ORAL COMMUNICATIONS

STROKE IN SICKLE CELL DISEASE: A COHORT STUDY ON ADULTS

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Stroke is a severe complication in sickle cell disease but studies are principally pediatric. We conducted a multicentric cohort study on adults with a history of symptomatic stroke. Sixty two patients were included (52 SS, 8 SC, 1 SßThal+ and 1 SβThal^0) with ninety-five strokes (72 ischemic, 17 hemorrhagic, 4 undetermined, one intracranial sinus thrombophlebitis and one medullar ischemia). The follow up was 759 patient- year, the sex ratio M/F was 0.87 and the mean age at the first stroke was 18±12 years [range: 1-49] in SS-SβThal^0 patients and 38±16 years [2-54] in SC patients. Ischemic strokes were more frequent during the first decade in SS patients but occurred principally after 30 years in SC patients, while hemorrhagic strokes were not observed before the age of 14 years. Thirty four recurrent strokes were observed in 19 SS patients and one SβThal$^+$ patient, associated with cerebral vasculopathy in 14 patients. Eleven patients died because of stroke (8 SS; 2 SC; 1 Sβtha^0), most of them were hemorrhagic (8/11) and inaugural (9/11). Cardiovascular risk factors were only observed in the SC population (4 high blood pressure, 2 diabetes, 1 obesity, and 1 dyslipidemia. In conclusion, strokes were principally ischemic with a greater frequency during childhood in SS patients and adults in SC patients, in contrast, death was almost exclusively due to hemorrhagic strokes. Vascular risk factors were only present in SC patients and recurrent strokes were only observed in SS patients.

INTERACTION OF LU/BCAM GLYCOPROTEINS WITH THE MEMBRANE SKELETON NEGATIVELY MODULATES CELL ADHESION TO LAMININ α5: ROLE ON THE INCREASED ADHESION PROPERTIES OF HEREDITARY SPHEROCYTOSIS RED CELLS

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We previously demonstrated that the erythroid laminin ·5 receptor, Lu/BCAM, interacts with the membrane skeleton through spectrin binding. We show now that this interaction negatively regulates K562 cell adhesion to laminin. Since hereditary spherocytosis (HS) RBC exhibit increased laminin adhesion (Wandersee, 2004), we investigated the role of Lu/BCAM-spectrin interaction in the adhesion properties of 2 splenectomized HS RBC exhibiting 40% spectrin deficiency. Under physiological flow conditions, HS RBC revealed exaggerated adhesion to laminin that can be abolished by soluble Lu/BCAM. Correlation between spectrin-deficiency and increased solubilisation of Lu/BCAM from the membrane skeleton suggested that the reinforced adhesion results from an impaired interaction between Lu/BCAM and the skeleton. While we previously reported that abnormal adhesiveness of sickle and Polycythemia vera RBCs is associated with phosphorylation of Lu/BCAM, we showed here that the phosphorylation status of Lu/BCAM is not regulated by association with the membrane skeleton. Hence, the mechanisms underlying Lu/BCAM mediated increased adhesion after spectrin binding disruption remained to be elucidated. Of note, abnormal adhesion to laminin was not observed when RBCs from one of the HS patients were analysed before splenectomy. Interestingly, a significant increased risk of adverse arterial and venous thromboembolic events in HS patients after splenectomy has been recently reported (Schilling, 2008). We assume that analysis of HS RBC from a large number of patients before and after splenectomy should allow us to test whether thromboembolic events might partially result from Lu/BCAM-mediated adhesion to laminin of HS RBCs that are no more cleared from the circulation after splenectomy.

CHARACTERIZATION OF IRON METABOLISM AND ANEMIA IN A MOUSE MODEL OF CONGENITAL ERYTHROPOIETIC PORPHYRIA

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Congenital erythropoietic porphyria (CEP) is a severe autosomal recessive disorder characterized by a deficiency in uroporphyrinogen III synthase, the fourth enzyme of the heme biosynthetic pathway. This defect leads to accumulation of type I isomers of porphyrins (uroporphyrins and coproporphyrins) in the bone marrow, followed by massive excretion. CEP is characterized by severe cutaneous photosensitivity and chronic hemolysis. We studied a murine model of CEP where the symptoms closely mimic those observed in patients. Hematological data of 14-month-old CEP mice confirm the microcytic and

hypochromic anemia, as well as severe reticulocytosis (25% of reticulocytes). FACS analysis is in process to characterize apoptosis of the different erythrocyte subpopulations in spleen and bone marrow. Tissue iron quantification shows important accumulation of iron in the liver (1600 µg/g of liver for CEP mice vs 208 µg/g for control) and in the kidney (650 µg/g for CEP mice vs 125 µg/g for control) and only moderate accumulation in spleen (1700 µg/g vs 1200 µg/g). Histological study confirms that iron accumulates mostly in liver macrophages and in proximal tubular cells of the kidney. Protein expression of heme oxygenase 1 (HO-1), ferroportin and ferritin is increased in the liver and the kidney of CEP mice. Absence of HO-1 induction in the spleen suggests that tissue iron accumulation mostly results from intravascular hemolysis rather than from an increase in erythrophagocytosis. Because the predominant site of metabolic expression of the disease is the erythropoietic system, bone marrow transplantation represents a curative treatment for patients with severe phenotype.

A NEW "REGIONAL MUTATION" OF THE BETA GLOBIN GENE: 4 FAMILIES FROM NORD PAS DE CALAIS ORIGIN WITH MINOR BETA THALASSEMIA

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Most of beta globin gene mutations are characteristics of Mediterranean populations. We reported (C Méreau et al SFH, Paris, 2001) a new beta globin mutation codons 8/9 (+AGAA); GAG.AAG.TCT (GLU-LYS-SER) → GAG.AAAGAAG, which corresponded to duplication of 4 bases of codons 7/8 involving a shift of the open reading frame starting from the codon 9 with a TGA stop codon in a patient with none other knew descent than Nord Pas de Calais area. We report here four families with this same mutation having no ancestry from the Mediterranean basin. This mutation, causing beta thalassemia heterozygote has never been described before. This is affecting four families without blood ties, all of them from Nord Pas de Calais origin. It could be regarded as a "regional" mutation concerning 21 patients: so a regional incidence of one case out of 200,000. Complementary studies are undertaken to specify genealogy of its appearance in our area.

PARADOXICAL HYDROXYUREA STIMULATION OF PRO-INFLAMMATORY CYTOKINE GENES EXPRESSION IN ENDOTHELIAL CELLS. RELEVANCE TO SICKLE CELL DISEASE

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In sickle cell disease (SCD), hemoglobin S (HbS) polymerization renders red blood cells (RBC) both fragile and rigid and accounts for anemia and vasoocclusive crises (VOC). Abnormal RBC adhesion to vascular endothelial cells (VEC), in a context of chronic inflammation, cell activation, and vascular tone abnormalities, plays a key role in triggering VOC. Hydroxycarbamide (HC) is the only drug with a proven efficacy at decreasing VOC occurrence. HC decreases HbS polymerization by inducing fetal Hb expression but also RBC adhesive properties. We studied HC effect on the other cellular partner of adhesion, i.e.VEC. HC-induced TrHBMEC (cell line from bone marrow microcirculation) transcriptome variations were analyzed by microarrays both in basal and pro-inflammatory conditions. The subtraction profile leads to the identification of 2,448 new potential target genes and we focused on those related to inflammation. Strikingly, it stimulates pro-inflammatory genes such as *IL1A, IL1B, IL6, IL8, CCL2, CCL5* and *CCL8* both at the mRNA and protein levels. Similar data were also obtained with HPMEC (from pulmonary microcirculation) and HUVEC (from umbilical vein macrocirculation) primary cells. The four interleukins are known to be elevated in SCD patients, but our study is the first to point out the potential implication of chemoattractant cytokines in this context. Our hypothesis is that HC propels the level of the pre-existing inflammation status of SCD patients over a threshold that results in a powerful cytokine-dependent hypothalamic-pituitary-axis mediated anti-inflammatory response.

SEVERE HEMOLYSIS INDUCED BY DIABETIC KETOACIDOSIS IN G6PD DEFICIENCY

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G6PD deficiency is the most frequent erythrocyte enzyme defect in the world. This deficiency is a model of a pharmacokinetic default whose expression is dependent on triggering agents: infection, oxidant drugs, fava beans. We report here three cases of diabetic patients with G6PD deficiency presenting with a severe hemolytic crisis after a diabetic ketoacidosis (DKA) episode. *Comments.* Case

No.1: D.M. female adult from West Africa, affected by a type 1 diabetes discovered on hyperosmolar hyperglycemic state. Equilibration of diabetes was difficult. Need for a transfusion after an acute hemolytic crisis. Three years later, a second hemolytic crisis occurred during a severe ketoacidosis coma triggered by an acute gastroenteritis episode. Discovery of a G6PD deficiency (activity: 1.8 IU /g Hb; N ≥ 8). Need a new transfusion. Greater sensitivity to acidosis. Case No. 2: K.M. African adult hospitalized for a ketoacidosis state of a very unbalanced type 2 diabetes (HbA1c= 14.9%). Hemolytic anemia with a normal hemoglobin profile (Hb=10.2 g/dl; MCV 94 fl; retic=104 giga/l; total bilirubin 18 µmol/L) ferritinemia=2150 µg/l; plasma haptoglobin <0.1 g/l. Discovery of G6PD deficiency (0.5 IU/g Hb). Hb fall (7 g/dL) requiring a transfusion. Treatment of diabetes by insulin. Case No. 3: K.T. African 11 months-old child hospitalized for ketoacidosis revealing a type 1 diabetes; HbA1c = 7.7%. Hb fall to 7.8 g/dL. Regenerative anemia with hemolysis. G6PD deficiency (3UI/g Hb). Treatment of diabetes by insulin pump because of young age. *Discussion*. DKA may precipitate hemolysis in G6PD deficient patients. For patients from countries at high risk for deficiency, we propose a systematic G6PD activity assay. Evidence of G6PD deficiency should prompted for a close follow-up of Hb levels during periods of diabetic unbalanced state. Mutation 376G/202A, responsible for moderate acute hemolysis, is the molecular defect involved in these 3 cases, including a female.

MALARIA INFECTION IN BLOOD DONORS IN YAOUNDE, CAMEROON

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Malaria is found in Cameroon with high prevalence. It can be transmitted through blood transfusion. There is paucity of data concerning transfusion transmitted malaria and the methods used for its prevention are not standardized. To determine prevalence of malaria infection and association with epidemiological and clinical data obtained from donors responses, a cross sectional study was carried out on blood donors recruited during October and November 2007 in two main hospitals in Yaoundé. We performed a microscopic examination of a stained thick and thin blood smear for the detection, quantification and specification of Plasmodium. A total of 493 donors were included, ageing from 17 to 55 years. There were 25,8% voluntary and 74,2% familial donors. 98% lived in different areas of the Yaoundé city. 6,5% of blood donors were detected positive for Plasmodium infection: 90,6% was *P. falciparum* and 9,4% was *P. malariae*. Parasite counts ranged from 80 to 800/µL with a median of 320/µL. Asexual forms were found in 75,9% of cases sexual forms in 24,1% of cases. We found no significant difference when comparing voluntary benevolent to familial donors concerning malarial infection. Significant associations were found between the absence of malarial infection, the practice of at least one preventive method and the last febrile episode within the last one to six months prior to blood donation. We found significant association between malaria infection and the last febrile episode within the month before blood donation. The risk of transfusion-transmitted malaria infection is high in Yaoundé, some epidemiological and clinical parameters seem to describe high risk donor profile.

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