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Therapeutic Effects of the Long-term Use of PAN Membrane Dialyzer in Hemodialysis Patients:
Efficacy in Old Dialysis Patients with Mild PAD

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Abstract

Background: AN69 dialyzer, a plate type dialyzer with a polyacrylonitrile membrane (PAN membrane) is reported to reduce symptoms in hemodialysis (HD) patients with complications such as poor nutritional status and peripheral arterial disease (PAD). Yet very few studies have investigated the long-term use of the PAN membrane or compared the solute-removal properties of the PAN membrane with those of the Type IV polysulfone membrane (PS membrane), the dialysis membrane most widely used. In the present study we compared the contaminant-removal properties of the AN69 membrane dialyzer with those of a Type IV PS membrane dialyzer and investigated the clinical effects of the long-term use of the former for elderly hemodialysis patients with mild PAD.

Methods: Cross-over trials with 2 week intervals for solute were conducted in 6 patients to compare the performance of the membranes in removing small molecular weight substances, β_2 microglobulin (β_2MG), amino acid (AA), and serum albumin (Alb). Next, the AN69 membrane was used for dialysis over a period of 72 weeks in 8 patients. The time course changes of Alb, the geriatric nutritional risk index (GNRI), the % creatinine generation rate (%CGR), the normalized protein catabolic rate (nPCR) and the dry weight (DW) were observed to evaluate the nutritional status. The time course changes of β_2MG, C-reactive proteins (CRP), LDL cholesterol (LDL), fibrinogens (Fib), nitrogen oxide (NOx), hemoglobin (Hb), ferritins, transferrin saturation (TSAT), dose of erythropoiesis-stimulating agents (ESA), and dose of iron were observed to evaluate the therapeutic effects of long-term use. Skin perfusion pressure (SPP) was measured at two points: once at the switchover to the AN69 membrane and once 72 weeks later.

Results: In cross-over trials, the AN69 membrane showed basically the same dialysis efficiency as the PS membrane in removing small molecular weight substances, but it removed significantly lower amounts of β_2MG. The AN69 membrane also showed significantly lower rates of AA removal rate and Alb leakage. The nutritional status was stably maintained during long-term use after the switchover to the AN69 membrane, and no significant increase of β_2MG was observed. Fib and NOx were both reduced, the latter to a significant degree. The

Hb values showed a good time course, with relatively high TSAT levels and low ferritin levels overall. SPP remained generally stable for 72 weeks.

Conclusion: The cross-over trial show the AN69 membrane eliminates less AA and Alb compared with the PS membrane. Judging from the therapeutic effects of the long-term use of the AN69 membrane, the membrane is effective for dialysis and has good biocompatibility in the treatment of elderly HD patients with mild PAD.
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Introduction

In hemodialysis (HD) therapy, blood and dialysate are brought into contact via a semi-permeable dialysis membrane in a dialyzer in order to remove unnecessary solute and excess water from the blood. The low molecular weight solute is removed by diffusion and the high molecular weight solute and excess water are removed by ultrafiltration. At present, only one dialyzer with multilayer dialysis membranes is being manufactured and used in Japan (AN69 membrane). Most of the dialyzers in use are designed with hollow fiber. Hollow fiber uses a concentration gradient with a blood compartment inside and dialysate chamber outside to generate a counter flow that maximizes the efficiency of diffusion transfer.

The solute-removal efficiency is determined not only by the rate of blood flow and rate of dialysate flow in the dialyzer, but also the dialyzer performance. The removal efficiency for low molecular weight solute depends on the blood flow rate, while that for high molecular weight solute mostly depends on the dialyzer performance. Three main factors determine the dialyzer performance: the area of the dialysis membrane, the size of the pores through which the solute passes from the blood side to the dialysate side, and the material composing the dialysis membrane.

The surfaces of some membrane materials can have electric charges, and solute can be removed by adsorption as well as diffusion and filtration. The targeted uremic toxins in the early days of HD therapy were low molecular weight substances such as blood urea nitrogen, creatinine, and uric acid. Later, after the publication of the medium molecular weight hypothesis in 1971 by Babb et al., the substances targeted for removal expanded from low molecular weight substances to high molecular weight substances[1]. Still later, in 1985, Gejyo identified the low molecular weight protein β_2MG (molecular weight: 11,800) as a cause of dialysis-related amyloidosis (DRA). Dialyzer makers took steps to remove β_2MG by increasing the pore diameter in hollow fiber membranes and developing a new generation of high-flux membranes[2]. Dialyzers in Japan are functionally classified into types I to V according to the rate of β_2MG clearance. According to a survey on the HD conditions of chronic dialysis patients by the statistical survey committee of the Japanese Society for Dialysis Therapy in 2008, Type IV–V dialyzers with high β_2MG clearance held a market share of more than 90%[3].

Back in the early days of HD therapy, membranes made of regenerated cellulose (RC), a natural material, were the mainstream for dialysis. In time, however, hydroxyl (OH) groups present on the surface of the RC membranes were found to play a role in the activation of a complement system resulting in transient leukopenia. To solve this newfound challenge to biocompatibility, manufacturers began producing biocompatible synthetic polymer membranes free of OH groups. Prominent among these were the PAN membrane (the only type of PAN membrane now manufactured and used in Japan is the AN69, a synthetic polymer membrane composed of acrylonitrile-co-methallyl sulfonate), the polymethlymethacrylate (PMMA) membrane, and the PS membrane.

The PS membrane, the most widely used dialysis member in Japan at present, had a market share of about 60% in 2008[3]. It has prevailed by dint of its asymmetric structure, excellent performance in removing β_2MG, and sharp fractionation properties. The uremic toxins targeted in dialysis therapy have now expanded to protein-bound uremic toxins in the α_1MG (a substance with a molecular weight of 33,000 thought to cause complications such as restless leg syndrome in dialysis patients) region, and even more high performance dialyzers are being developed. While β_2MG and Alb (molecular weight 69,000) are relatively easy to separate, α_1MG and Alb are not. In patients with good nutritional status and Alb synthesis capabilities, a certain amount of Alb leakage to remove protein-bound uremic toxins is tolerable (less than 2–4 g/HD). Yet the use of high-performance PS dialyzers on elderly patients or patients with poor nutritional status may either worsen the patient's nutritional status or hinder its improvement[4]. Further, PS membranes contain polyvinylpyrrolidone (PVP), a potentially allergenic hydrophilic agent, and bisphenol A (BPA), an endocrine disrupter[5]. The properties compromise the biocompatibility of PS membranes.

Arteriosclerosis is common in maintenance dialysis patients, and chronic inflammation may contribute greatly to its development. Some of the various factors causing chronic inflammation are related to problems with the HD itself, such as biological incompatibility of the dialysis membrane and contamination of the dialysate. This sustained inflammation can cause nutritional disorders and promote arteriosclerosis via mechanisms of the MIA (malnutrition, inflammation, and atherosclerosis) syndrome proposed by Stenvinkel in 1999[6]. Dialysis patients who exhibit high levels of the inflammatory cytokine also manifest a deteriorating nutritional status that influences their prognosis[7]. Hence, a dialysis membrane with better biocompatibility is awaited.

Recent papers on the PAN membrane (AN69 membrane) report that the membrane may improve the nutritional status of elderly and poorly nourished HD patients and improve the blood circulation of HD patients with PAD. The AN69 membrane has broader solute-removal properties than the PS membrane and reportedly reduces the loss of excess nutrients (Alb and AA). It also has the strongest negative charge (−100 mv) of all the dialysis membranes available. When blood comes into contact with the AN69 membrane, this negative charge pushes up bradykinin levels and may improves peripheral hemodynamics levels by increasing the production of nitric oxide (NO) in the vascular endothelial cells. The strong negative charge also leads to excellent adsorption of positively charged etiological agents. The AN69 membrane may inhibit chronic inflammation by strongly suppressing the production of inflammatory cytokines such as IL-6 and enhancing removal efficiency[7,8]. Further, the membrane is reported to lower the production of C3a[9] and offers better biocompatibility as a material free of PVP or BPA. The symmetric PAN membrane has a better adsorption capacity than the asymmetric PS membrane, but its inferior performance in removing low molecular weight proteins such as β_2MG may allow these proteins to accumulate over the course of long-term use.

Few studies have compared the efficacy with which the PAN and PS membranes remove solute, and reports on the use of PAN membranes over time frames of longer than one year are rarer still. Hence, many unanswered questions remain about the efficiency of dialysis with PAN membranes and the outcomes of long-term use for dialysis patients. Long-term observations are important for assessing the therapeutic effects for dialysis patients, as problems such as the hemoglobin cycling in patients receiving ESA medication must be examined. In the present study we compared the solute-removal properties of the AN69 membrane with those of the Type IV PS membrane. We also examined the clinical outcomes, biocompatibility, and other performance properties of the AN69 membrane over long-term use in order to assess the overall therapeutic effect in elderly hemodialysis patients with mild PAD.

Subjects and Methods

The subjects were twelve chronic maintenance hemodialysis patients receiving treatment at two of the clinics of our medical corporation. All of the subjects gave their written consent to participate after being apprised of the purpose of the study. The selection criteria were as follows: mild PAD, stage I or II symptoms according to the Fontaine classification; the use of a Type IV PS membrane dialyzer with a membrane area of 1.5−1.6 m² under typical hemodialysis conditions, and 4-hour hemodialysis three times a week at a blood flow rate of 200 mL/min. PAD was diagnosed according to physical examination and ankle brachial pressure index (ABI). The cardio-ankle vascular index (CAVI) and past episodes of blood vessel stenosis such as cardiac infarctions or cerebral infarctions were also taken into account. Results were expressed as mean±standard deviation (mean±SD) in all data analyses. A paired t-test was used to test for significant differences and a p value of less than 5% was considered significant.

Cross-over Trials of the PAN Membrane Dialyzer and PS Membrane Dialyzer

The solute-removal properties of the PS and AN69 membranes were compared in 6 patients whose hemodynamics were stable during hemodialysis and who exhibited no detectable anemia (mean age of 70.8±9.0 years; all males; 5 diabetic and 1 nondiabetic; mean duration of hemodialysis of 4.4±3.1 years). A Type IV PS membrane dialyzer APS-15 MD (1.5 m²) (Asahi Kasei Medical Co., Ltd., Tokyo, Japan) and a multilayer AN69 dialyzer H12-4000 (1.53 m²) (Gambro K.K., Tokyo, Japan) were switched every 2 weeks in a crossover trial to compare the following parameters: clearance (CL), reduction rate (RR), removal amount (RA), and clear space (CS) of low molecular weight solute comprising urea nitrogen (UN), creatinine (Cr), inorganic phosphorus (IP), and the low molecular weight protein β_2MG. The RR of amino acid (AA) and RA of Alb were also measured. The AA was assessed by measuring the total AA, essential AA (EAA), nonessential AA (NEAA), branched-chain AA (BCAA), arginine (Arg), and aromatic AA (AAA).

Evaluation of the Clinical Effect of the Long-term Use of the AN69 Membrane

The patients enrolled in the study were observed for 72 weeks to evaluate the long-term clinical efficiency of the AN69 membrane. Among the twelve subjects, four patients were excluded during the study because they had dropped out for reasons other than the side effects from application of the AN69 membrane. Eight patients (mean age of 72.1± 10.6 years; all males; 6 diabetic and 2 nondiabetic; mean hemodialysis duration of 5.0±3.4 years.) were protocol compatible. All patients were switched from a Type IV PS membrane with an area of 1.5−1.6 m², the membrane they normally used, to a multilayer AN69 membrane with an area of 1.53 m², and observed for the 72 week observation period. Serum Alb levels, GNRI, %CGR, nPCR, and DW were measured to assess nutritional status, and the levels of β_2MG, CRP, LDL, Fib, NOx (NO2⁻, NO3⁻) were measured before and during the observation period to collect various blood test data. Because of the difficulty in accurately measuring the gaseous mediator NO, the relatively stable metabolite NOx was measured to obtain an index of vascular endothelial function. Hb levels, ferritin levels, TSAT, ESAs, and changes in the dosage of iron were similarly observed over the 72 weeks to assess the state of renal anemia. The ESA drug dosage was calculated as a unit of epoetin alpha or epoetin beta. SPP, an index of peripheral hemodynamics determined noninvasively, was measured by laser Doppler flowmetry (PAD3000, Kaneka Medix Corp., Tokyo, Japan) immediately before and 72 weeks after switching to the AN 69 membrane. Measurements were obtained by attaching laser sensor probes to all measurement sites and wrapping each with a cuff. The pressure applied by the cuff avascularized the measurement site and then steadily dropped at a constant speed. The SPP was the cuff pressure at the point when the interrupted blood flow started to reperfuse. Normal levels are almost equal to the diastolic blood pressure. An SPP of less than 30 mmHg indicates

Table   Laboratory data from the cross-over trial. All data were assessed with results expressed as means ± standard deviations (mean ± SD).

RR, CS, RA of small molecular weight substance, β_2-MG, Alb

	membrane	UN	Cr	IP	β_2-MG	Alb
RR [%]	PS	67.1 ± 2.2	61.3 ± 3.1	48.0 ± 18.1	65.7 ± 5.4	
	AN69	66.1 ± 4.7	60.2 ± 3.6	45.0 ± 11.2	34.1 ± 3.2	
CS [L]	PS	22.4 ± 2.5	16.1 ± 1.9	16.4 ± 1.7	5.4 ± 0.7	
	AN69	22.4 ± 2.8	16.6 ± 1.9	15.3 ± 1.6	3.3 ± 1.0	
RA [mg/HD]	PS	13,388 ± 3,784	1,723 ± 270	713 ± 211	154 ± 23	205 ± 38
	AN69	11,725 ± 4,149	1,751 ± 244	686 ± 231	102 ± 9	493 ± 354
CL [mL/min]	PS	179.8 ± 6.2	157.5 ± 5.7	102.8 ± 6.3	55.0 ± 7.4	
	AN69	174.3 ± 11.6	151.9 ± 9.0	101.8 ± 8.5	19.2 ± 14.2	

RR [%] of AA

membrane	Total AA	EAA	NEAA	BCAA	AAA	Arg
PS	31.4 ± 10.8	20.4 ± 15.5	39.3 ± 8.0	31.6 ± 13.6	30.4 ± 15.2	47.1 ± 12.0
AN69	22.0 ± 9.8	9.9 ± 14.5	28.5 ± 8.1	12.5 ± 16.2	18.0 ± 11.0	38.1 ± 12.0

PS, PS membrane dialyzer; AN69, AN69 membrane dialyzer; CL, clearance; RR, reduction rate; RA, removal amount; CS, clear space; UN, urea nitrogen; Cr, creatinine; IP, inorganic phosphorus; β_2MG, β_2 microglobulin; AA, amino acid; EAA, essential AA; NEAA, nonessential AA; BCAA, branched-chain AA; Arg, arginine AAA, aromatic AA; Alb, albumin

critical limb ischemia, while wound healing requires an SPP of either 40 mmHg or more, or 50 mmHg or more in the case of maintenance HD patients[10]. All measurements in the present study were taken before the HD from the underside of the patient's toes as measurement sites, with the patient resting in a supine position.

The quality of the dialysate used at the two clinics was confirmed with a standard monthly test to ensure that the uncontaminated, ultrapure dialysate standard (bacterial count: less than 0.1 CFU/mL, endotoxin level: less than 0.001 EU/mL) stipulated by the Japanese Society for HD Therapy was met. Blood was collected in accordance with routine collection dates in order to avoid drawing blood in excessive amounts. Apart from the blood drawn for the usual tests, additional blood was drawn for the following assessments: 10 mL to calculate CL for comparison of solute-removal properties (4 mL for each arteriovenous circuit as a biochemical test and 2 mL for the arterial circuit as a blood count test), 5 mL to calculate the AA removal rate, 2 mL to measure Fib for verification of clinical efficiency, and 1 mL to measure NOx. Since this was a crossover trial where the membranes were switched every 2 weeks, the assessment of solute-removal properties

required a total of 30 mL of blood over 4 weeks. Over the course of the assessment period, 14 mL samples of blood were used to measure Fib seven times, and 5 mL samples were used to measure NOx six times. The total amount of additional blood collected during the 72 weeks of the study was no greater than about 50 mL per patient. The solute-removal properties were assessed in 6 patients whose hemodynamics remained stable during the HD and without severe anemia. All of the patients gave their informed consent to the assessment, and no part of the study protocol conflicted with the interests or rights of the patients.

Results

Comparison of the Solute-removal Performance
The AN69 membrane was about as efficient as the PS membrane in removing small molecular weight substances by dialysis. The creatinine clearance of the AN69 member was significantly lower than that of the PS membrane (**Table**, **Fig. 1**). The AN69 membrane was also noticeably less efficient than the PS membrane in removing the low molecular weight protein β_2MG. The AA removal rate and amount of Alb leakage were both
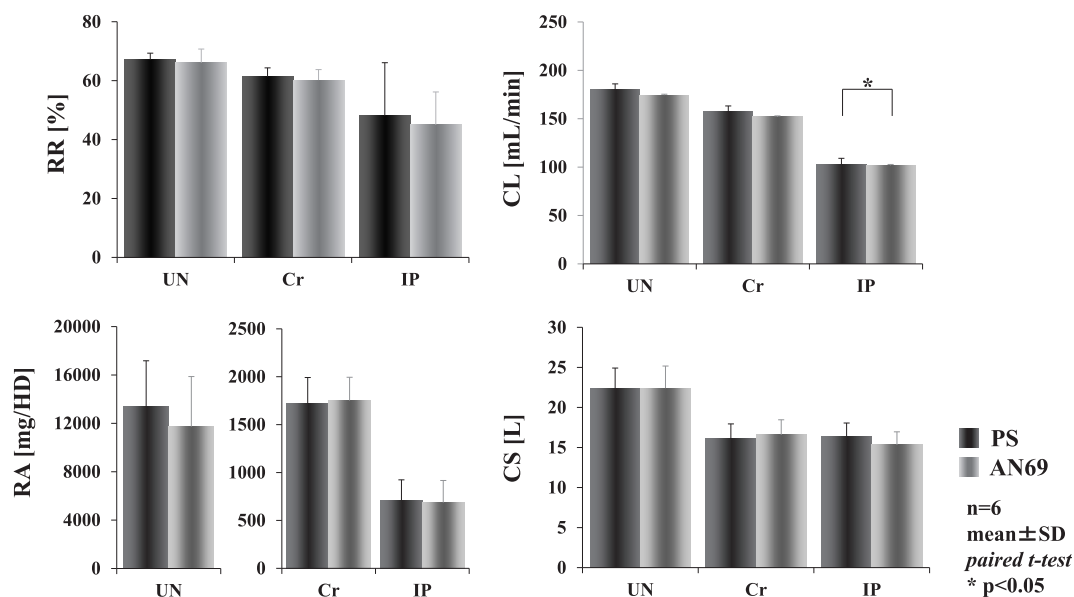
Fig. 1 Comparison of performance in removing low molecular weight substances
No significant differences between the membranes were found in the removal ratio (RR), removal amount (RA), or clear space (CS) of low-molecular weight substances. The membranes showed more or less comparable solute-removal properties. Clearance by the AN69 membrane was significantly lower than that of the PS membrane for only one substance, Cr.
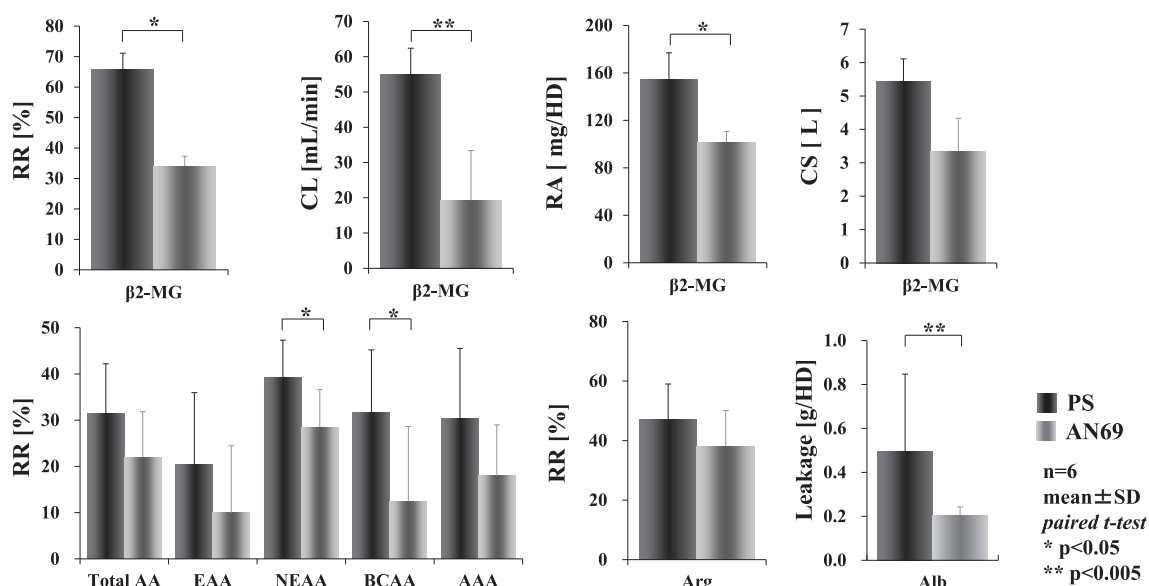

Fig. 2 Comparison of performance in removing β_2MG, AA, and Alb
For β_2MG, the AN69 membrane showed a significantly low RR, RA, and CL compared with the PS membrane. The AN69 membrane also showed a significantly lower RR for NEAA and BCAA in AA and Alb leakage.

suppressed to significantly low values (**Table**, **Fig. 2**).

Clinical Outcomes of the Long-term Use of the AN69 Membrane

Nutritional status

Serum Alb and the GNRI remained at good levels during the observation period. The GNRI stayed above 92, the target value for HD patients (**Fig. 3**).
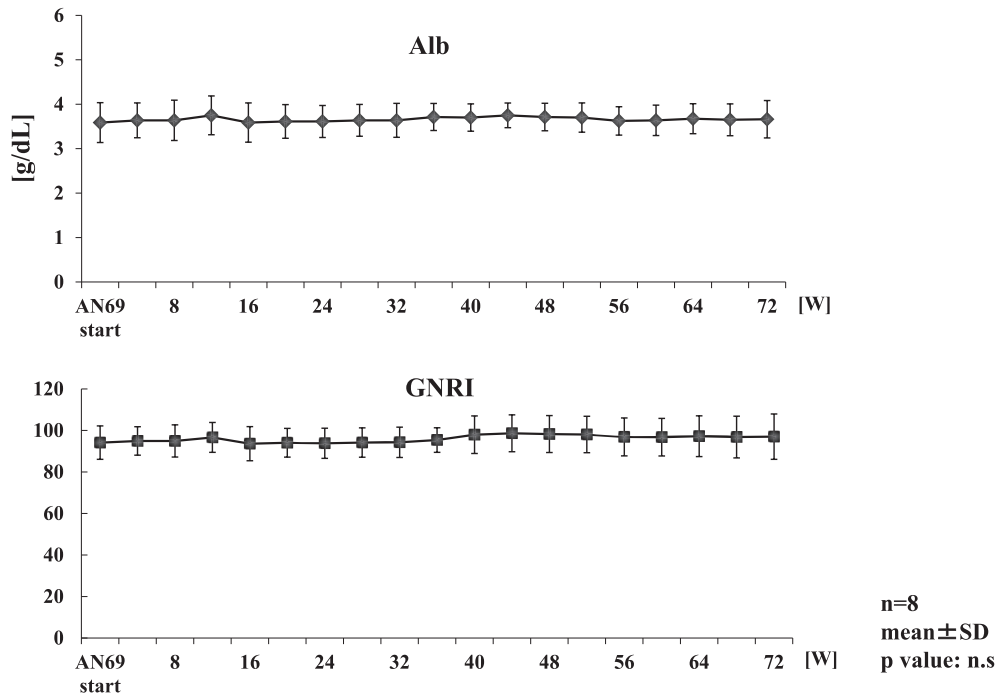
Fig. 3　Time course changes of GNRI and albumin levels
Serum albumin and GNRI remained at good levels. The GNRI kept remained above 92, the target value for hemodialysis patients. The albumin level was slightly increased at week 72 after the switchover to the AN69 membrane.
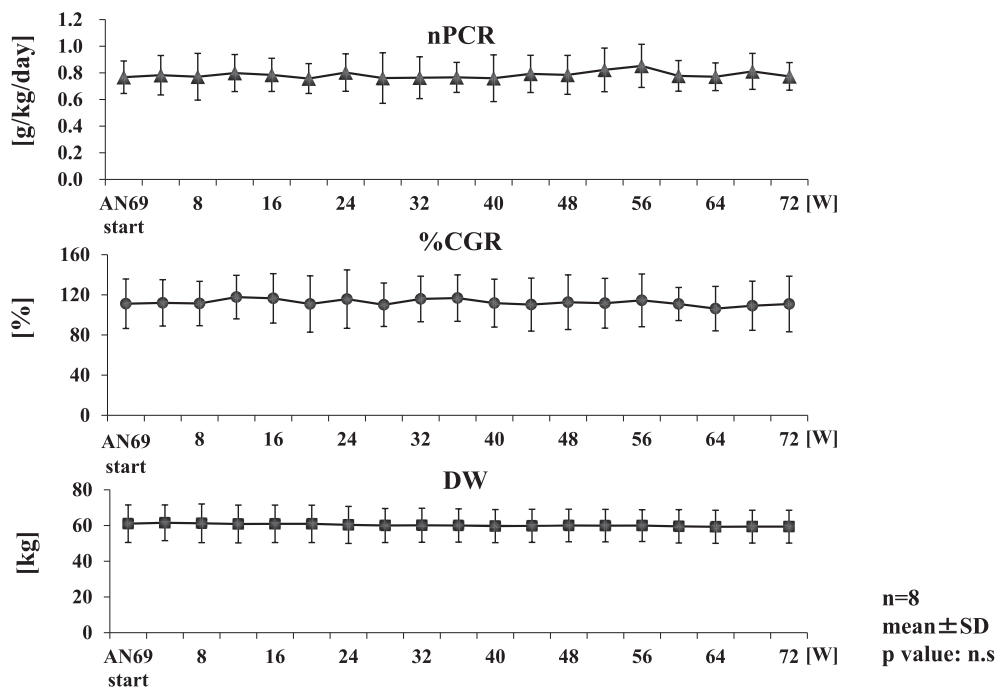

Fig. 4　Time course changes of %CGR, DW, and nPCR levels
The %CGR, DW, and nPCR remained comparatively stable.

Alb levels increased slightly from 3.59±0.45 g/dL at the switch of the membrane to 3.64±0.39 g/dL at 4 weeks, then remained at 3.6 g/dL or above for the duration of the study period, ending slightly higher by week 72 (3.66±0.42 g/dL). nPCR, %CGR, and DW remained stable. The nPCR was 0.768±1.22 g/kg/day at the membrane switch (slightly less than 0.8 g/kg/day, the target value for HD patients) and ended

β₂-MG

CRP

n=8
mean±SD
p value: n.s
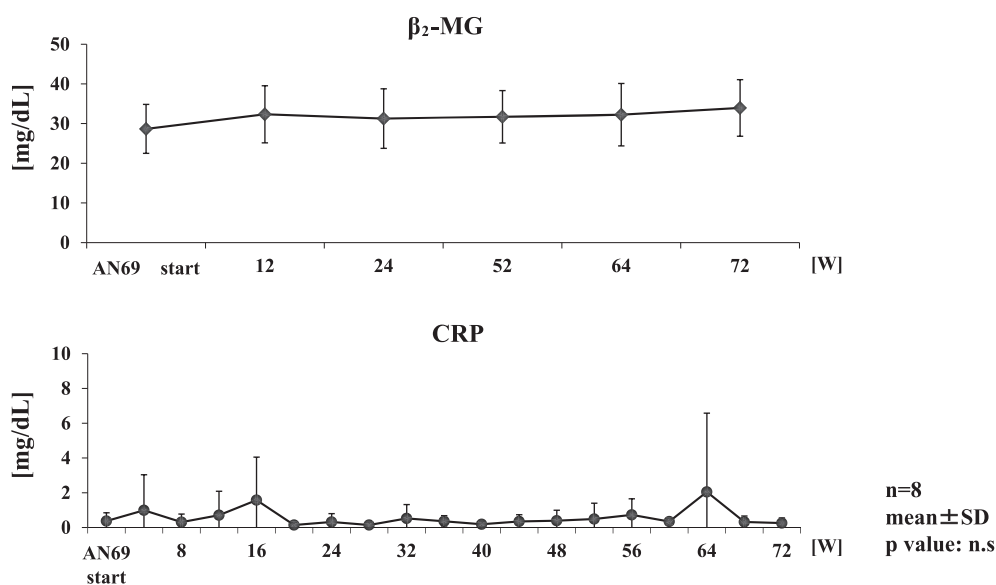
Fig. 5   Time course changes of β2MG and CRP levels
β2MG increased slightly but not significantly during the observation period. Some variations in CRP were noted halfway through the observation period, with slight increases at week 4, week 16, and week 64. Overall, however, CRP remained substantially low throughout the whole observation period and fell slightly at week 72.
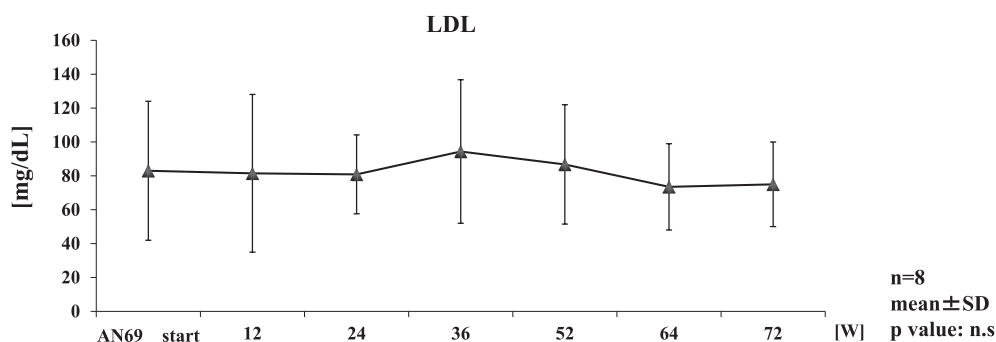
LDL

n=8
mean±SD
p value: n.s

Fig. 6   Time course change of LDL level
LDL decreased slightly with no significant difference.

slightly higher (0.774±0.104 g/kg/day) at week 72. The %CGR remained at a stable level just above 100, the target value for HD patients. DW remained relatively stable and fell slightly from 61.0±10.5 kg at the membrane switch to 59.4±9.2 kg at week 72 **(Fig. 4)**.

Laboratory data

β2MG rose slightly from 28.67±6.16 mg/dL at the membrane switch to 33.9±7.14 mg/dL at week 72, but the change was not significant **(Fig. 5)**. CRP decreased slightly from 0.37±0.48 mg/dL at the membrane switch to 0.26±0.29 mg/dL at week 72. Some variations of CRP were noted halfway through the observation period. The levels remained

substantially low throughout the observation period, with a slight climb to 0.99±2.04 mg/dL at week 4, another climb to 1.58±2.48 mg/dL at week 16, and another to 2.05±4.53 mg/dL at week 64 (every climb was associated with infectious disease) **(Fig. 5)**. Similarly, LDL showed only a slight decrease from 83±41 mg/dL at the membrane switch to 75±25 mg/dL at week 72, with no significant difference **(Fig. 6)**. Fib decreased significantly from 376.9±114 mg/dL at the membrane switch to 289.8±64 mg/dL at week 12 (P<0.05) and dropped further to 270±60 mg/dL at week 24 (P<0.05), with a significant decrease at week 72 (300±65 mg/dL)compared with before the switch (P<0.05). NOx decreased significantly from
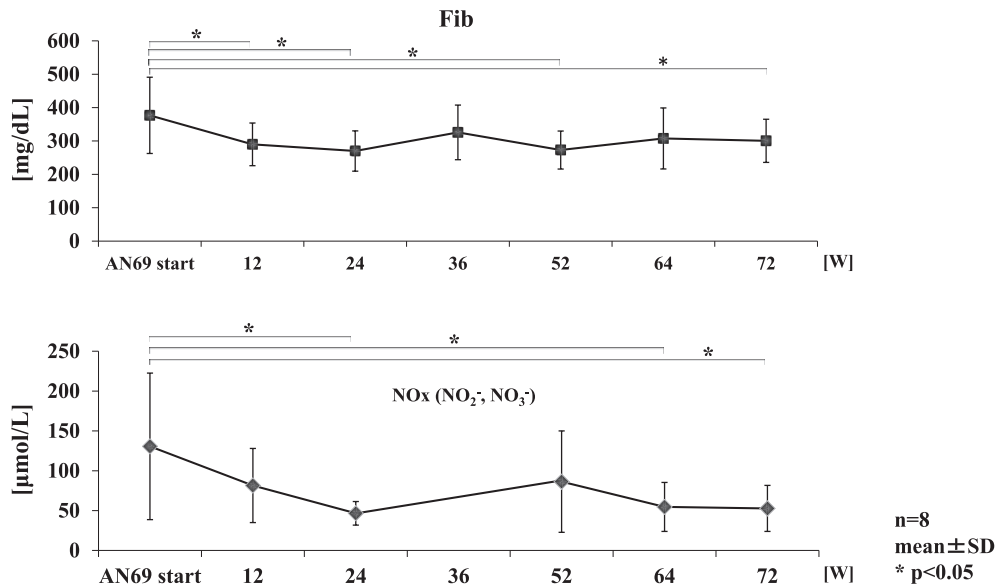
Fig. 7   Time course changes of Fib and NOx levels

Fib decreased significantly. NOx was significantly decreased at week 24 and almost remained at a normal level thereafter.
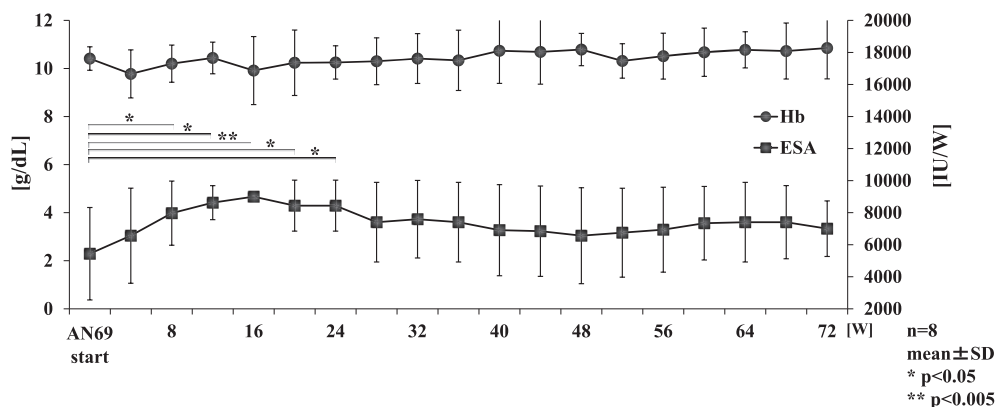

Fig. 8   Time course changes of the Hb level and ESA drug dosage

The Hb level remained above 10.0 g/dL for most of the analysis period. The ESA dosage increased up to week 16 then declined or remained almost unchanged up to week 72. The ESA drug dosage was calculated as a unit of epoetin alpha or epoetin beta.

130.6±91.9 μmol/L at the membrane switch to 46.6±14.8 μmol/L at week 24 (P<0.05), then increased to 86.4±63.6 μmol/L at week 52, but thereafter remained low until week 72 (52.8±28.9 μmol/L), with a significant reduction compared with before the switch to the AN69 membrane (P<0.05) **(Fig. 7)**.

Hb levels remained good throughout the observation period. The levels decreased from 10.4±0.5 g/dL at the membrane switch to 9.8±1.0 g/dL at week 4, and then increased to 10.2±0.8 g/dL by week 8. Then the levels remained at 10.0 g/dL or above, increasing at both week 48 (10.8±0.7 g/dL)and

week 72 (10.9±1.3 g/dL) compared with the levels before the switch to the AN69 membrane. The amount of ESA administered increased significantly from 5,438±2,884 IU/week at the membrane switch to 6,563±2,966 IU/week at week 4 (P<0.05). The amount rose further by week 16 (9,000±0 IU/week; P<0.05) but then steadily declined up to week 72 (7,313±1,870 IU/week) **(Fig. 8)**.

TSAT rose slightly from 13.7±6.4% at the membrane switch to 17.9±12.8% at week 36, then remained at roughly the same level for the rest of the study period (finishing at 20.4±9.9% at week 72).
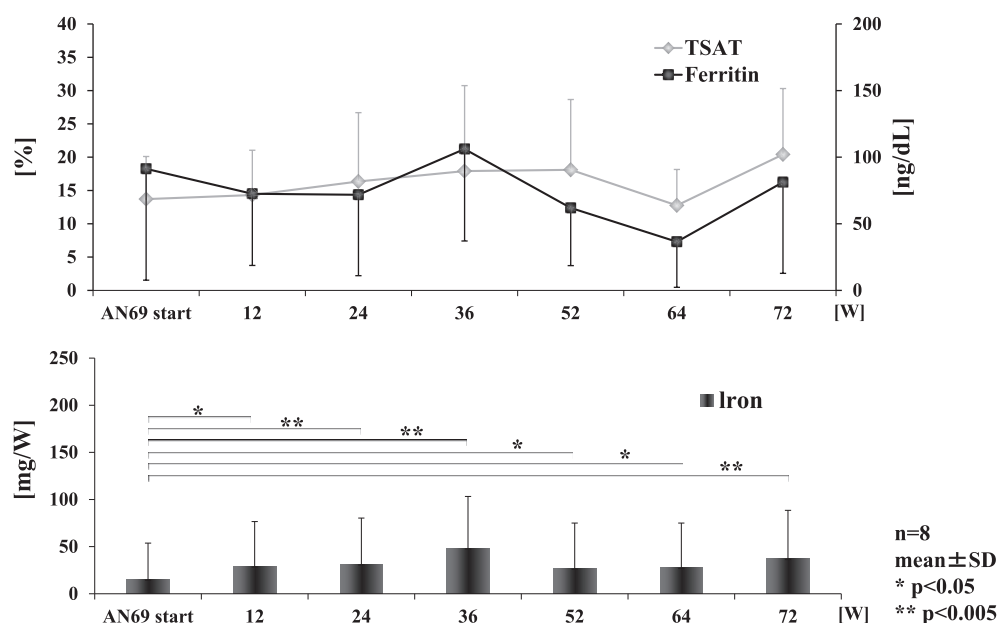
Fig. 9 Time course changes of TSAT, ferritin, and the iron dose
TSAT rose slightly up to week 36 and remained at roughly the same level after that. Ferritin was decreased at week 12 and remained at roughly the same level throughout the observation period, but TSAT remained relatively high and ferritin remained relatively low.
The iron dose was significantly increased at week 12 compared with that at the switchover to the AN69 membrane. The dose increased to a peak at week 36 and then declined up to week 64, but increased at week 72.

Ferritin decreased slightly from 91.3±83.6 ng/mL at the membrane switch to 72.5±53.8 ng/mL at week 12. Thereafter, the amounts rose or fell in correlation with the dose of iron, reaching 81.3±68.5 ng/mL at week 72. The TSAT and ferritin levels were both influenced by the iron dose but showed no significant variations throughout the observation period, with TSAT remaining relatively high and ferritin remaining relatively low. The iron dose rose to 29±47 mg/week by week 12, a level significantly higher than that at the switchover to the AN69 membrane (15±38 mg/week) (P<0.005). It then increased significantly to 48±55 mg/week (P<0.005) by week 36, fell slightly to 28±47 mg/week by week 64, and increased to 37±51 mg/week (P<0.005), a level significantly higher than that at the switchover to the AN69 membrane (**Fig. 9**), by week 72.

Peripheral hemodynamics

SPP was measured at the switchover to the AN69 membrane and then again at week 72, to assess the change in peripheral hemodynamics. SPP improved noticeably in both lower extremities of 1 of the patients but was exacerbated in one lower extremity of another patient. SPP remained generally stable up to week 72 in the other 6 patients, without significantly changing (**Fig. 10**).

Discussion

Comparison of Solute-removal Performance

In comparing the solute-removal performance in the crossover trial, we found that the AN69 membrane removed low molecular weight substances as efficiently as the Type IV PS membrane. We also confirmed that the AA removal rate and amount of Alb leakage were significantly inhibited with the former membrane. Our results suggest that the AN69 membrane may curb the deterioration of symptoms such as hypoalbuminemia, a complication often seen in aged patients on long-term HD. On the other hand, the significantly low level of β_2MG removed raises concerns over the long-term effect on patients. A more detailed examination of the results of long-term use is merited.
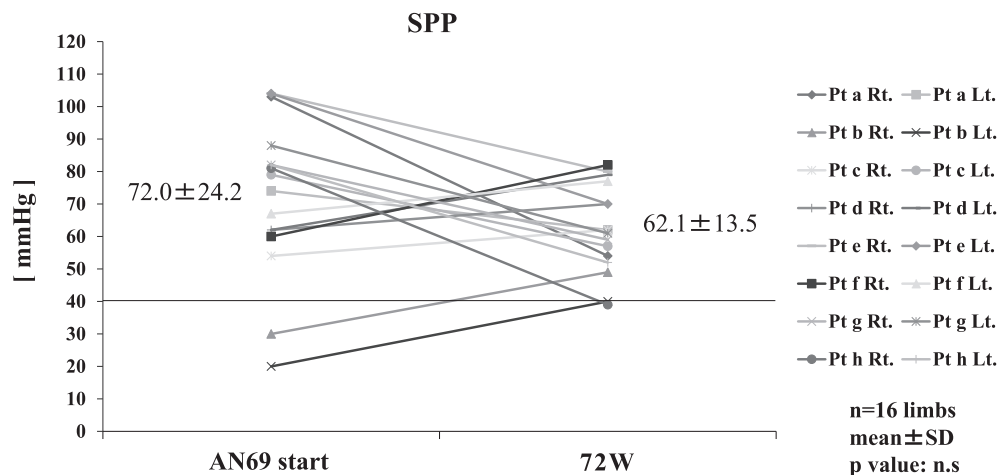
Fig. 10   Assessment of peripheral circulatory function by SSP
Two extremities showed significant improvement and one extremity deteriorated. The remaining 13 extremities were generally stable.

Nutritional Status

Every nutritional parameter was well maintained in our observation of the nutritional status and clinical efficiency. GNRI stayed at a good level above 92. Alb levels were slightly increased and remained above 3.5 g/dL, the target value for HD patients. nPCR increased slightly but still fell slightly short of the target value for HD patients, indicating a low protein intake that may have been partly due to the patients' ages. The %CGR remained at a good level above 100, and DW remained relatively stable. Satou et al. reported a similar improvement in GNRI at 3 months after the start of dialysis with the AN69 membrane[11] and Furuta et al. reported significant increases in Alb levels after 3 months[7].

The size barrier (molecular weight) and charge barrier (negative electric charge) of the AN69 membrane significantly limited the amount of albumin leakage. The large molecular weight of albumin (molecular weight 330,000) restricted albumin passage through the size barrier of the AN69 membrane. Further, the negative charge of albumin in blood and the negative charge of the AN69 membrane repel each other. Hence, the removal by filtration and adsorption appears to be low. Further, the highly efficient removal of IL-6 by the AN69 membrane can inhibit chronic inflammation, which in turn may promote Alb synthesis. The performance of the AN69 membrane in removing Alb and AA appears to be helpful in maintaining nutritional status. This clinical assessment of long-term use therefore suggests that the AN69 membrane may be effective and optimal for preventing MIA syndrome and other complications associated with long-term HD and aging.

In the earlier estimate, we expected the absorbing capacity of the AN69 membrane to be sufficient to remove β_2MG to a certain degree. We were concerned, however, that the β_2MG level could increase with long-term use as a consequence of the inferior performance of the AN69 membrane in removing solute by diffusion and filtration (a finding exhibited in the comparison of solute-removal performance in the cross-over trial in this study). Our results, though not significantly, showed slight increase in β_2MG level during the 72-week observation period. In any case, increases of β_2MG raise serious concerns regarding long-term effects on patients and merit careful clinical observation. Our careful evaluations revealed no symptoms of HD amyloidosis in any of our patients during the study. These results can be explained by the partially inhibited production of β_2MG through the long-term use of the AN69 membrane, a membrane capable of adsorbing inflammatory cytokines. Nonetheless, further study to elucidate this mechanism is called for.

Laboratory Data during the Long-term Use of PAN Membranes

CRP and LDL were found to be the primary factors responsible for the exacerbation of atherosclerosis. We observed the development of CRP and LDL during the study, as the patients suffered from atherosclerosis. CRP tended to fall slightly during the observation period, but not to a significant degree. The time course of LDL remained within normal limits, decreasing slightly but not significantly.

Fib decreased significantly. Fib, a plasma protein influenced by the rheological properties of the blood, plays a part in both blood clotting and hemagglutination. IL-6, an inflammatory cytokine produced during inflammation, promotes the synthesis of Fib by acting on the liver cells. We therefore added Fib as an index of the rheological properties of the blood and inflammatory state in this study. The molecular weight of Fib (330,000) is too large to readily fit through the size barrier of the AN69 membrane. The negative charge (−100 mv) of the AN69 membrane is thought to reduce Fib levels by adsorbing Fib, which has a positive charge. The inhibitory effect on Fib production may be due to the adsorption of inflammatory cytokines that act to induce Fib synthesis, such as IL-6.

NOx showed a significant decreasing trend throughout the entire observation period. NO_2^- and NO_3^-, referred to here as NOx, are oxidative metabolites of nitric oxide (NO), an essential endothelium-derived relaxing factor (EDRF). Endothelial nitric oxide synthase (eNOS) is activated by vasoactive substances in vascular endothelial cells such as bradykinin and generates NO and L-citrulline using L-arginine as a substrate. NO acts on vascular smooth muscle by enhancing the production of cyclic guanosine monophosphate (cGMP), which activates protein kinase G (PKG) and thereby relaxes the smooth muscle. NO, a gaseous mediator, is difficult to measure with high accuracy. We therefore decided to use NOx, a relatively stable metabolite of NO, as an index of vascular endothelial function. NOx reflects the amount of NO produced. The AN69 membrane increases production of bradykinin therefor may promote NO production.

Nevertheless, our study results showed a remarkably high concentration of NOx before the change to AN69 membrane, and the values gradually declined after the change. Other researchers have similarly reported high concentrations of NOx in the blood of CKD patients. In a study by Ooyama, et al., NOx values in the blood of HD patients rose above the reference values of healthy subjects. The NOx values decreased during maintenance HD in their patients and rose to high levels over the periods up to the succeeding HD treatments[12]. Overall, however, reports of long-term time course changes of NOx in the blood of maintained HD patients have been very rare. Increases of asymmetric dimethylarginine (ADMA), a chemical promoted by factors such as oxidative stress, acts protectively on the vascular endothelium and decreases the production of endothelial NOS-derived NO in patients with chronic renal failure. The inductive NOS-derived NO induced by inflammatory cytokines is remarkably increased in the same patients, however, which results in high concentrations of NOx[13–15]. In this study the average NOx value before the change to AN69 membrane in our subjects was 130.6±91.9 μmol/L, a markedly high level compared to the reference values of healthy subjects. The values gradually declined after the change of membrane and stayed virtually almost within the reference value for healthy subjects 24 weeks later. The strong negative charge of the AN69 membrane increases the production of bradykinin and may improve peripheral hemodynamics by promoting the production of endothelial NOS-derived NO. Yet there are speculations that the production of inductive NOS-derived NO may be inhibited, as the AN69 membrane also adsorbs inflammatory cytokines. In the result, abnormally high NOx values decrease. Further study is required.

Hb levels in the present study remained generally favorable (10–11 g/dL) and tended to increase slightly overall. TSAT remained relatively high and ferritin remained relatively low during the second half of the observation period.

The decreased renal function in HD patients increases blood levels of inflammatory cytokines

such as IL-6 and TNF-α, thereby eliciting a chronic inflammatory state. The various deleterious influences of HD therapy (bio-incompatibility of the HD membrane, endotoxin contamination, infection of the vascular access, etc.) not only reduce the survival time of red blood cells, but also cause various disorders of the iron metabolism. Infection and inflammation trigger the production of IL-6 and various other cytokines that promote the expression of hepcidin-25 in the liver and reduce the iron supply to the blood. These cytokines also increase the expression of ferritin, the phagocytosis of aged red blood cells, and the iron storage of macrophages, resulting in a state of intracellular iron overload. Hepcidin-25 decreases the expression of ferroportin, which inhibits the supply of iron from the macrophages to the blood and thereby depletes the stores of iron required for hematopoiesis. Thus, the abnormal iron metabolism leads to a state of iron overload within cells and iron deficiency in the blood. Moreover, the administration of erythropoiesis-stimulating agent (ESA) to HD patients can enhance erythropoiesis and thereby increase the demand for iron. Meanwhile, iron is lost through blood collection and residual blood in the HD circuit and dialyzer. Hence, a requisite amount of iron supplement is required to maintain hematopoiesis through the administration of ESA. Yet the abnormal iron metabolism mechanism described above leaves the HD patient prone to functional iron deficiency, a state where the iron in the blood remains insufficient even when sufficient stores of iron are available. ESA reactivity thus remains low in spite of frequent iron administration, as little of the iron administered is used for hematopoiesis[16–18].

In a crossover trial using the FBP-β and AN69 membranes, Hori et al. noted significant decreases of hepcidin-25 levels when using the AN69 membrane, along with increases in Hb levels and decreases of ferritin levels even when administering rhu-EPO and iron supplement at reduced doses[19]. The AN69 membrane may reduce the production of inflammatory cytokines such as IL-6 through adsorption and improve ESA hyporesponsiveness and the effective utilization of iron in hematopoiesis.

The dosage of iron significantly increased over time in the present study, compared with that at the switchover to the AN69 membrane. TSAT tended to be high and ferritin tended to be low after the switchover to the AN69 membrane. The dose of ESA was continuously increased over the period up to week 16, which increased the demand for iron and iron dose in parallel. The increased dose of iron also seemed to relate to the increase of CRP. Several increases of CRP were noted during the observation period. These changes, together with the inflammatory condition afterwards, lowered the effective utilization of iron. Later, after the highest increase of CRP was measured at week 64, TSAT went down in conjunction. This inflammatory condition seemed to worsen the effective utilization of iron and increase the dose of iron from week 64 onwards. Meanwhile, during the second half of the observation period TSAT remained relatively high and ferritin remained relatively low in the present study, and Hb levels tended to increase even when the amount of ESA administered remained unchanged. These findings suggested that the ESA reactivity was high and that iron was efficiently used. Hence, the AN69 membrane seems effective in improving abnormal iron metabolism. It may be possible to reduce the doses of ESA administered in the future through the long-term use of the AN69 membrane.

Assessment of Peripheral Hemodynamics

Observations of peripheral hemodynamics revealed a noticeable improvement in SPP in both legs of one patient and exacerbation in one leg of another patient. The patient whose peripheral hemodynamics significantly improved was a nondiabetic and the patient suffering from exacerbation was a diabetic with a long history of PAD. No decreases with the potential to cause clinical issues with regard to SPP were seen in the other 6 patients, and peripheral hemodynamics remained stable throughout the whole 72-week observation period. Skin perfusion pressure (SPP) is calculated from the microcirculation of the skin as an index of skin level blood flow. SPP is reported to be barely influenced by blood vessel calcification, a

condition often seen in diabetic or maintenance HD patients. The risk of Reynaud's phenomenon increases at an SPP of less than 50 mmHg, the lower limit for SPP in healthy individuals[10]. The present study revealed generally stable peripheral hemodynamics through the long-term use of the AN 69 membrane, with improved peripheral hemodynamics in a subgroup of patients.

Shirai et al. noted significant decreases in Fib levels and blood viscosity, together with an increase in Alb levels, after 6 months of use of the AN69 membrane[20]. Their results for Fib and Alb agreed with the trend we observed in the present study. The use of the AN69 inhibited membrane Alb leakage, and the decrease in blood viscosity via the adsorption of Fib may have helped improve the peripheral hemodynamics. Another factor underlying the improved SPP may have been the peripheral vasodilatory action of bradykinin, an action elicited by the negative charge of the AN69 membrane.

Conclusion

This cross-over trials show the AN69 membrane eliminates less AA and Alb compared with the PS membrane. And the evaluation of the therapeutic effects of the long-term use of the AN69 HD membrane suggested that the AN69 HD membrane has good biocompatibility and is optimal for the treatment of elderly HD patients with mild PAD. On the other hand, the increasing β_2MG levels, though not significantly, raise concerns over the long-term effects on patients and call for further observation.

Conflict of Interest: The authors have no conflicts of interest to declare.

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