Preanalytical issues in neonatology

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Neonates are subject to more preanalytical influences than any other population group. Many of these are related to the maturity of the infant and its adaptation to extra-uterine life.

The necessary need for capillary blood collection and processing of small quantities of blood introduce additional preanalytical factors. Healthy neonates experience greater changes in laboratory test results over a short period of time than any other population group.

Neonates are not a homogeneous group and many of the differences in test results are attributable to differences in maturity. This is the major, but not the only, factor that affects results in the neonate.

Thus, proper interpretation of test results in the newborn requires knowledge of his/her state of development as well as many other preanalytical variables, some of which are unique to the small volumes of blood that are frequently collected. An additional problem in the interpretation of laboratory data in neonates is the lack of well-characterized reference intervals.

Prematurity

About 75 % of the total weight of a premature newborn infant consists of water, whereas only 70 % of the total weight of the term neonate and 60 % of the adult's weight is water. In the newborn about 50 % of the total body water is extracellular fluid (ECF), compared with about 20-25 % in adults.

The normal newborn has an ECF compartment of up to 35 % of body weight, but loses 6-10 % per day in the first few days of life.

The average blood volume in premature babies is about 115 mL/kg, falling to 60-110 mL/kg in neonates and 75-110 mL/kg in infants [1]. The total blood volume is about 185 mL at 32 weeks of gestation, 299 mL at 36 weeks of gestation and 272-340 mL at birth.

The small blood volume in a neonate is associated with a high hematocrit and erythrocyte count, and the blood hemoglobin concentration is high, ranging up to 190 g/L.

The newborn is relatively less able than the older child or adult to maintain homeostasis of fluids and electrolytes and has difficulty in excreting water loads, and hyponatremia and hypernatremia are common. The mean plasma aldo-sterone in preterm infants may be as much as 2-5 times higher than in the term infant.

Change in hematocrit with age			
Days	%	Weeks	%
1	61	1 - 2	54
2	60	2 - 3	43
3	62	3 - 4	36
4	57	4 - 5	36
5	57	5 - 6	36
6	54	6 - 7	36
7	56	7 - 8	33

Table I. Modified from Matoh Z, Zaizov R, Vasaro I. Acta Paediatr Scand 1971; 60: 318.

Renal function in the newborn is immature and is frequently associated with a relatively large excretion of uric acid, and the serum uric acid concentration is also often high. Mild proteinuria is a normal event. Glomerular filtration rate, renal plasma flow and tubular function are less in the newborn but improve during the first few weeks of life. Urea clearance is low and the neonates concentrating ability is impaired. A transient increase in the blood urea nitrogen concentration may be observed in the first few days of life.

The neonate has a marginal capacity to excrete bilirubin and most preterm infants will exhibit hyperbilirubinemia evidenced by jaundice. UDP-glucuronyltransferase is an adaptive enzyme, with activity increasing in response to increased amounts of bilirubin over several days in both the preterm and mature infant. The concentration of most proteins, including albumin, is less in the premature infant than in the mature one and is often correlated with gestational age. The low availability of albumin binding sites in the neonate and increased concentration of unconjugated bilirubin cause hyperbilirubinemia. Salicylates, free fatty acids, heme pigments and lactate compete for albumin binding sites and may displace bilirubin from albumin. Treatment of babies for 6 hours with blue light intensive phototherapy will cause the bilirubin concentration to decrease by 25 %, but without effect on other analytes.

The serum calcium concentration may be low in the premature neonate primarily because of reduced parathyroid function, arising in part from the baby's exposure *in utero* to high calcium concentrations but also because of poor renal tubular responsiveness to parathyroid hormone. Preterm neonates, in particular, may not have the surge in parathyroid hormone secretion demonstrated by term infants after birth. Hypocalcemia may also follow ingestion of milk with a high phosphate concentration. Newborns are also prone to hypoglycemia, especially those with intrauterine growth failure, who are twins, or are infants of diabetic mothers, or who have erythroblastosis fatalis.

The causes are limited glycogen and fat stores in the first two categories and pancreatic islet-cell hyperplasia in the latter two. Hypocalcemia, hypoglycemia and hypomagnesemia often coexist. Insulin concentrations in the normal neonate are relatively low, and remain so during the first few hours of life. Growth-hormone concentrations are relatively high and increase in the immediate postnatal period. Glucagon, catecholamine, and adrenal corticoid concentrations increase in the immediate postnatal period.

In neonates, gestational age correlates well with the concentrations of thyroid-stimulating hormone. The concentration of serum thyroxine correlates with the gestational age and weight of infants greater than 33 weeks gestation, but not in more premature infants [2]. There is a good correlation in the infant at birth between its acid-base status and his/her Apgar score. This is also true for the fetal blood pH. Acidosis usually occurs in an infant in response to the stress of labor and delivery, but usually resolves within a few hours, primarily by pulmonary excretion of CO_2 and metabolism of non-volatile acids. The new born is prone to late metabolic acidosis.

The neonate has poorly established hemostatic mechanisms with minimal transfer of clotting factors

from its mother. Its capability to metabolize many drugs and ability to respond to many infections are reduced. Although the total leukocyte count in a newborn may be as high as 35,000 cells/μL, it may not increase in the presence of an infection.

Breast feeding

The plasma concentrations of albumin, phosphate, 25-hydroxy-vitamin D, and parathyroid hormone are less in neo-nates fed breast milk in those fed formula. In contrast, the concentration of ionized calcium is increased. Drugs may pass from the maternal circulation to the fetus during pregnancy. With the immature liver of the newborn, drugs are metabolized much more slowly than in the mature infant and adult with corresponding prolongation of their actions. Ingestion of anti-convulsants, in particular, by pregnant women may lead to hemorrhage in neonates as a result of vitamin-K deficiency. Perphenazine and some diuretics may produce the same effect. Anesthetic agents may cause methemoglobinemia. Most drugs ingested by lactating women appear in their breast milk so that exposure of the neonate may continue for a long time after birth. Neonates may consume up to one litre of breast milk per day so that a considerable amount of drug may be excreted, even if only present in breast milk in nanogram concentrations.

Sites for collection of blood

In neonates, blood is most often collected by skin puncture when infrequent tests are required, but in the very sick neonate blood for testing is most easily collected from an in-dwelling central line. If the latter is used, this should be performed only by an experienced physician or nurse. It is important to note that unless the line is well flushed with blood before that needed for the test is collected, the measurements of large-molecular-weight constituents, such as hormones, proteins and protein-bound constituents, and cells will show the influence of dilution, and other tests will be influenced by the composition of the infusion fluid. A general rule of thumb is that the blood comprising two or three times that of fluid in the IV line should be discarded before that coagulation testing is collected. If a relatively large volume of blood must be collected in the absence of an indwelling line, as for hormone analyses, venipuncture should be performed, preferably using a butterfly needle and syringe, since the strong suction of evacuated blood tubes may cause collapse of neonate's small veins.

Scalp blood may be collected in the delivery room or intensive care nursery for blood gas measurements. It is important to note that red- and white-cell metabolism may continue in the syringe used to collect the blood, leading to low pH and pO_2 and increased potassium, and pCO_2. Analyses must be performed within 10 minutes to minimize these effects.

Blood collection

It may be difficult to prepare a neonate for blood collection, and it should be noted that crying during collection of blood may be associated with increased glucose and lactate concentrations. Blood from a skin puncture is typically allowed to drip into the collection tube. In many neonatal nursing units, measurements are made at the point of care using whole blood. If this route is not pursued, plasma is generally preferred to serum, since a greater yield of plasma than serum can be obtained from the same volume of blood. Plasma has the additional theoretical benefits of being more representative of the *in vivo* state than serum and of having less risk of causing hemolysis and lysis of platelets. If the specimen is for blood pH or gases, the blood must be collected into a heparinized capillary tube, and the blood must be mixed for which a small metal mixer in the tube is often used most efficiently. The tube must be sealed and placed in ice water to reduce metabolism, with its attendant decrease in pH. Without such handling, blood pH decreases by about 0.005 every 10 minutes at ambient temperature [3]. The small volumes of blood used for testing specimens from neonates are prone to evaporation and efforts must be made to minimize the surface exposed to air, and testing of specimens should be performed rapidly. The tubes for specimen collection should always be capped before centrifugation. The concentrations of small

molecules, such as sodium, potassium, bicarbonate, and chloride have been showed to increase by about 30 % when blood is spun in a high-speed centrifuge without caps, compared with those in capped tubes. Even when uncapped specimens are aloud to sit at ambient temperature for an hour, the concentration of some analytes may increase by almost 10 % for blood volumes of about 0.1 mL, although the effect is much less with volumes of 5 mL or more [4].

Capillary blood

A preferred procedure to collect blood from babies has been described by the Pediatric Committee of the American Association for Clinical Chemistry [5]. Blood obtained by skin puncture is a composite of arterial, capillary and venous blood, but more resembles arterial blood than venous blood. The composition is influenced by the blood flow to the skin at the time of collection, and blood flow is enhanced by warming, since cold constricts both arterioles and capillaries [6]. The medial or lateral aspect of the plantar surface of the foot is the preferred site for collection of blood in neonates [7].

Puncturing of the skin to a depth of 2 to 4 mm in these sites lessens the risk of osteonecrosis. Gentle pressure without milking or massaging the area around the puncture site enhances the free flow of blood. The puncture site must be free from edema, since edema will cause the specimen to be contaminated by tissue fluid, with possible increase of plasma hemoglobin, potassium, and lactate dehydrogenase. After the first drop of blood is discarded, the blood for testing should be collected by gently touching the formed blood drops to the collection tube and allowing it to fill by capillary attraction. When the tube contains an additive or preservative, it should be gently inverted about 10 times to ensure adequate mixing without hemolysis.

Prior to puncturing the skin with a lancet, the skin to be punctured should be cleaned with 70 % isopropanol, which should be allowed to dry completely before the skin is punctured to minimize the likelihood of hemolysis. Using Betadine® (providone-iodine) as a cleaning agent is prone to cause increased concentrations of potassium, phosphate, chloride, CO_2 and uric acid [8]. The capillary-blood pH and concentration of glucose are higher than in venous blood because the venous blood reflects the action of tissue metabolism. The mean glucose concentration in capillary plasma is 0.5 mmol/L higher than in venous plasma, and that in capillary whole blood is 0.4 mmol/L higher than in venous whole blood. The concentrations of thyroid-stimulating hormone, thyroxine-binding globulin, and thyroxine in capillary blood of neonates are significantly higher than in venous serum. Often blood specimens to screen for inborn errors of metabolism are collected by skin puncture and applied to filter paper. If the test area is not completely filled with blood, falsely low concentrations of analytes may occur. Another cause of erroneously negative screening tests for inborn errors of metabolism is a failure to ensure that the neonate is appropriately challenged with an adequate amount of protein before the specimen is collected.

Hemolysis

Hemolysis induced during the specimen collection is more common in neonates than in other patients. Poorly performed skin punctures with tissue trauma are the most common cause. Hemolysis increases the concentration in plasma of constituents with a high intracellular content, but also causes optical interferences with various analytical instruments. It may also affect the reaction mechanism in some assays. The tests most affected by hemolysis are potassium, lactate dehydrogenase, aspartate and alanine aminotransferase (although the former is affected to a greater extent), creatine kinase, and triglycerides. The activities of alkaline phosphatase, amylase and glutamyltransferase may be decreased. Hemolysis interferes with diazo methods to measure bilirubin, but in different ways. The popular Jendrassik and Grof procedures yield progressively decreased concentrations with increasing concentrations of hemoglobin. In contrast, methods including 2,5-dichlorophenyldiazonium detergent yield falsely increased concentrations [9].

Hyperalimentation

Hyperalimentation enhances growth and positive nitrogen balance. Increased urinary catecholamine and plasma C-peptide concentrations occur. Intralipid has no effect on blood pH, pCO_2, and alveolar-arterial oxygen tension in neonates with normal respiratory function, although transcutaneous pO_2 is reduced. No effect has been observed on apolipoprotein A-I, cholesterol, lecithin: cholesterol acyltransferase in neonates. In ver small premature neonates the glucose concentration increases if a glucose infusion is combined with lipid. Since the fluid being infused contains many of the analytes measured to monitor the status of the patient, it is advised that infusions of electrolytes, carbohydrate-rich fluids, amino acids, and proteins are stopped for as long as one hour before collection of blood for testing.

Ideally, infusions of fat emulsions should be stopped for 8 hours before blood collection, primarily to avoid interferance by lipemia with analytical methods.

A more comprehensive compilation of the effects of preanalytical factors on neonates and others is avalable [10]. Yet from the incomplete examples cited here, it is readily apparent that physicians and laboratory staff need to become aware of the many possible analytical variables that can affect the proper interpretation of clinical laboratory test results.

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