



Mechanisms Involved in Hypersensitivity Reactions to Polysulfone Hemodialysis Membranes

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Abstract: Several cases of patients with anaphylactic or systemic hypersensitivity reactions to polysulfone (PS) hemodialysis (HD) membranes and tolerance to cellulose triacetate (CTA) membranes have recently been reported. To investigate the mechanisms involved in PS hypersensitivity, basophil, T cell, and complement activation were analyzed in acute-phase samples from two patients with systemic reactions to PS-based membranes. Basophil and T cell activation, as well as higher serum tryptase levels were detected in acute-phase samples compared with basal levels. Complement levels (C3 and C4) were decreased in acute-phase samples from PS-allergic patients to a higher extent than in samples from control donors taken at the same time points, indicating complement activation during the acute reactions. An experimental external circuit was established on pediatric membranes after rinsing with low or high priming volumes of saline solution, to analyze basophils, T cells, and complement activation in blood samples from 10 PS-allergic and 8 nonallergic HD patients upon

contact with PS-based or CTA membranes. Predialysis and postdialysis samples were collected. Basophils from PS-allergic patients exhibited increased degranulation, and T cells showed significantly increased activation after contact with PS-based membranes primed with low volumes of saline. No activation was detected in leukocytes from non-allergic patients under the same experimental conditions. Membrane priming with high volumes of saline abrogated activation of basophils and T cells. However, basophils from allergic donors showed significantly higher responses to Fcε stimulation after contact with PS membranes. Basophil degranulation and elevated serum tryptase levels in allergic patients during acute reactions support the systemic activation of mast cells and basophils during hypersensitivity reactions to PS-based membranes. A leachable component of the membranes might be responsible for cell activation in some patients. **Key Words:** Hemodialysis—Polysulfone membranes—Systemic reactions—Anaphylaxis—Hypersensitivity.

Adverse hypersensitivity reactions during hemodialysis (HD) sessions have been reported, with symptoms including malaise, hypotension, dyspnea, nausea or vomiting, and anaphylactic shock (1). Most type I allergic reactions have been reported in patients allergic to sterilizing agents such as ethylene oxide (EtO) (2). Hypersensitivity reactions

can also occur due to the bioincompatibility of the dialysis membranes; or they can occur in patients on dialysis with AN69 polyacrylonitrile membranes who were simultaneously receiving angiotensin-converting-enzyme inhibitors (ACEi), as a result of the accumulation of bradykinin (3). The incidence of adverse reactions during HD is currently lower with the avoidance of EtO as a dialyzer sterilizing agent, the use of AN69 membranes in patients treated with ACEi, and the increased biocompatibility of new dialyzers. In recent years, however, a series of cases have been reported in which unexpected hypersensitivity reactions occurred in patients dialyzed with polysulfone (PS) or

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TABLE 1. Demographic data of patients with adverse reactions during HD

Patient number	Age (years)	Sex	Months*	Problem membrane	Symptoms	Acute samples
P1	67	M	38	PS (Helixone)	Hypotension, dyspnea, desaturation	No
P2	65	M	0	PS (Helixone)	Hypotension, dyspnea, desaturation	No
P3	86	F	0	PS (Helixone)	Hypotension, dyspnea, desaturation	No
P4	64	M	4	PES (Polynephron)	Dyspnea, desaturation	No
P5	72	M	6	PS (Helixone)	Hypotension, dyspnea, desaturation	No
P6	84	F	5	PS (Helixone)	Hypotension, pruritus, abdominal pain	No
P7	53	F	8	PS (Helixone)	Hypotension, pruritus	No
P8	73	M	0	PS (Helixone)	Hypotension, dyspnea, desaturation	No
P9	74	F	0.5	PS (Helixone)	Abdominal pain, rash Dyspnea, [†] desaturation [†]	Yes [†]
P10	84	M	0	PS (Helixone)	Dyspnea, desaturation	No
P11	53	F	1	PS (Helixone)	Dyspnea, pruritus	Yes
P12 [‡]	60	F	0	Heparin reaction [†]	Dyspnea, desaturation	Yes

PS, polysulfone; PES, polyethersulfone.

*Time period using a PS hemodialyzer previous to the first hypersensitivity reaction.

[†]Second reaction.

[‡]P12 suffered a reaction to heparin while using a PS dialyzer.

polyethersulfone (PES) biocompatible membranes in the absence of other risk factors such as EtO or ACEi (4–12).

In this study, we investigated the possible mechanisms responsible for the hypersensitivity reactions to PS-based dialysis membranes. Based on clinical data, all PS-allergic patients tolerated the cellulose triacetate (CTA) membranes. Because it is unusual to be able to obtain samples during the acute phase of the allergic reaction, in addition to acute-phase samples we used an ex vivo approach to expose leukocytes from PS-allergic patients to PS-based and CTA membranes. This approach permitted us to explore possible implicated mechanisms including the immune response in both situations. Nonallergic patients were also studied in parallel for comparative purposes. Whole heparinized blood from cases and controls was circulated ex vivo through pediatric PS and CTA membranes, and the activation of various cellular and humoral components of the immune response was analyzed.

PATIENTS AND METHODS

Patients

The PS-allergic reaction was defined by clinical criteria when the patient suffered a premature or medium-term reaction during the HD session, consisting of chest pain, severe hypotension, and severe desaturation. All the patients were treated with high flux O₂ and, in several cases, hydrocortisone plus salbutamol were necessary. After treatment, blood pressure (BP) and O₂ saturation (O₂ sat) levels returned to normal. Two patients were rechallenged with the PS dialyzer, demonstrating intolerance. These two patients and the remainder were moved

to CTA dialyzers to continue their HD treatment, with no problems occurring during these sessions.

Twelve PS-allergic HD patients (P1–P12) and eight nonallergic HD patients (C1–C8) studied as control donors were recruited for the study. Demographic data of the allergic and nonallergic patients are shown in Table 1 and Supporting Information Table S1, respectively.

Two 50 mL blood samples were drawn from each patient to perform the study with PS or CTA membranes, with at least a 1-month delay between samples.

This study was conducted according to the principles expressed in the Declaration of Helsinki, and was approved by the Research Ethics Committee from La Paz University Hospital. Informed consent was obtained from all the patients.

Ex vivo hemodialysis membrane circuit

To expose the patient's leukocytes to the inner surface of the capillaries, mimicking a dialysis session, an ex vivo dialysis-like circuit was designed using pediatric HD membranes and shorter blood lines to circulate a minimal amount (50 mL) of blood through the capillaries (Supporting Information Fig. S1).

The dialysate compartment was completely filled with ultrapure “on-line” dialysis fluid and clamped. Therefore, there was no flow through the dialysate compartment. PS-based Helixone and pediatric CTA dialyzers (FX Paed Helixone 0.2 m² and Sureflux 30 L cellulose triacetate 0.3 m²) were primed with 50 or 1500 mL of ultrapure saline fluid to eliminate the components used to protect capillaries during manufacturing. The blood lines were simultaneously purged. Next, a heparinized blood sample of 50 mL

from the patients or the controls was progressively added to the blood lines, discarding the on-line fluid. Once the blood circuit was filled, it was maintained in recirculation for 1 h at 200 mL/min flow. Samples were taken before (pre-D samples) and after 1 h of circulation through the HD membranes (post-D samples).

A scheme of the experimental model and the determinations thereafter is shown in Supporting Information Fig. S2.

Basophil activation/degranulation test

Cell membrane expression of CD63 was analyzed by flow cytometry as a surrogate for basophil activation/degranulation (13). Heparinized whole blood was stimulated for 15 min at 37°C in the presence of IL-3 alone or with polyclonal goat anti-human IgE (1 µg/mL), used as a positive control. Before erythrocyte lysis, the cells were stained with CD123-APC, HLA-DR-PE, and CD63-FITC (Miltenyi Biotec, Bergisch Gladbach, Germany). The basophils were gated as FSC low/CD123⁺/HLA-DR⁻ leukocytes (Supporting Information Fig. S3). The frequency of basophils expressing CD63 was evaluated using a FACScalibur flow cytometer and CellQuest Pro software (BD Biosciences, San Jose, CA, USA). Basophil degranulation was expressed as % CD63⁺ basophils.

Flow cytometry analysis of T cell activation (expression of CD69 and CD25)

Heparinized whole blood was incubated with CD3-PerCP, CD4-FITC, CD8-APC, CD25-PE or CD3-PerCP, CD4-PE, CD8-APC, and CD69-FITC (Miltenyi Biotec) before erythrocyte lysis. The lymphocytes were gated as FSC low/SSC low/CD3⁺ leukocytes (Supporting Information Fig. S4). The expression of CD69 or CD25 was analyzed in selected CD4 or CD8 T cell subpopulations using a FACScalibur flow cytometer and CellQuest Pro software (BD Biosciences).

Serum tryptase and complement C3 and C4 serum levels

Serum tryptase and complement components C3 and C4 were evaluated after ex vivo blood circulation through HD membranes by the immunology laboratory, according to standard procedures.

RESULTS

Acute reactions

Acute-phase samples from two PS-allergic patients were obtained at the time of the adverse reaction. Half a month after starting hemodialysis sessions, approximately 20 min after initiating the HD session, patient 9 reported abdominal and chest pain. The HD session was stopped, the blood lines were discarded, and hydrocortisone was administered. The patient gradually improved (no acute-phase samples were collected from this episode). The HD session was restarted using a CTA membrane, without incident. Because the symptoms were not characteristic of an anaphylactic reaction, a few months later we decided to restart the HD with a PS dialyzer. A few minutes after starting the HD session, the patient reported dyspnea and showed low O₂ saturation. The HD session was stopped and the blood lines were discarded, and HD was restarted using a CTA membrane, with no incidents.

One month after starting dialysis sessions, and 30 min after starting HD, patient 11 reported malaise, feeling warm, progressive dyspnea, and pruritus. The HD session was stopped, the blood lines were discarded, and high-flux O₂ plus intravenous hydrocortisone were administered. The patient reported immediate improvement. The HD session was restarted with a CTA membrane, without incident.

Serum tryptase and complement levels were evaluated in the acute and 24-h samples, given elevated tryptase is one of the hallmarks of anaphylaxis (14) and complement activation has been previously reported in blood from patients undergoing PS-based membrane HD (15,16).

As shown in Table 2, tryptase levels were elevated during the acute adverse reaction, compared

TABLE 2. Serum determinations in patients with acute-phase samples

	P9		P11		P12 (Heparin)	
	Acute sample	24 h post-reaction	Acute sample	24 h post-reaction	Acute sample	24 h post-reaction
Tryptase (ng/mL)	32.2	25.0	7.73	5.97	4.85	ND
C3 (mg/dL)	73.6	119.0	77.4	109	129	126
C4 (mg/dL)	26.8	33.1	17	24.9	29.9	31

ND: No data.

TABLE 3. Serum complement levels in control and PS-allergic donors during HD

Patients	Complement C3 (mg/dL)			Complement C4 (mg/dL)		
	Basal levels	post-D 20'	%C3 degraded at 20'	Basal levels	post-D 20'	%C4 degraded at 20'
Nonallergic						
C1	119.0	103.0	13.45	26.8	26.6	0.75
C2	110.0	109.0	0.91	27.9	26.3	5.73
C3	115.0	105.0	8.70	35.6	33.3	6.46
Mean			7.68			4.31
Allergic	Basal levels	Acute sample		Basal levels	Acute sample	
P9	119.0	73.6	38.15	39.9	26.8	32.83
P11	109.0	77.4	28.99	24.9	17.0	31.70

with basal levels measured in the same patients 24 h postreaction, suggesting basophil and mast cell activation during the hypersensitivity reaction.

Some products of complement degradation such as C3a and C5a, also known as anaphylatoxins, bind to specific receptors in basophils and mast cells, which leads to cell activation and which can trigger anaphylactic reactions (17). To estimate the contribution of complement components to the anaphylactic reaction we measured C3 and C4 serum levels as surrogates of complement activation. The concentrations of C3 and C4 were decreased during the acute reaction in both cases. Although the complement was apparently not consumed overall during a regular HD session in nonallergic patients (data not shown), previous studies suggest that the complement is transiently activated at various levels in all patients during the first minutes after HD starts (15). To compare the levels of complement in the acute-phase samples and during regular HD sessions, blood samples were taken at 20 min of dialysis from three nonallergic control patients (C1–C3) using PS-based membranes. The percentage of C3 and C4 proteins degraded was much higher in the acute-phase samples than in any of the nonallergic patients analyzed, suggesting a specific activation of complement along the hypersensitivity reactions to PS-based membranes in both patients (Table 3). It is of

note that no decrease in complement levels was detected in the acute-phase samples from a patient who suffered a hypersensitivity reaction to heparin (Table 2).

We also evaluated the activation status of basophils and T cells during the acute reaction in these patients. As shown in Table 4, a strong activation of basophils and T cells occurred during the acute adverse reaction that was no longer detected in 24-h samples. No substantial changes were detected in samples from nonallergic patients analyzed at 20 min of dialysis (Supporting Information Table S2).

Analysis of basophil activation/degranulation on exposure to PS and CTA HD membranes

Anaphylactic reactions are triggered by vasoactive mediators released by mast cells and basophils upon cell activation and during the subsequent degranulation process (18). Upon activation basophils not only secrete bioactive mediators, but also upregulate the expression of some membrane proteins which can be detected on the cell surface by flow cytometry. CD63 is a secretory-granule associated protein which is exposed in the cell membrane along with basophil degranulation as a result of the fusion of the granule and plasmatic cell membranes. We evaluated membrane expression of CD63 as a surrogate for basophil activation/degranulation (13). To test

TABLE 4. Basophil degranulation and lymphocyte activation during hypersensitivity reactions

Patients	Activation markers	Acute sample	2 h post-reaction	24 h post-reaction
P9	% CD123 ⁺ CD63 ⁺	61.03	1.27	3.29
	% CD4 ⁺ CD69 ⁺	76.63	82.88	1.37
	% CD8 ⁺ CD69 ⁺	31.31	34.74	0.88
P11	% CD123 ⁺ CD63 ⁺	2.83	ND	1.90
	% CD4 ⁺ CD69 ⁺	0.66	ND	1.11
	% CD8 ⁺ CD69 ⁺	3.81	ND	4.81
P12 (Heparin)	% CD123 ⁺ CD63 ⁺	1.98	0.89	0.37
	% CD4 ⁺ CD69 ⁺	46.70	62.9	0.33
	% CD8 ⁺ CD69 ⁺	83.79	88.4	0.69

ND: No data.

whether some components of the PS-based dialysis membranes were able to stimulate basophil degranulation, blood from PS-allergic patients and control PS-tolerant donors was circulated through ex vivo HD circuits. The PS membranes were washed with 50 mL saline and heparinized whole blood was circulated for 1 h. A fraction of basophils ($CD123^+ / HLA-DR^-$ leukocytes) from the PS-allergic patients degranulated after this procedure, as assessed by augmented expression of CD63 on the cell surface (Supporting Information Fig. S3B, C). A statistically significant increase in degranulation was observed specifically in basophils from PS-allergic donors (Fig. 1A), whereas no effect was observed in basophils from nonallergic patients after circulation through PS membranes in this protocol (Fig. 1B).

It is of note that basophils from P4 who suffered an anaphylactic reaction to a PES-based HD membrane degranulated upon ex vivo circulation through the PS-based membrane (0.55% $CD63^+$ basophils in pre-dialysis samples vs. 2.55% $CD63^+$ basophils after 1 h circulation through the PS membrane).

To verify that cells were viable and able to degranulate, a positive control test was performed in which blood cells were further stimulated with IgE-specific monoclonal antibodies to activate Fc ϵ RI bound to IgE (13) (Supporting Information Fig. S3D,E). Basophils from both groups of patients were equally able to respond to this stimulus. In PS-allergic donors, a trend toward increased degranulation was observed after circulation through PS membranes (Fig. 1B). The differences were significant when only nine patients (P2–P9) were considered ($P = 0.0413$; paired t -test), whereas no significant differences were observed in the non-allergic patients (Fig. 1C,D).

In contrast, when CTA membranes primed with only 50 mL of saline were used, basophils from both PS-allergic and nonallergic donors underwent a strong degranulation process that led to a patent increase in the expression of CD63 on the cell membrane (Supporting Information Fig. S5A,B). This result led us to suspect that 50 mL priming volume was not enough to completely remove glycerol from the CTA membranes. When exhaustive washing of the CTA membranes with 1500 mL of

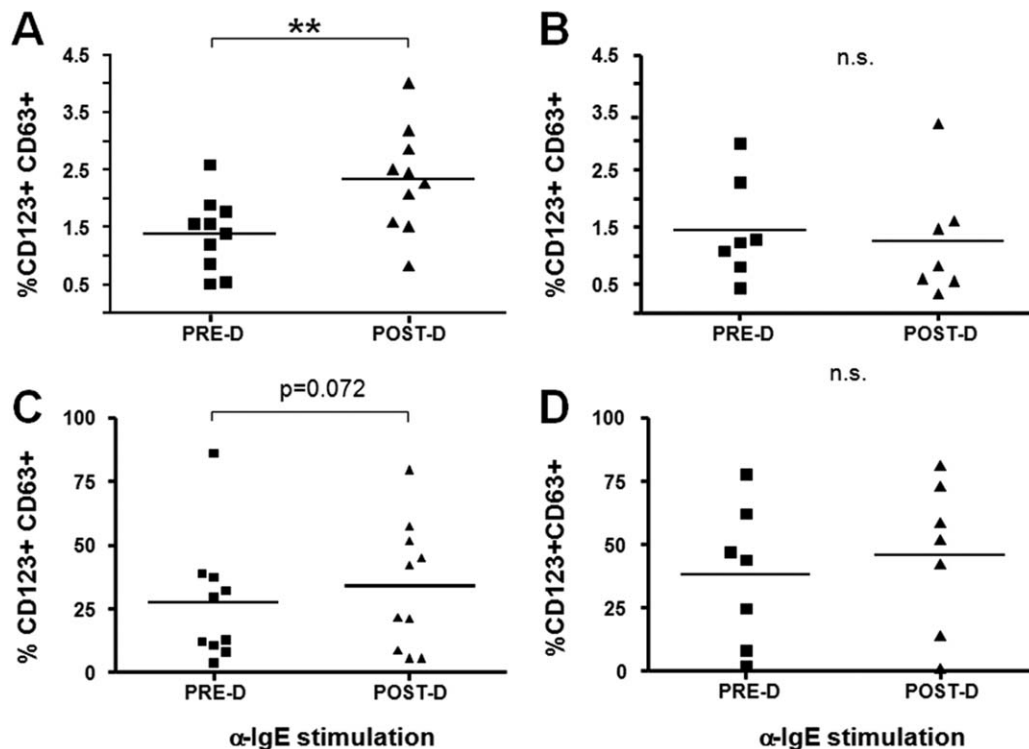


FIG. 1. Basophil degranulation on ex vivo blood circulation through low volume-primed hemodialysis membranes. (A) Basophil degranulation in allergic or (B) nonallergic donors at 1 h of circulation through PS membranes previously washed with 50 mL saline was analyzed by flow cytometry as the % of $CD63^+CD123^+HLA-DR^-$ leukocytes. (C) After undergoing the PS ex vivo circuit, blood cells from allergic or (D) nonallergic donors were stimulated with anti-IgE antibodies and basophil degranulation was analyzed. Data from 10 allergic donors and 7 nonallergic donors are shown. ** $P = 0.009$ (paired t -test); n.s.: not significant.

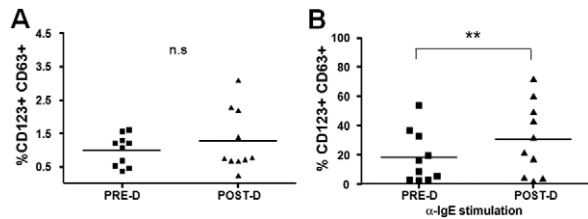


FIG. 2. Basophil degranulation in PS-allergic patients on ex vivo blood circulation through high-volume primed hemodialysis membranes. (A) Basophil degranulation in blood from 10 PS-allergic donors was analyzed before (Pre-D samples) and after (Post-D samples) 1 h of circulation through PS membranes previously washed with 1.5 L of saline solution. (B) Basophil degranulation was analyzed in the same blood samples after further IgE receptor stimulation. ** $P = 0.0024$ (paired t -test); n.s.: not significant.

saline was performed, basophils from the PS-allergic and nonallergic donors were no longer degranulated after undergoing the ex vivo CTA circuit (Supporting Information Fig. S5C,D), thus confirming that the glycerol remaining within the capillaries was responsible for the basophil activation in the previous setting.

In light of these results, we again tested the effect of PS-based membranes on basophils from PS-allergic donors after exhaustive membrane washing with 1500 mL of saline prior to blood circulation. Basophil activation on exposure to the PS membranes appeared to be dampened in allergic donors when the membranes were exhaustively washed, compared with the significant increase in degranulation levels when less volume was used to prime the PS membranes; however a trend toward increased degranulation was still observed in some patients (Fig. 2A).

Nonetheless, an increase in degranulation capacity was also observed in basophils from PS-allergic donors after contact with PS membranes when the cells were further stimulated through Fc ϵ RI cross-linking by anti-IgE antibodies (Fig. 2B). This effect was PS-specific, given that no differences were observed in the activation status of basophils from allergic or nonallergic donors on the CTA circuit after exhaustive priming with 1500 mL of saline solution and additional activation with anti-IgE antibodies (not shown).

These results suggest that an extractable component from the PS membrane might be responsible for basophil degranulation and release of soluble mediators thereafter, which might also “prime” basophils for an increased response to additional stimulating agents.

Analysis of lymphocyte activation on exposure to HD membranes

The activation of lymphocytes after contact with HD membranes in PS-allergic and nonallergic

patients was investigated through the analysis of CD25 and CD69 (19) expression by flow cytometry. We did not find any significant upregulation of CD25 in T cells from allergic or nonallergic patients on circulation through the PS or CTA membranes, even when they had not been exhaustively washed to fully remove preservatives (not shown). In contrast, both CD4 and CD8 T cells from PS-allergic patients specifically upregulated the early activation marker CD69 after blood circulation through the PS membranes when they had been washed with low volumes of saline. Supporting Information Fig. S4 shows flow cytometry analysis of T cell activation in a representative PS-allergic donor. A summary of data from several patients is shown in Fig. 3A. This activation was not detected after circulation through the CTA membranes (Fig. 3B), and was not observed after exhaustive washing of the PS membranes with 1500 mL of saline (Fig. 3C). Furthermore, T cell activation was not detected in nonallergic donors in any of the experimental conditions tested, regardless of the washing procedure of the HD membranes (not shown). These data further suggest a leachable component of the PS membranes as responsible for the hypersensitivity reaction.

Serum tryptase levels

Tryptase levels are most commonly evaluated as a readout of mast cell and basophil activation in allergic reactions (14). Basal serum tryptase levels were evaluated in PS-allergic and nonallergic donors prior to and after blood circulation through the PS or CTA membranes. Although differences were not statistically significant, a trend toward increased basal tryptase values was found in the allergic patients as compared with the nonallergic donors (Fig. 4). Serum tryptase levels were always lower after circulation through the HD membranes (Table 5). Similar results were observed in a regular HD session in patients with no adverse reactions to PS membranes (not shown). However, our attempts to detect the protein in the spent dialysate were unsuccessful. This suggests that the protein remains bound to the HD membranes, although it is possible that a certain amount of protein is transported across the HD membrane and the final concentration in the dialysate remains below the technique detection limits (1 ng/mL).

Serum complement levels

Lower serum levels of C3 and C4 were detected after 1 h of ex vivo blood circulation through PS membranes in allergic donors, regardless of priming

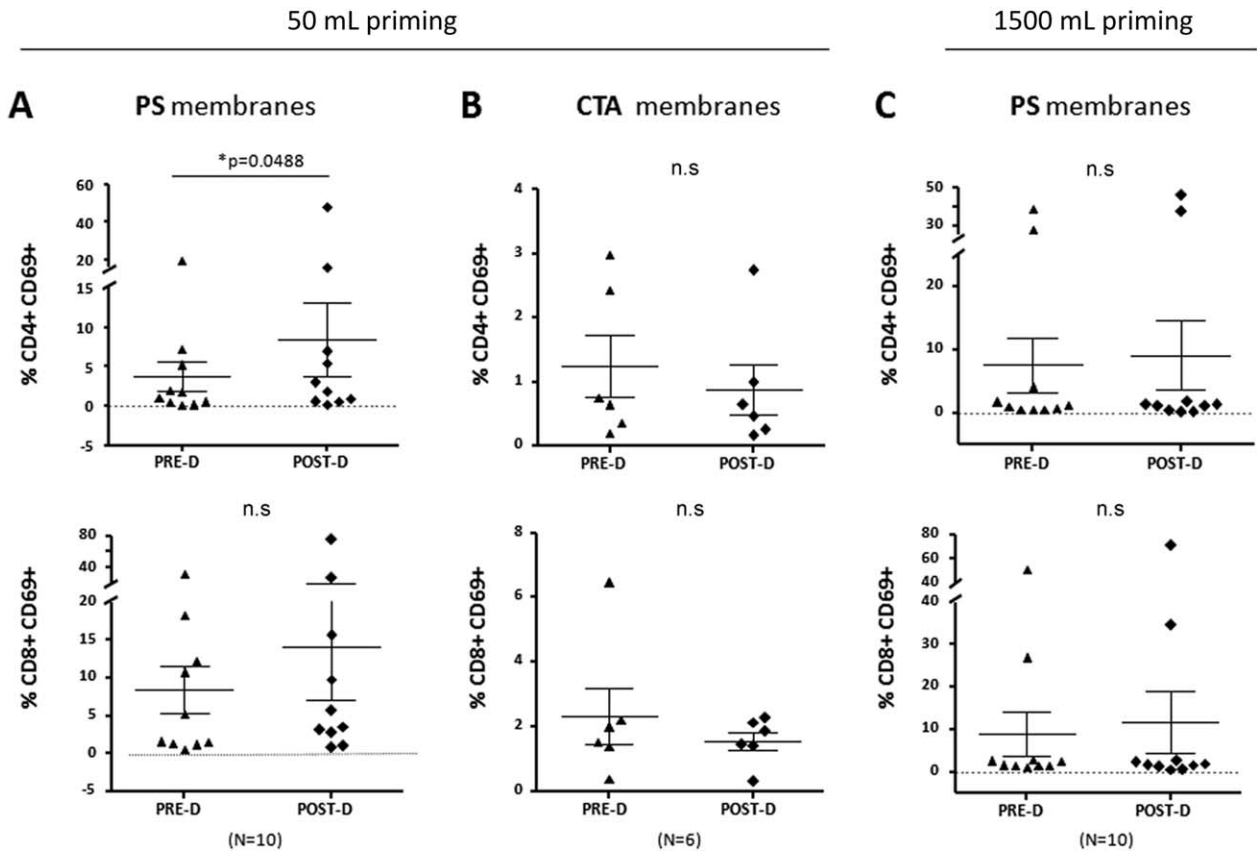


FIG. 3. T cell activation as assessed by flow cytometry analysis of $CD69^+CD4^+$ and $CD69^+CD8^+$ T cells. Blood from PS-allergic donors was circulated for 1 h through PS (A) or CTA (B) membranes previously washed with 50 mL of saline solution and (C) PS membranes previously washed with 1.5 L of saline solution. Differences between pre- and postdialysis samples were evaluated by the Wilcoxon signed rank test.

technique (Fig. 5A,B). Nonetheless, complement C3 and C4 levels were also decreased in serum from PS-allergic patients after 1 h of circulation through the CTA membranes under the conditions tested (Supporting Information Fig. S6), and similar results were obtained in samples from nonallergic donors (not shown).

DISCUSSION

The symptoms developed by the patients during adverse reactions to PS in the absence of EtO or other classical sensitizing agents suggest an anaphylactic/systemic reaction with the participation of mast cells and basophils (1). Our results obtained for the acute-phase samples in two patients with systemic hypersensitivity reactions to PS, regarding basophil degranulation, T cell activation, and degradation of C3 and C4 complement proteins prove the activation of the immune system. Moreover, elevated tryptase levels in acute serum samples from these cases are consistent with the participation of

tissue-resident mast cells and basophils (20). The clinical history suggests that the patients are sensitized to a specific component of the PS membrane, given PS-allergic patients tolerated HD sessions

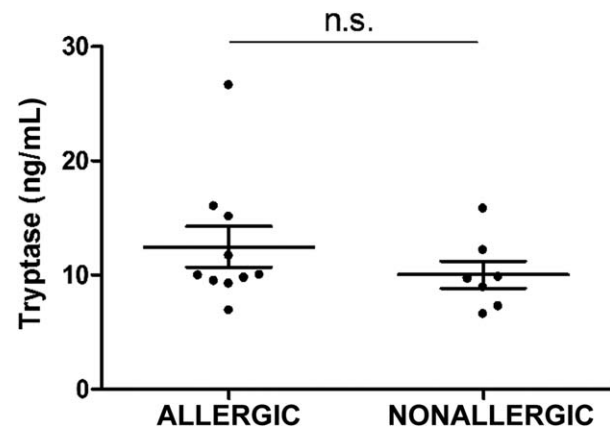


FIG. 4. Basal serum tryptase. Basal serum tryptase levels in PS-allergic and nonallergic patients treated with HD were measured in predialysis samples. n.s.: Not significant (Mann-Whitney *U* test).

TABLE 5. Serum tryptase concentrations in patients treated with HD

Sample	Serum tryptase (ng/mL) [mean values (range)]	
	PS-allergic (N = 8)	PS-nonallergic (N = 7)
PRE-D	11.46 (17.1–7.85)	8.69 (12.5–5.32) [†]
POST-D*	6.28 (10.7–1.48) [§]	4.72 (7.85–1.0) ^{†,§}

*Serum values after 1 h of circulation through PS membranes primed with 50 mL saline.

[†]Not significant when compared with PS-allergic patients.

[§]Not significant when compared with Pre-D samples.

with CTA membranes, similar to cases previously reported (4,6,9–12).

Ex vivo dialysis circuits using PS or CTA membranes were set to test this hypothesis. The cell membrane upregulation of CD63 is a marker of basophil degranulation and is widely used as a diagnostic tool to evaluate immediate hypersensitivity reactions to different allergens, including xenobiotics (13). Using this technique, we found that basophils from the PS-allergic patients were specifically activated after ex vivo circulation through PS membranes, especially when a low volume of saline solution was used to prime the dialyzers, whereas basophils from the PS-tolerant patients were not

activated under the same conditions. In addition, T cells from the PS-allergic patients were activated to express CD69 specifically upon circulation through the PS membranes only when a low volume of saline solution was used to prime the dialyzers. On the contrary, no significant changes were observed in T cells or basophils from nonallergic patients after ex vivo circulation through the HD membranes. Yet, the CTA membranes were not able to activate either T cells under any of the experimental conditions tested, or basophils after exhaustive membrane priming, in any group of patients. Altogether, these results indicate that PS-allergic individuals are sensitized to a component to PS membranes that is at least partially extractable. This leachable component from the PS-based membrane might be responsible for basophil degranulation and release of soluble mediators thereafter, which might also “prime” basophils for an increased response to additional stimulating agents. This component may enter the circulation during HD sessions and induce the systemic reaction possibly through the stimulation of tissue-resident mast cells in allergic patients.

Among the leachable components, hypersensitivity reactions to polyvinylpyrrolidone (PVP), a

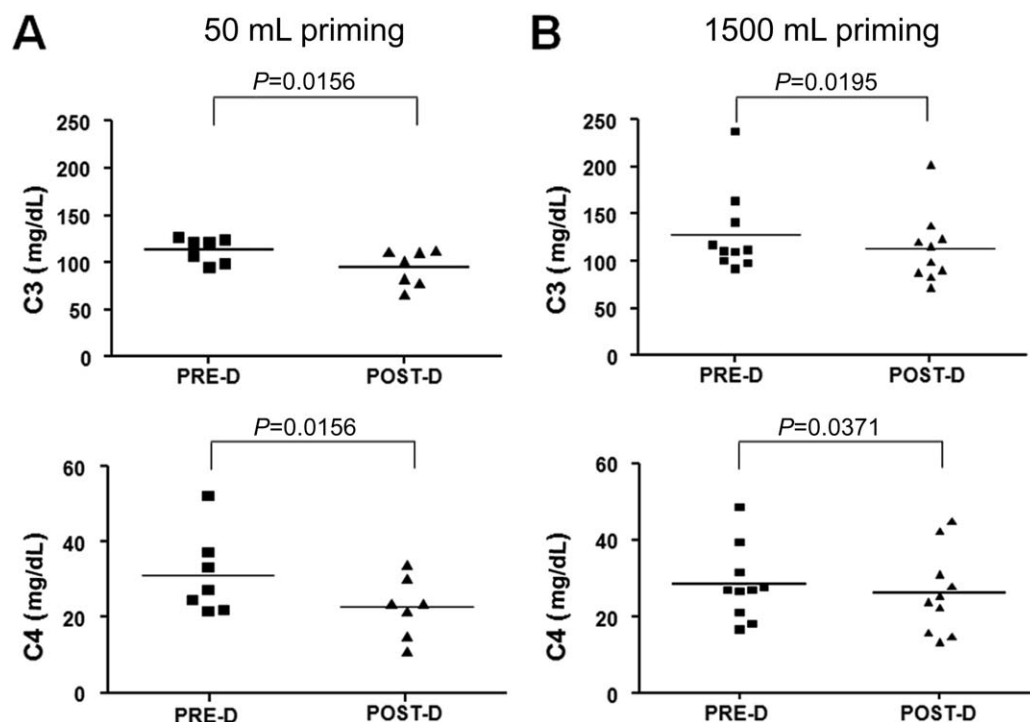


FIG. 5. Evaluation of complement levels in PS-allergic patients on ex vivo blood circulation through PS-based hemodialysis membranes. The concentrations of the complement proteins C3 (upper panels) and C4 (lower panels) were evaluated in serum samples from PS-allergic donors previous (Pre-D) and after circulation through PS membranes (post-D) primed with either 50 mL (A) or 1.5 L (B) of saline solution. Differences in complement levels in samples pre- and postdialysis were evaluated by the Wilcoxon signed rank test.

component used to diminish the hydrophobicity of the PS-based membranes, have been reported in different settings (21–23). PVP can be eluted from PS-PVP-based membranes under various conditions (24), thus being able to enter the circulation during HD. The absence of an anaphylactic reaction in a case of using a PS dialyzer with no PVP as an amalgamating agent suggested a significant role of this product as the cause of the patient's disorder (10). However, our efforts to detect PVP sensitization in our patients have been unsuccessful (data not shown). In this sense, a recent study reported that skin tests to PVP were negative in six PS-allergic patients (25). On the other hand, four of these six patients tested in the same study developed positive skin tests to effluxed priming fluid, suggesting that additional substances eluted from hemodialysis membranes, apart from PVP, could cause PS membrane hypersensitivity. Among these substances, bisphenol A (BPA) is one of the components of PS membranes which can be eluted from the dialyzer. However, only one patient in the above-mentioned study showed a positive skin test to BPA (25), supporting the hypothesis of additional extractables as sensitizing agents. In our study, patient 4 suffered an adverse reaction to a PES-based membrane, and basophils from this patient reacted to the PS-dialyzer during the ex vivo circuit. As PES membranes do not contain BPA, it is likely that other components common to PS and PES membranes caused the hypersensitivity reaction in this patient. However, the contribution of BPA in additional patients' needs further evaluation.

Anaphylaxis is usually triggered through mechanisms involving allergen-specific IgE and the high-affinity FcεRI on mast cells or basophils. Nonetheless, in addition to allergen-specific IgE, other potential immunologic mechanisms in anaphylaxis include the involvement of immune aggregates, IgG, IgM, platelets, and T cell secreted factors, and activation of the complement or coagulation systems (26).

Histamine is one of the primary vasoactive mediators released by mast cells and basophils on degranulation during anaphylactic reactions. However, the histamine half-life is very short, and tryptase levels are most commonly evaluated as a readout of mast cell and basophil activation in allergic reactions (14). Therefore, elevated serum tryptase levels are a hallmark of anaphylactic reactions, especially to parenteral antigens, and quantization of tryptase in peripheral blood is used for diagnosis of immediate type I hypersensitivity. Mast cells are likely the primary contributors to

serum tryptase levels during the acute reactions, in which systemic involvement occurs with massive activation of tissue mast cells, and which cannot be evaluated in the ex vivo dialysis model. Previous publications reported high basal tryptase serum levels in patients treated with HD (27), which might reflect the lack of clearance due to impaired renal function (28). We detected higher than basal tryptase levels in PS-allergic patients compared with PS-tolerant donors, although the differences were not statistically significant. However, elevated serum tryptase might reflect a predisposition to allergic sensitization in these patients. Moreover, our data indicate that tryptase is retained in the membranes during hemodialysis. This retention precludes the measurement of tryptase in serum or dialysate to evaluate the activation of basophils, which can also release low amounts of tryptase (20) during the ex vivo circuit.

Our findings of lower serum complement C3 and C4 levels during the acute reaction compared with regular HD sessions with the same dialyzer in PS-tolerant donors, indicate higher degree of complement activation and suggest that complement activation might also contribute to mast cell degranulation and release of mediators, given complement degradation products such as the anaphylatoxins C3a and C5a can contribute to mast cell activation and systemic symptoms. On the other hand, it has been reported that mediators released by mast cells, such as tryptase, can also contribute to complement activation and generation of anaphylatoxins (29). This might create an activating amplification loop enhancing the systemic symptoms, because anaphylatoxins themselves can stimulate mast cells and basophils to degranulate (30). In our ex vivo dialysis model, no substantial differences were detected in complement activation using PS or CTA membranes. This result suggests that complement activation in the acute anaphylactic reaction to PS is not the primary event leading to the hypersensitivity reaction. On the contrary complement activation in the PS-allergic patients might be a secondary event triggered by tryptase released upon massive systemic mast cell activation. Furthermore, anaphylatoxins might also enhance the activation of T cells (31).

Mast cells and T cell interactions have also been reported during anaphylactic reactions (32). Histamine receptors expressed on lymphocytes might modulate the cell activation status and be responsible for the CD69 expression in our ex vivo model. On the other hand, anaphylatoxins might also contribute to T cell activation during the acute anaphylactic

reactions in the patients. CD69 is known to be a very early activation marker, because it is promptly upregulated on all leukocytes upon activation (33). Importantly, it is upregulated on T cells at Ag encounter (19,34). In our cases, CD69 might reflect antigen activated T cells or the activation through anaphylatoxin receptors or by cytokines released by activated basophils or other immunocytes.

Immune-mediated mechanisms have been suspected in hypersensitivity reactions to PS-based HD membranes based on increased IgE (5,12), tryptase (11), and eosinophil (10) counts in some patients. Although we did not detect abnormal levels of total IgE in our cases (data not shown), specific IgE to some component of the HD membranes cannot be ruled out as responsible for basophil activation on FcεRI engagement. However, receptors stimulated by cationic substances and expressed by mast cells and basophils can be responsible for antibody-independent cell activation in some individuals (35). Different mechanism and sensitizing compounds may participate in the release of vasoactive mediators in individual cases.

CONCLUSIONS

Our data in acute-phase samples from patients with anaphylactic reactions to polysulfone membranes, as well as ex vivo exposure of blood from allergic patients to PS membranes confirm the participation of the immune system in systemic reactions to PS. Further research is needed to unravel the molecular events and specific sensitizing agents leading to anaphylactic reactions to PS membranes in different patients as well as the participation of additional immunocytes such as neutrophils and platelets.

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Author Contributions: TB, RS, and RSV conceived and designed the experiments. ARS, MPR, and FT performed the experiments. RSV, RDT, CC, EG, and MLT contributed samples, reagents, materials, and analysis tools. AR and TB analyzed the data. TB, RSV, RS, SQ, AMF, MLT, and JDO wrote the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Mechanisms involved in hypersensitivity reactions to polysulfone hemodialysis membranes

Supplementary Materials

Supplementary Table S1

Table S1. Demographic data of control donors

	Sex	Age (years)	Months on DP	Membrane
C1	Male	47	44	PS (Helixone)
C2	Female	83	42	PS (Helixone)
C3	Female	83	26	PS (Helixone)
C4	Male	64	25	PS (Helixone)
C5	Female	82	25	PS (Helixone)
C6	Male	68	56	PS (Helixone)
C7	Female	40	50	PS (Helixone)
C8	Male	67	80	PES (Polynephron)

Supplementary Table S2

Table S2. Basophil degranulation and lymphocyte activation during regular HD sessions in PS-nonallergic patients

Nonallergic patients		Pre-D	20 min	Post D
C1	% CD123 ⁺ CD63 ⁺	0.83	0.97	0.33
	% CD4 ⁺ CD69 ⁺	0.32	0.13	0.27
	% CD8 ⁺ CD69 ⁺	0.40	0.65	1.1
C2	% CD123 ⁺ CD63 ⁺	0.47	0.76	0.57
	% CD4 ⁺ CD69 ⁺	0.10	0.25	0.11.
	% CD8 ⁺ CD69 ⁺	1.23	1.17	0.76
C3	% CD123 ⁺ CD63 ⁺	1.56	0.78	0.95
	% CD4 ⁺ CD69 ⁺	0.53	0.46	0.44
	% CD8 ⁺ CD69 ⁺	2.07	2.03	2.20
C4	% CD123 ⁺ CD63 ⁺	0.50	0.54	0.72
	% CD4 ⁺ CD69 ⁺	0.28	0.26	0.29
	% CD8 ⁺ CD69 ⁺	1.50	1.25	1.30

Blood samples were evaluated immediately previous to HD (Pre-D) at 20 minutes, and at the end of the dialysis procedure.

“Ex-vivo” hemodialysis membrane circuit

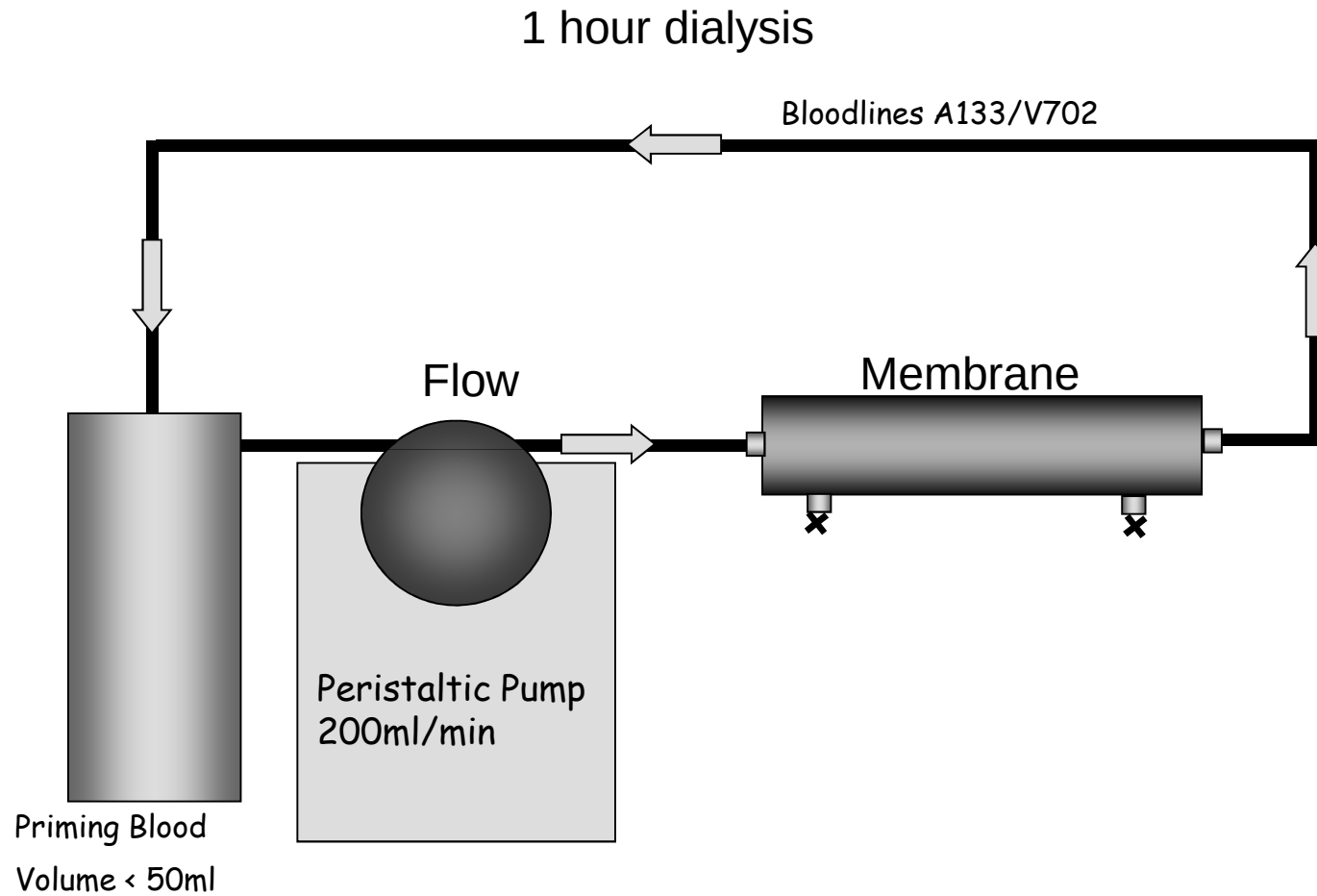


Fig S1. Schematic representation of the “ex-vivo” dialysis circuit

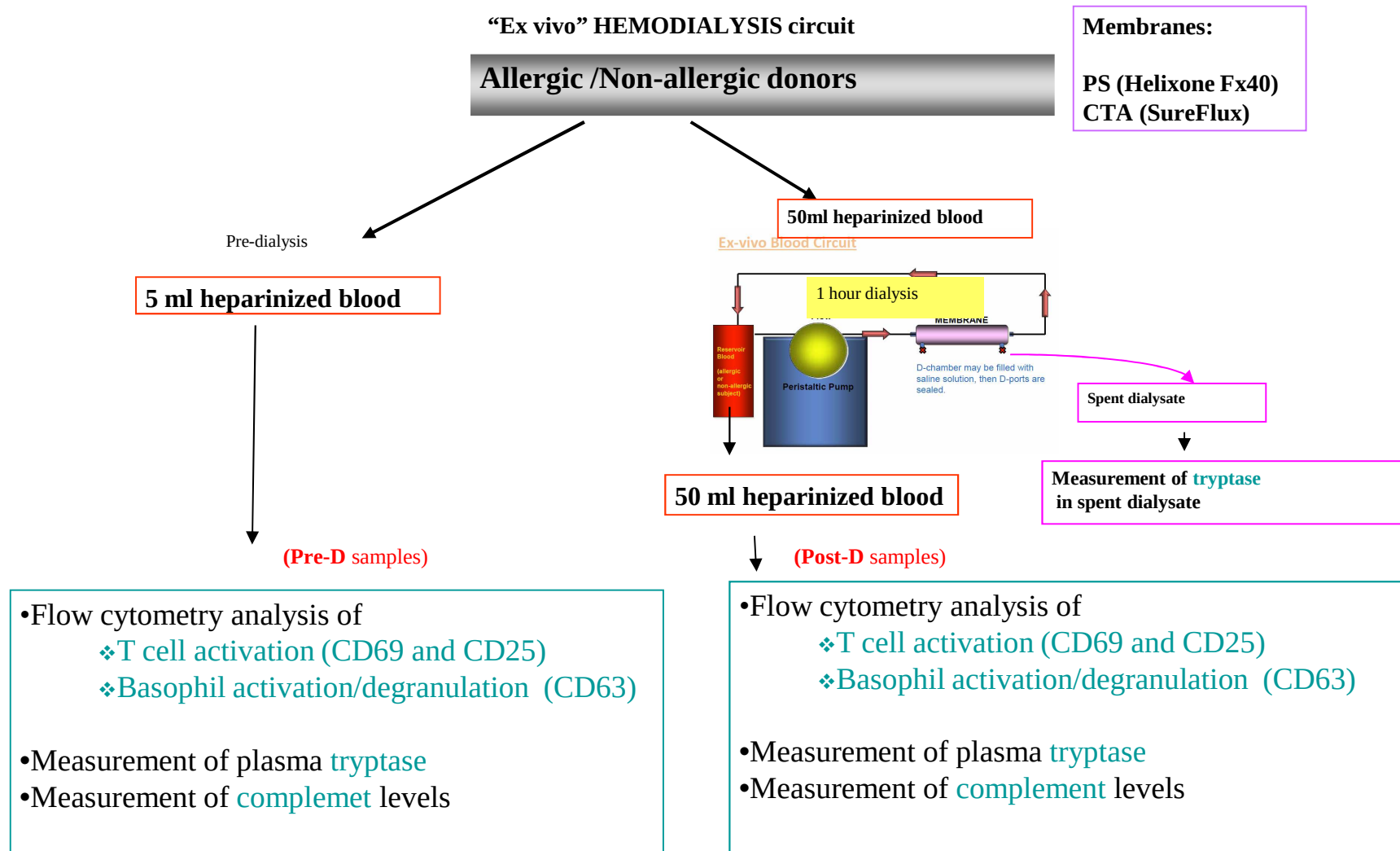


Fig S2. Scheme of experimental design and determinations performed

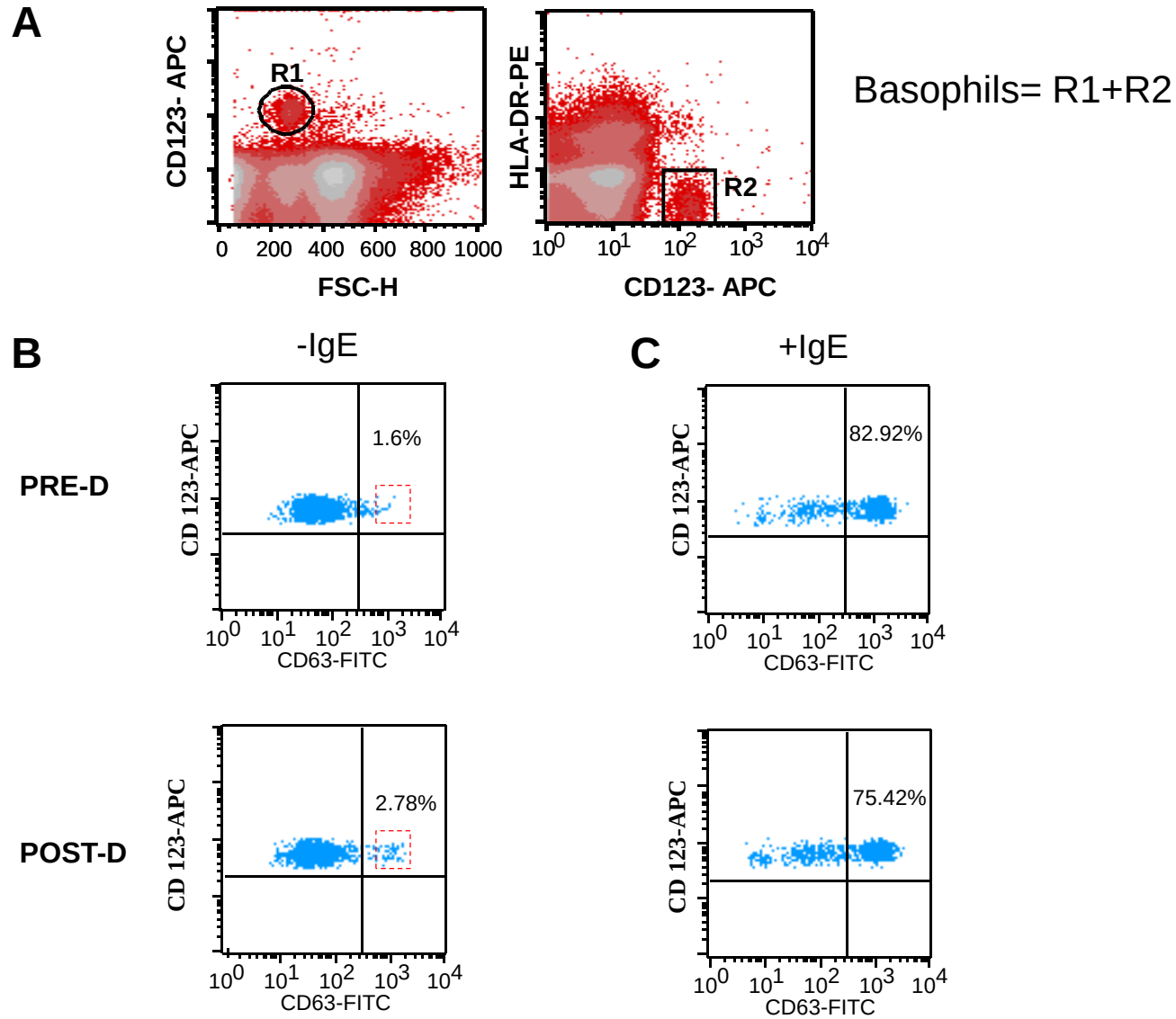


Fig S3. Flow cytometry analysis of basophil degranulation estimated as the frequency of CD63+CD123+HLA-DR- leucocytes in a representative polysulfone-allergic patient.

(A) Gating strategy to select basophils. (B) Cell surface expression of CD63 before (upper graph) and after (lower graph) blood circulation through a PS membrane. (C) Degranulation of basophils upon further stimulation of FcεRI previous (upper graph) or after (lower graph) circulation through PS.

Lymphocyte activation (CD69 expression assessed by flow cytometry)

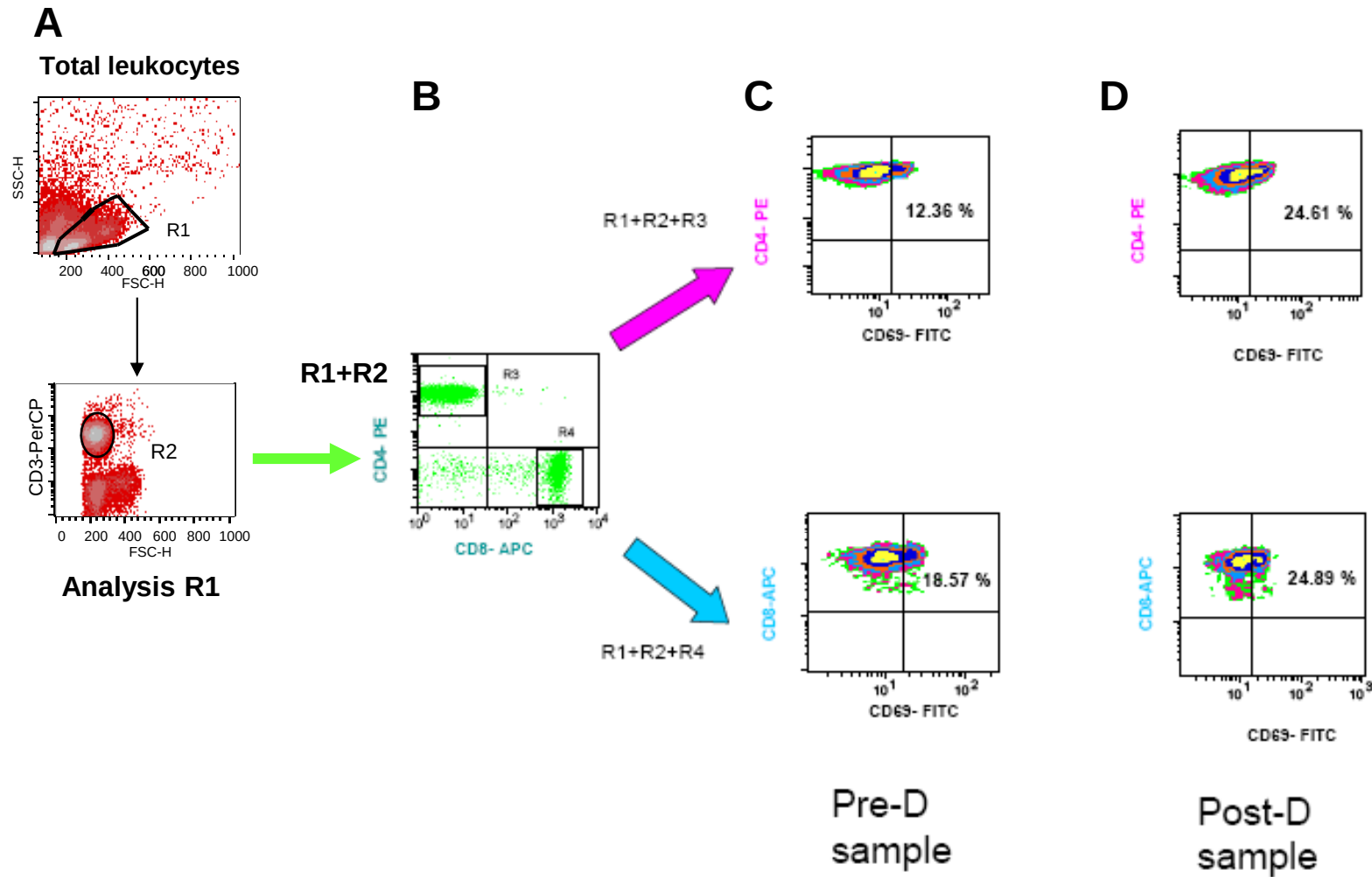


Fig S4. Flow cytometry analysis of T cell activation in a representative polysulfone-allergic patient.

(A) Gating strategy to select CD3⁺ T cells. (B) CD4 and CD8 expression in selected T cells. (C) Frequencies of CD69⁺ cells in CD4 or CD8 T cells prior to circulation through the PS membrane. (D) Frequencies of CD69⁺ cells in CD4 or CD8 T cells after 1 hour circulation through PS.

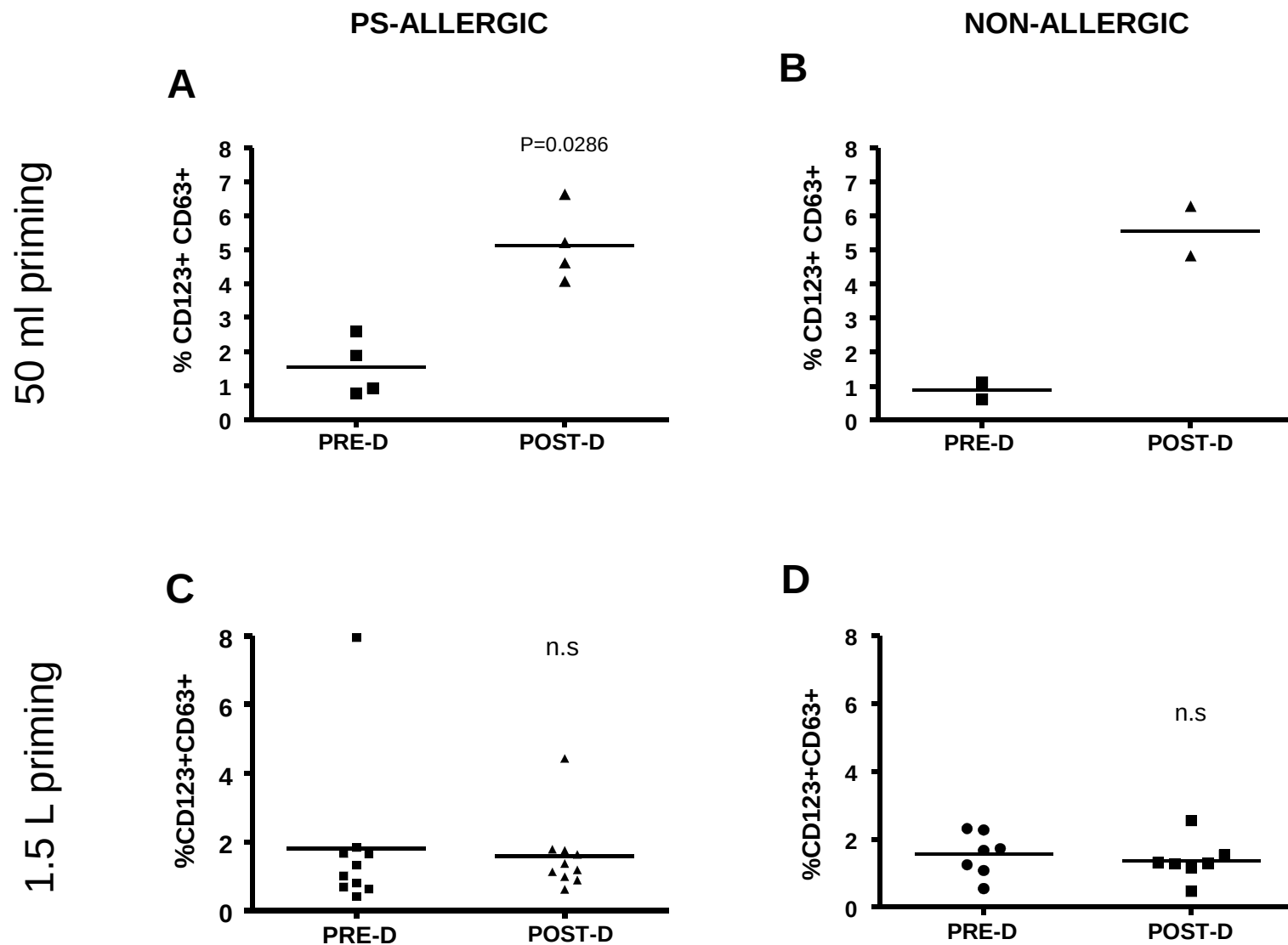


Fig S5. Basophil degranulation upon blood circulation through CTA hemodialysis membranes.

Basophil degranulation in PS-allergic (A, C) or non-allergic (B,D) donors upon 1 hour circulation through CTA membranes previously washed with 50 ml (A,B) or 1,5L of saline solution (C,D) was analyzed by flow cytometry as the %CD63+CD123+HLA-DR- leukocytes.

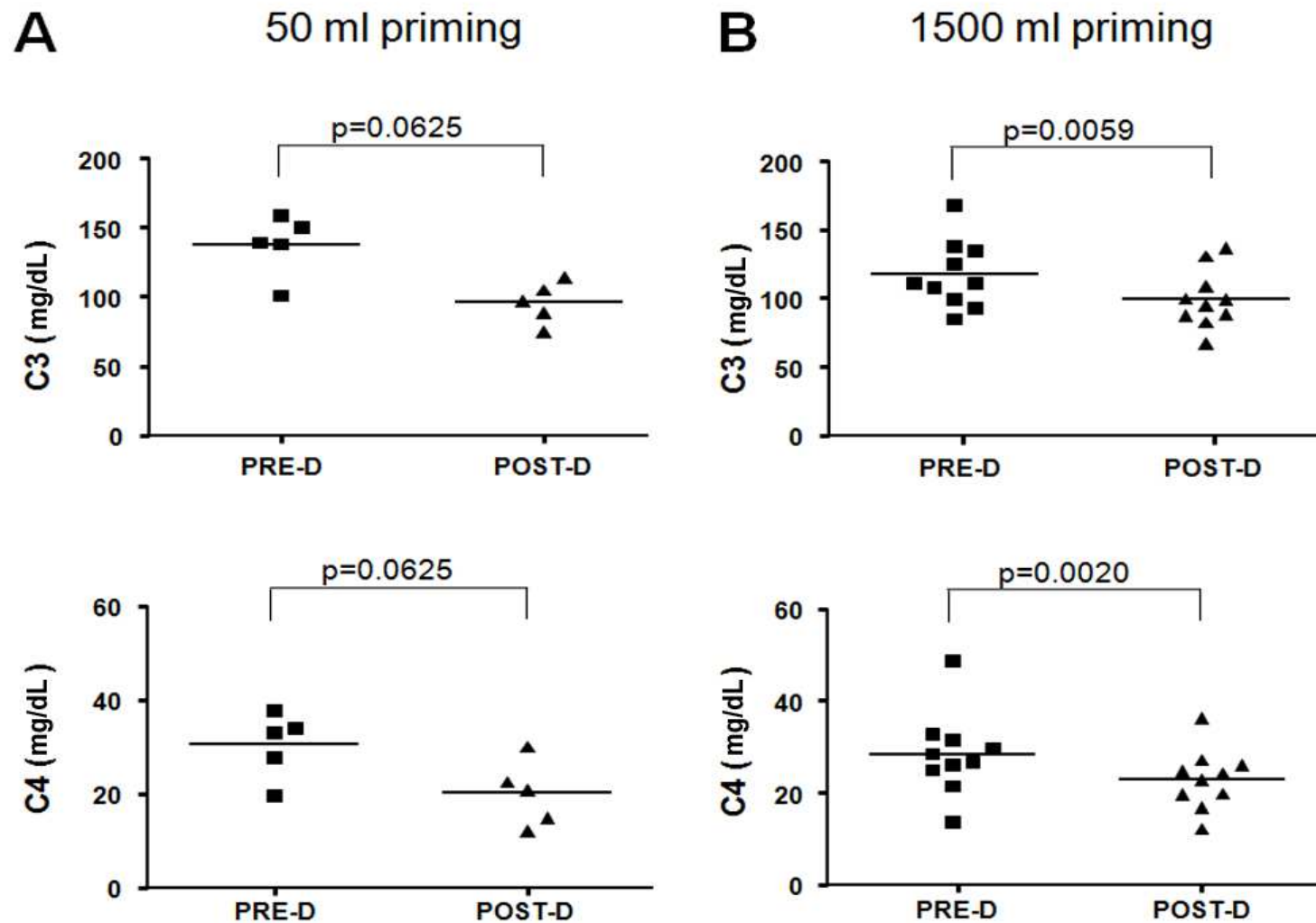


Fig S6. Evaluation of complement levels in PS-allergic patients on ex vivo blood circulation through CTA hemodialysis membranes. The concentrations of the complement proteins C3 (upper panels) and C4 (lower panels) were evaluated in serum samples from PS-allergic donors previous (Pre-D) and after circulation through CTA membranes (post-D) primed with either 50 ml (A) or 1.5 L (B) of saline solution. Differences in complement levels in samples pre- and postdialysis were evaluated by the Wilcoxon signed rank test.