POSTERS

PO-01
NEWBORNS FROM ANTI-RO/SSA POSITIVE MOTHERS: PRELIMINARY RESULTS FROM ONE YEAR ENROLLMENT

L. Barberis, D. Farina, P. Galletto, P. Cigna, G. Gomirato

Divisione Ospedaliera di Neonatologia – TIN, A.S.O. OIRM – S.Anna - Torino, Italy

Pregnancies in women with autoimmune disease (AD) are considered at risk for pregnancy outcome and neonatal health. The transplacental passage of maternal anti-Ro/SSA antibodies is associated with neonatal lupus syndrome, featuring congenital heart block in utero and other clinical manifestations in newborn, such as cutaneous lesions, haematological and liver disorders. *Methods.* In the period February 2005 – April 2006, out of 13 newborns from mothers with AD enrolled in our Center, we considered 7 newborns from 5 Ro/SSA positive mothers to evaluate neonatal outcome. One mother was diagnosed with systemic lupus erythematosus (SLE), 3 with undifferentiated connective tissue disease and the other one with essential thrombocythemia. Three pregnancies were induced by assisted reproduction technique and 2 out of 3 were multiple: one was twin (intrauterine death of one fetus) and one was triplet. All pregnancies were prophylaxed with low molecular weight heparin, plus ASA in 3/5. One was treated with hydrossichloroquine. Serial fetal echocardiograms were done during pregnancies. In the newborn ECG, whole blood cell count, liver enzymes and specific maternal antibodies determination were performed in the first days of life and later, according to a specific protocol.

Results. Pregnancy outcome is described in Table 1.

Table 1.

Pregnancy number	5
Multiple pregnancies	2
Living newborns	7
Full term birth	2
Premature birth <37w	1
Premature birth <34w	4 (1 triplet; 1 single with placental insufficiency)
Gestational age (weeks)	32-41
Weight (g)	1550-3220

Fetal echocardiographic surveillance showed normal intracardiac conduction velocity in absence of cardiac malformation in all cases. No physical abnormality was detected at birth; the triplets and the other preterm infant (32 weeks) needed O2-supplementation in the first days of life. One term newborn, with elevated anti-Ro/SSA level, showed prolongation of QTc interval (0,48") at the age of 25 days, without clinical involvement. The triplets, from the mother with SLE, had asymptomatic neutropenia (in the absence of anti-neutrophil antibodies), recovered with the disappearance of maternal antibodies in two cases. Follow-up of all infants is still ongoing and is scheduled for the first year of age. *Conclusions.* Our preliminary data are encouraging for the outcome, but confirm the presence of some clinical abnormalities in newborns, suggesting the importance of a multidisciplinary approach and a standardized follow up of these patients.

PO-02
HEMATOLOGICAL ABNORMALITIES DURING THE FIRST WEEK OF LIFE AMONG NEONATES WITH DOWN SYNDROME: DATA FROM A MULTIHOSPITAL HEALTH-CARE SYSTEM

S.E. Wiedmeier,[1] E. Henry,[2] D. Walker,[2] R.D. Christensen[3]

[1]LDS Hospital and the Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT, USA; [2]Institute for Health Care Delivery Research, Salt Lake City, UT, USA, [3]Intermountain Healthcare Clinical Research, McKay-Dee Hospital Center, Ogden UT, USA

Aims. Various hematological abnormalities have been reported among neonates with Down syndrome. Thrombocytosis, thrombocytopenia, polycythemia, neutrophilia, transient myeloproliferative disorder (TMD), and congenital leukemia have all been reported. The two largest case series previously reported involved 63 and 31 cases. To acquire hematological data from a larger case series, we obtained all CBCs done during the first week after birth on all neonates with Down syndrome born during the past five years in a multihospital healthcare system. *Methods.* We performed an historic cohort analysis of all neonates with Down syndrome who had one or more CBCs recorded during their first week of life, and were cared for in an Intermountain Healthcare (IHC) hospital, with dates of birth from January 1, 2001 through December 31, 2005. *Results.* During the five-year period, 145,522 live births were recorded at the 18 hospitals. Down syndrome was recognized in 226 (prevalence estimate, 1 in 644). One hundred fifty-eight (70%) of these had one or more CBCs obtained before the seventh day. Neonates who did vs. did not have a CBC in the first week had a similar gestational age, birth weight, percentage who were LGA and SGA, and length of stay. Neutrophilia was the most common hematological abnormality detected, with 80% of absolute neutrophil counts above the upper limit of normal for age. Six percent (9/158) had blasts identified on the blood film and three, where this was persistent, were referred to the pediatric hematology service for further evaluation. The next most commonly detected abnormality was thrombocytopenia, with 66% of platelet counts <150,000/uL, and with 6% of counts <50,000/uL. The mean platelet volume did not correlate with the platelet count, but tended to run slightly large (9.2±1.3 fL), with 24% of values above 10 fL. Only one had a platelet transfusion. Polycythemia was the next most common hematological abnormality detected, with 33% of hematocrit values above 65% or hemoglobin concentrations above 22 g/dL. Six had a reduction transfusion. One patient had significant anemia (hematocrit <15%) and received an erythrocyte transfusion. One had neutropenia associated with an infection after bowel surgery. *Conclusions.* Neutrophilia, thrombocytopenia, and polycythemia were the most common hematological abnormalities observed among neonates with Down syndrome. Anemia, thrombocytosis, and neutropenia were not more common than among neonates who do not have Down syndrome. Hematological abnormalities were so common in this group that it seems reasonable to recommend that one or more CBCs be obtained on all neonates with Down syndrome.

PO-03
THREE STUDIES TESTING DARBEPOETIN ADMINISTRATION TO ANEMIC NEONATES

T.L. Warwood, R.K. Ohls, S.E. Wiedmeier, D.K. Lambert, C. Jones, S.H. Scoffield, N. Gupta, P. Veng-Pedersen, R.D. Christensen

Intermountain Healthcare Clinical Research, McKay-Dee Hospital, Ogden, UT and LDS Hospital, Salt Lake City, UT, the University of New Mexico School of Medicine, Albuquerque, the Division of Neonatology, University of Utah School of Medicine, Salt Lake City, UT, and the College of Pharmacy, University of Iowa, Iowa City, IA, USA

Background. Darbepoetin is longer acting and more potent than recombinant erythropoietin (rEpo). In adults with the anemia of renal insufficiency, less frequent dosing and lower costs are advantages of Darbepoetin over rEpo. However, until our three recent studies, no publications have assessed potential advantages of Darbepoetin over rEpo among anemic neonates. *Methods.* In our first study we gave a single subcutaneous (SC) dose of darbepoetin of either 1 µg/kg or 4 µg/kg to 12 anemic preterm infants and measured pharmacokinetics and erythropoietic effects. In our second study we gave a single IV dose of 4 µg/kg to 10 neonates (using a 4 h infusion) and measured pharmacokinetics and erythropoietic effects, comparing these with the SC results from our first study. In our third study we gave SC (n=5) or IV (n=5) dosing of 4 µg/kg and compared urinary loss of drug, as well as erythropoietic effects. *Results.* We observed that the $t^1/_2$ of darbepoetin is 26 h after SC dosing and 10 h after IV dosing (the $t^1/_2$ in adults is 48 h after SC dosing and 24 h after IV dosing). The erythropoietic effect, judged by increases in Immature Reticulocyte Fraction, Absolute Reticulocyte Count, and Reticulocyte Percentage, is greater after a 4 µg/kg dose than after a 1 µg/kg dose. Similar to rEpo, the volume of distribution (Vd) of Darbepoetin is large in neonates, averaging 0.77 L/kg (range 0.18 to 3.05) and the clearance (Cl) is rapid, averaging 52.8 mL/hour/kg (range 22.4 to 158.0). Increased reticulocytes are seen 48 h after SC or IV dosing, but they generally do not peak until 96 h after dosing. SC and IV dosing give similar magnitudes of reticulocyte response at 96 h. Essentially no urinary loss of drug occurs following either SC or IV dosing. *Conclusions.* Darbepoetin dosing in neonates requires a higher unit dose/kg and a shorter dosing interval than are generally used for anemic adults. This is because in neonates Darbepoetin has a shorter $t^1/_2$, a larger Vd, and more rapid Cl than in adults. If a neonate who is to receive Darbepoetin has an IV in place, the drug can be given IV, thus avoiding any discomfort associated with the SC injection with anticipated efficacy equal to SC dosing. We speculate that Darbepoetin has advantages over rEpo in the NICU, in that it is likely more cost-effective than rEpo and requires less frequent dosing.

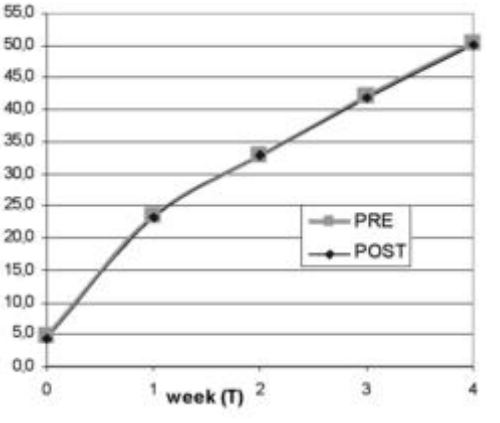
PO-04
EXTRACELLULAR POTASSIUM CONCENTRATION AFTER IRRADIATION IN RBC UNITS FOR NEONATAL TRANSFUSION: A PRELIMINARY STUDY

A. Tiburzi[1], M. Motta[2], S. Braga[1], M. Bertolassi[1], M. Rocchi[1], M. Marini[1]

[1]Blood Bank Service and [2]Neonatal Intensive Care Unit, Spedali Civili, Brescia, Italy

Background. Multiple stored packed cells from a single donor for serial transfusion have been recommended to reduce the number of donor exposures in VLBW infants. Moreover in these infants, irradiation of red blood cell (RBC) components to prevent the transfusion-associated graft-versus-host disease it is also recommended. The red cell sodium-potassium ATPase pump does not function well at 4°C, and potassium leaks out of RBCs during refrigerated storage. Reported concentrations of free potassium in the supernatant of RBCs at the end of their storage period range from 50 to 80 mmol/L. The rate of leakage increases after irradiation and may rises, if transfusion are delayed, two time values of not irradiated unit. For this reason concerns have been raised that infusion of stored RBCs could cause transient hyperkalemia in the neonatal transfusion recipient. *Objectives:* We designed a study to determine the kinetic of exracellular potassium increase of stored RBC units following irradiation and to estimate the time period within the extracellular potassium concentration remains within safe limits for neonatal transfusion. *Methods.* Ten RBC units were analyzed before and after irradiation, within two hours, for the determination of the pre- and post-radiation exracellular potassium at the following storage days: 1 (T0), 7 (T1), 14 (T2), 21 (T3) and 28 days (T4) from the collection. *Results.* The average of extracellular potassium concentration have been collected in the following table and represented in the adjacent diagram.

	Pre irradiation	*Post irradiation*
T0	4,34	4,79
T1	23,29	23,39
T2	33,00	33,00
T3	41,83	42,17
T4	50,25	50,50



The graphic clearly evidence that extracellular potassium concentration does not increase significantly within the first two hours after irradiation at any time of storage.

Conclusion: Our results suggest that transfusion of stored RBCs within two hours after irradiation does not increase the risk of hyperkalemia. It will be recommended to irradiate the stored RBC units for neonatal transfusion recipient just before the administration.

PO-05
CHANGES IN RBC TRANSFUSION PRACTICE IN A NICU: 2 RECENT YEARS IN COMPARISON

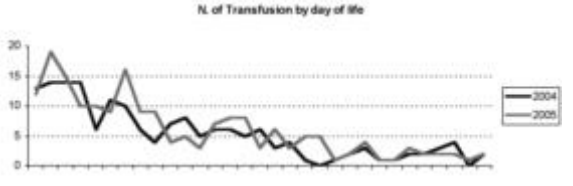
S. Malguzzi, V. Dell'Orto, C. Farina

TIN – San Gerardo Hospital, Monza, Italy

Objectives. Anemia develops in increasing numbers of critically ill newborn and they receive multiple red blood cell (RBC) transfusions. Despite their need for prolonged medical treatment, VLBW infants presently receive fewer RBC transfusions as a result of the growing awareness of transfusion risks and improvement of neonatal care. *Methods.* RBC transfusion practices and clinical outcomes in newborn of a NICU were analyzed retrospectively in the last two years: 2004 and 2005. *Results.* Progressive declines in RBC transfusions, donor exposures, and transfusion volumes occurred concurrently with decreases in morbidity and mortality rates. Transfusions per infant (mean ± sd) declined as shown in the table below.

	2004			2005					
	n	m	ds	n	m	ds	chi	t	p
transfused	48			58					
transfusions	200			227					
mean transfusions		4,23	3,59		3,9	3,3		0,49	0,62
GA (wks)		29,94	4,79		29,32	4,09		0,719	0,47
BW (g)		1464,1	910,16		1240,7	735,38		1,39	0,16
GA <= 32 wks	33 (68%)			49 (84%)			2,86		0,09
GA <=28 wks	22 (46%)			33 (57%)			0,88		0,35
BW <=1500 g	33 (68%)			47 (81%)			1,52		0,23
BW <=1000 g	21 (44%)			30 (52%)			0,4		0,54
newborns on CPAP	27			41			1,79		0,18
mean duration CPAP (h)		292,95	303,15		268,02	249,43		0,37	0,71
newborn on MV/HFO	22			27			0,66		0,42
mean duration MV/HFO (h)		215,71	234,54		217,44	281,13		0,023	0,98
newborns O2tp	26			35			0,19		0,66
mean duration O2tp (h)		34,43	114,46		29,25	106,44		0,18	0,85
newborns on TPN	40			51			0,15		0,69
mean duration TPN (h)		18,55	13,74		18,8	13,3		0,078	0,93
surfactant treated	27			24			0,16		0,68
PDA pharm. Treated	15			17			0,0000		0,99
EPO treatment	11			37			16,1		0,0000
EPO mean doses		8,73	5,71		12,24	5,43		1,86	0,07
ROP (any grade)	5			11			0,9		0,34
neo IVH (any grade)	17			16			0,43		0,51

The distribution of RBC transfusions given by day of life among the study years did not change (Figure 1); 84% and 82% of RBC transfusions were given within the first month, when infants are sickest, especially in the first week. All infants weighing 1 kg or less in both the years received transfusions. EPO treatment was significantly increased.



Conclusions. Overall administration of neonatal transfusions has decreased in the last year, most likely because of multiple factors. Most RBC transfusions were given to infants weighing 1 kg or less in the first weeks of life, EPO treatment was promoted, blood loss redutions and microsamples of blood was enanched on the group of VLBW infants. The temporal changes observed in transfusion patterns emphasizes the importance of future studies evaluating transfusion interventions and preventive strategies.

PO-06
ENHANCED RESISTANCE TO YERSINIA ENTEROCOLITICA INFECTION IN A NOVEL MURINE NEONATAL MODEL OF BACTERIAL INFECTION

A. Echeverry, B. Adkins

Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL USA

Neonates are considered highly susceptible to gastrointestinal infections. This susceptibility has been partially attributed to immaturity in immune cell function. However, many questions remain about how immunity is established in early life, especially to pathogens encountered through the gut mucosa. To begin to understand this phenomenon, we have developed a model system to study infection of murine neonates with the gram negative enteropathogenic bacteria *Yersinia enterocolitica*. Using the natural orogastric (o.g) route of transmission, we compared the susceptibility of seven day old and adult mice of the BALB/c and C57BL/6 strains to *Y. enterocolitica* infection by determining their respective LD50 values. Remarkably, we found that neonatal mice infected with two highly pathogenic *Y. enterocolitica* strains have enhanced survival compared to adult mice. The LD50 values in both strains of mice were at least ten times higher for neonates than for adults. When adjusted for their respective weights, neonates were at least 40 fold more resistant than adults. Bacterial colonization experiments indicated that the enhanced resistance of neonates was not due to failure of the bacteria to colonize the intestinal tissues of neonates; the levels of viable *Y. enterocolitica* in the intestine were consistently high for at least the first 10 days post infection, showing that there is prompt and sustained colonization of the neonatal intestine. In addition, bacteria could be readily detected in the neonatal mesenteric lymph node (MLN). However, translocation of the bacteria to other tissues in neonates was minimal. In striking contrast, *Y. enterocolitica* readily disseminated to the spleen and liver of adult mice. These results led to the hypothesis that *Y. enterocolitica* infection during neonatal life may be controlled by a highly efficient intestinal immune system which acts to limit bacterial spread beyond the gut. To investigate this idea, we examined infiltration of the MLN by innate phagocytes. We found a marked increase in the percentage of neutrophils (Gr-1hi CD68int) and macrophages (Gr-1lo-intCD68hi) in the neonatal MLN, but there were no significant changes in the influx of these cells into the adult MLN. The observation that *Y. enterocolitica* is contained in the intestine and MLN of neonates and does not reach periheral tissues is consistent with the observed increase in innate phagocytes. The combined results from these experiments support our main hypothesis that the intestinal immune system of neonates rapidly mobilizes phagocytes which confine the bacterial infection to the gut, resulting in a high level of resistance. The knowledge acquired through this neonatal model of infection will give major new insights into the impact of enteropathogens on the development of the neonatal immune system. The long term goal of these studies is to design mucosal immunization or intervention strategies to improve disease outcome or to prevent gastrointestinal infections in the pediatric population.

PO-07
MARKERS OF NEONATAL SEPSIS

G. Corona, A. Bonarrigo, N. Decembrino, R. Blandino, G. Gagliostro, G. Giorgianni*, G. De Pasquale, I. Barberi

*Department of Pediatrics, Neonatal Intensive Care Unit -*Department of Biochemistry University of Messina, Italy*

Introduction. Sepsis are an important cause of morbidity in the first month of life. The incidence is around 1-10/1000 newborns, and up to 160/1000 VLBW newborns <1500 g. Many different markers have been used for the diagnosis of sepsis, although no one has reached the ideal 100% sensitivity and 100% positive predictive value. Some tests, however, used either alone or in combination, may give an early reliable indication of neonatal sepsis. *Study objective.* The aim of this study was to evaluate in our UTIN sensitivity and specificity of the newest markers of sepsis (fibronectine - citokine and procalcitonin) as compared to the traditional indexes of inflammation (PCR, GB, ANC) for the early identification of newborn with sepsis, with the purpose to avoid an excessive antibiotics use and to reduce the mortality and morbidity in infected newborns. *Patients and Methods.* In this prospective study we evaluated 49 newborns, divided into 3 groups based on sepsis risk (high, medium and low risk), clinically evaluated with the Sepsis-Score. Our protocol included: Procalcitonin serum levels assay; blood cell counts, Absolute Neutrophil Counts (ANC); blood culture; measurement of cytokines (IL-1β, IL6 and TNFα), and fibronectine serum levels; Clinical examination every 24 hours; evaluation of Sepis-Score and laboratory investigations every 72 hours. The determination of Procalcitonine was done at 24, 48 and 120 hours using an Immunoluminometric method LUMITEST. The PCR was evaluated by Immunonefelometric test. The ANC was evaluated with an Electronic Counter. The cytokines was determined with ELISA test. Fibronectine was evaluated with the technique of Radial Immunodiffusion (RID). *Results.* Of the 49 newborns studied, 31 (63.56%) had Procalcitonine levels >0,5 ng/ml. This high value persisted in 23 newborns (74.19%) that later developed sepsis, 16 were in group A (69.56%) and 7 in group B (30.44%). Only 18 newborns (78.26%) were positive for bacterial and fungal infection. Initial PCR values were not significantly different in the examined groups. A significant difference was only found at 48 hours of life in group A ($p<0.5$). There were no significant differences among the groups of White Cells and Absolute Neutrophils Counts ($p=0.16$). Serum IL-6 levels at 24 hour was not significantly increased Vs Fibronectine in the 3 groups of newborns (p=n.s.). *Conclusions.* In our study Procalcitonine demonstrated an early and sensitive marker of sepsis, as compared to PCR, WC, ANC, Fibronectine and Cytokine, in high risk newborns in TIN.

PO-08
A STRANGE CASE REPORT OF NEONATAL ERYTHROBLASTOSIS

M. Testa*, S. Barella**, V. Fanos*

**Istituto di Patologia e Terapia Intensiva Neonatale, Puericultura e Nido Università di Cagliari; **Centro Regionale per le Microcitemie- Regione Sardegna, Italy*

F.C., born full term by uncomplicated vaginal delivery, birth weight 2400 grams, presents at birth a mild respiratory distress and anemia: Hb 11.2 g%; Ht 37.7%; MCV105 fl; RBC 3.580.00; WBC 20.000 with presence of circulating nucleated red blood cells; after 12 hours of life the baby is transferred to our NICU. At admission heart rate, blood pressure and respiratory rate are normal; there is no more cardiorespiratory distress, but liver and spleen are very enlarged. The complete blood count confirms the anemia: Hb 12.5 g%; Ht 40%; RBC 3.750.000; WBC 49.800; the baby's blood smear reveals a great number of nucleated erythroblasts, so the correct WBC count is 16.600 with 33.200 nucleated red blood cells. The aspecific infection tests are negative (CRP= 16 mg% and white-cell differential count= 0.05) and so is the emocolture. A congenital infection is excluded: TORCH, aspecific IgM, V.D.R.L., MHA-TP and FTA-ABS, search for Parvovirus B19 specific antibodies and DNA are performed, without any positive result. The plasma-serum and urine amino acids are normal and any kind of metabolic disease is excluded. The hemoglobin electrophoresis of mother's blood shows a normal level of HbF, so fetomaternal transfusion didn't occurred. The DNA study in the baby's blood excludes the most frequent Mediterranean mutations for α-thalassemia. At present more tests are going on to detect rare mutations. The liver and spleen ecography shows an evident hepatosplenomegaly, without macroscopic structural modifications. Brain and heart ultrasonography are normal. On the third day of life the erythroblastosis is decreased but still evident (WBC 40.551; nRBC 10.949; RBC 3.910.00; Hb 12.5 g%; Ht 39%; reticulocytes 11.76%); the circulating nucleated red blood cells disappear only after ten days of life. After three weeks the hepatosplenomegaly suddenly becomes less evident and after some few days it is completely regressed. The baby is discharged at four weeks of life, without a real diagnosis. An unbalanced synthesis of globin chains is the last hypothesis to be checked to solve this strange case.

PO-09
ELECTROCARDIOGRAPHIC ABNORMALITIES IN INFANTS FROM MOTHERS WITH ANTI-SSA/RO ANTIBODIES

C. Rodríguez-Pérez[1], M. Motta[1], A. Tincani[2], A. Lojacono[3], G. Chirico[1]

[1]Department of Neonatology and NICU, [2]Department of Rheumatology and Clinical Immunology, [3]Department of Obstetrics and Gynecology, Spedali Civili, Brescia, Italy

Objective. To evaluate the incidence of electrocardiographic abnormalities in neonates born from mothers with connective tissue disease (CTD) and positive for anti-SSA/Ro antibodies. *Patients and Methods.* Electrocardiograms (EKGs) prospectively obtained from 51 infants born from CTD anti-SSA/Ro positive mothers were compared with those obtained from 50 control infants born from mothers with CTD and anti-ENA negative. *Results.* No infant showed sinus bradycardia. Heart rate values were similar in the two groups. PR interval values were similar in the two groups. Incidence of 1st degree atrioventricular block (AVB) at birth was significantly higher in study group than control group (five infants in study group vs no infants in control group; $p = 0.023$). AVBs spontaneously reverted or remained stable during the first year of life. The incidence of QT interval prolongation was not significantly different between the two groups. Mean corrected QT (QTc) value of infants born from anti-SSA/Ro positive mothers was slightly prolonged as compared with the control group (0.404 ± 0.03 sec vs 0.395 ± 0.02 sec), although the difference was not significant ($p = 0.060$). The correlation between the PR and the QTc interval values at birth was significant in study group ($r = 0.3279$; $p= 0.0188$), while no significant correlation was found in control group ($r = 0.1581$; $p= 0.3718$). *Conclusions.* Infants exposed to anti-SSA/Ro antibodies had a statistically significant higher incidence of 1st degree AVB. Sinus

bradycardia was not clinically apparent, suggesting a lack of sinoatrial nodal involvement. A slight difference between the QTc interval values of the two groups was found, however more data are needed to rule out or to confirm an interference between the anti-SSA/Ro tranplacental passage and ventricles repolarisation in infants.

PO-10
INTRACELLULAR CYTOKINES IN BREAST MILK

A. Gasparoni, L. Ciardelli, A. Avanzini, F. Garofoli, G. Giudici, M. Stronati, G. Chirico

Department of Neonatology and Neonatal Intensive Care, Spedali Civili, Brescia; Research Laboratories, and Division of Neonatal Intensive Care, IRCCS Policlinico S. Matteo, Pavia, Italy

Introduction. Of the several factors with immunologic, hormonal, enzymatic and trophic activity, cytokines are believed to play a significant role in the immune-modulation and immune-protection of breast milk. Most of the cytokines that are known to be deficient in the neonate, particularly in preterm infants, have been found in significant amounts in breast milk. We evaluated cytokine production in breast milk by CD4 and CD8 lymphocytes, expressing either the CD45RA or the CD45RO antigen. The same cytokines were assayed on cord blood (CB) lymphocytes of term infants. *Methods.* We assayed IL-2, IL-4, IFN-γ and IL-10 production by CD4 and CD8 lymphocytes in 18 breast milk samples obtained within 2-5 days after delivery, and on cord blood (CB) lymphocytes of 10 term infants. For intracellular cytokines evaluation CD3-TC, CD8-Fitc, CD4-Pe, IL-2-Pe, IL-4-Pe, IFN-γ-Pe, Il-10-Pe, isotype-specific anti-mouse IgG1 or IgG2, and anti-rat IgG2a were used. For intracellular cytokine analysis, mononuclear cells (MC) were stimulated with PMA and Ionomycin. Simultaneously, Brefeldin was added for disrupting intracellular Golgi-mediated transport, to allow cytokines to accumulate, yielding an enhanced cytokine signal. Thereafter, MC were stained with CD3-TC for T cells, CD8-FITC for T cytotoxic subpopulation and CD4-Pe for T helper subpopulation. Phenotypic analyses of the fluorescent stained cells were performed by three-color flow cytometer using a Becton Dickinson FACSCalibur flow cytometer and analysed with Cell Quest software. *Results.* Milk lymphocytes were 100% CD45RO (memory) and CB lymphocytes 87% CD45RA (naïve) positive. Results are expressed as percentage of producing cells (Mean±SD):

	CD4 milk	*CD8 milk*	*CD4 CB*	*CD8 CB*
% of total	42.6±27.1	48.8±25.4	72.4±8.6	20.1±4.5
IL-2	75.8±15.0	62.1±25.7	51.3±10.0	23.2±8.2
IL-4	17.6±23.5	5.6±5.0	0.35±0.38	1.68±1.88
IFN-γ	77.2±13.1	89.5±11.1	0.8±0.5	4.6±1.8
IL-10	1.0±1.5	2.1±5.1	0.4±0.3	2.3±1.5

Conclusion. Our data showed a substantially higher percentage of cytokines producing cells in breast milk than in cord blood. In particular, high level of IFN-γ was found in breast milk, as opposed to the very low level found in cord blood. Recent studies reported that intact human milk leukocytes adhered to the gastrointestinal mucosa and remained there for up to 60 hours, providing cell-mediate immunity to offspring via breast feeding. Therefore, we can speculate that the high percentages of Th1-type cytokines (IL-2 and IFN-γ) found in human milk may play an important role in the differentiation of neonatal T cells toward a preferential Th1, rather than Th2, pathway providing an important defence mechanism against infection.

PO-11
HAPTOGLOBIN (HP) DOSAGE IN THE FIRST WEEK OF LIFE IS NOT PREDICTIVE OF INFECTION DURING PERINATAL PERIOD

L. Bolgia*, M. Della Ventura§, L. D'Angelo§, A. Dinatale*, F. Palmesi*, F. Dituri*

**Department of Neonatology, §Department of Immunohaematology, Fatebenefratelli S. Giovanni Calibita Hospital, Rome, Italy*

Haptoglobin (Hp), is a highly polymorphic protein, that binds haemoglobin and accomplishes several other functions, such as protection from free radicals, inhibition of nitric oxide release from vessel wall, inhibition of prostaglandin synthesis, angiogenesis stimulation and Ig-like function. Hp is an acute-phase protein; its concentration increases during an inflammatory response. After binding of Hb to Hp, iron becomes less available for bacterial growth; this may represent an aspecific mechanism of defence against infections. In addition, Hp seems to have specific defence ability, when interacting with B cells, T cells, monocytes, neutrophils and endothelials cells. Hp has been studied as acute-phase protein along with other proteins for diagnosis of sepsis during neonatale age.

The aim of our study was to assess if Hp can be considered as an early, sensible and predictive index of infections from the first days of life, as it is usually absent from serum of the majority of healthy term infants. Its concentration usually increases in first week and reaches normal levels during the first month. We compared 20 healthy babies (first group) to 30 babies (second group) with infection (half of them were preterm). Most of sick neonates were considered high risk babies due to prenatal complication (PROM, positive vaginal culture, maternal fever). In all subjects we performed CBC, band cells count, CRP and Hp at the 1st , 3 rd and 7th day of life. We also performed microbiological culture of blood, urine, gastric aspirate, pharynx, external ear and conjunctiva. The sick newborns had fever or other symptoms of infection and all showed abnormal value of CRP (> 1 mg/dL), abnormal CBC counts with band-to-total neutrophil ratio > 0.14 and at least 3 positive coltures at 1st and 3 rd day of life. Sign of infection, following antibiotic therapy, underwent a progressive normalization towards the 7th day of life. We did not note any significant difference of Hp levels in these newborns before and throughout the therapy; the ranges of Hp levels in this group were 10-40 mg/dL at the 1st day, 7-45 mg/dL at the 3rd day and 6-47 mg/dL at the 7th day. The group of healthy babies (first group) showed similar values of Hp at the 1st, 3 rd and 7th day of life (range 6-50 mg/dL). In newborns of any gestational age with infection or sepsis, we could not find a sensible variation of Hp levels (p not significant), probably due to the interference of the physiologic hemolysis of this age, therefore its dosage may not be considered an useful index of infection during the first days after birth.

PO-12
AN INDIVIDUAL APPROACH TO THE TREATMENT OF ANEMIA OF PREMATURITY WITH ERYTHROPOIETIN

F. Dituri

Department of Neonatology, Fatebenefratelli S. Giovanni Calibita Hospital, Rome, Italy

Therapy with recombinant human erythropoietin (r-HuEPO) is effective in treating anemia of prematurity. Indeed, the erythropoietin-treated patients require fewer transfu-

sions and receive smaller cumulative volumes of transfused blood. Many centers routinely administer erythropoietin with a quasi-standardized modality; moreover, this therapy is associated with supplementation of iron, vitamin B12, folic acid and proteins. In the past years nevertheless we (and others) showed that the premature infants show much variable serum levels of endogen erythropoietin; indeed, some of them produce an insufficient amount of this growth factor, while others may sometimes produce appreciable or quite high EPO levels. We evaluated 12 preterm newborns (first group, EG 27-31 weeks) and 14 full term babies (second group, EG 38-42 weeks). We measured in each group the value of endogenous serum erythropoietin (mU/mL) at 3 days of life; subsequently, at 10 days of life, we assayed the value of reticulocytes (%) and calculated the following index: reticulocytes (%) X patient Ht / expected Ht.

Results: in the first group the level of endogenous erythropoietin was 50.3 ±93 mU/mL (range 2.1-340) and the reticulocytes range was 1.6-5.3%; in the second group the level of endogenous erythropoietin was 39.3 ±20 mU/mL (range 11.1-92.1) and the reticulocytes range 2.0-4.5%.

Discussion: according to the normal range (confidence interval of 95%) the preterm infants showed much variable levels of serum erythropoietin (DS ±90 mU/mL), while the term babies had a smaller dispersion (DS ±20 mU/mL). The reticulocytes index was always normal in the group at term; on the contrary, it showed an inadequate bone marrow prodution in the preterm infants with the lowest values of endogenous erythropoietin. These data seem to suggest the advantages of routine serum endogenous erythropoietin measurement, before starting with r-HuEPO therapy, in addition to CBC, platelet and reticulocytes counts, ferritin, total proteins, liver and kidney function test investigation. The method (ELISA) is fast and requires only a few µLs of serum. This approach, could improve the pharmacologic supplementation with recombinant erythropoietin and allow for the personalization of treatment to the single patient.