

# First Clinical Experience with an Adjunctive Hemoperfusion Device Designed Specifically to Remove $\beta_2$ -Microglobulin in Hemodialysis

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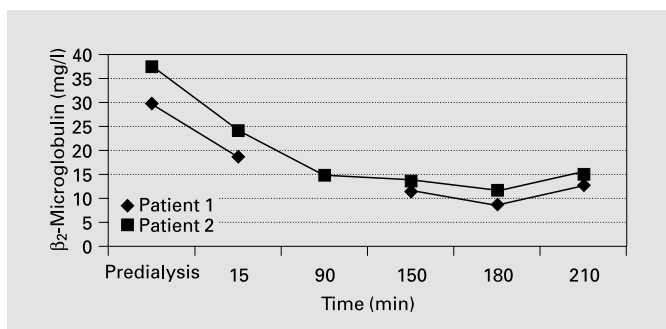
## Introduction

$\beta_2$ -Microglobulin, a middle molecular weight protein (11.8 kD), has increasingly been the focus of attention in its role as the precursor of dialysis-related amyloidosis (DRA) [1]. Current evidence favors the alteration of  $\beta_2$ -microglobulin by glycosylation [2], and subsequent deposition of glycosylated  $\beta_2$ -microglobulin as amyloid fibrils in many tissues, particularly in large joints (shoulders), and in the carpal tunnel. DRA in chronic dialysis patients can produce bone cysts, severe carpal tunnel syndrome and crippling arthritis [3]. While there is poor correlation between blood concentrations of  $\beta_2$ -microglobulin and DRA, there is direct correlation of this complication with duration of dialysis. Moreover, the disposition of  $\beta_2$ -microglobulin, follows a 3-pool kinetic model with distribution in plasma water, extracellular fluid and a slow equilibration compartment [4].

Dialyzers constructed with cuprophane dialysis membranes (cellulosic), long the standard therapy for chronic dialysis, do not reduce  $\beta_2$ -microglobulin concentrations and may in fact alter the conformational structure of  $\beta_2$ -microglobulin, promoting amyloidosis. On the other hand, noncellulosic (e.g. polyacrylonitrile and polysul-

fone) dialyzer membranes may reduce  $\beta_2$ -microglobulin concentrations during treatment, by a combination of adsorption (the majority) to the membrane and by convective removal (the minority), the latter of which is dependent on high rates of ultrafiltration. The efficiency of the latter membranes is not considered optimal, and usually adsorption is complete early in the dialysis procedure. Moreover, dialyzer reuse significantly impairs the removal of  $\beta_2$ -microglobulin [5].

Hemoperfusion devices containing adsorbents have been used to enhance 'middle molecule', amino acid, and creatinine removal, in dialysis patients, using nonspecific agents such as activated charcoal [6]. A porous resin hemoperfusion device has also been shown to reduce  $\beta_2$ -microglobulin concentrations during extracorporeal treatment, with improvement in the clinical manifestations of DRA [7]. In this study we present a new adsorbent device with improved biocompatibility by virtue of a polymer coating [8], designed specifically to adsorb  $\beta_2$ -microglobulin. In vitro and in vivo data are reported as far as efficacy and biocompatibility performances are concerned. We report the case of two long-term hemodialysis patients who volunteered to undergo combined hemodialysis/hemoperfusion at a single session, in order to assess the



**Fig. 1.**  $\beta_2$ -Microglobulin concentration throughout the session of combined hemoperfusion-hemodialysis.

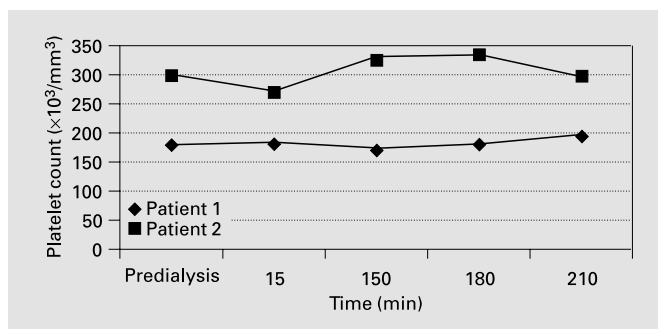
effects of the hemoperfusion device on  $\beta_2$ -microglobulin removal, as well as hemocompatibility.

### Materials and Methods

The combined hemodialysis/hemoperfusion procedure was approved by the Ospedale San Bortolo Institutional Review Board for Investigation in Human Subjects. The procedure and risks were explained to both patients prior to combined treatment. The device will be subject to trial in human subjects in the USA and Italy under an Investigational Device Exemption (IDE) by the US Food and Drug Administration.

The cylindrical hemoperfusion devices were constructed of polysulfone and contained 300 g hydrated polystyrene resin beads coated with polyvinylpyrrolidone sealed with end-caps (BetaSorb<sup>TM</sup>, RenalTech International, New York, N.Y., USA). The devices were steam sterilized, inspected, primed prior to use with sterile normal saline containing 1,000 IU heparin, and placed in line with the dialysis circuit, upstream of the dialyzer. Combined hemodialysis/hemoperfusion was performed with a Fresenius 4008B controlled ultrafiltration dialysis machines (Fresenius AG, Bad Homburg, Germany), using an FH80 (polysulfone high flux) dialyzer (Fresenius AG). Blood flow rate was maintained at the patient's customary values (380 and 405 ml/min, respectively) during the dialysis period, as was dialysate flow rate (500 ml/min).

Pressures (mm Hg) across dialyzer and hemoperfusion were measured at timed intervals to detect potential flow disturbances within the device. Heparin anticoagulation was given as an intravenous bolus (2,000 IU) at the beginning of the procedure, continued by infusion of 1,000 IU/h and supplemented where appropriate to maintain an



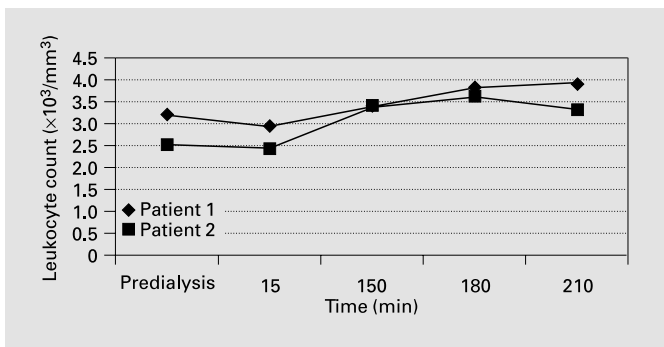
**Fig. 2.** Platelet count during different moments of the combined hemoperfusion-hemodialysis session.

activated clotting time (ACT) >120 s. Blood samples were drawn from the dialyzer lines before and after the hemoperfusion device, and after the dialyzer to assess the contribution of the dialyzer and hemoperfusion component to changes in platelets, leukocytes,  $\beta_2$ -microglobulin and albumin concentrations.

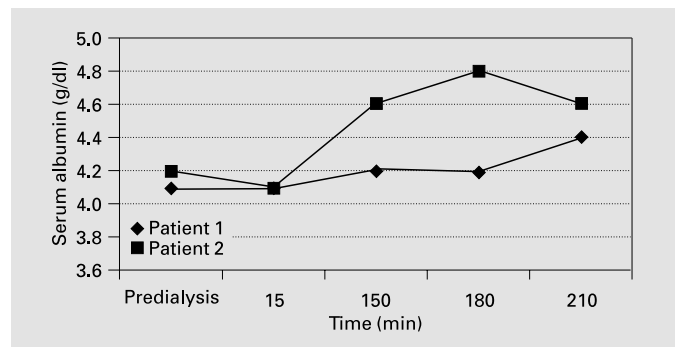
Vital signs were measured at 30-min intervals. The combined procedure was carried out for 3 h and an additional blood sample was drawn 30 min after the procedure was discontinued for determination of  $\beta_2$ -microglobulin concentration rebound. Patient 1 was a stable 64-year-old white male with adult polycystic kidney disease who had been on dialysis 15 years, with a history of a failed transplant 3 years earlier. Patient 2 was a stable African male aged 41 years who had been on dialysis for 8 years.

### Results

The combined hemoperfusion/hemodialysis procedure was well tolerated in both patients, with neither exhibiting changes in vital signs attributable to the addition of the hemoperfusion device.  $\beta_2$ -Microglobulin concentrations were reduced substantially (79 and 69% in patients 1 and 2 respectively) during the combined hemodialysis/hemoperfusion procedure (fig. 1). Rebound in  $\beta_2$ -microglobulin concentration of 49 and 31% respectively in patient 1 and 2 from the nadir was observed (fig. 1). Platelet and leukocyte counts remained stable, as did serum albumin (uncorrected for ultrafiltration required for routine patient management during treatment) (fig. 2, 3). Serum albumin concentrations are depicted in figure 4. The complete setup of the combined hemoperfusion-hemodialysis treatment is displayed in figure 5.



**Fig. 3.** Leucocyte count during different moments of the combined hemoperfusion-hemodialysis session.



**Fig. 4.** Serum albumin concentration throughout the session of combined hemoperfusion-hemodialysis.



**Fig. 5.** The combined hemoperfusion-hemodialysis treatment mounted on a dialysis machine. The adsorbent cartridge is placed in series with the hemodialyzer just before it.

Pressure changes measured by both standard transducers, as well as a pressure transducer connected to a computer through an analog interface, across the hemoperfusion and hemodialysis devices remained stable throughout the procedure (uncorrected for changes in blood volume).

## Discussion

DRA is not corrected by standard, nor high-flux dialysis. Serum  $\beta_2$ -microglobulin concentrations do appear to be reduced after a period of high-flux dialysis following a period of low-flux dialysis, although normal concentrations of  $\beta_2$ -microglobulin are never achieved in long-term dialysis patients. Retention of  $\beta_2$ -microglobulin occurs as renal function declines. Continuous ambulatory peritoneal dialysis patients also exhibit elevations in  $\beta_2$ -microglobulin concentration [9]. Transplantation is associated with a fall in  $\beta_2$ -microglobulin concentration when near normal renal function is achieved.

Leypoldt et al. [10] have demonstrated that there is a survival advantage to reduced  $\beta_2$ -microglobulin concentrations in dialysis patients, prompting investigation of methods to remove  $\beta_2$ -microglobulin.

Using a hemoperfusion column containing 350 ml of a porous resin, Homma et al. [7] have demonstrated substantial removal of  $\beta_2$ -microglobulin, with partial regression of DRA over 6–13 months. While our primary interest is in  $\beta_2$ -microglobulin reduction, the adsorptive resin used here is also capable of removing other ‘middle molecular’ weight toxins, such as TNF- $\alpha$ , and IL-1 $\beta$ .

To our knowledge, the biocompatibility of the adsorbent resin used in this device, as measured by platelet and leukocyte counts, is superior to devices used currently or abandoned. This property encourages the application of hemoperfusion for continual use in dialysis to achieve sustained reduction in  $\beta_2$ -microglobulin, to measure kinetics of  $\beta_2$ -microglobulin behavior, and to study the effects of  $\beta_2$ -microglobulin removal on prevention and reversal of dialysis related amyloidosis.

Lornoy et al. [12] have recently shown that after 10 years of either hemodiafiltration (which removes about

340 mg  $\beta_2$ -microglobulin per session), or biocompatible membrane hemodialysis that  $\beta_2$ -microglobulin-associated bone disease is present in 25%, and carpal tunnel syndrome in 12.5%.

Clinical hemodialysis using the synthetic membranes Arylane (Hospal Renal Care, Lyon, France) or Fresenius Polysulfone (Fresenius Medical Care, Bad Homburg, Germany) in addition to removing  $\beta_2$ -microglobulin (170 and 110 mg per session, respectively), is also associated with moderate removal of other small molecular weight proteins (10 and 7 g, respectively) [13]. The minimal adsorption of albumin in our study suggests that there is no added removal of protein as described in the above study. This has clinical benefits in reducing the contribution of protein removal to malnutrition.

We have shown that a 53% reduction of  $\beta_2$ -microglobulin can be achieved using synthetic membranes (T-sulfone, Toray, Japan) [14]. In the long term this resulted in a

lower predialysis  $\beta_2$ -microglobulin concentration in hemodialysis patients treated with synthetic high-flux membranes compared to those treated with low-flux membranes. Our preliminary results with the coupled hemoperfusion-hemodialysis technique suggest that a further reduction should be expected after 6–12 weeks of this combined therapy.

## Conclusions

The substantial reduction of  $\beta_2$ -microglobulin concentrations in two end-stage renal disease patients with a hemoperfusion device as part of a hemodialysis procedure, without changes in formed elements of blood, encourages us to pursue formal evaluation of the device as a treatment for DRA or its prevention.

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