POLYMETHYLMETHACRYLATE MEMBRANE REDUCES SERUM LEVELS OF SOLUBLE CD40-LIGAND, AN INDEPENDENT PREDICTOR OF CARDIOVASCULAR EVENTS IN HEMODIALYSIS PATIENTS

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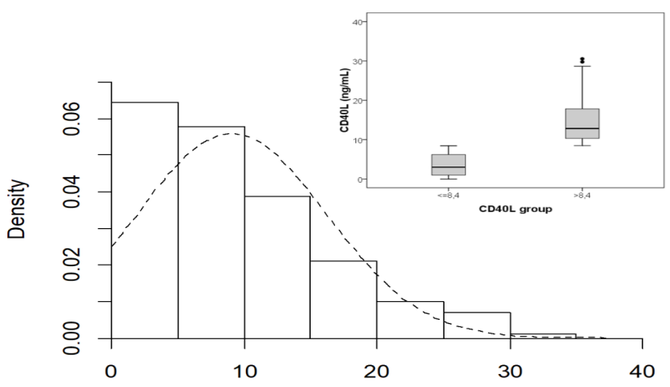
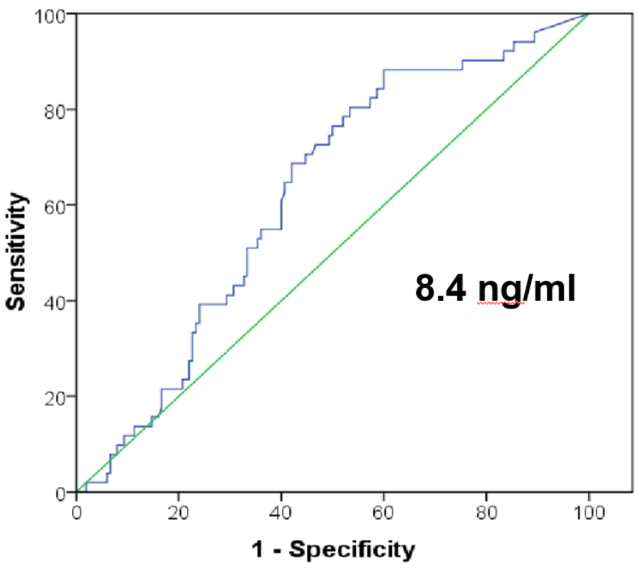
Background and Aim: Inflammation and oxidative stress are key factors for the incidence of premature vascular aging and for an increased rate of cardiovascular events in hemodialysis (HD) patients. The RISCAVID study has previously demonstrated that the soluble form of the costimulatory molecule CD40Ligand (sCD40L) is involved in inflammaging and cardiovascular events of HD patients. The aims of the present study were: 1) to confirm the role of sCD40L as an independent predictor of cardiovascular events about 10 years after RISCAVID; 2) to evaluate the effect of polymethylmethacrylate (PMMA) membrane on sCD40L serum levels and on CD40-CD40L pathway-related Endothelial Cell (EC) dysfunction and Vascular Smooth Muscle Cell (VSMC) calcification.

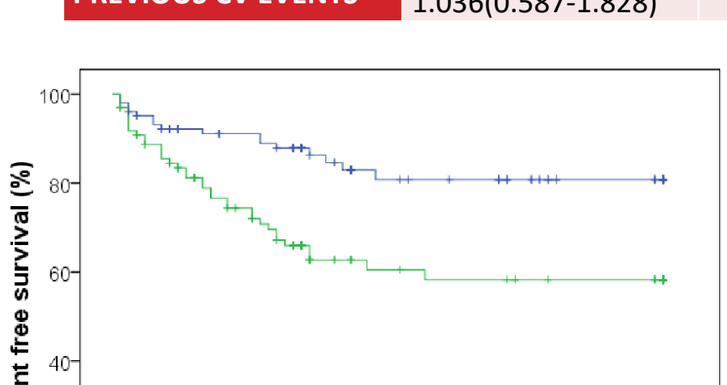
Methods: 201 patients treated with high-flux bicarbonate HD were evaluated for sCD40L (ELISA), Indoxyl sulfate (IS) and p-Cresyl sulfate (pCS) (HPLC-MS) serum levels and followed for at least 24 months to evaluate the incidence of Major Adverse Cardiovascular Events (MACE). Moreover, 54/201 patients with sCD40L serum levels higher than the median value were randomized for 9 months as follows: Group 1 (n=27): T0-T3 months PMMA; T3-T6 polysulfone (PS); T6-T9 PMMA; Group 2 (n=27): T0-T3 months PS; T3-T6 PMMA; T6-T9 PS. In both groups, sCD40L levels were correlated with standard clinical and laboratory parameters. In a subgroup of patients, the mass removal of sCD40L was investigated in two different dialysis sessions (PMMA vs PS). *In vitro,* the biological effects of sera drawn from HD patients enrolled in the study on EC dysfunction and VSMC calcification were also studied.

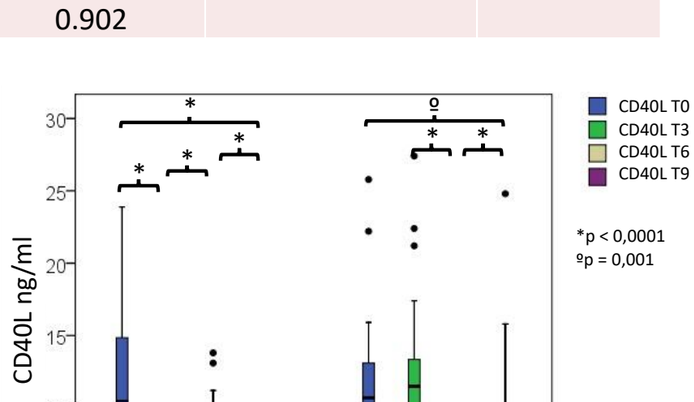
Results: By univariate and multivariate analyses, we found that a serum level of sCD40L higher than 8.4 ng/ml was an independent predictor of MACE in a follow-up of at least 24 months. In addition, IS and pCS serum levels correlated with sCD40L and the *in vitro* stimulation of platelets with IS and pCS enhanced sCD40L release. In HD patients, the shift to PMMA membrane resulted in a significant reduction of sCD40L levels in both groups, whereas hepcidin levels were significantly reduced only in Group 1 at 9 months. Moreover, mass removal of sCD40L with PMMA was significantly higher than that observed with PS in 2 different HD sessions in 2 consecutive weeks. *In vitro,* incubation of EC and VSMC with sera collected from patients after switching from PS to PMMA showed a significant reduction of EC dysfunction and VSMC calcification through a decreased activation of the CD40-CD40L pathway. In particular, PMMA induced a significant decrease of EC injury (ROS production, endothelial-to-mesenchymal transition, monocyte adhesion, inhibition of angiogenesis) and VSMC calcification (red alizarin staining and Runx-2 mRNA/protein expression).

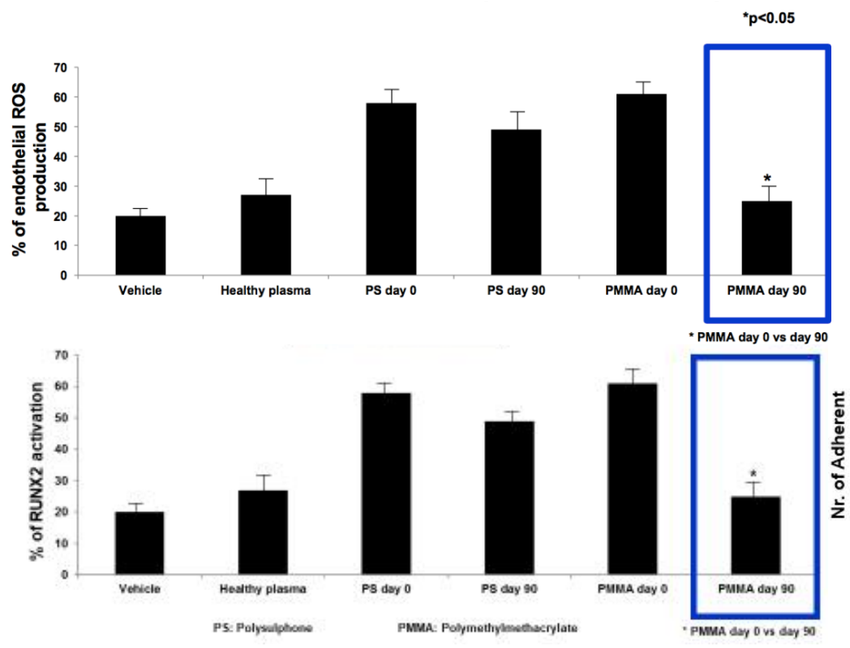


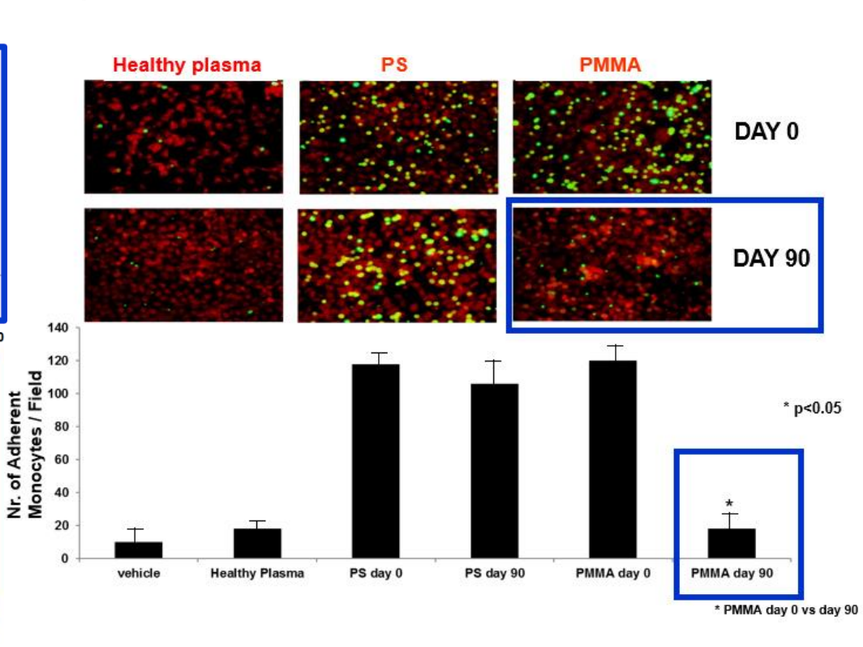


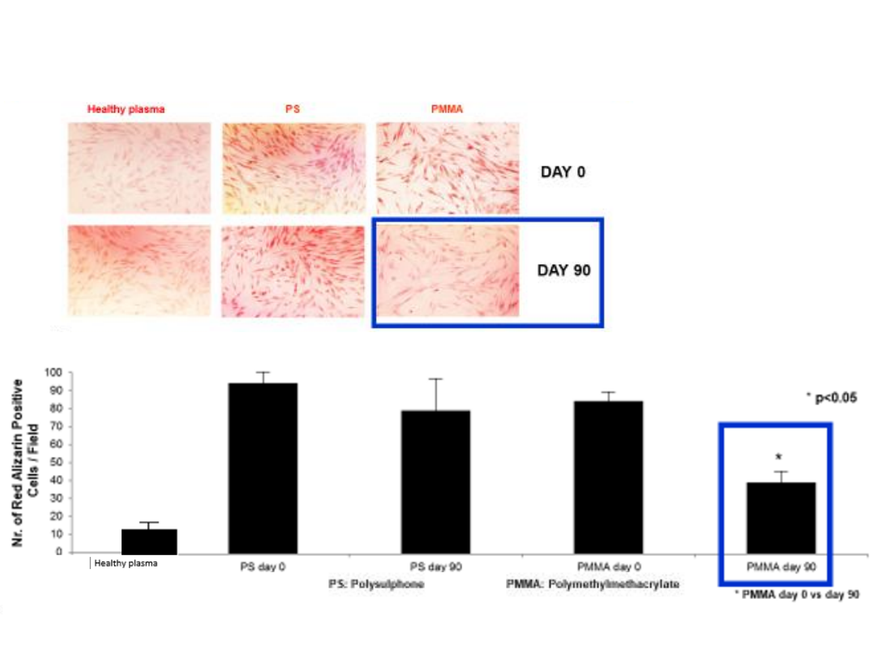
	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
VARIABLES	HR (95% CI)	p-value	HR (95% CI)	p-value
sCD40L > 8.4 ng/ml	2.677(1.480-4.841)	0.001*	2.542(1.403 4.606)	0.002*
AGE (years)	1.030(1.005-1.055)	0.017*	1.031(1.004-1.058)	0.010*
HD DURATION (months)	0.999(0.993-1.004)	0.672		
SEX (male/female)	1.416(0.806-2.488)	0.227		
DIABETES	1.416(0.806-2.488)	0.036*	1.567(0.899-2.733)	0.113
HYPERTENSION	1.811 (1.04-3.153)	0.096		
DYSLIPIDEMIA	2.384(0.858-6.625)	0.683		
SMOKING	1.123(0.253-1.962)	0.902		
PREVIOUS CV EVENTS	1.036(0.587-1.828)	0.902		











Conclusions: We herein observed that sCD40L is an independent predictor of MACE in HD patients. Moreover, the protein-bound uremic toxins IS and pCS increased sCD40L serum levels by enhancing its release from platelets. PMMA membrane significantly and stably reduced sCD40L serum levels under the high-risk cut-off of 8.4 ng/ml. *In vitro* studies confirmed that the switch to PMMA decreased EC dysfunction and VSMC calcification concomitant to sCD40L reduction. PMMA membrane should be considered as a novel therapeutic strategy to limit early vascular aging of HD patients.