Working Group on Perinatal and Paediatric Microcirculation—WGPPM

Lectures

Blood Cell Deformability—Why Does it Matter in Microcirculation?

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The function of microcirculation is to achieve the exchange of gases, fluids and substances between blood and tissues. By decreasing the diameter of the capillaries and the spacing between them the transport processes are made more efficient for two reasons: 1. The same blood volume will have an increased area of contact with the tissue and 2. the transport distance within the capillaries and in the tissues will be minimized (1). However, the smallest calibre of capillaries must still permit the passage of erythrocytes and other blood cells. Red cells with resting diameters of 8µm are able to enter capillaries with diameters of 3µm because of their unique ability to assume various shapes in a very short time. Thus, red cell deformability is an important prerequisite for narrow and closely arrayed capillaries. The time for marked extensional deformation, for the passage of a red cell through a capillary and for deoxygenation is approximately 0.1 s (2, 3). Moreover, red cell deformation accelerates deoxygenation of red cells (2). This may be explained by closer contact of red cells and vessel wall, by a smaller stagnant layer on the cell surface and by mixing of the hemoglobin during cellular deformation.

Red cell deformability also affects platelet aggregation and platelet adherence to the vessel wall (5). Platelet adherence may be increased as a result of decreased red cell deformability, increased red cell size and increased hematocrit. Platelet aggregation at high shear is enhanced by increased red cell deformability and by increased hematocrit.

Cellular deformability determines the ability of the red cell to survive in the microcirculation (3). Rigid cells (e.g., spherocytes, aged red cells) are destroyed in the spleen. In most types of hemolytic anemias, the final destruction of red cells in the spleen is due to impaired cellular deformability.

Which pediatric disorders are associated with both decreased red cell deformability and disturbed microcirculation? The best known example is sickle cell anemia. Irreversible sickled red cells cannot deform at all and, therefore, plug narrow capillaries. Subsequent red cells are blocked, they release their oxygen, become sickled and plug adjoining vessels, thereby initiating a sickle crisis.

Red cells from preterm and term neonates and adults show similar deformability when studied under defined shear conditions (4). Decreased filterability of neonatal red cells is probably due to their larger volume. In neonates, decreased red cell deformability has been observed in septicemia and necrotizing enterocolitis. This may contribute to impaired circulation in these disorders. Moreover, red cells in infants of diabetic mothers are moderately less deformable than in normal neonates.

In children, red cells may be less deformable because of iron deficiency. This may be hazardous if the hematocrit is increased as in patients with cyanotic congenital heart disease. These patients are highly susceptible to cerebrovascular accidents when polycythemia is associated with iron deficiency. Moreover, red cell deformability may be diminished in children with diabetes, hypertension and M. Raynaud and contribute to the long-term vascular complications in these diseases.

Little is known about the meaning of leukocyte deformability (6) for microcirculation. Neonatal leukocytes are less deformable than adult leukocytes when studied by means of micropipettes and filter techniques. There is also evidence that leukocytes become more rigid in neonatal septicemia. Moreover, leukocytes may be involved in the pathogenesis of vascular complication in diabetes and peripheral vascular diseases. In children with leukemia, circulatory problems have been observed when the leukocyte count exceeds 50,000/µl.

Therapeutic approaches to improve disturbed red cell deformability include simple removal of rigid red cells by exchange transfusion. Leukocytes can be removed by leukopheresis or exchange transfusion. Pentoxifylline is the only drug known to decrease the deformability of red cells. At high concentration, it also decreases the rigidity of leukocyte by up to 50% (6). In spite of extensive use of pentoxifylline in adults, no experience appears to exist for pediatric patients.

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Refractory Early Anaemia of Prematurity—Recognition, Physiological Basis and Management

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The refractory anaemia of prematurity (REAP) is one of the multiple non-physiological complications of preterm delivery and reflects the inadequacies of the blood oxygen transport system for physiological needs *ex utero*. These infants depend for a prolonged period on predominantly fetal red cells of poor viability and high oxygen affinity so that haemoglobin concentration and P50 are low (3). This results in low values of 'available oxygen' (2), during the first three or so months after birth. During this period oxygen requirements for growth, notably of the brain, are high and are intensified by cardio-respiratory and infective complications. The inadequacy of the erythropoietic response to hypoxia is partly explained by inappropriately low erythropoietin concentrations, perhaps also a feature of immaturity (1). This haematinic-refractory anaemia would be compounded by hypoxia from other causes and by spontaneous and iatrogenic blood losses in the early weeks of life. The resulting hypoxia is clinically recognisable by increased respiratory and heart rates at rest or on slight exertion, raised oxygen consumption and poor feeding and growth. These symptoms and signs respond to red cell transfusion, the immunological and infective hazards of which are incompletely understood. The longterm clinical effects of impaired oxygen availability in the first months are unknown, for example, on the individual's ultimate intellectual potential.

Objectives in optimising management of REAP

- (1) Define adequate 'available oxygen' values by appropriate clinical investigations in preterm infants during the first few months of life, e.g. until one year post-conception.
- (2) Seek means of making 'available oxygen' adequate to prevent short-term and any long-term adverse clinical effects. Preferably avoid donor red cell transfusion.
- (3) When red cell transfusion is required, e.g. for replacement of investigational blood losses and/or for the prevention and correction of anaemia, avoid hazards of donor red cell transfusion, e.g. immunological and infective.

Possible means of achieving optimal management of REAP (assuming minimal blood losses and optimal haematinic supplies)

- (1) Define and secure optimal placento-fetal transfusion on delivery, avoiding circulatory overload!
- (2) Collect and store placental blood for autologous replacement of blood losses.
- (3) Optimise the newborn's whole-blood and red cell rheological properties to minimise haemolysis and so jaundice, perhaps circulatory stagnation *in vivo* and certainly anaemia.
- (4) Optimise fetal haemoglobin function for oxygen transport.
- (5) Study control of timing and rate of fetal to adult erythropoietic switch and of resumption of erythropoiesis after its post-natal shutdown: bring forward adult erythropoiesis in preterm infants. (Drugs may be developed to achieve some of these goals).

Pertinent investigations:

- (1) Laboratory and clinical assessment of adequacy of oxygen supply from blood. 'Available oxygen' calculation; erythropoietin assay. Infant's oxygen consumption at rest and under stress, e.g. in a cool environment. Estimation of cardiac output and calculation of mixed venous oxygen tension.
 - (2) Placental blood. Placento-fetal transfusion - assess blood volume at birth in relation to subsequent anaemia. Placental blood - collect, store and assess usefulness in autologous transfusion to replace investigational blood losses.
 - (3) Infant's blood's Hb-O₂ dissociation - may be pharmacologically modifiable.
 - (4) Infant's red cell deformability - assess at birth and prospectively in relation to later rate of fall of Hb concentration and development of jaundice.
 - (5) Fetal-adult erythropoietic switch. Assess fetal/adult erythropoietic balance at birth and the changes across period of REAP.
 - (6) Ultimate assessments - long-term studies needed of red cell transfusion and its complications, e.g. immunological and infective, and of the pathological significance of the REAP in influencing the development of the individual's ultimate intellectual potential.
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Neonatal Polycythemia and Hyperviscosity Syndrome

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Infant hematocrit varies with both gestational age and postnatal age. It has been assumed that the rise in hematocrit which occurs from first to third trimester is an adaptation to increase oxygen needs and the relative intrauterine hypoxia. Three decades ago newborns with markedly elevated hematocrits and serious cardiorespiratory distress were described. Often the symptomatic infant was the recipient of a twin-to-twin transfusion. These early case reports are the first documented cases of what is now described as neonatal polycythemia.

In 1966, Baum (1) identified an elevated viscosity in blood from infants who were polycythemic. This elevation in viscosity was believed to decrease peripheral blood flow and hence might explain the pathology associated with neonatal polycythemia. A new condition, neonatal hyperviscosity, was described.

It is common but inaccurate to equate neonatal polycythemia and neonatal hyperviscosity. The definitions of each are specific and, although overlapping, do not exactly define the same population.

Neonatal polycythemia is most commonly described as a venous hematocrit of 65%. Levels as low as 60% or as high as 70% have also been suggested. Variations in sample site or postnatal age compound the difficulty in defining neonatal polycythemia. Linderkamp (3) and Gattl (2) have demonstrated the variability between capillary and venous hematocrit values. Shohat (4) was able to demonstrate dynamic changes in both venous hematocrit and viscosity measurements. By two hours of age there is a linear correlation between cord blood hematocrit and venous hematocrit. Thus, cord blood hematocrit may be a reliable technique for initial screening. However, this technique may miss infants who become polycythemic as a result of acute placental transfusion.

Neonatal hyperviscosity refers to a characteristic of blood which increases with hematocrit. Several other factors also contribute to the viscosity of whole blood. Among these are large plasma proteins and stiffness of the red blood cell membrane. Newborn plasma has a lower viscosity compared to adult plasma since the concentration of plasma proteins is lower in the newborn. Standards for viscosity measurements are available from several authors. Hyperviscosity has been defined as a value more than two standard deviations from mean cord viscosity. Whole blood viscosity is dependent on the shear rate studied. Viscosity measurements are therefore given over several shear rates. Use of the viscosity standards available suggest that 2-6% of newborn infants have hyperviscosity. While hematocrit and viscosity are closely correlated the contribution of other factors to viscosity make it impossible to equate the two conditions. At the higher hematocrit levels all polycythemic infants are hyperviscous. Some infants without polycythemia have abnormal whole blood viscosity. Some polycythemic infants are hyperviscous. In the remainder of this review the clinical signs and outcome associated with the two conditions will be discussed.

Infants with polycythemia and hyperviscosity often present with cardiorespiratory signs which mimic cyanotic congenital heart disease. Tachypnea, tachycardia, cyanosis, and apnea have all been described as well as pulmonary hypertension.

Central nervous system signs are common including the non specific findings of irritability, rapid state changes, vasomotor instability, lethargy, poor tone, and poor feeding. Early reports of polycythemic infants frequently reported seizures. Prospective screening of infants has identified few with neonatal seizures. Obvious central nervous system hemorrhage is also uncommon.

More recently there has become increasing evidence that polycythemia/hyperviscosity is a frequent cause of necrotizing enterocolitis (NEC) when it appears in the term infant. Polycythemic infants undergoing partial plasma exchange with fresh frozen plasma seem particularly vulnerable to developing a spectrum of gastrointestinal injuries. Pneumatosis intestinalis has also been seen in many of these infants with a range of gastrointestinal signs.

Renal failure, thrombocytopenia, and various other hematologic findings have also been reported. Hypoglycemia is commonly associated with polycythemia and may be of greater magnitude than appreciated since the determination of plasma glucose overestimates whole blood glucose. Peripheral blood flow may be severely compromised and has improved following plasmapheresis.

Outcome following neonatal polycythemia/hyperviscosity is largely dependent upon the method used to identify the subjects. Seriously compromised infants as originally described are not the rule. Most infants have symptoms that are less obvious. The most symptomatic infants in early studies had developmental delays and neurologic handicaps. Prospective screening identifies a group of infants for whom the outcome remains controversial. Several authors have failed to show significant impairment at 1-6 years. Our studies suggest that there are clear differences between polycythemic infants and a comparison group at two years of age.

Partial plasma exchange appears to improve sensory outcome. All studies of prospectively identified polycythemic infants suggest that mental development is not significantly affected.

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Oxygen Radicals and Tissue Damage in the Perinatal Period

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Posthypoxic tissue damage could be caused by oxygen free radicals generated by hypoxanthine (Hx)-xanthine oxidase (XO) system. Hx which is accumulated in hypoxia is washed out into the circulation in the posthypoxic reperfusion period. If XO is present the superoxide radical, O_2^- , is formed: $Hx + O_2 \xrightarrow{XO} \text{Urate} + O_2^-$.

We have suggested that this mechanism is responsible, at least partly, for several conditions in perinatal medicine such as bronchopulmonary dysplasia, necrotizing enterocolitis and retrolental fibroplasia. If this hypothesis is correct we are dealing with a common pathogenetic mechanism leading to one disease affecting different organs.

To explore this hypothesis we have mainly studied the lung. In summary we have found: 1) The combination of 100 percent oxygen and intravenous oxygen given 48 h damage the rat lung. The typical changes found are hemorrhage and edema. Furthermore, in spite of a normal lung surfactant profile the surfactant function was abolished indicating that surfactant was inactivated either by peroxidation or by the induction of surfactant inhibitor. 2) XO applied into the airways of guinea pigs acutely induced lung hemorrhage and edema. The lung compliance was dramatically reduced. One single application of XO induced long term effects on the lungs as well. After 2 days thickening of alveolar septae and proliferation of type 2 cells were observed. Furthermore, there was an increased number of alveolar macrophages. 7 and 14 days afterwards the histological changes seemed to have normalized, lung compliance, however, was still significantly lowered. 3) Superoxide dismutase given together with XO protected against XO.

The experimental data show that the hypoxanthine-xanthine oxidase system potentially destroys the lungs under experimental conditions.

Red Cell Aggregation in the Neonate

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The viscometric characteristics of neonatal blood show distinct differences from those of adults

(1,2); in particular it is found that at a given haematocrit neonatal blood generally exhibits lower viscosity at all shear rates. In part this is due to the lower plasma viscosity that is also found in the neonate. The effect of this plasma difference can be removed by calculating relative viscosity, and when this is done the high shear rate ($>5s^{-1}$) values of the two types of blood become similar but the low shear rate difference persists. Since it is usually accepted that the elevated low shear rate viscosity of adult blood is due to rouleaux formation, it is to be expected that the latter will be reduced in the neonate and this has been confirmed experimentally.

The cause of the reduced aggregation must lie in the red cell or in the plasma or both. However plasma exchange experiments indicate that adult and neonatal cells aggregate similarly when suspended in adult plasma, while both show reduced aggregation in neonatal plasma (2). Of the aggregating agents in adult blood fibrinogen is by far the most important, so experiments have been performed in which both cell types were suspended in solutions of purified adult fibrinogen. Again they showed similar degrees of rouleaux formation. The conclusion is therefore that the reduced aggregation found in neonatal blood is not due to cellular differences, but rather to variations in the composition of the plasma.