 prescriptions/year. Most medications were antimicrobials, and within this category, of the penicillin group. Oral medications represented 85.3% of prescriptions, given mostly to children <2 years of age and to boys (all P<0.001). Girls received more clindamycin and hormones than boys (P<0.001). Most of the drugs of small therapeutic index were of the category affecting the CNS. There was an excess of use of theophylline in children <2 years. The smallest number of prescriptions was given to children from low- income communities, and the largest, to children from affluent communities (P<0.001). It was prominent the lack of prescriptions of methylphenidate ("Ritalin") in low-income communities.

*Conclusion*s: The differences in the prescription of medications to children of low-income *vs* affluent communities may reflect differences in the availability/accessibility of medical services and/ or differences in parental awareness to their children medical problems. Findings of this study may be used in CME programs for primary physicians aiming at the improvement of drug therapy to children in the community.

P15
Urinary Metabolites of Paracetamol (APAP) in Children with Burns: A Preliminary Study

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Background: Paracetamol (APAP) is one of the most widely used medications in children. Children with burns receive APAP for analgesia and antipyresis. Changes that occur as result of burns affect the metabolism and the pharmacokinetics of many drugs. The metabolism of APAP has not been described in children (or in adults) with burns.

Methods: Seventeen hospitalized children with major burns (>10% of body surface area) who received APAP regularly in therapeutic doses (10–15 mg/kg q 6 h) were studied. Control groups were:
(a) 16 children with no burns, who received APAP for fever
(b) 6 normal adult volunteers

Urine samples were obtained from burn children during the acute (days 1–2) and the diuretic (days 5–7) phases; from control children after 1-5 APAP doses, and from the adult group on days 2 and 5–7 of APAP administration (500 mg every 6 hours). Urinary APAP and its 2 major metabolites: glucuronide (G) and sulfate (S), were measured by HPLC.

Results: The urinary G/S molar ratio was significantly smaller
(a) in burn children in days 1–2 (0.52±0.36) than in days 5–7 (0.9±0.6; P=0.03)
(b) in the acute phase in burn children than in control children (0.8±0.36; P=0.027)
(c) in burn and in control children than in the adult group (P <0.001)

Conclusion: The observed changes in APAP metabolism:
(a) confirm developmental changes in G formation
(b) indicate a need of further therapeutic evaluation of APAP in children with burns

P16
Easy and Reliable Renal Function Assessment is Mandatory Before Drug Administration in Children

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Background: The marked changes in glomerular filtration rate (GFR) that occur during maturation significantly affect the renal elimination of drugs. Pronounced changes in GFR are seen in sick infants, profoundly affecting the pharmacokinetics of drugs. The accurate assessment of GFR before administering drugs to children is thus mandatory. Among all available methods, the standard clearance of inulin (Cin) remains the best estimate of GFR, but is technically demanding. Creatinine (creat) is commonly used to assess GFR, but its production varies considerably intra- and interindividually, especially in paediatric patients as it is proportional to muscle mass which increases with linear growth. Much publicity is being made around plasma cystatin C (PcysC), a 13 kDa protein recently proposed to estimate GFR. The present prospective study was therefore designed to determine which parameter (creatinine clearance = Ccreat; creat; Formula GFR = K height (cm)/creat (mmol/l), with K = 46; PcysC) best discriminates impaired from normal renal function, as assessed by Cin.

Methods: Ninety nine children (51 M / 48 F) with a median age of 8.3 years (1.0 –17.9) were studied. Using a cutoff for Cin of 100 ml/min 1.73 m^2, 54 children had impaired GFR (Cin <100). Children with impaired (Cin <100) or normal GFR (Cin ≥100) were comparable for age, height, weight and body mass index.

Results: Logistic regression showed that Ccreat was the best parameter to discriminate between impaired and normal GFR, followed by the Formula GFR, PcysC and creat. PcysC was a significantly better marker than creat.

Conclusion: These results show that PcysC reflects GFR better than creat. However, logistic regression showed the K height/creat formula to have a better discriminating power than PcysC or creat alone. The use of this formula thus appears to be the easiest and most accurate method for assessing GFR when urine collection is not available. Because the cost of PcysC dosage is at least twice that of creat in most institutions, simply measuring height instead of PcysC will also be cost effective.

P17
Haptenation of Sulfonamide Reactive Metabolites to Cellular Proteins: Implications for Adverse Drug Reactions

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Background: Adverse drug reactions are a major problem complicating medical therapy. The pathogenesis of many severe adverse drug reactions, notably hypersensitivity reactions, is poorly understood. The sulfonamides are associated with severe hypersensitivity reactions. The initial pathogenesis appears to be due to bioactivation of the parent drug to a reactive intermediate and subsequent propagation by the immune system. The determinants of the immune response are not known.

Methods: We explored the formation of sulfonamide haptens in Molt-3 and HEPA 1C1C7 cells after incubation with sulfamethoxazole (SMX), the hydroxylamine of sulfamethoxazole (SMX-HA) or the nitroso of sulfamethoxazole (SMX-NO).

Results: Haptenation was demonstrated with SMX-HA and SMX-NO but not SMX; this occurred at concentrations below that associated with toxicity (significant haptenation was seen at 25 to 50 _M). Thus, haptenation occurred presumably onto viable cells. Haptenation occurred rapidly; haptenation of cell surface proteins was demonstrated within 5 minutes; this did not occur indiscriminately; confocal microscopy demonstrated haptenation on to specific sites on the cell membrane. We found that haptentation was significantly inhibited by thiols and other anti-oxidants (P<0.05).

*Conclusion*s: Sulfonamide-specific haptens were rapidly internalized by what appeared to be a caveole-dependent process. It appears that sulfonamide reactive metabolites haptenated specific cell surface proteins that are rapidly internalized. Understanding the specific protein target(s) for haptenation and how these haptens are processed will be important in understanding the immune mediation of sulfonamide hypersensitivity adverse drug reactions.

P18
Artificial Intelligence (AI) Prediction of β-Lactam Antibiotic Toxicity

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Background: Adverse drug reactions (ADRs) are generally divided into two categories, type A and type B. Type A reactions are dose-related, predictable and usually non-life threatening. Type B reactions are non-dose related, unpredictable and often lethal. Better prediction methods for drug-response relationships would minimize patient exposure and potential drug toxicity. A new approach, artificial intelligence, to predict toxicity was investigated with a small number of the b-lactam antibiotics.

Methods: Three controls were randomly chosen, and their peripheral blood mononuclear cells (PBMCs) were collected. A total of 29 beta-lactam antibiotics were incubated with PBMCs at the following concentrations: 6.25, 62.5, 625 uM for 2 hours. MTT assay was used to determine cell viability. The results from the MTT assay were used to train the AI system. The trained AI system was tested with a previously unseen subset of beta-lactams. AI predictions were recorded and compared to the *in vitro* assay results.

Results: The AI system was able to predict all six toxic and 23 non-toxic compounds correctly.

Conclusions: AI can successfully predict the toxicity of b-lactam antibiotics. The AI system is an effective predictive tool that can be applied to non-linear relationships often encountered in a biological setting.

P19
Sulfamethoxazole Hydroxylamine (SMX-HA): Necrotic or Apoptotic Cell Death in Lymphocytes?

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Background: Adverse drug reactions (ADRs) to sulfamthoxazole (SMX) occur in 2-3% of the general population, in comparison to 40% in HIV infected individuals. ADRs range from uticarial rash to systemic involvement. SMX-HA has been implicated in ADRs to SMX. To compare the contribution of apoptosis *vs.* necrosis we compared the apoptotic reaction of peripheral blood mononuclear cells (PBMCs) from sensitive patients compared to non-sensitive controls.

Methods: Isolated PBMCs were incubated with varying concentrations (25,50,100,200,400,800 μM) of SMX-HA for 2 hours. Cells were examined with propridium iodide and annexin V staining with FACScan to determine apoptotic *vs* necrotic cells.

Results: PBMCs from SMX sensitive patients (P=5) showed a significantly (P<0.05) increase in overall cell death (>15%) paired with a decreased apoptotic profile compared to the cells of controls (P<0.05).

*Conclusion*s: The lower amounts of apoptotic death with concurrent higher overall total cell death among patient cells *vs* those of controls suggests that apoptosis might be a protective mechanism with respect to cellular injury produced by reactive drug metabolites, while in sensitive patients increased necrotic cell death might trigger an undesired immune response manifested by an adverse drug reaction.

P20
Drug Compatibility in the NICU

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Background: Pharmacological treatment of sick infants is becoming increasingly complex. Many drugs are inadequately tested in neonates, and this "orphan drug" problem is compounded by the lack of data regarding drug compatibility and interactions.

Purpose: Our purpose was to create a handy reference on drug compatibilty for daily use in the NICU. The focus was stability during mixing or co-infusion through Y-connectors.

Methods: Data on drug compatibility were collected from literature. The main reference was L.A. Trissel "the Handbook of Injectable Drugs" with over 2000 references. Other sources were Neonatal Drug formulary (1997), Paediatric drug therapy handbook & formulary (1995-1996) and Neofax (2001).

Results: Data on drug compatibility were assembled for 10 commonly used NICU drugs. A 2x2 table was constructed providing the permissible concentrations, solubility and duration of stability. For a surprising number of drug combinations no data on compatibility are available. This problem constitutes an added layer of complexity to multi-drug treatment in neonates, where the issue of "orphan drugs" is already a significant problem.

Discussion: Prescription of unlicensed/offlabel medication exposes children to greater risks. Lack of awareness has caused therapeutic mishaps. We have constructed compatibility table for some of the most common drug in the NICU, and believe that this may contribute to increased security in drug treatment in neonates. Even though information about many of these drugs exists it is not readily accessible when needed. Further work is in progress as far as providing similar user information regarding the more complex issues of pharmacokinetic and pharmacodynamic interactions.

P21
Identification of Cellular Proteins Targeted by Reactive Sulphonamide Metabolites and their Relation to Adverse Drug Reactions

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Background: Mechanisms of many adverse drug reactions (ADR's) remain unclear. One possibility is described by the hapten hypothesis, which postulates formation of a covalent complex between drug and protein potentially visible to cells of the immune system. Reactive and toxic metabolites of Sulphamethoxazole (SMX), which are sulphamethoxazole-hydroxylamine (SMX-HA) and nitroso of sulphamethoxazole (SMX-NO) have been shown to covalently bind extra and intracellular proteins *in vitro*. This finding suggests that presentation of a drug-protein antigen is possible. The goal of this study was to identify and sequence cellular proteins haptenated by SMX-HA or SMX-NO, as they may have antigenic potential.

Methods: MOLT-3 cells incubated with 0.01% DMSO or 100μM SMX, SMX-HA or SMX-NO were lysed and soluble proteins subject to SDS-PAGE and both silver staining and western blot analysis with a polyclonal rabbit anti-SMX antibody. Protein bands of interest were excised from gel, proteins eluted and further separation was achieved by RP-HPLC through a Zorbax 300 SB-C18 column at 30°C using an acetonitrile gradient from 5% to 50%. Protein detection was by UV absorbency at 280 nm. Protein fractions were collected and re-analyzed by SDS-PAGE with both silver staining and western blotting. Bands representing haptenated protein were excised and processed for partial identification using surface enhanced laser desorption/ionization-time of flight-mass spectroscopy (SELDI-TOF-MS) by Ciphergen Biosystems Inc.

Results: Western blotting of primary gels showed at least 15 protein bands in both SMX-HA and SMX-NO treatment groups and that banding intensity was greater following SMX-NO treatments. Our RP-HPLC method has allowed successful separation of proteins of similar molecular weights and has proven to be a very useful tool in purification of a mixture of proteins. SELDI-TOF-MS analysis has provided several potential identifications of protein candidates, which are currently being sequenced by N-terminal sequencing techniques.

Conclusion: Sulpha-specific hapten formation provides a potential target for immune response in hypersensitivity ADRs.

P22
Evaluation of the Palatability of Medication for Children: Differences between Adults and Children and Availability of Assessment Tools

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Background: Compliance is a key determinant of therapy, and palatability is a major factor effecting whether children take their medication. Traditional taste evaluations use adult volunteers, but this may not be reflective of children's preferences. Tools to evaluate palatability of medication in children have been lacking.

Methods: We developed a tool to evaluate palatability of medication in children using a facial hedonic scale coupled with a 10 cm visual analogue scale. This tool was tested using eight different antibiotics in a group of 80 children aged 5 to 10 years of age and 20 adults.

Results: Clear differences in taste between the antibiotic formulations were demonstrated with good internal consistency (P<0.01). There were significant differences between the selection of worst tasting antibiotic between adults and children (P<0.029).

*Conclusion*s: Palatability of medications can be evaluated in children directly without the need for surrogate testing in adults. The use of adults to test palatability for preparations intended for children may give results which are not applicable for children and thus may give a false sense of the drug's acceptability for children. Drugs intended for use in children should be tested in children.

P23
Off-Label Drug Prescribing for Paediatric Outpatients as recommended in a Local Treatment Guideline

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Background: Off-label prescribing for children has been shown to be widespread in various clinical settings in Europe and is generally believed to be associated with a lack of drug safety and quality. However, there is increasing awareness that off label drug use might eventually be required and it seems inappropriate to restrict pharmacotherapy only to licensed drugs. We therefore compared the proportion of off-label prescribing with the adherence to a local treatment guideline in a Swedish population of 350,000 paediatric outpatients.

Methods: We ranked all drugs which were prescribed for children younger than 16 years of age in the Stockholm County in the year 2000, by the number of prescription items and restricted the retrospective, descriptive analysis to drugs that accounted for 90% of total prescribing (DU 90%). The proportion of off-label drug prescribing was calculated for different therapeutic groups with respect to age, formulation, and route of administration using the *"Swedish Physician's Desk Reference"* as the primary reference source. We also assessed the proportion of recommended drug prescribing according to the evidence-based treatment guideline *"Kloka Listan"* ("The Smart List") which is provided and distributed free of charge to all prescribing physicians by the County Council.

Results: Among the 317 drugs accounting for 90% of total prescribing the average off-label proportion amounted to 20.7% and was found to be widely different comparing therapeutic groups. Dermatologics, otologics, and ophthalmologicals were most frequently prescribed off-label but almost 2/3 of these prescription items corresponded to recommended drugs which is a higher adherence than in adults. In addition, the extent of recommended drug prescribing varied independently from the respective off-label proportion between different therapeutic groups.

Conclusion: This study suggests that off-label prescribing for paediatric outpatients might frequently be appropriate and children should not be denied access to drugs which are clearly beneficial despite a lack of paediatric labelling. Nevertheless the current licensing situation is unacceptable since it does not allow a proper dose adjustment for many drugs thereby putting children at unnecessary risks.

P24
Application of Population Pharmacokinetics to Cladribine and some Observations in Children

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Background: The nucleoside analog Cladribine, 2-Chlorodeoxyadeno-sine (CdA), is used for treatment of a variety of indolent B- and T-cell lymphoid malignancies. The primary aim of this study was to evaluate the population distribution of pharmacokinetic parameters in patients undergoing treatment with CdA, to detect influence of different covari-ates on the pharmacokinetic parameters and to evaluate the results in relation to previously presented data.

Methods: This pharmacokinetic study presents the results of a retro-spective population pharmacokinetic analysis based on pooled data from 163 patients including three children with age 7, 18 and 18 years. Nonlinear mixed effect modeling was applied for the pharmacokinetic analysis of the data.

Results: A three compartment structural model best described the dispo-sition of CdA. Clearance was found to be 35 L/hour/73 kg for the pop-ulation, with a weak correlation to weight 1.2%/kg and a large inter-individual variability. The half-life for the terminal phase was 17 hours. The three studied children had all pharmacokinetic parameters within the variability of the adults.

Conclusion: Individualized dosing on basis of body surface area or weight does not represent an improvement in this study as compared to administer all patients a fixed dose in adults. The body weight increases more than ten times between a neonate and a 10-years-old child. Body weight or body surface area may be of importance in younger children for dose calculations because of the large differences in body size even though the correlation was weak for clearance and weight.

P25
German Survey of Special Formulations and Dosages for Paediatric Wards Prepared by Clinical Pharmacies: A Retrospective Study

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Background: Several surveys could demonstrate a large proportion of drug applications in children in an unlicensed or off-label manner. This poses children at high risk for adverse events since a significant coherence between unlicensed / off-label drug use and adverse drug reactions was found. The preparation of special formulations and dosages for paediatric use represent two categories for an unlicensed drug use in children. Aim of the present study was to determine special formulations and the amount of different dosages prepared by pharmacies for paediatric wards within Germany.

Method: Based on a pilot-study a questionnaire with 96 active substances asking for special formulations and different dosages was send to 56 pharmacies and children hospitals in all parts of Germany. Further substances and dosages could be added by the pharmacies.

Results: 32 special formulations could be identified. In most of these cases liquid formulations were prepared. We could determine a "top 10" of substances which were changes in liquid formulations in most pharmacies. 90 active substances were modified in different dosages. In these cases up to 10 different dosages were not uncommon. The widest range of dosages could be found in diuretics and corticosteroids.

*Conclusion*s: The results demonstrated that the preparation of special formulations and dosages for paediatric wards is common practice in German clinical pharmacies. The present study elucidated the need for active substances which could be easily given over a wide dose range. Therefore further studies with easy to dose formulations (*e.g.* liquid) are needed.

P26
The Use of Gentamicin in the Neonate

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Background: This study aimed to develop a dose regimen achieving safe and effective serum concentrations of gentamicin in the neonatal patient.

Methods: Gentamicin serum concentrations achieved using the traditional dosing regimen of 2.5mg/kg given 24, 18 or 12 hourly depending on post-conceptional age (PCA) (Regimen 1) were audited. Results were evaluated and the dosing regimen was modified to 4mg/kg loading dose then 3mg/kg 36,24 or 18 hourly depending on PCA (Regimen 2). Further modification to 4mg/kg 36 (<32/40) or 24 hourly (>32/40) (Regimen 3) followed. All drug levels were taken at the second dose. Predose levels were taken immediately before the next dose, post dose levels one hour after dose administration.

Results:
Desired predose levels of <2 mg/l were achieved for Regimens 1, 2 and 3 respectively:
- Patients <28 weeks PCA: 78%; 100%; 100%
- Patients 28–31 weeks PCA: 62%; 92%; 100%
- Patients 32–35 weeks PCA: 47% ; 72%; 92%
- Patients 36 weeks and above PCA: 68%; 100%; 100%

Desired post dose levels of 5-10 mg/L were achieved:
- Patients <28 weeks PCA: 68%; 31%; 100%
- Patients 28–31 weeks PCA: 42%; 56% ; 98%
- Patients 32–35 weeks PCA: 67%; 74% ; 92%
- Patients 36 weeks and above PCA; 68%; 92% ; 90%

Conclusions: Changes to the neonatal gentamicin dosing regimen provided significant improvements in the rates of desirable serum concentrations achieved. Safe and effective therapy with gentamicin is now being administered to the majority of babies.

P27
Validating the Derbyshire Children's Pain Tool: A Pilot Study

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Background: The Derbyshire Children's Hospital Paediatric Pain Chart (DPC) is the current pain assessment tool used at the Derbyshire Children's Hospital. It was originally devised as a simple pain tool for use in the clinical area, it is applicable for use in children of all ages within the postoperative setting. The pain assessment chart encompasses pain assessment by utilising facial expression, body movement and verbal expression. An exploratory study was performed to define its reliability and validity.

Methods: The research nurse (VP) assessed 40 children aged 1–5 years undergoing minor and intermediate surgery comparing the Toddler Pre-schooler Post-operative Pain Scale (TPPPS) and the DPC. Assessments were performed preoperatively and for four hours postoperatively. Any analgesia administered postoperatively was recorded.

Results: All the children scored 0 preoperatively with both pain scales thus demonstrating known groups validity. There were 116 dual assessments by the Research Nurse using both pain scales. There was a strong correlation (r 0.89) demonstrating convergent validity. There was a significant correlation between 182 joint assessments by the research nurse (VP) and the nursing staff using the DPC (Spearmans rank correlation 0.81). Construct validity was demonstrated by a fall in the mean pain scores from 1.8 to 0.1 following analgesia in 19 children.

Conclusions: This exploratory study suggests the DPC holds construct, convergent and known groups validy and is a reliable pain assessment tool for children aged 1–5 undergoing minor and intermediate surgery.

P28
CYP3A5 Variant Alleles in Caucasians

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Background: Enzymes of the cytochrome P450 3A (CYP3A) family catalyse the metabolism of over 50% of currently prescribed drugs. This family consists of CYP3A4, 3A5, 3A7 and 3A43. CYP3A4 is believed to be the major contributor to the total CYP3A content. Based upon protein studies, approximately 70% of Caucasians do not express CYP3A5. This finding was recently correlated to a genetic polymorphism: *CYP3A5*3*. CYP3A5 may represent up to 50% of total CYP3A protein in individuals polymorphically expressing *CYP3A5*, and may therefore have a major role in variation of CYP3A-mediated drug metabolism. Using sequencing techniques, variant alleles *2 to *7 were described for *CYP3A5*. Detection of *CYP3A5* variant alleles, and knowledge about their allelic frequency in specific ethnic groups, is important to establish the clinical relevance of screening for these polymorphisms in order to optimise pharmacotherapy.

Methods: In a group of 500 healthy Dutch Caucasian donors, the allelic frequency of the *CYP3A5*2, *3, *4, *5, *6* and **7* alleles were determined using newly developed polymerase chain reaction-restriction fragment length polymorphism assays (PCR-RFLPs).

Results: The frequency of the defective *CYP3A5*3* allele was 91%; the **2* and **6* allele were found in 1.0% and 0.1% of the Caucasians, respectively. The *CYP3A5*4, *5* and **7* alleles were not detected.

Conclusion: Based upon its allelic frequency, we conclude that screening for the *CYP3A5*3* allele in the Caucasian population is extremely relevant. In addition, screening for the *CYP3A5*2* allele may be taken into consideration in individuals heterozygote for the *CYP3A5*3* allele. The *CYP3A5*4, *5, *6* and **7* alleles have low allelic frequencies which do not support initial screening.

P29
DDAVP-Toxicity Due to Prolonged Half-life in 9 Patients With Nocturnal Enuresis

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DDAVP is a frequent used drug in patients with monosymptomatic nocturnal enuresis (EN). Side-effects are rare. DDAVP is classically administrated in the evening 30 min to 1h before sleeping, and no fluid intake is allowed overnight. In the past years we have observed several children, where we suspected prolonged bioactivity of DDAVP nasal spray (dose 10–20µg).

Aim of the study: To investigate the characteristics of these patients.

Study-population: 4F/5M (age 6–15 years). Exclusion-criteria: renal diseases or drugs interfering with renal function.

Methods: 24h urine-collection in 8 portions for volume/min, osmol, creat. Daytime and nighttime were each divided in 4 ± equal periods of time. Values were compared with 200 controls (I)out of a normal population, and 50 children with proven nocturnal polyuria before (IIA) and after introduction of DDAVP (IIB).

Results: 4/9 had symptoms of water-intoxication with vomiting and headache, and proving hyponatriemia. In 9/9 urinary osmolality remained >850 mosmol/l during 15h, and in 2 during 18h after administration, despite high fluid intake. In 5/9 we documented inappropriate dilution capacity. The hypo-osmolar polyuria in the remnant time is suggestive for a correction mechanism.

Conclusion: Although DDAVP is a very safe drug, there is a risc of water-intoxication, when high fluid-intake during daytime is promoted, secondary to a prolonged bioactivity of DDAVP in a small percentage of patients.

P30
Tolteridine Versus Oxybutinine in ADHD

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Instable detrusor and small bladder syndrome may benefit from a treatment with anticholinergic drugs together with a bladdervolume-training and urotherapy. Because there is a known co-morbidity between ADHD-syndrome and bladderdysfunction, anticholinergics may also be prescribed in this patients. With oxybutinin however we have seen in the past many side-effects. Tolteridine has definitely less side-effects in normal children, but if this is also the case in ADHD children is not known. Over the last years the drug has been prescribed in such patients, despite of the non-registration in children, because of the unacceptable high frequency of side-effects of oxybutinine.

Aim of the study: Comparative study between Tolteridine (2mg/day) and oxybutinine (15mg/day) in children with ADHD.

Methods: Case-control-study. Patients were matched for age and sex. Registration of side-effects and effect (during 3 months).

Study-population: Age 5–14 years, 30 children in each group, predominantly boys.

Results: In the oxybutinin group 12 patients stopped the drug <3m, because of side-effects (hypernervositas, concentration-disorder). In nine other patients there was significant worsening of the ADH-symptoms according to the parents. In the detrusitol-group five children interrupted therapy <3 m: two for the price, one for accomodation-problems, two for worsening of ADHD-symptoms. Only in three other patients there was suspicion of more ADHD-symptoms. There was no difference in effect on the bladder-function.

Conclusion: The side-effect-ratio between Tolteridine and oxybutinine is definitely in favour of Tolteridine in children with ADHD.

P31
DDAVP-Dependency in Enuresis During Long Term Follow Up: Myth or Reality?

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A decade ago, the introduction of DDAVP in the treatment of monosymptomatic nocturnal enuresis (MNE) seemed a major progress in the field. On the short term, high recurrence rate is well known. The long term effects are ,however, unknown. The aim of the study was a long-term follow up study of a cohort of MNE patients.

Methods: Retrospective study of DDAVP-efficacy and dependency during long term follow up of two patient-groups, participating between 1993 and 1995 in two prospective randomised placebo controlled studies. Patients were recontacted in 2001. Study-population $P = 101$ (68M).

Results: Initial DDAVP response correlated with nocturnal polyuria and a maximal bladdervolume (BV) larger than BV for age ($P<0.005$). Patients were divided in two groups after the randomisation period. 56 initial DDAVP-responders: four are lost for follow up. All other patients became full-continent, 40 had 1 relapse, 21 had >3 relapses, five DDAVP dependent in 2001, 19 nycturia regularly overnight, 38 received DDAVP monotherapy, 18 patients received associated alarm to obtain full continence.

45 initial non DDAVP-responders: three not continent within the first 2 years of follow up. The majority needed a combination-therapy, but all achieved drug-independence and full continence in 2001. 18 relapse, nine with >3 relapses, nine nycturia.

Conclusion: The initial DDAVP responders have an early response, a higher relapse-rate, 10% remains DDAVP dependent and 1/3 has nycturia. The initial DDAVP non-responders need often a combination-therapy, need more time to respond to therapy, but have a lower relapse-rate and have lower drug-dependency.

P32
Determinants for Prescribing of Drugs below the Minimum Licensed Age

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Background: In the light of undesired effects that unlicensed and off-label drug use might have, it is necessary to study determinants which affect prescribing of such drugs. Prescription of drugs to children who are younger than the minimum licensed age may carry the highest risk of adverse reactions.

Methods: To obtain insight into the factors that affect prescription of drugs below the minimal licensed age in children we conducted a population-based case-control study, which was nested in a cohort of 13426 children. The children were aged between 0 and 16 years, and were registered in the Integrated Primary Care Information (IPCI) project, a longitudinal observational general practitioners database in the Netherlands. Cases were all children who received a drug prescription below the minimum licensed age. To each case we matched up to four controls based on GP practice and patient age. As potential risk factors we evaluated use of health care resources, and acute and chronic morbidity.

Results: We identified 447 cases who were matched to 1355 controls. Cases consulted their GPs significantly more often during the preceding half-year, had more drug prescriptions, and had more specialist referrals than controls. Respiratory diseases were the most important determinants for the prescription of drugs to below the minimum licensed age in children. In adolescents, migraine and other headaches were the most important reasons.

*Conclusion*s: This study showed that children suffering from respiratory disease or migraine have the highest risk of receiving a drug prescription below the minimum licensed age. Regulatory authorities and the pharmaceutical industry should be stimulated to improve evaluation of drug efficacy and safety in children.

P33
Opioid Withdrawal in Critically Ill Infants and Children: Validation of a Paediatric Abstinence Score

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Background: The objectives of this study are:
– to construct and validate a paediatric clinical observation scale of opioid withdrawal in critically ill infants and children
– to determine incidence and risk factors of withdrawal associated with prolonged infusion of sufentanil in paediatric intensive care population

Design: Prospective clinical trial.

Setting: Paediatric intensive care unit of a tertiary care centre.

Patients: 29 consecutive patients aged 3 months to 15 years (median:13 months) who had received sufentanil IVC >5 days; 2 patients were excluded due to unrelated medical complications.

Methods: Sufentanil was interrupted abruptly if infusion lasted <216 hours and tapered if infusion lasted >216 hours. We constructed a withdrawal scoring system based on symptoms and signs reported in the medical literature. The children were assessed by the nursing team every 3 hours during the first day and every 6 hours over the following 48 hours. Concomitantly and independently, the medical team evaluated the children clinically and prescribed a standardized treatment with oral methadone if they thought that withdrawal was present.

Results: Underlying conditions were cardiac surgery (23), severe pneumonia (3), and near-drowning (1). Withdrawal occurred in 21 patients (71%).The mean cumulative dose of sufentanil was 260 µg/kg (63-1244) and mean length of infusion was 247 hours (120–495). Cumulative dose of sufentanil >294 µg/kg and sufentanil infusion >266 hours were risk factors for the development of withdrawal (P=0,01 and P=0,001 respectively). The scoring system was validated by comparing scores with clinical diagnosis of experienced doctors. A cut-off for the decision of substitutive treatment was discussed.

P34
Effect of Ibuprofen Prophylaxis on Early Lung Inflammation and Respiratory Morbidity in Preterm Infants

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Background: Data of the possible effect of ibuprofen-lysine on lung inflammation and respiratory morbidity in preterm infants are scarce and not conclusive. Therefore, we evaluated the effect of ibuprofen-lysine on the inflammatory response in the lung and possible effect on respiratory morbidity.

Methods: Study population: neonates with a gestational age <31 weeks, receiving mechanical ventilation after birth were randomized (double blind) to receive either ibuprofen-lysine (10–5–5 mg/kg; 10 mg/ml; q 24h) or saline (1–0.5–0.5 ml/kg; q 24h) i.v. with the first infusion <6 hours of life.Tracheal aspirates, taken within 2 hours after birth and on day 3, were processed for cytokine determination (TNF-alfa, IL-1, IL-6, IL-8, IL-10 and IL-12). Oxygen days and ventilation days were also registered.

Results: We included 46 neonates, of which 24 received ibuprofen-lysine (52%). Fewer small for gestational age infants (8% vs 32%, P=0.045) and more surfactant doses (1.2 vs 0.9 doses, P=0.02) were observed in the ibuprofen-lysine group. No differences in cytokine levels between the two groups were found. Multivariate survival analysis revealed a trend towards longer duration of ventilation (hazard ratio [HR]: 0.29, 95% confidence interval [95%CI]: 0.80–1.06, P=0.06).

Conclusion: Neonates receiving ibuprofen prophylaxis do not have a different cytokine profile in the lung but have a trend towards more ventilation days. The latter should be confirmed in a larger randomized trial.

P35
Analgesic Efficacy of Rectal Acetaminophen Versus Rectal Diclofenac in Children Undergoing (Adeno)Tonsillectomy

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Introduction: (Adeno)Tonsillectomy [(A)TE] is considered a very painful and traumatic procedure. In general acetaminophen (APAP) is prescribed for postoperative pain relief; despite inconsistency in efficacy reports. Diclofenac is suggested as an alternative. Therefore, we conducted a study aiming at comparing the analgesic efficacy of rectal APAP and rectal diclofenac in children undergoing (A)TE in day-stay surgery.

Methods: A RCT was performed in 58 children receiving either APAP (40 mg/kg loading dose, 30 mg/kg 8-hourly maintenance dose) or diclofenac (2 mg/kg loading dose, 1 mg/kg 8-hourly maintenance dose) rectally. APAP loading dose was administered 2 hours before surgery, diclofenac loading dose was administered one hour before surgery. Analgesic efficacy was assessed every 15 minutes during the first hour postoperatively using validated pain scores (VAS, COMFORT and POCIS). After the first hour patients were assessed 1-hourly until discharge. Morphine (5 _g/kg) was administered intravenously when VAS ≥4cm or POCIS ≥4.

Results: Median (25–75th percentile) age was 4 (3–5) years. Both the APAP and the diclofenac group consisted of 29 patients. There was no difference in analgesic efficacy between APAP and diclofenac while the number of patients receiving morphine did not differ between both groups. Coagulation was performed in 14 patients. These children had a lower risk on experiencing painful periods (VAS ≥4 cm) in the first hours postoperatively; odds ratio (95% CI): 0.86 (0.76–0.97). Primary bleeding was experienced by only two patients both receiving APAP.

*Conclusion*s: Diclofenac does not provide more adequate pain relief after (A)TE compared to APAP. Coagulation decreases the risk of experiencing painful periods during the first hours postoperatively. Our data show no increase in incidence of primary bleeding due to the administration of diclofenac, despite its effect on platelet aggregation.

P36
Acetaminophen Concentrations in Cerebro-Spinal Fluid in Children

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Introduction: Acetaminophen (APAP) is one of the most widely used analgesics for mild postoperative pain. Most research is aimed at defining an analgesic APAP plasma concentration. However no correlation was shown between APAP plasma concentrations and analgesic effect. Since the analgesic effect of APAP is thought to be caused by inhibition of the prostaglandin synthesis in the central nervous system, we aimed at measuring APAP levels in cerebro-spinal fluid (CSF) and compare them with plasma APAP levels.

Methods: Serum and CSF APAP concentrations were determined in 35 children 0–9 years of age undergoing elective ventriculo-peritoneal drain insertion. Patients received a rectal APAP loading dose of 16.2–59.0mg/kg 1.5 hours preoperatively. Paired samples of blood and CSF were collected for serum APAP concentration and CSF APAP concentration analysis during the insertion of the ventriculo-peritoneal drain.

Results: Median (25–75th percentile) age was 8 (3–41) months. Median (25–75th percentile) APAP loading dose was 32.3 (25.3–43.6) mg/kg. Median (25–75th percentile) APAP serum and CSF concentrations were 7.5 (1.7–14.3) and 4.5 (1.1–8.5) mg/l.

Conclusion: These are the first data showing APAP CSF concentrations in children with an intact blood-brain barrier. APAP CSF levels are somewhat lower compared to APAP serum levels, possibly due to a delayed onset of the appearance of APAP in CSF.

P37
Non Specific Colitis in Hyper-PGE Syndrome (HPS) under Long-Term Treatment with Indomethacin: Report of Two Patients

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Case reports: We care for about 50 HPS-patients which are all treated with indomethacin since several years. We here report about two patients out of this cohort with the endoscopic, histologic, and radiologic diagnosis of chronic non-specific colitis. No typical signs of Crohn's disease or ulcerative colitis were found (See Table P37).

Case 1: Treatment of the colitis consisted of budenoside, mesalazine, and azathioprine. Budenoside-reduction resulted in an immediate relapse. Reduction of the indo-dose brought a short period of recovery but diarrhea recurred. After 3 years of frustrating trials to treat the colitis as well as the HPS indo was replaced by rofecoxib (0.7 mg/kg/d), a selective COX-2 inhibitor with probably less gastrointestinal side-effects. However, a short period of recovery was followed by a relapse. Finally, all COX-inhibitors were stopped for 2 months resulting in a normalization of bowel habits but aggravation of HPS symptoms. Recently, low-dose treatment with rofecoxib (0.17 mg/kg/d) was re-introduced without intestinal side effects so far, but benefitial effects on HPS symptoms.

Case 2: The treatment of the colitis consisted of mesalazine and budenoside. Indo was withdrawn for 10 days leading to a deterioration of HPS and finally replaced by rofecoxib (0.7 mg/kg/d). Under this regimen, the topic steroids were stopped without relapse. The patient is now well recovered, had returned to normal body weight and normal bowel habits.

Conclusion: Long-term treatment with indo is associated with the risk for non-specific colitis. If COX-2 inhibitors are more beneficial than conventional NSAIDs such as indo remains to be evaluated, especially with respect to the probably physiologic role of COX-2 in the human colon.

P38
Lisinopril: Pharmacokinetic and Pharmacodynamic Characteristics

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Aim of the study: To evaluate lisinopril pharmacokinetic and pharmacodynamic characteristics in children (1-18 y) with and without decreased GFR.

Methods: Lisinopril 1mg/kg followed by bloodpressure monitoring at time, 0, 30, 60, 120, 180 min, 6h, 12h and 24h. Plasmasamples were drawn at time 0, 60, 120, 240min and 24h. Daytime was divided in 3 time-periods for urine-collections, with measurement of GFR (creatinine), and excretion of Na, K, Cl and urinevolume/min. Urinary metabolites of lisinopril were measured to evaluate renal excretion/metabolisation.

Results: In the eight patients with normal GFR, the pharmacokinetic curve is comparable to the findings in adults. In four children with decreased GFR there is a very important increase of area under the curve with a higher maximal plasma concentration. Bloodpressure decreased significantly, but only after 4h, and persisted for 24h, despite the lower plasmaconcentrations at 24h. Pharmacodynamic effects on renal function and excretion were significant, but minimal after 12h.

Conclusion: The PK-study demonstrates that the PK-prophyle in children is comparable to adults, however in presence of decreased GFR, we should adapt doses earlier. The pharmacodynamic effects are not directly related to the plasmaconcentrations, but probably to the tissue activity.

Table P37.

	Sex	Genetics	Start of indo treatment (age) treatment	Duration of indo	Indo dose (mg/kg/d)	Age at colitis manifestation
Case 1	Male	ROMK	11 weeks	7 years	1-3	4 years
Case 2	Female	NKCC2	1 year	17 years	1-5	18 years

P39
Association of Deptropine Use with Neuropsychiatric Events in Children

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Background: Published case reports associated the antihistamine deptropine, which is unlicensed but regularly used for allergic rhinitis and asthma in children, with neuropsychiatric events. We conducted a retrospective cohort study to investigate this association.

Methods: By using the computerized medical records of the Integrated Primary Care Information (IPCI) database we identified all prescriptions for systemic antihistamines issued to children between 1 and 10 years of age during the study period 1st January 1995 and 1st January 2000. The occurrence of hallucinations, confusion, agitation, aggressive behaviour or ataxia during use of antihistamines were assessed by medical reviewers who were blinded to exposure. The risk of any of these neuropsychiatric events during use of deptropine was compared to the risk of such events to other antihistamines who were also prescribed for respiratory indications.

Results: The final study cohort comprised 1657 users of deptropine and 4515 users of other antihistamines among which promethazine, ketotifen and loratidine were most frequently used. The incidence rate of neuropsychiatric reactions was 5.6 (95% CI: 1.6–19.3) times higher during use of deptropine than during use of other antihistamines. Deptropine was associated with an excess rate of 3.1 cases per 100 person-years of exposure. The incidence of neuropsychiatric reactions was 3.8 fold higher in the first episode of use than in subsequent episodes and was highest for children 2–10 years of age.

*Conclusion*s: Our study demonstrated that deptropine use is associated with an increased risk of neuropsychiatric events in children. As this drug is not licensed for use in children and therapeutic alternatives are available, its use should be discouraged.

P40
Off Label and Unlicensed Drug Use in the Cardiology Department at the University Children Hospital in Belgrade

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Background: Use of drugs in an off label or unlicensed manner to treat children is a widespread phenomenon in the Europe and USA. The incidence of unlicensed and off label prescribing in paediatric practice in Yugoslavia has not been studied.

Method: This study was designed to assess the extent and nature of off label and unlicensed drugs use in paediatric cardiology inpatients. In a retrospective study, drug prescriptions in the paediatric cardiology ward during two years period were reviewed. Data were collected and analyzed by special software created for this purpose.

Results: Children (P=544) were aged from 4 hours to 18 years. One or more off label and unlicensed prescriptions were given to 414 (76%) patients. Of 2130 prescriptions given during the two year period, more than one half were unlicensed (11.58%) or off-label (47.17%). Children aged 2–11 years received most of the unlicensed drug prescriptions (16.78%). On the other hand neonates, who didn't receive unlicensed drugs, are leading (64%) in the use of off label drugs.

Conclusion: This study shows that the problem of off label and unlicensed drug use exists in Yugoslavia too. These findings imply that the phenomenon of off label and unlicensed use of drugs in children seems to be correlated rather to the deficiency of paediatric drugs formulation on the global market, than to the socioeconomic status of the country.

P41
Safety-Monitoring in Dose-Response Ace-Inhibitor Studies in Hypertensive Children

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Since the FDA decided in 2000 that every drug of which data were available in children, could obtain a prolongation of their registration for 6 m, the number of studies in children has increased exponentially. However the protocols, inclusion criteria and monitoring are not adapted to paediatric needs, but are a simple copy of the FDA demands for adults. We participated in a prospective randomised placebo controlled study with lisinopril.

Aim of the study: Critical appraisal of inclusion-, exclusion-criteria and monitoring of a placebo-controlled cross-over study with lisinopril (dose-response-study).

Methods: It was a three-branch design with different doses between 0.625 mg, 2.5, and 10 mg in children <50 kg, and doubled when >50kg. Each patient received different doses. Monitoring of bloodpressure and renin+ aldosteron-levels.

Results: Only 5/74 patients of our children with hypertension were elegible for inclusion, limiting the clinical relevance of these kind of studies. Although there was a dose-response effect on blood pressure, some patients had extremely high renin-levels >2000µU/ml with severely suppressed aldosteron suggesting an overdosing, despite the fact that hypotension was not observed. No other side-effects were objectivated.

Conclusion: This data indicate that monitoring of only bloodpressure during dose response studies with antihypertensive drugs in hypertensive children, with still highly reactive and compliant vessels, may lead to overestimation of safetiness. Definitely the study-protocols derived from adult studies will lead to the wrong conclusions. Therefore study-protocols must be adapted.

P42
Cyclooxygenase-2 in Renal Development and Physiology

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Prostanoid metabolism is initiated by the enzyme Cyclooxygenase (COX) which can be blocked by nonsteroidal antiinflammatory drugs (NSAID). Two isoforms of COX are known. COX1 is expressed constitutively in most tissues including the kidney. COX2 is usually absent but can be induced by various stimuli. In contrast to most other tissues COX2 is also constitutively expressed in the kidney. The use of mice with targeted disruption of either COX gene and isozyme selective NSAIDs allowed the identification of the crucial roles of COX2 in renal development and physiology.

Identical to COX2 ko mice, administration of a COX2 selective NSAID results in severe cortical dysplasia, characterized by a reduction in glomerular number and size. Coexpression of COX2 and prostacyclin-synthase in the nephrogenic cortex may indicate that prostacyclin acts downstream of COX2 to promote nephrogenesis.

Salt-depletion and/ or administration of furosemide in animal species induces COX2 expression in the macula densa, which stimulates secretion of renin. In patients with Hyperprostaglandin E2-Syndrome (HPE), who are the genetic counterpart to chronic furosemide treatment, we could demonstrate enhanced expression of COX2 mRNA and protein in cells of the macula densa. Administration of a COX2 selective NSAID Vioxx™ reduced excessive salt- and water losses as well as renin secretion in HPE patients to control levels. Colocalization of COX2 and microsomal Prostaglandin E synthase (mPGES) in the macula densa clearly indicates that PGE2 stimulates renin secretion in HPE patients.

Our data demonstrate that a COX2 selective NSAID should not be used in pregnant women. To avoid gastrointestinal side affects seen with conventional NSAIDs such as Indomethacin a COX2 selective NSAID might be advantageous in patients with HPE.

P43
A Survey of Paediatric Adverse Drug Reaction Reports in Sweden 1988-2000

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Background: Paediatric patients are often prescribed drugs without adequate documentation in this group to secure the correct use of the drug. The dosing strategy is often extrapolated from adult recommendations and drugs are many times used in an off-label manner. In this retrospective study we studied the frequency and type of reported paediatric adverse drug reactions (ADRs) in Sweden over a period of 13 years (1988–2000). Our aims were to identify and describe the reported drugs and types of ADRs in paediatric patients.

Methods: Data from spontaneous reports to the Swedish ADR register (SWEDIS) age groups 0–15 years was analysed for the period 1988–2000. Only reports with "certain", "probable" or "possible" causality assessment were studied.

Results: The total number of reports over the period was 4961. More than 50% were reports of ADRs related to vaccines with some variation between certain years due to alterations in the vaccine development program for infants. The number of serious ADRs was 883 or 14% of the reported 6715 ADRs (more than one ADR related symptom can be reported in one ADR report). The three most reported group of drugs in the 883 reported serious ADRs, according to the ATC (Anatomical Therapeutic Chemical) classification, were J07 (vaccines), J01 (antibacterials for systemic use) and N03 (antiepileptics). If vaccines were excluded the reporting frequency was stable at approximately 170 reports annually. The most frequent ADR diagnosis was "application site reaction" (1716) followed by "fever" (766) and "exanthema" (455). The number of reports / 1000 prescriptions in year 2000 was 0.40 in children compared to 0.23 in adults (vaccines excluded).

Conclusion: Over 50% of the ADR reports in paediatric patients are related to local reactions and fever in connection to vaccines. The number of spontaneous ADR reports is quite stable over the period when vaccines are excluded. There were almost twice as many paediatric ADR reports/ prescription compared to adults (vaccines excluded). Further studies are needed to clarify the underlying factors to this observation.

P44
Optimal Sampling Strategies for the Assessment of Inulin Clearance in Children

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Background: Assessment of inulin clearance is a generally accepted method to determine the glomerular filtration rate (GFR) in patients with renal disorders. In our hospital two methods are used: the continuous intravenous infusion method (gold standard) and the single injection method. The single injection method has several advantages (no collection of urine is necessary and no overnight hospitalisation is required). After administration of an iv bolus dose of inulin 12 blood samples are drawn during a time-period of 240 min and a concentration-time-curve is constructed. For practical and convenient application in children it is important that the number of sampling times for the determination of GFR is minimal. The aim of this study was to develop and validate optimal sampling strategies with only 2–4 blood samples and to reduce the total sampling time of 240 min for the estimation of inulin clearance following a single injection in children.

Methods: Complete inulin plasma concentration-time curves of 154 patients were available. All patients received a single intravenous dose of Inutest® (5000mg/1.73m^2) and blood samples were collected at 0, 10, 20, 30, 45, 65, 90, 120, 150, 180, 210 and 240 minutes after injection. The curves were divided in an index (P=100) and a validation data set (P=54). A population pharmacokinetic model was developed for the index set using 'nonlinear mixed effect modelling' (NONMEM). Optimal sampling times were selected based on the 'D-optimality criterion'. From these optimal sampling times 14 sampling strategies were designed. For the validation data set individual Bayesian estimates of clearance were generated using the derived population pharmacokinetic model and 2–4 optimally sampling times. The predictive performance of the Bayesian sampling strategies was assessed by calculating the mean relative prediction error (MPE) as measure of bias and the root mean relative squared prediction error (RMSE) as measure of precision.

Results: The strategies with samples taken at 10/30/90/240 min, 10/30/240 min, 10/90/240 min, 30/90/240 min and 90/240 min produced good predictions of clearance of inulin with a bias less than 3% and not significantly different from zero and a precision less than 15%. Reduction of the total sampling time of 240 minutes resulted in a decrease of predictive performance.

Conclusion: At least two samples and a total sampling time of 240 min are required for adequate Bayesian estimation of inulin clearance in children using the inulin single injection method.

P45
Nonparametric Population Pharmacokinetic Analysis of Amikacin in Neonates, Infants and Children

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Background: The therapeutic and toxic effects of amikacin are known to depend on its plasma concentration, but the pharmacokinetics of this drug in neonates, infants and children and the influence of clinical and biological variables have only been partially assessed.

Methods: Therapeutic drug monitoring data collected from 155 patients (49 neonates, 77 infants and 29 children) receiving amikacin were analyzed by a nonparametric population-based approach, the nonparametric maximum likelihood (npml) method. We assessed the effect of gestational and postnatal age, weight, apgar score, plasma creatinine and urea concentrations on pharmacokinetic parameters. There is no specific formulation of amikacin for neonates and infants. We therefore used an error model to account for errors due to dilution during preparation of the infusion.

Results: The covariates that reduced the variance of plasma clearance and distribution volume by more than 10% were postnatal age (43 and 28% respectively) and body weight (30.4 and 17.4 respectively). The expected reduction of clearance was about 10% for plasma creatinine. The other covariates studied (apgar scores, plasma urea concentration, gestational age, sex) were found to have little effect. Simulations showed that a smaller percentage of patients had a Cmax/MIC ratio greater than 8 with a 15 mg/kg b.i.d. regimen than with a 15 mg/kg once a day regimen, for MIC values of 1 to 8 mg/l.

P46
Retrospective Analysis of Efficacy and Tolerability of Tolterodine in Children with Overactive Bladder

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Objective: To evaluate the efficacy and tolerability of tolterodine in children with an overactive bladder, treated in a single enuresis centre.

Materials and methods: We performed a retrospective analysis of a database of a total of two hundred and fifty-six patients (175 boys and 81 girls, age range 3 years to 17 years, mean age 8.33 years) with urodynamically confirmed bladder overactivity. The children received tolterodine tartrate (dose range of 0.5–4mg orally) replacing anticholinergic drugs (oxybutinin cloride or oxyfencyclimine-hydrocloride P=205, group 1) or as initial therapy (P=51, group 2). Tolerability was assessed from adverse events by a standardised questionnaire. Efficacy assessment was based on micturition diary variables, mean change of maximum bladder capacity and number of incontinent episodes/24h.

Results: The mean treatment time was 9.32 months with a range from 1.5 months to 23.4 months. The final dose was 0.1 mg /kg orally daily, divided into two doses. In group 1, 60.4% switched to tolterodine due to serious adverse events during treatment with non-selective antimuscarinic drugs. Central nervous system disorders (81%) were the most common adverse events, 26.2% showed flushing, 12.2% had abnormal visual accomodation and 25.2% had gastrointestinal complaints (constipation, diarree, abdominal pain). Withdrawal of the non-selective antimuscarinic drug resulted in total recovery from adverse events. The remaining 39.6% of group 1 switched tot tolterodine due to lack improvement in micturition variables. Introduction of tolterodine in group I and II caused no serious adverse events. Nine patients (3.5%) reported side effects and only 2 discontinued treatment. There were no reports of flushing, hyperpyrexia and troubles of visual accomodation. In group 1 we observed a mean decrease in urgency by 38.7%, a mean increase in maximal bladdercapacity by 33.6% and the number of incontinence episodes decreased by 64.8%. In group 2, we observed equivalent values with a significant (P<0.001) change in maximal bladdercapacity (49.7%), incontinence episodes (64.8%) and micturition episodes / 24 h.

*Conclusion*s: The results of this retrospective analysis suggest that tolterodine is well tolerated in children and offers an effective treatment for urinary symptoms due to overactive bladder. Tolterodine is superior to non-selective antimuscarinic drugs, with respect to adverse events, allowing more compliance and more effective treatment in children.

P47
Intrathecal Aminoglycoside Therapy for Pseudomonas Aeruginosa Meningitis in a Child

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We report the case of a 8-month-old boy, who developed *Pseudomonas aeruginosa* infection of the central nervous system (CNS) following a neurosurgical procedure. Although the bacteria was sensitive to many antibiotics, no improvement could be achieved with intravenous antibiotics (including carbapenems, aminoglycosides and quinolones). We also feared the co-infection of the Rickham device for ventriculo-peritoneal bypass, which could have led to mandatory removal of this device, with many concerns in this malnourished child. We then shifted to intrathecal aminoglycoside therapy for 11 days, with clinical and biological cure.

Initial doses were empirically chosen (5 mg q24h) and then adapted (10 mg from day 3 to day 11), depending on CSF aminoglycosid concentration obtained at H24. Our goal was to obtain CSF concentration 5–10 times higher than *Ps. aeruginosa* MIC (at least 15–20 µg/ml).

Intrathecal use of antibiotics is an uncommon way of treating CNS infections due to resistant infectious agents. Even powerful *in vitro* antibacterial agents may be useless *in vivo* because of their lack of diffusion at the site of infection. Only a few studies show efficiency of this way. Little is known about required doses and duration of treatment, especially after the neonatal period. Addition of various single experiences may help to define guidelines for this alternative choice for treating meningitis.

P48
The Triptans, a Significant Therapeutic Advance in Migraine Treatment, Still Not Labeled For Children Over 10 Years After Introduction

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We investigated plausibility of a common argument that lack of licensed and labelled medicines for children is due to a small size of the paediatric market. We performed a case study of the triptans, the most recent significant group of medicines introduced for the treatment of migraine, a common condition in adults and children.

Migraine is one of the most common types of recurrent pain in children. In developed countries 3–7% of children and 10% of adolescents (>8 million individuals) suffer from migraine. Of the attacks 30–60% can be controlled with simple analgesics; for the rest other treatments are needed. As migraine is often familial, parents usually know, and use triptans with success themselves.

The triptans are serotonin 1 receptor agonists, specially developed for the treatment of migraine attacks. Sumatriptan, the first triptan, was launched in 1991. Since then six other triptans (zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan, and frovatriptan), have received marketing authorisation for adults. Their efficacy is well documented. The triptans are considered a significant therapeutic advantage in the treatment of migraine in adults, and they have gained a considerable market share (estimated US triptan market value over 1000 million US$).

Five controlled trials of sumatriptan in the paediatric age group have been published (two as abstracts); three of them investigator initiated (one as an abstract). Less or no paediatric data at all are available for the other triptans. Sumatriptan nasal spray, authorised for 12 to 17 year-old adolescents in Australia in 2002, is the only triptan labelled in the paediatric age group.

The case of the triptans indicate, that a common condition in children treatable by the very same products already on the market for adults, has not proven commercially attractive enough for the pharmaceutical industry to license their products in this age group. Even studies are few and to a large extent investigator initiated.

Table P49.

	I	II	III	p
Patients	334	340	306	0.67
GA (weeks): mean	35.6	35.5	35.3	0.8
(range)	(24–42)	(24–42)	(24–42)	
Hospitalisation days: mean	12	12	13	0.6
(range)	(2–128)	(1–153)	(1–104)	
Deaths	15	7	9	0.18
Sepsis events	16	13	10	0.6
Colonisation strains (all sites incl.)				
Enterobacteriacae (n)	533	566	492	
Group 1 (%) (E. Coli), Proteus mirabilis)	34.4	48.4	43.6	
Group 2 (%) (Klebsiellae, Citrobacter koseri)	26.0	29.7	34.6	
Group 3 (%) (Enterobacter Citrobacter)				
gr. Freundii Morganella Proteus vulgaris	39.7	21.5	21.7	0.001

P49
Influence of Antibiotic Policy on Bacterial Epidemiology in a NICU

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Introduction: Widespread use of empiric broad-spectrum antibiotics (ABs) for early-onset infection in neonates can select multi-resistant bacteria. The bacterial epidemiology for early-onset neonatal infection has been remarkably stable along the last decades. We postulated that switching to narrower spectrum ABs in empiric antibiotic policy could modify the selective pressure among *Enterobacteriacae* and decrease the emergence of resistant inductible bacteria.

Design: Twenty two months prospective epidemic survey comparing 3 periods (I,II,III) of different ABs empiric treatments for early-onset infection.

Methods: I (7 months): Ampicilllin+Amikacin; III (7 months): Penicillin+Amikacin; II (in between, 8 months) equal mix of both treatments. Systematic bacterial screening included blood culture and multiple swabs on admission. Multiple swabs and faece culture were repeated once a week. Additional cultures were taken when clinically indicated. Antibiograms were done with the Viteck2 System, (Biomerieux). Data on all hospitalized neonates were collected during the study course.

Results: (See Table P49). A trend toward a decrease of *Enterobacteriacae* resistance to all AB's but Piper. and Cotrimo. is also observed. The three periods were comparable in terms of length of treatment, morbidities, neonatal complications.

*Conclusion*s: Switching empiric AB's policy for early-onset infection in a NICU to narrower spectrum AB's does significantly modify bacterial population among *Enterobacteriacae*. It is associated with a trend toward a decrease in AB's resistance in that bacterial population without increase of morbidities.

P50
Effects of N-Nitro-L-Arginine Methyl Ester on the Renal System of Neonatal Piglets During Endotoxemia

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Background: Nitric oxide is believed to be a major vasoactive mediator involved in kidney perfusion during septic shock of the neonate. Our objective is to gain a clearer understanding of the mechanism involved in kidney hypoperfusion observed during sepsis by examining the effects of N-nitro-L-arginine methyl ester (NAME), a nitric oxide synthase inhibitor, on the kidneys of neonatal piglets during endotoxic shock.

Design/Methods: Piglets were randomized into a control group (P=5), which was resuscitated with 0.9% NS, or an experimental group (P=5) that received NAME at a rate of 50 mg/kg/min after endotoxic shock was induced by injecting 0.06 mg/kg of endotoxin. Mean arterial pressure (MAP), glomerular filtration rate (GFR), and urine output (UOP) were plotted at baseline, 1 hour, 2 hours and 3 hours with renal blood flow (RBF) and renal vascular resistance (RVR) calculated at baseline, 1 hour and 3 hours via a radioisotope technique. UOP was monitored via a suprapubic cystostomy. At completion, piglets were sacrificed, and kidneys removed, weighed and counted for radioactivity. Statistical significance was determined by Student's t-test.

Result:s (P<0.05 for 1 hour, 2 hours, or 3 hours *vs* Baseline^ and NAME *vs* fluid*)(See Table P50).

*Conclusion*s: Inhibition of nitric oxide with NAME produces a detrimental effect on renal function with a significant drop in UOP, GFR, RBF AND RVR when compared to fluid resuscitation alone. Nitric oxide has an important role in maintaining kidney function during endotoxic shock in a neonatal piglet model.

Table P50.

	0.9% NS (*n*=5)				NAME 50 mcg/kg/min (*n*=5)			
	Base-line	**1 hour**	**2 hours**	**3 hours**	**Base-line**	**1 hour**	**2 hours**	**3 hours**
UOP	0.20 ±0.03	0.18 ±0.09	0.54 ±0.06∧	0.12 ±0.08	0.77 ±0.43	0.30 ±0.14∧	0.15 ±0.10∧*	0.04 ±0.03∧*
GFR	0.20 ±0.05	0.20 ±0.05	0.21 ±0.05	0.11 ±0.04∧	0.24 ±0.03	0.18 ±0.14∧	0.11 ±0.06∧*	0.04 ±0.03∧*
RBF	1.84 ±0.38	1.72 ±0.26		0.97 ±0.34∧	1.88 ±0.60	0.96 ±0.16∧*		0.36 ±0.21∧*
RVR	2.49 ±0.32	2.76 ±0.87		3.18 ±0.18	2.03 ±0.64	3.94 ±0.63∧*		6.41 ±1.48∧*
MAP	85±8	80 ±11	67±10	74±12	77±6	85±10	59±6∧	64±17

P51
Functional Analysis of Romk Mutations: Correlation with the Clinical Phenotype

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Background: Mutations in the renal potassium channel ROMK lead to a life-threatening salt-losing tubular disorder with antenatal onset due to defective salt reabsorption in the medullary thick ascending limb of Henle's loop, the Hyperprostaglandin E syndrome/antenatal Bartter syndrome. Clinical characteristics include fetal polyuria leading to polyhydramnios and preterm delivery. Postnatally, affected patients show severe salt- and water-wasting and marked hypercalciuria with consecutive nephrocalcinosis.

Methods: After heterologous expression in Xenopus oocytes and a human kidney cell line 19 naturally occurring ROMK mutations were analyzed by two electrode voltage clamp analysis in oocytes and immunofluorescence in oocytes and HEK293 cells.

Results: We identified 14/19 ROMK mutants that did not reach the cell surface indicating defective trafficking. High expression levels rescued 6/14 ROMK mutants leading to membrane-positive staining and significant K^+ currents. The remaining 5/19 mutants showed either decreased K^+ currents which is most likely due to defective regulation and/or putative conformational changes or resulted in inframe premature stop mutations.

Conclusion: In contrast to previous reports most of the investigated ROMK mutations displayed a trafficking defect that might be rescued by pharmacological agents acting as molecular chaperones. As already shown for CFTR or vasopressin receptor mutations, inframe stop- and trafficking-mutations are targets for successful pharmacological restoration, e.g. by application of aminoglycosides, butyates and/or specific agonists/ antagonists. The clinical relevance of an at least residual K^+ conductance is reflected by the mild phenotype and late clinical onset of a patient harboring a homozygous ROMK mutation which shows a significant residual function in electrophysiological recordings. This indicates that even partial restoration of ROMK mutants might effectively alleviate the disease phenotype.

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P52
Comparative Efficacy of Paracetamol-Sucrose Syrup and Sucrose Alone for Procedural Pain in Preterm Neonates

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Objective: To determine whether paracetamol (15mg/kg orally) in sucrose syrup is effective compared to sucrose 28% alone and placebo in reducing pain during heel lance procedure in preterm neonates.

Design: Prospective, randomized, placebo – controlled trial.

Subjects, inclusion criteria and methods: Preterm neonates (*P*=90): 27–35 weeks gestation, birth weights: 770–2600 gr (41 females, 49 males) were randomized and divided into three groups (*P*=30/group):

- Group 1: paracetamol (15 mg/kg/dose orally + sucrose 28% given 30 minutes before heel prick)
- Group 2: sucrose (28%) syrup alone
- Group 3 placebo.

Outcome variables included physiologic parameters – heart rate (HR) (primary outcome), respiratory rate (RR) and oxygen blood saturation (O_2 sats by pulse oxymetry) were measured during three periods: before, during heel prick and during heel squeeze. Drug treatment groups were compared with placebo using chi square test, ANOVA and MANOVA.

Results: Both paracetamol and sucrose group had heart rate acceleration lower than placebo during heel squeeze (F=9.5, *P*<0.01). Respiratory rate also had a lower increase in the paracetamol and sucrose group compared to placebo during heel prick (F= 7, *P*<0.01) and during heel squeeze (F=8.2, *P*<0.01). Both paracetamol and sucrose groups had a higher oxygen saturation compared to placebo group wherein oxygen saturation decreased during heel prick (F=12, DF=2, *P*<0.01) and heel squeeze (F=20, DF=2, *P*<0.01) (See Table P52).

Conclusion: Paracetamol in sucrose syrup and sucrose 28% alone attenuated pain responses due to heel prick with equivalent effects. Sucrose with or without paracetamol appears effective and may be sufficient to minimize acute pain during heel pricks in newborns.

Table P52.				
Variables	**Study Phase**	**Group 1 Paracetamol+ sucrose 28% (*n*=30)**	**Group 2 Sucrose 28% alone (*n*=30)**	**Group 3 Placebo (*n*=30)**
HR,beats/min	Before	127 ± 17	133 ± 22	125 ± 22
	Heel prick	132 ± 19	137 ± 21	139 ± 20
	Heel squeeze	139 ± 20*	139 ± 28*	147 ± 17
RR(bpm)	Before	27 ± 11	25 ± 10	27 ± 11
	heel prick	28 ± 12*	27 ± 11*	37 ± 12
	Heel squeeze	30 ± 11*	30 ± 18*	41 ± 12
Oxygen sats%	Before	98 ± 2	98 ± 4	97 ± 2
	Heel prick	98 ± 2*	97 ± 5*	93 ± 3
	Heel squeeze	97 ± 5*	96 ± 5*	90 ± 3

Values are expressed as mean ± SD, *denotes P<0.05 compared to placebo.

P53
Furosemide in Preterm Infants Treated With Indomethacin for Patent Ductus Arteriosus (PDA)

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Background: Concurrent administration of furosemide and indomethacin in preterm infants treated for PDA is controversial. Furosemide may protect against indomethacin induced nephrotoxicity, but also may impair ductal closure. Because a Cochrane review in 1998 mentioned only three randomised trials that assessed the effect of furosemide on renal function in preterm infants with PDA, we aimed to evaluate this in our population.

Methods: Multicenter case control study of 64 preterm infants <32 weeks treated for symptomatic PDA with indomethacin i.v. 3 0.2 mg/kg, q12 hr. 32 infants with furosemide (3 1 mg/kg iv) as co-medication (IF-group) were compared with 32 matched controls without furosemide (IN-group). Patient characteristics, ductal closure rate and renal side effects (urine output, serum creatinine and sodium concentration) were registered.

Results: Neonatal characteristics were comparable (BW: 1040 ± 270 *vs* 1050 ± 270 g; GA: 27.9 ± 1.5 *vs* 27.9 ± 1.5 wk; day of therapy: 3.7 ± 2.0 *vs* 3.5 ± 1.9 d; respectively IF *vs* IN). The ductal closure rate was similar in the two groups (IF 88% *vs* IN 75%). In the furosemide group there was a significant increase in serumcreatinine (33 ± 14 *vs* 13 ± 21 µmol/l; *P*<0,001) and decrease in serumsodium (–8.5 ± 6.3 *vs* –3.3 ± 6.0 mmol/l; *P*=0,004) but no difference in urine output as compared to the control group.

Conclusion: Additional use of furosemide to indomethacin did not compromise ductal closure, but did result in a significant increase in serum creatinine and hyponatremia without influencing urine output.

P54
Surfactant Replacement Therapy in Term Infants with Respiratory Failure

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Background: Term infants with respiratory failure may have secondary surfactant deficiency. We aimed to determine the response to two types of exogenous surfactant administration in term infants with respiratory failure which was not caused by the idiopathic respiratory distress syndrome.

Methods: Infants with gestational age >36 weeks ventilated from birth with supplemental oxygen concentration >60%, were randomly assigned to receive endotracheal bolus instillation of either bovine lung lavage surfactant (Alvofact) or bovine lung minced surfactant (Survanta). Perinatal characteristics, diagnoses, complications and respiratory evolution (oxygen supplement, mean airway pressure, oxygenation index; at baseline and +1 and +4 hours) were registered.

Results: 66 infants received Alvofact and 53 Survanta. Mean birthweight (2750 vs 2930 g), gestational age (36.4 vs 36.9 weeks) and baseline ventilatory support were comparable between groups. In the Alvofact group, a significantly greater decrease of oxygen supplement was observed 1 hour after the instillation (–26% vs –17%; P = 0.04) and a smaller dose of surfactant needed to be administered (83 vs 100 mg /kg; P = 0.001). No significant differences were observed with regard to complications and outcome. Irrespective of type of surfactant, the amelioration of the respiratory parameters was comparable among the different diagnostic groups (pneumonia and/or septicemia, meconium aspiration, persistent pulmonary hypertension of the newborn).

Conclusion: Alvofact causes a faster positive respons than Survanta. Surfactant is an effective adjunctive treatment in term infants with respiratory failure which is not caused by the idiopathic respiratory distress syndrome.

P55
Intestinal Colonisation In Preterm Infants

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Intestinal colonization in preterm infants was studied in two groups of premature infants: 14 infants suffering from gastrointestinal bleeding, an early stage of NEC (referred as pre-NEC infants) and 19 healthy preterm infants. Each pre-NEC infant was matched with one or two healthy control infant(s) with regards to gestational age (30±3 weeks) and age of sampling (median postnatal age 25 days, range 14–78 days). For pre-NEC infants, feces were collected at the beginning of the bleeding. Quantitative studies of the aerobic and anaerobic flora were performed. Calprotectin, a marker of inflammatory disease in the lower gastro-intestinal tract, was measured in feces by the means of an ELISA (Calprest, Eurospital, Italy. Results were statistically analyzed using the χ^2 test or exact Fisher test for flora analyses and the Mann and Whitney test for calprotectin levels.

Analyses of the gut microflora underscores the paucity of the bacterial flora with 3–4 species by infant. Frequent colonisation with antibiotic resistant microorganisms and microorganisms from the environment, *e.g*. coagulase negative staphylococci or clostridia was observed. Gut colonisation was similar between the two groups, except for *Bifidobacterium*, *i.e*. none of the pre-NEC infants were colonized, whereas 7/19 healthy preterm infants harbored high levels of bifidobacteria (*P*=0.04). Concerning calprotectin, although non significant, higher level was observed in the pre-NEC infants (1724 ± 1355 µg/g of feces) as compared with the healthy ones (896 ± 572 µg/g of feces).

These results focus on the aberrant colonisation of the preterm infants, associated with a high level of calprotectin, even without gastrointestinal disease. Bifidobacteria was demonstrated in several studies to have immunomodulating effects. Then, the significant difference in bifidobacteria colonisation between the two groups raise the question of the modulation of the gut microflora in preterms by probiotics, prebiotics or both.

P56
Relationships Between Maximal, Minimal Blood Concentrations, AUC of Cyclosporin A and Acute GVHD in Paediatric Stem Cell Transplantation

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Background: Despite recent advances in prophylaxis, the success of allogeneic stem cell transplantation (SCT) is offset by the risk of graft-*versus*-host disease (GVHD). Cyclosporine A (CsA) efficiency is inconstant because a high pharmacokinetic variability during the first month following the transplantation. Moreover, relationships between CsA exposure during the early post-transplant period and its efficiency in aGVHD prophylaxis remains largely unknown. The aim of this study was to compare relationships between: maximal, minimal CsA blood concentrations (T_{max}, T_{min}), AUC and occurrence of aGVHD.

Methods: 67 children aged from 3 months to 18 years received a SCT from unrelated donors (*P*=34) or familial donors (*P*=33) for treatment of leukaemia (*P*=36), severe aplastic anemia (*P*=12), hemoglobinopathies (*P*=4), inborn errors of metabolism (*P*=11) and immunodeficiencies (*P*=4). GVHD prophylaxis consisted of CsA alone (*P*=16) or associated to a short course of methotrexate (*P*=48). Antithymocyte globulins were used if the donor is unrelated. CsA whole blood concentration was measured twice weekly during the early post-transplant period, by an immunoenzymatic assay (EMIT). CsA regimen was monitored by Maximum A Posteriori (MAP) bayesian fitting procedure from day +1 until end of therapy. Retrospectively, mean T_{max}, T_{min} and AUC corresponding to first two weeks post-transplant were estimated by MAP bayesian fitting procedure and compared between patients developing grade II–IV and those developing no or grade I aGVHD.

Results: Incidence of aGVHD was 41.8% (grade I: *P*=16, grade II: *P*=6, grade III: *P*=2, grade IV: *P*=4). Tmin were lower in patients who developed grade II–IV aGVHD *versus* those developing no or mild aGVHD (week 1 post-transplant: 52±9 *versus* 100±6ng/ml, *P*=0.008, (week 2: 61±9 *versus* 99±7ng/ml, *P*=0.016). Patients with mean T_{min}>85ng/ml during the first two weeks post-transplant developed only grade I (*P*=3) or no aGVHD (*P*=18). Patients with T_{min}<85ng/ml developed grade II–IV (*P*=12), grade I (*P*=11) or no aGVHD (*P*=18). T_{max} were similar in two groups: respectively 480±72 *versus* 514±42ng/ml, *P*=0.337 (week 1), 371±70 *versus* 462±34ng/ml, *P*=0.443 (week 2). AUC were similar in two groups: 45±19 *versus* 49±7 ng/ml h, *P*=0.275 (week 1), 25±5 *versus* 37±4ng/ml h, *P*=0.100 (week 2).

Conclusion: Our results had shown a strong association between T_{min} during the first two weeks after transplant and occurrence of aGVHD, despite a diversity in characteristics of patients, donors and in immunosuppressive drugs associated to CsA. On the other hand, T_{max} and AUC were not associated to aGVHD. In order to monitor CsA therapy following paediatric SCT, our results suggest that T_{min} should be used rather than AUC or T_{max}.

P57
Real Time Quantitative RT-PCR of CYP3A in Placenta

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Background: CYP3A is present in the placental but little is known on variations of expression with term or maternal treatment. Corticostéroids are known to induce the expression of cytochrome P450 3A, the predominant cytochrome P45O in hepatic cells. In a prospective study, we compared CYP3A4, 3A5 and 3A7 mRNA expression in human placenta of normal term pregnancies and betamethasone treated pregnant women.

Methods: Total mRNA was extracted with the use of RNA-PLUS™. After a reverse transcription (RT) reaction, a real time mRNA quantification was performed by the ABIPRISM 7700 SDS technology. The method used a fluorogenic probe to generate sequence-specific fluorescent signals during PCR. The initial amount of target mRNA in each sample is estimated using a standard curve with known amounts of plasmids containing the cDNA of interest. Forward and reverse primers and the fluorogenic TaqMan probe were designed by Primer Express software (PE Biosystems). The difficulty in the choice was the high sequence homology between the 3 CYP genes (>80%). TaqMan probes were used for CYP 3A5, CYP3A7 and a MGB-probe was used for CYP3A4. The use of 18S allowed normalisation of variation in reverse transcriptase efficiency in the cDNA reactions and differences in the amount of mRNA added to each reaction.

Results and conclusion: CYP3A4 was detected at a very low level (0–100 copies/50ng cDNA). CYP3A5 has a heterogeneous expression (0–1000 copies/50ng cDNA). CYP3A7 was detected at a very high level ranging from 0 to 10000 copies per 50 mg/cDNA. Term and corticoid treatment appeared as determinant factors for this high expression.

P58
Expression of CYP3A and P-gP in Human Placenta: A Prospective Controlled Study

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Background: The ontogeny of cytochromes P450 3A (CYP3A) in the human liver showed an increase in CYP3A4, the adult form from birth to childhood together with a corresponding decrease in CYP3A7, the main fetal form. CYP3A is known to be present in the placental syncytiotrophoblast but data on variations of expression with term or maternal treatment are very limited.

Methods: In a prospective control study, we collected placentas from normal term pregnancies and pregnancies treated with betaméthasone because of preterm labor. We analysed the expression of CYP3A and P-gP in normal and treated biopsies by immunoblot studies performed using a polyclonal (Pr Beaune, Paris) and a monoclonal (Pr. Kremers (Liège) rabbit anti human CYP3A antibody, and a mouse anti human monoclonal P-gp antibody (C494, Dako-Denmark) on formalin-embedded tissue sections.

Results: CYP3A and P-gP were expressed in almost all the samples, whatever the term and treatment. CYP3A expression was located in the syncytiotrphoblast, endothelial cells and cytotrophoblast. P-gp was expressed with high intensity in the membrane border of endothelial cells.

Conclusion: A complementary study quantifying specific CYP3A mARN using a real time qantitative RT-PCR is currently performed. These findings will lead to a better comprehension of the placental metabolism of drugs administered during pregnancy.

P59
Antenatal Treatment with Corticoids Affects the Expression of Dopamine D1-Receptor mRNA in the Adrenals of Developing Rabbit

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Background: Antenatal treatment with corticoids is widely used in the aim of enhancing pulmonary fetal maturation. The adrenal gland plays a key role in perinatal adaptation by releasing dopamine and other catecholamines. It is a target organ of glucocorticoids, but the effects of an antenatal exposure to a corticoid treatment on the dopaminergic system are not known. The aim of the study was to determine the effects of antenatal exposure to betamethasone on the expression level of dopamine D1 receptor (DA D1-R) mRNA expression in adrenal gland during development.

Methods: New-Zealand white pregnant rabbits were given either 0.1mg/kg betamethasone or 0.1ml/kg saline 0.9% at days 25 and 26 of gestation for fetal analysis and days 27 and 28 for postnatal analysis. The spontaneous delivery took place on 30 or 31 days of gestation. The fetus were delivered by cesarean section at 27 days of gestation. DA D1-R mRNA expression was determined at 4 developmental ages using Northern blot analysis: fetus (27 days of gestation), 1-day old, 25-day old and adult. Rabbits were allocated to their respective groups (treated or untreated) according to the maternal treatment (betamethasone or saline respectively).

Results: We have shown first that DA D1-R mRNA was expressed in rabbit adrenals from the fetal period to adulthood and this expression was not age-dependent. Moreover, antenatal corticotherapy induced a significant increase in DA D1-R mRNA levels of 20%, 15% and 8% in fetuses, 1-day old and 25-day old rabbits compared to the untreated groups (P=0.003, 0.037 and 0.007 respectively). This increase was not observed in adulthood.

Conclusion: These findings suggest that an antenatal treatment with corticoids induced an over-expression of DA D1-R mRNA levels in rabbit adrenal gland, over-expression observed just after administration, sustained like a printing effect but disappearing at long term.

P60
Acetaminophen Metabolism in Paediatric Patients with Oncologic Disease Measured by Capillary Electrophoresis

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The analgetic and antipyretic drug acetaminophen is widely used in paediatric oncology. Acetaminophen is extensively metabolised in the liver by three main pathways: sulphation, glucuronidation and cytochrome P450-dependent oxidation. The cytochrome P450-dependent oxidation results in the formation of the hepatotoxic N-acetyl-*P*-benzoquinone (NAPQI), which is detoxified by conjugation with glutathione. Saturation of the glucuronidation and sulphation pathways or induction of cytochrome P450 enzymes results in an increased oxidation of acetaminophen to NAPQI. Paediatric cancer patients receive multiple anticancer drugs interacting with conjugation and oxidation, which are supposed to interact with the metabolism of PCM. In order to evaluate the PCM metabolism in cancer patients we established a simple, sensitive and rapid method for determination of acetaminophen, acetaminophenglucuronide, acetaminophensulphate, acetaminophen-cysteinate by capillary electrophoresis. In the ongoing study we compare urinary excretion and serum concentrations of PCM and its metabolites in paediatric patients with and without anticancer therapy.

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P61
Off-Label and Unlicensed Drug Use in Neonatal Wards of Three Different Italian Hospitals

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Background: To measure the off-label (OL) use of drugs in the Italian, neonatal hospital ward setting, also among three different speciality levels (NICU III, NICU II, and 1 non-NICU), and to understand for which indications neonates remain uncovered, and therefore unprotected, due to inadequate knowledge of drug effects in this population.

Methods: Prescriptions given to all premature and underweight newborns admitted to the neonatal wards of three different hospitals in Italy between December 1998-February 1999, who received at least one drug prescription, were analysed for OL and unlicensed use.

Results: 143 infants were included in the study and received a total of 720 prescriptions (72 different drugs), given for 46 indications. The most common drugs were vitamin K (109 prescriptions), vitamin D (63), and ampicillin (56), and the most common indications were prophylaxis and infections. OL prescriptions made up 79.9% of the total and involved 97.2% of children, while unlicensed prescriptions made up 19.2% of the total number of prescriptions. Of the five most represented therapeutic subgroups in the study, those with the highest OL use rates were systemic antibacterials (73.1%), anti-haemorrhagics (88.1%), and diuretics (100%), and together accounted for 72% of all OL prescriptions. No significant difference was found in the rates of OL drug use among the different speciality levels, but a significant one was found in the greater use of unlicensed drugs in the level III NICU with respect to the other two levels (in addition to caffeine, NICU III prescribed midazolam and extemporaneous preparations of vitamins D and C). 83% of the indications for which drugs were prescribed involved OL prescriptions. The most common OL use category was dosage (47.4% of all OL prescriptions), followed by lack of paediatric license (40.7%). The level III and II NICUs prescribed more drugs OL for dose, while the non-NICU for lack of paediatric license.

Conclusions: Despite some differences found between speciality levels, OL use was high everywhere. It should also be noted that, despite the relatively few therapeutic necessities of patients in neonatal wards (only 46 indications in this study), in 83% of the cases these patients received drugs for which insufficient information exists for their age group, putting them at a higher risk also of adverse reactions. A more rational drug use is necessary and should begin with regulatory intervention to increase the number of studies carried out in this population.

P62
From Proposal to Reality

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Background: The longstanding, underprivileged position of children throughout Europe with respect to medicine calls for strategies aimed at the promotion of the rational use of drugs in paediatric patients. Clinical trials have a fundamental role in obtaining this, since they are the key to distinguishing useful from harmful treatments. However, it is difficult to identify the already insufficient (in number and, at times, in method) paediatric studies carried out and to thus implement the knowledge derived from them. The development of a drug therapy trials registry specific to the paediatric field would therefore be valuable, but, despite the remarkable efforts made to create numerous, diverse databases addressing the needs of the biomedical research community in the past few years, none have involved such a registry. A registry of completed and ongoing clinical trials in children would be a useful resource for planning new studies, promoting communication and collaboration among researchers, facilitating patient access and recruitment into trials, preventing trial duplication and inappropriate funding, and identifying the therapeutic needs of children that remain neglected. It would also allow for active monitoring of new or evolved knowledge of drug therapies. The aim is to create an online registry that will collect essential data on paediatric clinical trials conducted in European member states, and will monitor the outcome of the studies (results, conclusions and publications).

Methods: The authors have formed a network characterised by different, complementary clinical backgrounds and research experiences to facilitate the creation of a clinical trials registry. The members will identify planned and ongoing trials through communication with ethical review boards, national associations of paediatricians, pharmaceutical companies, *etc*, and the data will then be collected into one database. Members will be responsible for monitoring progress in their country and in countries allocated to them in order to keep the register up to date. The register will be put up on the internet, with a friendly interface so that trial data will be available to all.

The members are ready to begin working on the registry and are currently seeking funding.

P63
Not Appropriate Anti-Asthmatic Drugs Prescription in the Italian Paediatric Population

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Background: Asthma is the most common chronic disease in children and it represents a huge burden on the family and society. An adequate pharmacological treatment is essential in preventing disease progression. In this context, anti-asthmatic drug prescription could be an indicator of quality of care.

Methods: In order to evaluate the use of the anti-asthmatics, the 977,000 paediatric prescriptions in 16 Italian local health units (417,559 children under 14 years) during 2000 were analysed. Data were collected from the ARNO database, a system that merges information regarding out-of-hospital prescriptions, the population and the community setting into a single database.

Results: 92,980 children (22%) received at least one anti-asthmatic drug prescription. The prevalence rate of prescriptions was higher in children under 1 year (34%) and significantly decreased with increasing age (χ^2_t = 9,200; P<0.0001). The prevalence rate was significantly higher in boys than in girls (χ^2= 452; P<0.0001). 72% of children treated with anti-asthmatic drugs received prescriptions of less than three boxes. Beclomethasone (prevalence 15%), salbutamol (7%), flunisolide (4%) and fluticasone (2%) were the most prescribed drugs. There was a 2:1 ratio of corticosteroids to bronchodilators prescriptions. 98% of beclomethasone boxes and nearly all flunisolide boxes were prescribed as nebulised suspension, a formulation licensed in Italy, but not in other countries such as France, Germany, UK or *USA*.

Conclusion: These data suggest that, in Italy, anti-asthmatic drugs are prescribed not only for asthma, but also for the treatment of acute diseases such as upper respiratory tract infections. In fact, the prevalence of prescription is higher than the estimated prevalence of asthma (9% in children under 14 years); anti-asthmatic drugs were prescribed especially to children under 5 years and for short term therapies. Moreover, inhaled corticosteroids were prescribed more than bronchodilators, often as nebulised solutions. Many children were exposed to inhaled corticosteroids as treatments for which there is no evidence of efficacy. Efforts are therefore needed for improving the rational use of drugs for children.

P64
Nephrogenesis and Antenatal Exposure to Cyclosporine A (CsA) in Rabbits

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Background: The number of pregnancies in transplanted women administered CsA is increasing. However CsA is a nephrotoxic agent crossing the placenta. So, the assessment of the effect of an antenatal exposure to CsA on nephrogenesis is mandatory.

Methods: Three groups of New Zealand White rabbits were studied according to their antenatal exposure to CsA:

Group 1 (P=11): the pregnant rabbits were subcutaneously administered CsA (10 mg/kg/d) from d14 to d18 of gestation *i.e*. at the outset of renal nephrogenesis

Group 2 (P=14): the pregnant rabbits were administered the vehicle of CsA (cremophor) from d14 to d18 of gestation

Group 3 (P=13): control group

Renal histology was performed at birth and at one month of age. After an acid maceration of kidneys, the total number of nephrons was measured in the one month-old rabbits.

Results: The number of rabbits per litter, the incidence of stillborn, the birthweight and the postnatal weight gain were similar amongst the three groups.

The number of nephrons was not significantly different in groups 3 and 2 (190.000 ± 26.000 *vs* 200.000 ± 25.000 nephrons/kidney). As compared with the control group, group 1 presented a reduced number of nephrons (150.000 ± 19.000 (P <0.01). In group 1, histological analysis at birth showed an interstitial inflammation, cortical fibrosis, lipid droplets within the basal area of tubular epithelial cells and hypertrophy of glomeruli in the deepest cortical layer (341 +/- 27 *vs* 266+/- 6 pl; P <0.01). At one month of age (mature kidney) histological analysis showed an interstitial fibrosis, a focal and segmental glomerular sclerosis and an hypertrophy of deep glomeruli (443 ± 23 *vs* 347 ± 22 pl; P<0.01).

Conclusion: This in vivo study showed that in utero exposure to CsA impaired nephrogenesis in the rabbit. The consequences of this oligonephronia on renal function have to be assessed.

P65
Consequences on Renal Function of Antenatal Exposure to Cyclosporine A (CsA) in the Rabbit

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Background: The number of pregnancies in transplanted women administered CsA is increasing. However, CsA is a nephrotoxic agent crossing the placenta. Our previous results showed that an antenatal exposure to 10 mg/kg/d of CsA from day 14 to day 18 of gestation impaired nephrogenesis in rabbits. The number of nephrons was reduced by 21%. An hypertrophy of glomeruli in the deepest cortical layer, a glomerular sclerosis and an interstitial fibrosis were also observed. Therefore this study was performed to investigate CsA effect on renal function in New Zealand White rabbits.

Methods: Two groups of rabbits were studied according to their antenatal exposure to CsA:
Group 1: control group
Group 2: pregnant rabbits received subcutaneous injection of 10 mg/kg/d of CsA from d14 to d18 of gestation
In each group, newborn rabbits were divided in 4 subgroups according to age: 0ne-month-old; 11-week-old; 18-week-old (young adult rabbits) and 35-week-old (mature adult rabbits) rabbits. In each subgroup, mean arterial pressure, diuresis, creatinine plasma levels, clearance of creatinine, proteinuria and kidney weight were measured.

Results: At one month of age, no difference was recorded between the 2 groups. Diuresis was slightly increased in group 2 at 11 weeks of age but was subsequently similar in the two groups. At 11 weeks, group 2 presented an increase in both kidney weight (+21%) and an systemic blood pressure (+17%). At 18 weeks, group 2 significantly presented a decrease in creatinine clearance by 20% and an increase in: kidney weight by 21%, plasma creatinine level by 14%; protein urinary concentration by 100%; arterial blood pressure by 12%. At 35 weeks, group 2 significantly presented a decrease in creatinine clearance by 63% and an increase in: kidney weight by 27%, plasma creatinine level by 19%; protein urinary concentration by 100% and arterial blood pressure by 52%.

Conclusion: Beyond the neonatal period, antenatal exposure of rabbits to CsA induced alterations in renal function, renal hypertrophy and systemic hypertension. These pathological conditions worsened with increasing age. So, the nephronic reduction induced by antenatal exposure to CsA was associated with a marked renal dysfunction at adulthood.