BIOLOGICAL EFFECTS OF POLYMETHYLMETHACRYLATE (PMMA) MEMBRANE ON SERUM LEVELS OF SOLUBLE CD40-LIGAND, A MIDDLE MOLECULE INVOLVED IN ATHEROGENIC INFLAMMATION AND CARDIOVASCULAR MORTALITY OF HEMODIALYSIS PATIENTS

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OBJECTIVES

Soluble CD40-Ligand (sCD40L) is a 36 KDa middle molecule known to play a key role in cardiovascular disease in hemodialysis (HD) patients. RISCAVID study demonstrated an increased cardiovascular risk in patients with sCD40L serum levels higher than 7.6 ng/ml.
The activation of the CD40/CD40L pathway plays a key role in endothelial cell (EC) dysfunction and in vascular calcification through osteoblastic differentiation of vascular smooth muscle cells (VSMC). The aim of this study were to evaluate:

1)the effect of PMMA membrane on sCD40L levels in HD in a multicenter trial in Italy;
2)the mechanisms of adsorption-based sCD40L removal by PMMA;
3)the effect of PMMA-induced sCD40L removal on endothelial dysfunction and VSMC calcification.

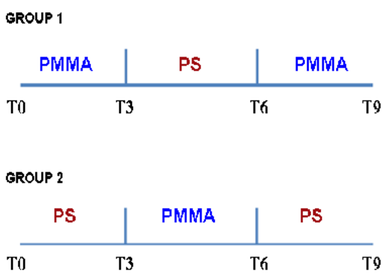

Figure 1: schematic representation of study planning in Group 1 and 2.

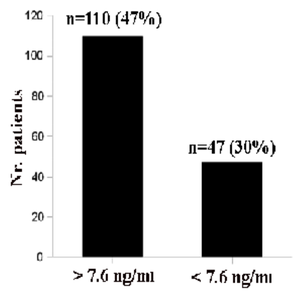

Figure 2: ELISA evaluation of serum CD40-Ligand in the whole BHD population enrolled in the study.

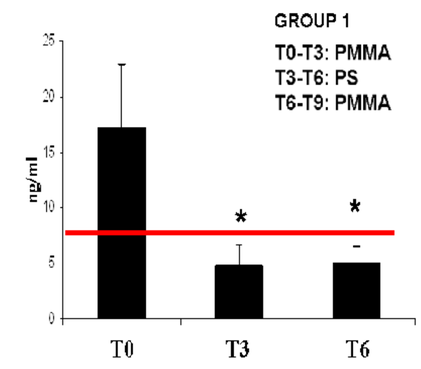

Figure 3: time-course ELISA evaluation of serum CD40-Ligand levels in Group 1. *p < 0.001: T6, T3 vs. T0

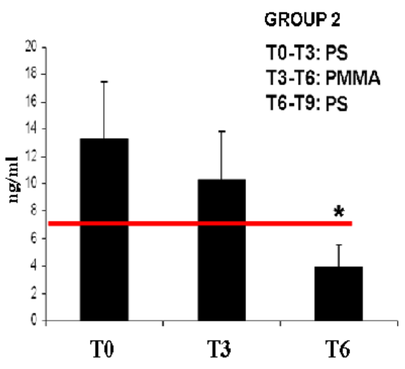

Figure 4: time-course ELISA evaluation of serum CD40-Ligand levels in Group 2. *p < 0.001: T6 vs.T3,T0

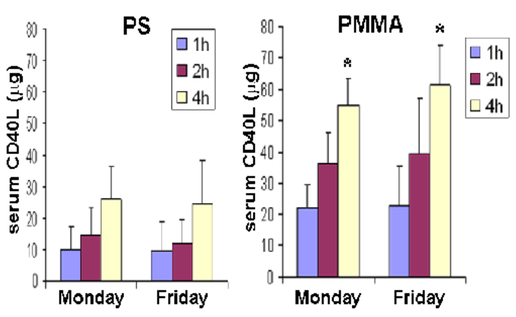

Figure 5: ELISA evaluation of sCD40-Ligand mass removal (expressed in µg) in 5 patients treated for 1 week with PS and for the following week with PMMA. Data show sCD40-Ligand mass removal after 1, 2 or 4 hr of dialysis in the first (Monday) and last (Friday) session of the week. *p < 0.001 4h vs 1h

Mass removal calculation:
[(1000 x body weight before HD x 1/13) x (sCD40-Ligand before HD)] -
[(1000 x body weight before HD x 1/13 x Hct before HD/Hct after HD start) x (sCD40-Ligand after HD start)]

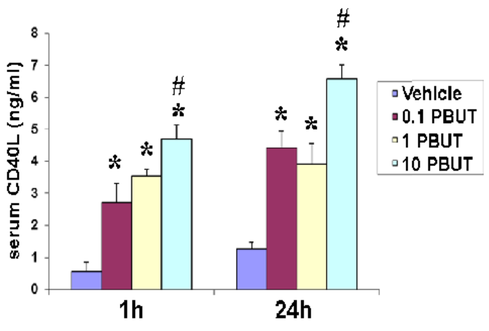

Figure 6: ELISA evaluation of serum CD40-Ligand in supernatants of human platelets incubated with the protein-bound uremic toxins (PBUT) p-cresyl sulfate and indoxyl sulfate.
*p < 0.001: 0.1, 1, 10 PBUT vs. Vehicle;
# p < 0.001: 10 PBUT vs. 0.1 , 1 PBUT, Vehicle

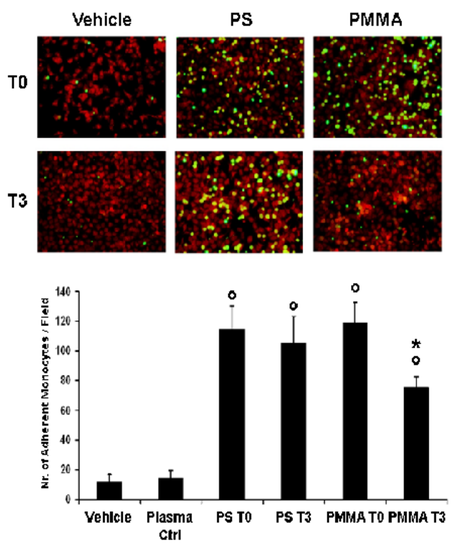

Figure 7: representative micrographs (upper panels) and relative count (lower panel) of *in vitro* leukocyte adhesion on EC monolayers incubated with sera collected at different time points. *p < 0.001: PMMAT3 vs. PMMAT0, PST3, PST0, Plasma Ctrl, Vehicle; °p < 0.001: PMMA T3, PMMA T0, PS T3, PS T0 vs. Plasma Ctrl, Vehicle.

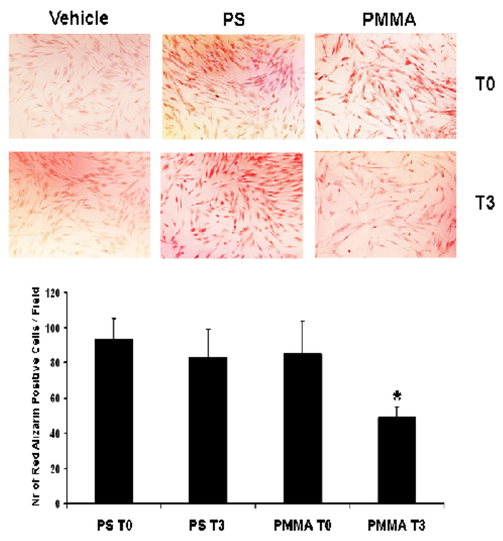

Figure 8: representative micrographs (upper panels) and relative count (lower panel) of *in vitro* leukocyte adhesion on osteoblastic differentiation (red alizarin staining) of VSMC incubated with sera collected at different time points.
*p < 0.001: PMMA T3 vs. PMMA T0, PS T3, PS T0

METHODS

A total of 157 bicarbonate hemodialysis (BHD) patients were evaluated for sCD40L serum levels by ELISA; 30 patients were randomized for 9 months as follows (Figure 1):

Group 1: T0-T3 months PMMA; T3-T6 months polysulfone (PS); T6-T9 months PMMA;

Group 2: T0-T3 months PS; T3-T6 months PMMA; T6-T9 months PS.

In both groups, sCD40L levels were measured each month and correlated with clinical parameters.

***In vitro,* sCD40L release from platelets activated by the protein bound uremic toxins (PBUT) p-cresyl sulphate and indoxyl sulphate was evaluated. Biological effects of sera drawn from HD patients on EC dysfunction (NO release, angiogenesis, leukocyte adhesion) and VSMC calcification (red alizarin staining) were also studied.**

RESULTS

110/157 HD patients (70%) presented sCD40L serum levels higher than 7.6 ng/ml (Figure 2). In both group 1 (Figure 3) and group 2 (Figure 4), shift to PMMA membrane resulted in a significant reduction of sCD40L levels. Of interest, sCD40L levels remained lower than 7.6 ng/ml after 2-3 months from a new shift from PMMA to PS, suggesting the presence of a sort of "drag" effect.
Experiments aimed to evaluate sCD40L mass removal at the start and 1, 2 and 4 hrs of HD suggested a direct adsorption of this molecule by PMMA (Figure 5). Mass sCD40L removal was about 50-60 µg with PMMA vs. 10-20 µg with PS in 3 different HD sessions in 2 consecutive weeks (first week PS, second week shift to PMMA).
***In vitro* data revealed that PBUT induced a significant increase of sCD40L from platelets (Figure 6). Preliminary results by HPLC-MS suggested a possible adsorption of PBUT by PMMA (not shown). Last, incubation of EC and VSMC with sera collected from patients after switching from PS to PMMA (T0 vs. T3) showed a significant reduction of EC dysfunction evaluated as NO bioavailability, angiogenesis on Matrigel-coated surface (not shown), leukocyte adhesion (Figure 7) and of VSMC osteoblastic differentiation leading to enhanced calcification processes (Figure 8).**

CONCLUSIONS

PMMA membrane significantly and stably reduced sCD40L serum levels under the cut-off of 7.6 ng/ml. Our data suggested a dual mechanism of sCD40L reduction by PMMA: first, a direct adsorption to the membrane; second a potential adsorption of PBUT that we herein demonstrated to increase sCD40L release from platelets. Independently from the adsorption mechanisms, sera drawn from HD patients after switch to PMMA induced a decreased EC dysfunction and VSMC calcification in concomitance with sCD40L reduction, suggesting a potential cardiovascular protective effect.

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Dialysis. Protein-energy wasting, inflammation and oxidative stress.
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DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA AUSTRIA MAY 21st-24th, 2016
PosterSessionOnline.com