**POTENTIAL MECHANISMS LINKING DEFECTS IN METHYLENE TETRAHYDROFOLATE REDUCTASE (MTHFR) AND CRYPTOGENIC STROKE PREDISPOSITION. A PRELIMINARY STUDY.**

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The association of thrombophilic states and interatrial defects, mostly represented by patent foramen ovale (PFO) involves one third of the population and may predispose to cryptogenic stroke. A similar prevalence is observed for genetic defects in MTHFR, a key enzyme in the folate cycle. Abnormalities in the MTHFR activity result in a methyl surcharge that impairs cell survival. In endothelial cells, defects of MTHFR may trigger endothelial dysfunction, that in turn enhances pro-thrombotic conditions and affects the endocardial septum integrity. This study was planned to evaluate whether a correlation exists, in terms of severity, between the genotype of the MTHFR mutation, the phenotype of the interatrial septum and the degree of endothelial dysfunction, based on the following premises: A. Interatrial septum phenotype encompasses increasing severity spectrum among: 1)Septum integrum (SI); 1b) Septal aneurysm (SA); 2) PFO with right to left shunt (RtoL PFO); 3) PFO with left to right shunt (LtoR PFO); 4) Ostium secundum defect (OS). B. The most common point mutations of the MTHFR gene are in C677 and A1298 positions, and combination of these mutations may progressively affect enzyme function (expressed as residual enzymatic activity; REA): 1) A1298C heterozygosis maintains a 80% REA; 2) C677T heterozygosis accounts for 65% REA; 3) A1298C homozygosis (1298 C/C) for 60% REA; 4) A1298C/C677T double heterozygosis (677 C/T+1298 A/C) and 5) C677T homozygosis (677 T/T) both maintain a 30% REA. C. Disruption of the folate cycle promotes both homocystein accumulation and deficiency in the cellular methyl carrier S-adenosyl-methionin. As alternative methyl acceptors, cells recruit the asymmetric dimethyl-arginine (ADMA), which is a potent inhibitor of the endothelial nitric oxide synthase (eNOS). Both homocystein plasma levels (p-Hcy) and L-arginine/ADMA ratio are plasmatic markers of endothelial dysfunction, and potential predictors of stroke and microangiopathy-related cerebral diseases.

On 15 patients (8 males and 7 females, aged 33±8 years) hospitalized because a history of stroke, plasma levels of Hcy, L-arginine and ADMA, and MTHFR genotype were evaluated. Characterization of interatrial septum phenotype was assessed by transcranial Doppler coupled with transesophageal echocardiography.

Eight (8) patients were MTHFR homozygotes (5 677 T/T and 3 1298C/C), 4 were heterozygous (2 677C/T and 2 1298A/C), 3 were double heterozygous. The severity of the interatrial phenotype correlated with the severity of the MTHFR genotype: the OS patients (n=2) both carried the 677 T/T mutation; the LtoR patients (n=6) carried a 677T/T mutation (n=3), a double heterozygosis (n=2), or a 1298 C/C mutation (n=1); the RtoL patients (N=6), carried a double heterozygous (n=1), a 1298 C/C mutation (n=2), and a 677 C/T heterozygosis (n=2); the SI patients (n=2) carried the wild type MTHFR (n=1) or the 1298 A/C mutation (n=1). P-Hcy were higher in OS patient (14,1±0,55 µmol/L) with respect to RtoL PFO (8,6±0,54; $p<0,05$) and SI patients (8,5±0,55; $p<0,01$). The Arg/ADMA ratio decreased inversely to the severity of interatrial defects, with values of

153,5±19,67 in SI patients; 131,1±12,19 in RtoL PFO patients; 78,5±8,81 in LtoR PFO patients ($p<0,05$ among groups) and was inversely related to the severity of MTHFR genotype (OS $p<0,01$ vs. SI; $p <0,05$ vs. all intermediate groups). Thus, based on these preliminary findings, the severity of MTHFR mutation correlates with the degree of endothelial dysfunction and the severity of interatrial septum phenotype. Of note, genetic defects in MTHFR may exacerbate adverse effects of pharmacological therapies targeting folate production (including methotrexate), thus underlying the importance of studies evaluating the overall consequences of MTHFR genetic variations.