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References

Neopterin Concentrations in Cord Blood: A Single-Cohort Study of Paired Samples from 541 Pregnant Women and Their Newborns, Harald Schennach,³ Christian Murr,¹ Clara Larche¹, Werner Streif,² Erika Pastner,³ Daniela Zulnurn,¹ Diethar Schönzter,¹ and Dietmar Fuchs⁶ (¹ Central Institute for Blood Transfusion, and Departments of ² Pediatrics and ³ Gynecology, University Hospital Innsbruck, A-6020 Innsbruck, Austria; Institutes of ⁴ Medical Chemistry and Biochemistry and ⁵ Hygiene and Social Medicine, Leopold-Franzens University, and ⁶ Ludwig Boltzmann Institute for AIDS Research, Fritz Pregl Strasse 3, A-6020 Innsbruck, Austria; * address correspondence to this author at: Institute of Medical Chemistry and Biochemistry, Leopold-Franzens University, and Ludwig Boltzmann Institute for AIDS Research, Fritz Pregl Strasse 3, A-6020 Innsbruck, Austria)

Neopterin, a product of interferon-γ-activated monocyte-derived macrophages, is a sensitive indicator of cell-mediated immune activation (1). In humans, increased concentrations of neopterin in serum and urine have been found in various malignant disorders and autoimmune diseases as well as during allograft rejection episodes and viral infections, including HIV type 1 (2–8). Serum neopterin concentrations have also been investigated during pregnancy and in the neonatal period (9–11).

In this study, serum neopterin was measured in women with uncomplicated pregnancies, and concentrations were compared with cord-blood concentrations after delivery. A total of 541 women with a median age of 29.0 years (range, 15.5–44.3 years) who delivered at the University Hospital Innsbruck between October 1997 and July 1999 and who had all examinations during pregnancy performed at the same institution were included in the study. All of them took part in the Austrian healthcare program called “Mutter-Kind-Pass”, which is recommended to every pregnant woman and is supported by the public health system. This program includes at least five gynecologic examinations and one internal medical investigation during pregnancy. In addition, all pregnant women are tested for antibodies against rubella virus, Treponema pallidum, and Toxoplasma gondii and are screened for hepatitis B surface antigen. None of them had medical or obstetric complications. All pregnancies were uncomplicated singleton gestations that produced (with one exception) healthy term infants (290 males and 251 females), whose growth was appropriate for gestational age. In keeping with customary healthcare practice in Austria, the development of all the children was checked by medical investigations at least five times beginning with the neonatal period up to the age of 14 months. In addition to this routine program, EDTA-blood samples collected from all newborns by heel lancing in the first week after birth were tested for cytomegalovirus (CMV) by the qualitative Amplicor CMV test (Roche Molecular Systems). This PCR assay amplifies a 365-bp fragment of the CMV polymerase gene and has a limit of detection of ~1000 copies/mL (12).

Blood samples were drawn by venipuncture of the mother in the 28th week of gestation. Immediately after delivery, blood samples were drawn by puncture of the umbilical artery of the cord before the placenta was discarded. The blood was allowed to clot at room temperature, and serum was obtained by centrifugation at 3220g for 15 min. Neopterin analyses were performed within 1 day after blood collection. Serum neopterin was measured by a commercially available ELISA (ELItest Neopterin; BRAHMS Diagnostica) with a detection limit of 1 nmol/L neopterin and an interassay CV ranging from 3.9% to 8.2% (13). Upper reference limits (95th percentiles) for neopterin concentrations are age-dependent and range from 8.7 nmol/L (19–75 years) to 13.5 nmol/L (<19 years) and 19.0 nmol/L (>75 years) as described previously (13). The study was approved by the local ethics committee, and consent was obtained from all participating women before all procedures were performed.

Correlation between variables was assessed by the nonparametric Spearman rank correlation method because the distributions of observed values were generally

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nongaussian. Differences in distributions of laboratory variables among patient groups were tested for significance by the nonparametric Mann–Whitney test.

Shown in Fig. 1 are the neopterin concentrations of healthy pregnant women in the 28th week of gestation (median, 5.4 nmol/L; interquartile range, 4.4–6.7 nmol/L; 95th percentile, 9.0 nmol/L) and in cord blood (median, 13.0 nmol/L; interquartile range, 10.7–15.4 nmol/L; 95th percentile, 20.9 nmol/L) of healthy newborns. There was a weak but statistically highly significant correlation between neopterin concentrations in maternal blood during the 28th week of gestation and cord blood [Spearman rank correlation ($r_s$) = 0.1889; confidence interval, 0.1038–0.2712; $P < 0.0001$; $n = 541$].

All except four neonates had negative results by CMV-PCR. One of these four neonates had clinical signs of CMV infection; the others were free of symptoms during a follow-up of at least 2 years. In comparison, cord-blood neopterin values of newborns who were positive by CMV-PCR showed a significantly higher median neopterin value of 22.5 nmol/L (Mann–Whitney test, $U = 412.0; P = 0.034$). In contrast, neopterin concentrations in maternal serum samples obtained during the 28th week of gestation did not differ between those who delivered healthy newborns and those whose newborns were positive by CMV-PCR (Mann–Whitney test, $U = 933.5$; not significant).

This study provides reference values for neopterin concentrations in cord blood and from pregnant women, with the 95th percentiles defined as the upper reference limit. The serum neopterin values we observed in women during the 28th week of gestation were similar to those reported in a previous investigation (9). In general, neopterin values increase with pregnancy up to the third trimester and are higher than in nonpregnant women (9, 14). Because neopterin production reflects cellular immune activation (2), it might be hypothesized that during pregnancy immunogenic stimuli are increasingly induced by the placenta and the fetus (15). Cord-blood neopterin concentrations may reflect immune activation of the fetal compartment, which has previously been found to be isolated from the maternal compartment with respect to neopterin metabolism (16). This isolation between compartments is somehow contradicted by the positive correlation between maternal and cord-blood neopterin concentrations in our study, but this correlation was rather weak and has not been found in other studies (10). The measured cord-blood neopterin concentrations are slightly lower than those reported in an earlier investigation (11). In agreement with a comparable investigation (10), they are approximately three times higher than maternal serum neopterin concentrations at the beginning of the third trimester. Because neopterin is not metabolized and will be excreted in its native form by the fetal kidneys, neopterin may accumulate in the amniotic fluid and possibly also in the fetal compartment (10). Cord-blood neopterin concentrations are a result of maturation of the fetal immune system and may reflect peripartum inflammatory processes or noninfectious cytokine activation processes attributable to exposure of the fetal immune system to exogenous immunogens.

Fetal infections may lead to increased neopterin concentrations in cord blood. This is supported by the higher median neopterin concentration in the four CMV-PCR-positive newborns compared with the negative control group. CMV infection can be important in immunosuppressed patients and during pregnancy; thus, in newborns, CMV may cause asymptomatic infection, prematurity, growth retardation, and illness, including acute hepatitis infection, fever, pneumonitis, encephalitis, deafness, chorioretinitis, hematologic disorders, and death. Congenital CMV infections remain the leading viral cause
of congenital malformations in the developed world (17). Because neopterin concentrations sensitively detect acute viral infections, one possible application of neopterin measurements would be the detection of unrecognized infections such as those by CMV (18,19). In immunocompromised adults, neopterin determinations are already a valuable tool in addition to CMV antibody determinations for estimation of the severity of CMV infection (20). In our study, the only newborn with a symptomatic CMV infection had a cord-blood neopterin concentration that was slightly above the 95th percentile of the healthy control group. Nevertheless, the number of CMV-infected newborns was too low for definite conclusions. Further investigations with a higher number of CMV-infected newborns will be necessary to clarify the potential diagnostic impact of neopterin determinations in cord blood for estimation of severity of CMV infection.

The cord-blood neopterin concentrations of CMV-PCR-positive newborns in general were in the high end of values for the general population but were not extraordinarily high. Therefore, the differences between healthy newborns with neopterin concentrations at the low or high end of the scale remain unclear, and one can only speculate whether other infectious agents or metabolic differences are involved or whether such differences may be related to the maturation status of newborns. Higher neopterin concentrations have also been described in preterm infants compared with term infants (11).

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References

Bias and Random Error in Retinol Measurements of Laboratories in Countries with Populations with Mild to Severe Vitamin A Deficiency, Paul J.M. Hulshof,1* Jitske T. Brouwer,1 Jan Burema,1 and Clive E. West1,2 (1 Division of Human Nutrition and Epidemiology, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands; 2 Division of Gastroenterology, University Medical Centre Nijmegen, 6500 HB Nijmegen, The Netherlands; * author for correspondence: fax 31-317-483342, e-mail paul.hulshof@staff.nutepi.wau.nl)

Vitamin A deficiency is common in many developing countries. Population-based surveys suggest that as many as 175 million children of preschool age are vitamin A-deficient (1). Especially in sub-Saharan Africa, severe subclinical deficiency (retinol values ≤0.70 μmol/L) often exceeds 30%. In these countries, laboratories should be able to measure the biochemical indicators of nutritional status to acceptable standards. The aim of the present study was to determine the proficiency of selected laboratories, particularly in Africa, in measuring retinol in serum.

The study was performed between September 1999 and February 2000. Laboratories in Africa with the capability to measure vitamin A in serum were located with help from WHO headquarters and country offices, UNICEF headquarters, and through our contacts. Sixteen of the 35 invited laboratories were able to participate in the study: 12 within Africa (including Ethiopia, South Africa, 4 laboratories); Tanzania, Zambia, Gabon, Ghana, Nigeria, Egypt, and Morocco), and 4 outside Africa (including Guatemala, Indonesia, 2 laboratories), and Vietnam). Participants were offered a total honorarium of US $200. All laboratories claimed that they could measure retinol in serum. The