Pro-calcifying analysis of uraemic serum from patients treated with medium cut-off membrane in a prospective, cross-over study

Ciceri P, et al. Pro-calcifying analysis of uraemic serum from patients treated with medium cut-off membrane in a prospective, cross-over study. *Clinical Kidney Journal*. 2020:1-10. doi: 10.1093/ckj/sfaa216.

BACKGROUND

The retention of a large number of solutes that are normally excreted or metabolized by the kidney is responsible for the symptoms typical in uraemic patients. These molecules are defined as uraemic toxins and can be classified into three groups: small water-soluble molecules, middle molecules and protein-bound toxins. Recently, efforts were put towards developing dialysis membranes that allow the removal of large middle molecules without clinically relevant albumin loss. These membranes are the medium cut-off membranes that allow the removal of middle molecules up to ~50,000 Da.

Patients affected by ESRD have an increased morbidity and mortality due to many complications, mainly represented by cardiovascular diseases. In all the causes that induce cardiovascular diseases, an important role is played by vascular calcification (VC), which modifies arterial pulse wave velocity and pressure, leading to hypertension, left ventricular hypertrophy and heart failure. In end stage renal disease (ESRD) patients, VC affects principally the tunica media and vascular smooth muscle cells (VSMCs) and is due to different factors, among which the main ones are high phosphate levels and uraemic toxins.

Phosphate stimulates VSMCs to deposit calcium in the extracellular matrix. In this process, VSMCs loose muscular markers and start to express an osteoblastic phenotype, behaving as simil-osteoblasts. Besides the trans-differentiation, high phosphate promotes calcification by causing apoptosis, with the release of apoptotic bodies loaded with calcium that work as VSMC calcification machinery. Another process that induces calcification exacerbation is necrosis, as the massive release of calcium after rupture of the cellular membrane triggers and participates in calcium deposition.

OBJECTIVE

- To investigate whether different dialysis treatments, e.g., **MCO** membrane or high flux dialysis membranes, could have an impact on calcium deposition.
- To characterize uraemic serum composition to find which components are differentially removed by dialysis treatment that could play a role in modulation of the serum pro-calcifying effect.

METHODOLOGY

Study Design

Twenty prevalent hemodialysis (HD) patients participated in this prospective, open-label, controlled, cross-over pilot study. The study was undertaken at the dialysis unit at the University of Milan (Italy) from 1 October 2017 to 31 December 2018. Consecutively unselected male and female adult patients with ESRD on HD were eligible for participation in the study. Patients were divided into two groups (A and B) with similar mean age, male:female ratio and dialytic vintage. As a cross-over design, patients in Group A were treated with **HDx** therapy with the **Theranova** dialyzer for the first three months of the study then switched to traditional bicarbonate dialysis for the remaining three months. Patients in Group B were treated with bicarbonate dialysis for the first three months and then switched to **HDx** therapy for the remaining three months. See Figure 1. Sera samples from both groups were collected at 1, 2, and 3 months. The pro-calcifying effect of uraemic serum in a well-established in vitro model of high-phosphate (Pi)-induced calcification in VSMCs was then analyzed.

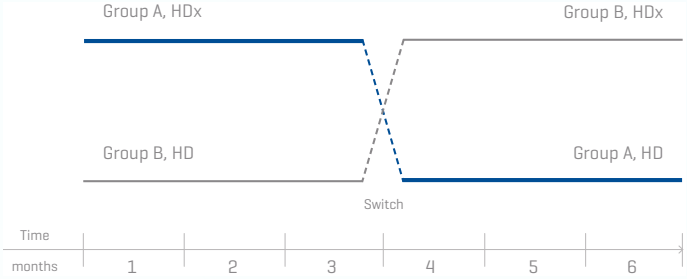

FIGURE 1. Study Design. **HDx** therapy: expanded hemodialysis. HD: hemodialysis. Figure adapted from Ciceri, et al.

Induction of Calcification/Quantification of Calcium Deposition

Rat VSMCs were obtained by enzymatic digestion, and were routinely subcultured in a growth medium. Quantification of calcium deposition was performed by staining, followed by perchloric acid destaining after high propidium iodide (Pi) treatment. Calcium content was determined colorimetrically at a wavelength of 450 mn. Protein content was assessed by a bicinchoninic acid (BCA) protein assay kit and calcium deposition was normalized to protein content and expressed as absorbance (optical density (OD)) per milligram of protein.

Detection of Apoptosis and Necrosis

Cytoplasmic histone-associated deoxyribonucleic acid (DNA) fragments were detected with a cell death detection Enzyme-Linked Immunosorbent Assay (ELISA) plus kit as a quantitative index of apoptosis 4 days after high Pi treatment. Necrosis was detected with the same kit.

VSMC Osteochondrogenic Shift

Seven different molecules, either inducers or inhibitors of calcification, involved in VSMC simil-osteoblast transformation were tested.