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COMPARATIVE ANALYSIS OF TRACE ELEMENTS CONCENTRATIONS IN DIALYSIS FLUIDS BEFORE AND AFTER DIALYZER

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Abstract

Disturbances of mineral metabolism are one of the many complications observed in patients with renal failure. Fluids used in dialysotherapy may introduce elements to a patient's body. On the other hand, some trace elements may be removed. Dialysis fluids contain chlorides of calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K), but they may also be contaminated by toxic metals. In the first part of our work the amounts of Mg, Ca, zinc (Zn), and iron (Fe) were determined in samples of hemodialysis fluids just before and after a dialyzer. The concentration of copper (Cu), aluminium (Al), lead (Pb), cadmium (Cd), and chromium (Cr) were measured by the atomic absorption spectrometry method (AAS) in a graphite furnace (GFAAS) in the same dialysates. The average concentration of Cu in dialysis fluids before and after dialyzer was 13.51 and 10.51 μg L^{-1} respectively, Al – 8.72 and 7.88 μg L^{-1}, Pb – 24.03 and 22.81 μg L^{-1}, Cd – 1.09 and 1.07 μg L^{-1}, Cr – 5.91 and 6.28 μg L^{-1}. Except for Cr ($p < 0.05$), the comparison of concentrations of all the measured elements before and after haemodialysis did not show any significant differences. Positive significant correlations between the element concentration before and after a dialyzer were found for Al ($r = 0.33030$ and Cd ($r = 0.7496$). In the samples of dialysis fluids of patients who had been dialyzed for less than one year, a negative balance of elements was found, except Pb. The balance was positive in patients who had been dialyzed for more than a year. Our findings also show statistically significant negative correlation between duration of dialysis treatments and Al concentration. Statistically, the examined dialysis fluids seem to be safe for dialyzed patients. However, individual data show that it is important to control elemental levels in dialyzates used for many years to prevent some complications in these patients.

Key words: dialysis fluids, trace elements, atomic absorption spectrometry.

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ANALIZA PORÓWNAWCZA STĘŻEŃ PIERWIASTKÓW ŚLADOWYCH W PŁYNACH DIALIZACYJNYCH PRZED I ZA DIALIZATOREM

Abstrakt

Zaburzenia w gospodarce mineralnej to jedno z wielu powikłań obserwowanych u pacjentów ze schyłkową niewydolnością nerek. Płyny stosowane w dializoterapii mogą do organizmu dostarczać pierwiastki, ale mogą je także z niego usuwać. W składzie płynów dializacyjnych znajdują się chlorki Ca, Mg Na i K. Mogą one także zawierać inne metale będące ich zanieczyszczeniami. W pierwszym etapie pracy oznaczono stężenie Ca, Mg, Zn i Fe w 38 próbkach płynów dializacyjnych przed i za dializatorem. Stężenie tych pierwiastków nie zmieniło się w sposób istotny statystycznie, ale stężenie Zn było o ok. 7% wyższe w płynie po zabiegu, a w kilku z nich stwierdzono podwyższone stężenie Fe. Celem pracy było oznaczenie w tych samych dializatach stężeń pierwiastków śladowych istotnie wpływających na funkcje organizmu. Porównawcze oznaczenia stężenia miedzi (Cu), glinu (Al), ołowiu (Pb), kadmu (Cd) i chromu (Cr) wykonano metodą spektrometrii absorpcji atomowej z atomizacją w piecu grafitowym (GFAAS). Zastosowane procedury analityczne zapewniają dobrą czułość i precyzję metody. Średnie stężenie Cu w płynach przed i za dializatorem wynosiło 13,51 i 10,51 µg L^{-1}, Al – 8,27 i 7,88 µg L^{-1}, Pb – 24,03 i 22,81 µg L^{-1}, Cd – 1,09 i 1,07 µg L^{-1}, Cr – 5,91 i 6,28 µg L^{-1}. Z wyjątkiem Cr (p<0,05), stężenie pierwiastków w płynach przed i za dializatorem nie różniło w sposób statystycznie istotny. Dodatnie istotne korelacje między zawartością pierwiastków przed i za dializatorem wyznaczono dla Al (r = 0,3303) i Cd (r = 0,7496). W próbkach płynów dializacyjnych pacjentów dializowanych w czasie krótszym niż rok stwierdzono, z wyjątkiem Pb, ujemny bilans pierwiastków, a powyżej roku – dodatni. Stwierdzono ujemną istotną korelację między czasem trwania dializoterapii a stężeniem Al.

Badane płyny dializacyjne, uwzględniając ocenę statystyczną, nie zwiększają ekspozycji pacjentów dializowanych na oznaczane metale ciężkie. Z analizy danych indywidualnych wynika jednak, że płyny te, ze względu na systematyczne stosowanie w ciągu wielu lat, mogą wywierać wpływ na status mineralny pacjenta, i dlatego też pod tym kątem powinny być kontrolowane.

Słowa kluczowe: płyny dializacyjne, pierwiastki śladowe, spektrometria absorpcji atomowej (GFAAS).

INTRODUCTION

Mineral disturbances which accompany the chronic renal failure (CRF) may be affected by several factors i.e. impaired intestinal absorption and distribution, reduced renal function and urine excretion, hormonal disorders, restricted and unbalanced diet, medication and dialysis treatment. Dialysis osteomalacia, anaemia, cardiovascular diseases, and mental disorders are frequent and serious complications of renal dysfunction, which are observed in dialyzed patients. It has been shown that many elements, including Ca, Mg, Fe, Zn, Cu, Se, Al, Si, Cr and Sr, take part in the pathogenesis of kidney failure complications. Abnormalities of the elemental status have been reported in serum, blood, erythrocytes and tissues (e.g. osseous tissue, hair and brain) of dialyzed patients. Some elements (Al, Cr, Fe, Si, and Sr) excessively accumulate in the body, while others (Ca, Se, and Zn) may demonstrate their deficiency (ŠČANČAR et al. 2003). Medical treatment for renal insufficiency includes pharmacological treatment, special diet, and dialysotherapy. Dialysis fluids may introduce contribute bioelements and toxic metals to a patient's body. On the other hand, some trace elements may be removed. So far, most of the reports have dealt with the Al and Cr concentration in fluids used in haemodialysis and peritoneal dialysis. Aluminium may be responsible for intoxication of dialyzed patients caused by high content of this element present in water used for preparation of dialysis fluids. Between 517-1275 μg Al L^{-1} was determined post mortem in serum of 4 patients treated with aluminium contaminated dialyisis fluids. Additionally, significant amounts of this element were discovered in the liver, bone, and brain of these patients (WOLF et al. 2002). Some authors (ŠČANČAR et al. 2003, MIURA et al. 2002, ŠČANČAR et al. 1999, MILAČIČ, BENEDIK 1999) determined other elements, such as Ca, Mg, Zn, Cu, Fe, Ni, Co, Pb, Cd, Mn, Si, Se, and Rb both in dialysis fluids and in concentrates. It has been shown that dialysis fluids influence the mineral balance in a patient's organism.

Because of a difficult and complicated dialysis fluids matrix (e.g. carbodydrates, large amounts of chloride ions) and trace amounts of most elements present in dialysates (except Ca, Mg, K, and Na), the authors used various techniques of the preliminary chemical treatment of the specimens, for example preconcentration, coprecipitation, addition of high amounts of acids. Instrumental analyses were carried out by neutron activation analysis (NAA) and particle-induced X-ray emission (PIXE), but generally by atomic absorption spectrometry (flame atomic absorption spectrometry FAAS and graphite furnace atomic absorption spectrometry GFAAS) (ŠČANČAR et al. 1999, MIURA et al. 2002, ŠČANČAR et al. 1999, MILAČIČ, BENEDIK 1999, BERKKAN, ERTAŞ 2004, SARACOGLU et al. 2003, ELÇI et al. 1997).

In the study (DŁUGASZEK, KARBOWIAK 2007) the Ca, Mg, Zn, and Fe concentrations were determined in haemodialysis fluids before and after a dialyzer. There were statistically non-significant differences between the concentrations of the examined elements, although the amount of Zn was 7% higher in dialysis fluid after the operation.

As for a possible contamination of dialysis fluids contamination by toxic metals, it seems rational to investigate both transport of elements to a patient's body and their removal from the tissues. Moreover, little is known about these mechanisms at present.

Our present study has been undertaken in order to investigate the Cu, Al, Pb, Cd, and Cr content in fluids used in dialysotheraphy and to compare the metal concentrations before and after a dialyzer.

MATERIALS AND METHODS

The material included fluids used during 38 haemodialyses in patients with chronic renal failure: 22 men (aging 39-87 years) and 16 women (aging 43-80 years). The samples were taken before and after a dialyzer. The specimens of dialysate fluid were obtained from the Department of Internal Medicine of the Military Clinical Hospital in Bydgoszcz.

Capillary dialyzers equipped with cuprophan or polysulfone membranes were used in the dialysis treatment. The dialysate flow was 500 mL min^{-1}, and blood flow rate was 250-300 mL min^{-1}. The dialyzer surface was 1.1, 1.3 or 1.5 m^2 depending on the medicalrecommendations. The conventional dialysate fluid contained approximately 1.25 mmol Ca L^{-1} and 0,5 mmol Mg L^{-1} (values declared by producer). The collected samples of dialysate fluid were initially mineralized with suprapure 65% HNO_3 acid. Afterwards, they were diluted with acid (1 + 1) and the mixtures in closed vessels were allowed to stand for 24 h. Next, the samples were diluted with deionized water, so that the final concentration of HNO as a matrix modifier was 16%.

During the collection of dialysis fluids samples, storage (-20°C), mineralization, and performing instrumental analysis, a possible risk of contaminating the samples contamination was controlled. All the glassware, vessels, plastic tubes and pipette tips were treated with 6 mol L^{-1} HCl and 6 mol L^{-1} HNO_3. For the analyses, suprapure reagents and deionized water (18 M Ω cm^{-1}) were used.

In solutions of dialysis fluids prepared as described above (in two duplicate series), concentrations of copper, lead, cadmium, aluminium, and chromium were determined by the atomic absorption spectrometry method using a spectrometer AVANTA Σ (GBC) equipped with a graphite furnace GF3000, autosampler PAL 3000 and Ultra-Pulse deuterium corrector. The concentrations of Cu, Pb, Cd, Al, and Cr were measured by the graphite furnace atomic absorption spectrometry technique (GFAAS). The analyses were done in pyrolitic graphite tubes in argon atmosphere as a purging gas. The volume of a sample injected to the graphite furnace was 20 µL. The peak area mode was used for the calculation of the tested element concentration. Instrumental parameters and analytical characteristics of the procedures are shown in Table 1. In order to test the accuracy of the determination of the concentrations of the elements, two calibration methods were applied: calibration curve and standard addition method, as well as two reference materials: SRM 1577b and NCS 81002 (DŁUGASZEK, SZOPA 2007).

The results are expressed as means and medians. The data were analyzed using normality Shapiro-Wilks test and Fisher test verifying homogeneity of variances. The differences between independent groups were assessed by t-Student, Cochran-Cox and Kolmogorow-Smirnov (for non-normally

Table 1

Instrumental parameters and analytical characterization of used methods

Element	Pyrolisis/ atomization temperature (°C)	Wavelenght (nm)	Calibration range[1] (ng mL^{-1})	Limit of detection[3] (ng mL^{-1})	Sensivity[4] (pg)	Precision (%)
Cu	800/2300	324.7	2.5-10.0	0.4	2.6	8.2
Pb[1]	900/2000	283.3	1.5-15.0	0.81	5.8	6.8
Cd[2]	600/1800	228.8	0.15-1.50	0.06	0.36	6.7
Al	1400/2400	309.3	3-20	0.40	8.6	9.1
Cr	1000/2500	357.9	0.5-5.0	0.51	1.44	3.2

Modifiers used:
[1] $NH_4H_2PO_4$
[2] NH_4NO_3
[3] limit of detection (LOD) defined as 3SD (n = 10)
[4] characteristic mass defined as amount of element giving an absorbance of 0.0044

distributed parameters) tests. Correlation analysis between variables was performed using Pearson's test. The level of statistical significance was at $p<0.05$. For statistical analysis a Statistica software package was used.

RESULTS AND DISCUSSION

The results are shown in Tables 2, 3 and 4, where the differences between concentrations of the elements before and after a dialyzer in separate samples of dialysis fluids and the mean concentration of these metals are presented. Moreover, the effect of the dialysis treatment time (below and above 1 year) on amounts of elements in the dialysate leaving a dialyzerwas assessed. Both these results and Pearson's correlation coefficient (r_{xy}) between the time of dialysotheraphy and the concentration of elements in dialysate after a dialyzer are shown in Table 4.

In the examined dialysis fluids, the mean Cu concentration is comparable before and after a dialyzer. However, in three samples its concentration before a dialyzer was much higher than in the others. In the same samples the Cu concentration after a dialyzer was lower and similar to that in the other samples of dialysis fluids. Moreover, in these samples the Fe concentration (before a dialyzer) was also much higher, about 10-fold higher (216.1, 299.2, and 548.6 µg L^{-1}), and, like the Cu levels, the Fe concentration after a dialyzer was at the same level as in the other samples. This means that the dialysis fluids may have been contaminated with these metals and the contaminants may have been retained in a patient's organism. The correla-

Table 2

Differences in concentration of elements (µg L^{-1}), after and before a dialyzer

No	Cu	Al	Pb	Cd	Cu
1	- 7.63	- 2.98	- 21.5	+ 0.04	+ 2.85
2	- 7.24	+ 2.48	+ 2.8	+ 0.59	+ 0.29
3	+ 0.57	- 5.55	- 0.9	+ 0.07	+ 2.27
4	- 2.61	+ 6.58	- 2.1	+ 0.05	+ 3.56
5	+ 4.85	- 1.32	- 0.9	+ 0.03	+ 0.35
6	+ 4.49	+ 3.05	+ 1.0	- 0.07	+ 2.28
7	+ 0.64	+ 2.96	- 0.4	- 0.03	+ 2.48
8	+ 2.25	+ 1.92	- 3.8	+ 0.12	- 1.40
9	+ 2.06	+ 1.14	- 2.6	- 0.08	+ 0.74
10	+ 5.22	+ 2.36	+ 0.2	- 0.06	+ 2.02
11	- 1.74	- 0.38	- 4.3	- 0.19	- 0.37
12	+ 4.63	- 3.26	+ 3.4	+ 0.09	+ 0.97
13	- 4.76	- 6.32	+ 2.0	+ 0.02	+ 1.40
14	- 3.13	- 7.27	- 3.5	+ 0.10	+ 0.81
15	+ 10.29	+ 6.46	+ 2.0	+ 0.07	+ 0.93
16	+ 6.45	+ 0.35	+ 2.1	- 0.04	+ 0.20
17	- 3.15	+ 0.36	- 1.6	- 0.06	- 10.54
18	+ 0.76	+ 4.45	+ 3.4	+ 0.15	+ 0.34
19	- 13.45	+ 2.79	- 6.4	- 0.04	- 0.92
20	+ 17.7	- 4.99	+ 6.7	- 0.02	+ 3.08
21	- 1.07	- 4.44	- 10.4	+ 0.03	+ 0.13
22	+ 6.82	- 2.68	- 6.4	- 0.04	+ 2.78
23	+ 4.48	+ 2.54	- 1.3	- 0.13	+ 0.93
24	+ 4.8	- 11.33	+ 1.1	- 0.03	+ 0.62
25	+ 6.78	- 5.39	- 5.4	- 0.07	+ 0.02
26	- 9.07	- 3.76	+ 12.3	+ 0.09	- 1.50
27	- 1.46	+ 0.67	- 4.1	- 0.01	+ 0.90
28	- 23.95	- 1.75	- 3.7	- 0.28	- 1.60
29	- 1.4	- 3.95	+ 1.8	- 0.10	+ 6.23
30	- 4.82	+ 2.30	- 1.6	- 0.15	+ 1.34
31	+ 2.14	- 5.36	- 1.7	+ 0.14	+ 1.18
32	- 11.4	- 5.66	- 4.5	- 0.02	- 0.98
33	+ 6.78	+ 10.24	- 0.1	+ 0.17	+ 0.79
34	+ 0.2	- 5.21	- 5.2	+ 0.30	+ 0.50
35	- 23.83	+ 1.88	+ 9.5	- 0.20	+ 0.34
36	- 44.68	- 6.63	+ 6.2	- 0.64	+ 0.53
37	- 43.49	- 0.47	- 11.1	- 0.37	- 3.42
38	+ 5.36	+13.86	+ 7.2	- 0.02	- 0.73

Table 3

Concentration of elements (μg L^{-1}) before and after a dialyzer

Element	Cu		Al		Pb		Cd		Cr	
	before	after	before	after	before	after	before	after	before	after
Mean	13.51	10.51	8.27	7.88	24.03	22.81	1.09	1.07	5.91	6.28 $p<0.05$
Median	9.07	9.65	6.83	7.53	23.50	22.85	1.13	1.14	5.79	6.41
S.D.*	12.46	5.20	4.18	4.65	4.58	4.12	0.22	0.29	1.92	1.03
Range (min.- max.)	3.19 59.68	2.68 30.19	2.63 17.89	1.68 21.09	15.10 42.9	13.30 32.6	0.58 1.37	0.42 1.78	2.32 14.89	4.07 8.27
Quartiles (Q_1, Q_3	5.19 18.14	7.18 12.72	5.36 10.07	4.12 9.75	21.2 25.9	19.4 25.4	0.99 1.24	0.88 1.29	4.87 6.22	5.52 7.02

* – standard deviation

Table 4

Concentration of elements (μg L^{-1}) before and after a dialyzer, below (n = 12) and above one year of dialysis treatment (n = 26)

Element	Cu		Al		Pb		Cd		Cr	
	before	after	before	after	before	after	before	after	before	after
< Year										
Mean	14.21	12.36	10.10	8.24	23.40	24.86	1.15	1.11	5.80	6.10
Median	13.67	10.3	9.49	9.05	23.50	25.3	1.35	1.27	5.88	6.23
S.D.*	9.56	6.77	4.54	4.47	2.86	2.74	0.19	0.31	0.90	0.90
Difference		-1.85		-1.86		+1.46		-0.04		+0.30
> Year										
Mean	8.97	9.87	7.45	7.71	24.31	21.85	1.06	1.05	5.75	6.36
Median	7.59	9.09	6.08	7.03	23.80	21.65	2.00	1.08	5.70	6.52
S.D.*	4.96	4.49	3.82	4.81	5.21	4.34	0.24	0.28	2.46	1.10
Difference		+0.90		+0.26		-2.46 $p<0.05$		-0.01		+0.61
$r_{(xy)}$	-0.162		-0.469 $p<0.05$		0.137		0.415		0.098	

* – standard deviation

tion between the Cu concentration in our dialysates before and after a dialyzer was weak ($r = 0.0857$).

The Cu content in concentrates used in haemodialysis determined by Elçi et al. (1997) was 3.5 ± 0,1 µg L^{-1}, and that reported by Saracoglu et al. (2003) ranged from 6.7 to 81.0 µg L^{-1}. In turn, the Cu concentration in spent continuous ambulatory peritoneal dialysis (CAPD) fluids ranged from 6.3-94.9 µg L^{-1} (Milačič, Benedic 1999), and according to Ščančar et al. (2003) it was 4.9-32.5 µg L^{-1}. In concentrates used in peritoneal dialysis, the amount of 0.006 µmol Cu/L was noted, but in spent dialysates the Cu concentration ranged within 0.120-0.549 µmol L^{-1}. This suggests that Cu clearance from the serum of dialyzed patients is possible (Ščančar et al.1999).

According to the recommendation of the Association for the Advancement of Medical Instrumentation (AAMI) (Mactier 2007), the Cu concentration in water used for preparing dialysis fluids should not exceed 0.1 mg L^{-1}.

In the analyzed dialysates, an averaged Al concentration did not exceed the acceptable maximum level for water i.e. 0.01 mg L^{-1} (Mactier 2007). The mean concentration of this element in the dialysis fluids flowing out of a dialyzer was lower than before a dialysis session, but the median value was respectively higher. Statistically significant positive correlation between the Al amount in the dialysis fluids before and after a dialyzer ($r = 0.3303$, $p<0.05$) suggests that Al would not be retained in a patient's organism.

The mean concentration of Al reported by Joffe et al. (1989) in spent CAPD fluids was 9.0 µg L^{-1} (3.6-16.3 µg L^{-1}). Other authors (Ščančar et al. 2003) have shown that the Al migration from a patient's serum to dialysates during peritoneal dialysis is possible, as they compared concentration of this element in fresh CAPD fluids (< 1.0 µg L^{-1}) and in spent CAPD fluids (1.1--8.6 µg L^{-1}), and < 0.037 µmol L^{-1} vs 0.130 µmol L^{-1} – 0.478 µmol L^{-1}, respectively (Ščančar et al.1999). Y. Miura et al. (2002) also noticed higher Al level in 5 samples of dialysates after a haemodialysis treatment.

In our study, the mean Pb concentration in the dialysis fluids was slightly lower (about 6%) after a dialyzer, but only in 14 out of the 38 samples the Pb concentration was higher in dialysates leaving a dialyzer. Moreover, the correlation between the Pb level in dialysis fluids before and after a dialyzer was weak ($r = 0.0061$).

In the haemodialysis concentrates Saragoclu et al. (2003) measured 3.6--61 µg Pb L^{-1}, but Elçi et al. (1997) reported 1.67 ± 0.05 µg Pb L^{-1}. Other authors (Berkkan, Ertaş 2004) using two atomic absorption techniques (flow injection hydride generation atomic absorption spectrometry FI-HGAAS and electrothermal atomic absorption spectrometry ETAAS) have shown 10-70 ng Pb L^{-1} in 12 samples of dialysis concentrates. In accordance to the AAMI Standards (Mactier 2007) the Pb acceptable concentration in water is 0.005 mg L^{-1}.

We found that the Cd concentration in dialysates is comparable both before and after a dialyzer. Statistically significant positive correlation between Cd levels in dialysis fluids before and after a dialyzer (*r* = 0.750, *p*<0.0001) suggests that Cd is not transferred to a patient's blood. These results are in agreement with the data presented by SARAGOCLU et al. (2003) – 0.1-0.3 μg L^{-1} and ELÇI et al. (1997) – 0.83 ± 0.04 μg L^{-1}. The acceptable maximum value for Cd concentration in water using for dialysis fluids preparation is 0.001 mg L^{-1} (MACTIER 2007).

In almost 80% samples the mean Cr concentration in the examined dialysates flowing out of a dialyzer was significantly higher than before a dialyzer (*p*<0.05). The median value was also higher (approximately 10%). Negative correlation coefficient (*r* = –0.185) suggests that this element may cross semipermeable membrane of a dialyzer and may be transferred to a patient's blood.

Other authors (MILAČIČ, BENEDIK 1999) found 0.2-1.9 μg Cr L^{-1} in spent CAPD fluids, and 5.1 – 39 μg Cr L^{-1} in concentrates used in haemodialysis (SARAGOCLU et al. 2003). With respect to the AAMI Standards (MACTIER 2007), the acceptable maximum concentration of Cr is 0.014 mg L^{-1}.

In our study, when analyzing the concentrations of metals in dialysates from patients dialyzed less than 1 year we observed a tendency to retain almost all the examined elements, with the exception of Pb, but during the next years of a haemodialysis therapy this tendency decreased, probably as a result of exceeding the capacity of tissues to accumulate elements.

The examined dialysis fluids seem to be safe for patients' health. They do not contribute significant amounts of heavy metals. On the other hand, considering the cases of dialysis fluids samples contaminated with Cu and Fe and the fact that the dialysotheraphy is continued for many years, it is necessary to monitor concentrations of bioelements and heavy metals in fluids used in dialysotheraphy to prevent intoxication of patients with toxic metals.

CONCLUSIONS

1. In the examined dialysis fluids, concentrations of the determined elements did not exceed the recommended maximum concentrations of chemical contaminants in water for dialysis (AAMI), excluding Pb.

2. Differences in the concentrations of Cu, Al, Pb, and Cd before and after a dialysis treatment were not significant. However, the concentration of these elements was higher in dialysis fluids before a dialyzer. On the basis of these results, it could be concluded that some amount of these elements may have been retained in a patient's organism. The opposite was observed in the case of Cr, whose level significantly increased in postdialysis fluids (*p*<0.05).

3. Statistically significant positive correlations for the Al and Cd concentrations in fluids before and after a dialyzer may indicate that transport of both elements to a patient's body via dialysis fluids is restricted.

4. Our findings have shown that the presence of Pb in dialysates requires special attention, because it is likely that this element may cross a dialyzer membrane.

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