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Bilirubin Removal by Plasmafiltration-Adsorption: Ex vivo Adsorption Kinetics Model and Single Case Report

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Keywords
Bilirubin · Adsorption · BS330 · Plasmafiltration-adsorption

Abstract
Background: Extracorporeal removal of bilirubin in patients with severe liver dysfunction is a key blood purification strategy. We conducted an ex vivo study to assess the quantitative capacity to remove bilirubin from plasma of a novel adsorptive cartridge. ***Methods:*** We studied a downscaled module of the BS330 Plasma Bilirubin Adsorption Column Cartridge (Jafron Biomedical, Zhuhai City, China) to minimize the plasma requirement in an ex vivo circulation using a solution of hyperbilirubinemic plasma. We measured the bilirubin concentration gap (ΔC) between inlet (Cp_{in}) and outlet (Cp_{out}) of the unit and we calculated the removal ratio (RR) as mass adsorbed at different time points. Moreover, we compared the ex vivo model with the bilirubin adsorption kinetics in a patient with acute on chronic liver failure treated with the BS330 cartridge. ***Results:*** Bilirubin concentration change across the cartridge at 30 min was 16.5%, and cartridge saturation was reached at 750 min. We used a mini-module downscaled to 1:3 and containing approximately 131 g of BS330 sorbent beads: the device retained 759 mg of bilirubin with a RR of 78.1% and a RR of 42.6% at 120 min. Thus, the adsorption capacity was 5.76 mg of bilirubin per gram of sorbent. Bilirubin adsorption kinetics in our clinical case with a full-scale unit shows a coherent trend with a total bilirubin mass adsorbed after 180 min of 470 mg. ***Discussion:*** Our findings provide the first assessment of bilirubin adsorption in an ex vivo model of plasma perfusion and can be used to design interventional studies in humans, providing guidance for an adequate prescription of treatment frequency and duration.

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Introduction

Several toxins accumulate in acute and chronic liver failure because of impaired organ function. This results in signs and symptoms associated with negative outcomes and possible distant organ dysfunction [1]. One such toxin is bilirubin. Removal of bilirubin may alleviate signs and symptoms of liver dysfunction but may also act as an index for the removal of other compounds that accumulate in patients with liver failure.

Conjugated and unconjugated bilirubin, due to their molecular weight and albumin binding, respectively, cannot be removed by classic dialysis membranes, and thus, alternative techniques are indicated such as plasma exchange or plasmafiltration-adsorption [2]. However, while solute clearances, sieving coefficients, and hydraulic permeability have been extensively studied in dialysis membranes, bilirubin kinetics have not been sufficiently elucidated in sorbent units, and accurate isotherms to detect adsorption capacity and saturation over time have not been established. Our study aimed to analyze the bilirubin removal capacity of a specific sorbent cartridge (BS330, Jafron Biomedical Co. LTD) and to establish the optimal time of application in relation to saturation of the sorbent beads.

Materials and Methods

We conducted an ex vivo study simulating plasma perfusion with the BS330 sorbent cartridge. Moreover, we present a single case report of a patient admitted to the ICU of San Bortolo's Hospital (Vicenza, Italy) and treated with plasma adsorption pPerfusion (PAP) with the BS330 cartridge. The study was conducted according to the Declaration of Helsinki.

Cartridge

The BS330 is a new disposable Plasma Bilirubin Adsorption Column by Jafron Biomedical. The anion-exchange resins with styrene divinyl benzene inside the BS330 are expected to electrostatically bind to bilirubin molecules and achieve highly specific adsorption of the bilirubin [3]. The resin in the cartridge has been reported to have binding sites for bilirubin, so that bilirubin detaches from albumin and moves toward the resin [4]. Using a downscaled module of this cartridge, we set up an ex vivo circulation experiment in which a solution of hyperbilirubinemic plasma was pumped into the circuit and through the cartridge.

Bilirubin Solution

The bilirubin solution was prepared using synthetic bilirubin powder (Sigma-Aldrich Product Number B4126). This product consists of three different α-isomers, which are unconjugated. The powder is insoluble in water; therefore, the solution was prepared in the hospital pharmacy of our center.

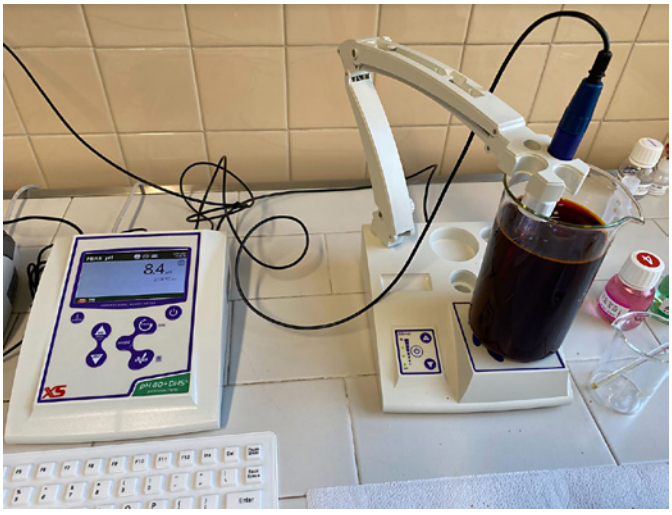

Fig. 1. Bilirubin solution preparation. pH of final solution was 8.4.

A 2% aqueous solution was prepared as follows: 940 mg of bilirubin powder was suspended in 100 mL of 0.1 M NaOH to dissolve the product and reaching pH 12.1. Subsequently, approximately 6.5 mL of 1 M HCl and 2.5 mL of 0.1 M HCl were added to reach a final pH of 8.4. Sterile water for injection was added to reach a final volume of 470 mL (shown in Fig. 1).

We used a bag of 510 mL of plasma, taken from the Department of Transfusion Medicine in San Bortolo Hospital, and stored in a refrigerator at temperature of 4°C. The plasma bag was decanted into a pharmaceutical glass bottle and incubated with the bilirubin solution obtained from the hospital pharmacy, reaching a final volume of 1 L of diluted plasma and a total bilirubin concentration of 92.8 mg/dL.

Mock Plasma Adsorption Perfusion Circuit

A dedicated machine for in vitro simulation, named GALILEO, equipped with actuators (pumps) and sensors was used to perform the treatments (shown in Fig. 2). A peristaltic pump circulated plasma inside the inflow line, from the reservoir through the sorbent cartridge, and back to the reservoir inside the outflow line. From the BS330 cartridge, we obtained a minimodule downscaled to 1:3 containing 131.6 g of sorbent's beads. Ports for drawing 2 mL of samples were placed in inflow and outflow lines at an equal distance from the cartridge. The circuit was primed with a saline solution that was subsequently discarded. The hyperbilirubinemic plasma was circulated at a flow rate 30 mL/min according to an in vivo plasma adsorption perfusion treatment protocol. The pharmaceutical glass bottle (reservoir) was placed on a magnetic heat stirrer to keep a constant temperature of 37°C (shown in Fig. 2).

The total duration of the experiment was 810 min. We collected a total of 84 plasma samples of 2 mL, reducing the volume of plasma in the reservoir to 832 mL at the end of the treatment.

Samples were collected at baseline, at 10 and 30 min from the beginning of the treatment, and every 30 min afterward. At each time, three samples were taken: one from the reservoir and two from the circuit (before and after the cartridge).

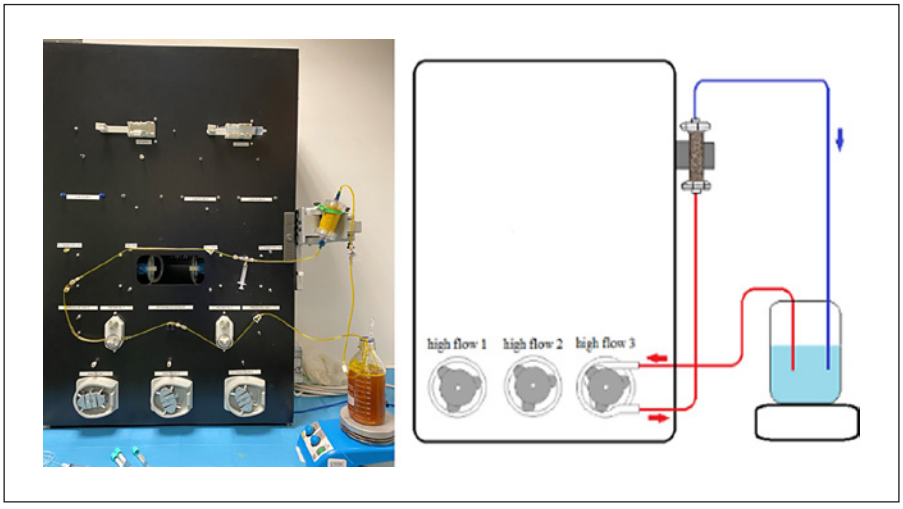

Fig. 2. Upward, mock plasma adsorption perfusion circuit with hyperbilirubinemic plasma circulating from the reservoir through the minimodule cartridge; below, the schematic representation of GALILEO machine with mock perfusion circuit.

In order to quantify the cartridge adsorption capacity, specific parameters were assessed. The adsorption trend was evaluated as the bilirubin concentration gap (ΔC) between inlet (Cp_{in}) and outlet (Cp_{out}) lines:

$$\Delta C = Cp_{in} - Cp_{out}$$

Removal ratio at a given time point was calculated as follows:

$$RR(t_x) = \frac{C(t_0) - C(t_x)}{C(t_0)} \times 100$$

where $C(t_0)$ represents bilirubin concentration before circulation, $C(t_x)$ represents bilirubin concentration at that time point in the reservoir.

Mass adsorbed at a given time point was calculated as follow:

$$Mass_{ads}(t_x) = Mass_{injected} \times RR(t_x)$$

where $Mass_{injected}$ is the initial amount of bilirubin in the reservoir and $RR(t_x)$ the removal ratio at that time point.

Total Bilirubin Determinations

Since our bilirubin solution was composed only of indirect bilirubin, the determination of the total bilirubin concentration was representative of the unconjugated bilirubin mass added to the solution. The bilirubin concentration was determined in 84 lithium heparinized plasma samples, and a quantitative determination was obtained using the ILab650 system (Instrumentation Laboratory Werfen, Milano, Italy). The principle for total bilirubin measurement was a modified Jendrassik-Grof assay. The ILab650 system automatically dilutes samples with a bilirubin value over 70.0 mg/dL.

Single Case Report

We report the case of a 71-year-old male patient admitted to our emergency department due to fever, malaise, and jaundice with total bilirubin concentration of 29.6 mg/dL. The patient was admitted to the ICU for septic shock and multi-organ failure. E.Coli was isolated from blood and urine cultures. Patient had chronic HBV infection and developed acute on chronic liver failure. PAP treatment with the BS330 cartridge was performed for 180 min with a plasma flow rate of 25 mL/min. A retrospective analysis of bilirubin adsorption kinetics in this clinical case was performed to evaluate if it was comparable to our ex vivo model. Plasma total bilirubin was evaluated at 60, 120, and 180 min. Bilirubin mass adsorbed was calculated from a plasma volume of 3.168 L. Blood volume was calculated with Nadler's formula [5].

Results

Adsorbed Mass Quantification

The bilirubin concentration in the plasma bag was 0.1 mg/dL, and after addition of 940 mg of synthetic bilirubin, the bilirubin concentration at T0 was 92.8 mg/dL. After 810 min of circulation though the resin cartridge, the bilirubin concentration was 20.3 mg/dL, calculated in a volume of 832 mL. The ΔC values provided information on the residual capacity of the cartridge to adsorb bilirubin.

Saturation was reached at T750, when the value of ΔC was zero. At this time point, we could notice a significant change in plasma color (shown in Fig. 3).

Subsequent fluctuations in bilirubin concentration (T780 and T810) were probably due to detachment of bilirubin from the binding sites, as usually seen when the condition of equilibrium is reached (shown in Table 1). Total bilirubin mass adsorbed was 759 mg, with a RR of 78.1% (shown in Table 1).

The RR was close to 50% after 3 h of circulation; afterward, RR increased but a significantly slower rate and reached 78% of the total bilirubin added to plasma at the end of the experiment, after 13.5 h. This behavior is in line

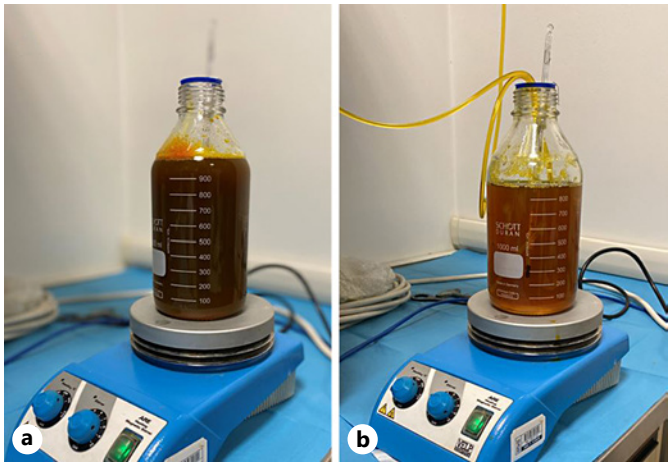

Fig. 3. Hyperbilirubinemic plasma solution at baseline (**a**) and at the end of the treatment (**b**).

with the dynamic adsorption curve (shown in Fig. 4) indicating a fast and efficient removal of bilirubin by BS330 at the beginning of perfusion: more than a half (411 mg out of 759 mg) of the total bilirubin mass adsorbed was achieved the first 120 min (shown in Table 1).

Our experiment was conducted using a minimodule downscaled to 1:3 containing 131.6 g of BS330 sorbent's beads that restrained 759 mg of bilirubin. Therefore, the adsorption capacity of the resin can be estimated at 5.76 mg of bilirubin per gram of sorbent.

In our experiment, bilirubin removal per hour after 180 min shows a progressive reduction over time (see Table 1, bilirubin removal per hour can be calculated as the difference in mass adsorbed at different time points). The total bilirubin mass adsorbed after 180 min was 470 mg.

The retrospective analysis of the bilirubin adsorption kinetics in our clinical case shows a similar trend with a total bilirubin mass adsorbed after 180 min of 487 mg. Results are shown in Table 2. The performance in vivo is coherent with our in vitro model, and bilirubin kinetics follows the curve in Figure 5.

Discussion/Conclusion

In our experiment, we evaluated the plasma adsorption capacity of BS330 cartridge using a minimodule and a dedicated machine for extracorporeal treatment simulation. We set up an ex vivo circulation experiment in which a solution of hyperbilirubinemic plasma was pumped into the circuit and through the cartridge and we assessed the adsorption trend with the bilirubin concentration gap (ΔC) between inlet (Cp_{in}) and outlet (Cp_{out}) lines. Saturation of the cartridge was reached at approximately 12 h, with a total bilirubin mass adsorbed of almost 80% of total. Our experiment shows a fast and efficient removal of bilirubin during the first hours of perfusion with more than a half of the total bilirubin mass adsorbed at 120 min. The analysis of the bilirubin adsorption kinetics in the clinical case showed a trend similar to our ex vivo model.

Acute liver failure (ALF) and acute on chronic liver failure (AoCLF) have a high mortality rate [6] and, in affected patients, endogenous toxins such as bilirubin and biliary acids accumulate. Although bilirubin may have a physiologic role as an antioxidant, elevations of indirect, unconjugated bilirubin are potentially neurotoxic and nephrotoxic (bile casts, acute tubular necrosis) [7]. Extracorporeal blood purification therapy for the removal of exogenous and endogenous toxins is a rapidly evolving area. Recently, both in vitro and in vivo studies, have described the ability of different sorbents to remove bilirubin [8, 9]. Different extracorporeal systems (MARS, Prometheus, single-pass albumin dialysis, plasma exchange) are available to remove bilirubin from blood [10]. However, these treatments have high costs and require highly qualified and trained personnel. Through its intrinsic capacity to bind and transport molecules, plasma is the best fluid to perform a purification process. Plasma adsorption perfusion (PAP) is a technique of therapeutic apheresis in which the plasma is separated from the blood on a first column and then filtered on a bilirubin-adsorbing column [11]. When compared to plasma exchange (PEX), PAP does not require administration of blood products, allows preservation of clotting factors or drugs, and it is not associated with some adverse effects that accompany PEX [12]. This technique, using the bilirubin adsorber BS330, has proved effective in reducing bilirubin levels [3].

Unconjugated bilirubin is nonpolar and lipid soluble. Its albumin binding limits its migration from the vascular space and its glomerular filtration, avoiding its precipitation and deposition in tissues [13]. Albumin has a molecular weight of approximately 68 kDa, and therefore, it is impermeable to conventional CRRT membranes. However, it can be adsorbed during hemoperfusion, making possible the removal of albumin-bound toxins such as bilirubin. Studies [14, 15] have suggested that the BS330 cartridge can detach bilirubin from albumin and toward the resin which binds electrostatically to bilirubin and bile acids in a selective manner. This discriminative ability relies on the size of the pores in the resin, at 30–40 nm. This mechanism has potentially two beneficial effects: re-

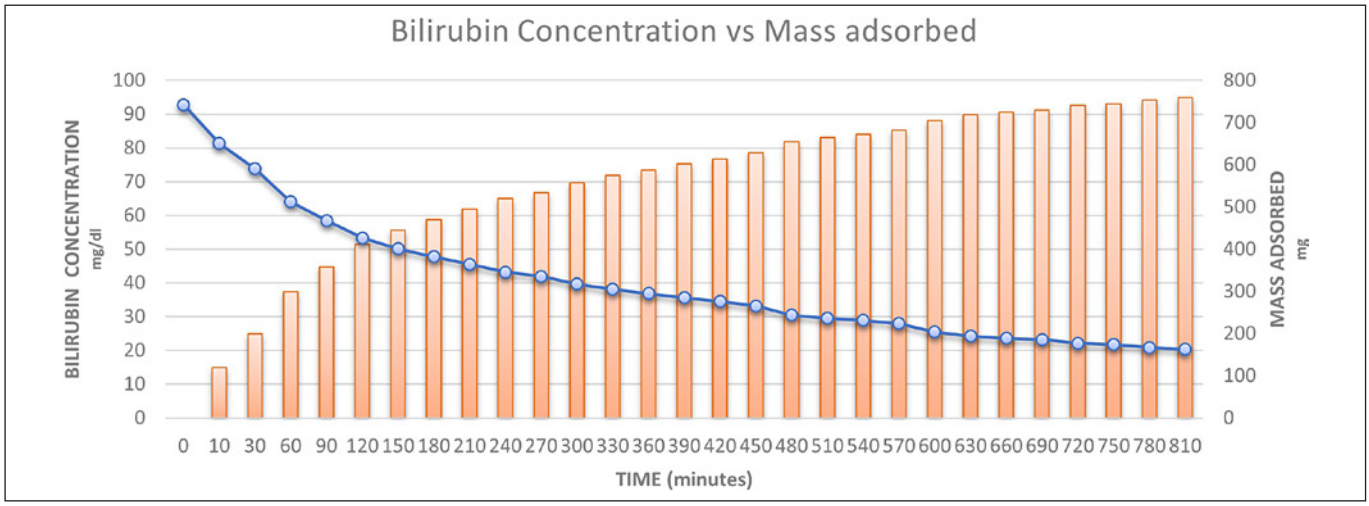

Fig. 4. Bilirubin adsorption kinetics. The curve represents the fall of bilirubin concentration during time; columns represent total mass adsorbed at each time point.

Table 1. Experimental data on bilirubin kinetics during mock in vitro plasma adsorption perfusion treatment

Time, min	Reservoir, mg/dL	Pre, mg/dL	Post, mg/dL	ΔC, mg/dL	Volume, mL	Mass adsorbed, mg	RR, %
T0	92.8	–	–	–	1,000	–	–
T10	81.3	83.0	63.2	19.8	994	120	12.4
T30	73.8	74.0	61.8	12.2	988	199	20.5
T60	64.0	64.1	57.5	6.6	982	300	31.0
T90	58.5	58.4	52.6	5.8	976	357	37.0
T120	53.3	53.6	49.7	3.9	970	411	42.6
T150	50.1	50.7	47.2	3.5	964	445	46.0
T180	47.8	48.0	45.5	2.5	958	470	48.5
T210	45.5	45.6	43.2	2.4	952	495	51.0
T240	43.2	43.7	41.2	2.5	946	519	53.4
T270	41.9	41.8	39.9	1.9	940	534	54.8
T300	39.7	40.1	38.3	1.8	934	557	57.2
T330	38.1	38.6	36.8	1.8	928	574	58.9
T360	36.9	36.9	35.9	1.0	922	588	60.2
T390	35.6	35.8	34.6	1.2	916	602	61.6
T420	34.5	34.5	33.6	0.9	910	614	62.8
T450	33.2	33.6	30.6	3.0	904	628	64.2
T480	30.5	30.6	29.9	0.7	898	654	67.1
T510	29.5	29.6	28.8	0.8	892	665	68.2
T540	28.9	28.9	28.1	0.8	886	672	68.9
T570	28.0	28.2	27.5	0.7	880	682	69.8
T600	25.5	25.3	24.4	0.9	874	705	72.5
T630	24.2	24.5	23.9	0.6	868	718	73.9
T660	23.6	23.5	23.2	0.3	862	725	74.6
T690	23.2	23.2	22.7	0.5	856	729	75.0
T720	22.1	21.9	21.2	0.7	850	740	76.2
T750	21.7	21.1	21.1	0.0	844	745	76.6
T780	20.9	20.9	20.3	0.6	838	753	77.5
T810	20.3	20.3	20.2	0.1	832	759	78.1

Reservoir, bilirubin concentration in plasma contained in reservoir; Pre, bilirubin concentration in inflow line; Post, bilirubin concentration in outflow line; ΔC, bilirubin concentration gap; Mass adsorbed, bilirubin mass adsorbed during treatment; RR, removal ratio.

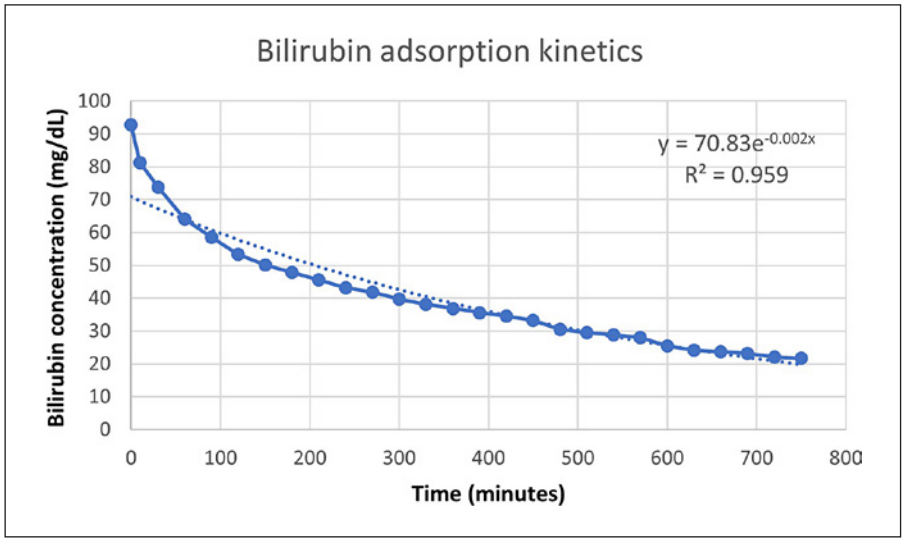

Fig. 5. Bilirubin adsorption kinetics in BS330 minimodule over time. Blue dots represent experimental data obtained from the ex vivo circulation, dashed line is the obtained trendline described by the equation $c(t)=C_0e^{-kt}$. The $R^2 = 0.959$ points out that the model well describes experimental data, where $c(t)$ is the concentration expected at time t, C_0 is the initial concentration, k is a constant parameter of the system.

Table 2. In vivo versus in vitro comparison: bilirubin adsorption kinetics during 180 min of PAP treatment with the BS330 cartridge

	T60 (mg/h)	T120 (mg/h)	T180 (mg/h)	Total mass adsorbed at T180 (mg)
In vivo treatment	298	139	69	487
In vitro experiment	300	111	59	470

moving bilirubin and simultaneously making albumin molecules available again to bind other toxins. Moreover, the presence of albumin seems to determine a decrease in adsorption capacity, probably due to its competition with the resin for bilirubin adsorption [14]. In other adsorption experiment carried out with the same synthetic bilirubin, authors added bovine serum albumin to the solution in order to simulate the physiologic condition of bilirubin in plasma [4].

In our experiment, we did not use albumin because the main purpose was to quantify the mass of bilirubin that brings the BS330 cartridge to saturation. However, in pathological condition where the levels of unconjugated bilirubin rise to a level capable of saturating its albumin binding capacity, the unbound concentration rises in plasma. Given its ability to cross membranes and bind to tissues, free bilirubin has a greater distribution volume and therefore, much more complex adsorption kinetics. Considering the results of the retrospective analysis of our clinical case, however, bilirubin adsorption kinetics seems to be similar to our ex vivo model. Our experiment was performed by circulating 1 L of hyperbilirubinemic plasma through a minimodule downscaled to 1:3 containing 131.6 g of sorbent's beads. In the clinical case we presented, PAP treatment was performed with a similar Qp (25 mL/min) but plasma volume (3.186 L) and sorbent mass (BS330 cartridge) were three-fold greater. Therefore, in our experiment, the total plasma volume was circulated through the sorbent cartridge more times. Thus, we can expect greater depuration efficiency when the treatment is performed ex vivo.

In conclusion, we believe our experiment represents the first attempt to describe free bilirubin kinetics during plasma adsorption. As such, it provides a better understanding of the BS330 cartridge's ability to achieve bilirubin binding and can help predict in vivo bilirubin adsorption kinetics in patients undergoing PAP treatment for hyperbilirubinemia.

Acknowledgments

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Statement of Ethics

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' family members/next of kin.

Conflict of Interest Statement

Prof. Claudio Ronco in the last 3 years has been consultant, medical advisor, or part of the speaker bureau receiving fees from the following companies: Asahi Medical, Aferetica, Baxter, B. Braun, Biomerieux, Bioporto, Cytosorbents, ESTOR, Fresenius Medical Care, GE Healthcare, Kaneka, Medica, Medtronic- Bellco, Nipro, Spectral, Toray, and Jafron. Silvia De Rosa has been consultant for Estor. Matteo Marcello, Anna Lorenzin, Massimo De Cal, Michela Zorzi, Marco Salvatore La Malfa, Valentina Fin, Alessandra Sandini, Francesco Fiorin, Rinaldo Bellomo, Monica Zanella have no COI to disclose.

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Author Contributions

Matteo Marcello: conception of the study, interpretation of data, and drafting the article; Anna Lorenzin: conception of the study and interpretation of data; Massimo De Cal: laboratory analysis; Michela Zorzi, Marco Salvatore La Malfa, and Valentina Fin: providing chemical and technical content; Alessandra Sandini and Francesco Fiorin: providing technical content; Silvia De Rosa: intensive care unit support and interpretation of data; Rinaldo Bellomo: revision of the article and interpretation of data; Claudio Ronco and Monica Zanella: providing intellectual content of critical importance to this work. All the authors approved the final version of this paper.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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