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KINGA MUSIAŁ[1], DANUTA ZWOLIŃSKA[1], KRYSTYNA SZPRYNGER[2], MARIA SZCZEPAŃSKA[2]

Soluble Adhesion Molecules During a Single Dialysis Session in Children and Young Adults on Chronic Hemodialysis*

Rozpuszczalne cząstki adhezyjne podczas pojedynczej sesji dializacyjnej u dzieci i młodych dorosłych przewlekle hemodializowanych

[1] Department of Pediatric Nephrology, Silesian Piasts University of Medicine in Wrocław, Poland
[2] Department of Pediatrics, Clinic of Nephrology, Endocrinology and Metabolic Diseases of Childhood, Silesian School of Medicine, Zabrze, Poland

Abstract

Background. Immune system impairment in patients on hemodialysis (HD) may partly result from bioincompatibility of dialysis membranes.
Objectives. The aim of the study was to analyze the impact of a single dialysis session and the type of dialysis membrane on soluble(s) adhesion molecules, thus evaluating their value as biocompatibility markers.
Material and Methods. Serum sL-selectin and sVCAM-1 concentrations were assessed by ELISA. These parameters and the sL-selectin/sVCAM-1 ratio (L/V) were examined in children and young adults on maintenance HD: 14 with cuprophane (CU), 10 with vitamin E-modified cellulose (VE), and 8 with polysulfone (PS) membranes, as well as in 15 controls. Baseline laboratory test results, age, BMI, and time of therapy were also evaluated and regression analysis was performed.
Results. Linear correlations were found between sL-selectin, L/V, and the type of dialyzer before and after dialysis session. The correlation coefficient for the linear regression equation after HD was higher than before the session (sL-selectin: $R = 0.6$ vs. $R = 0.5$, L/V: $R = 0.7$ vs. $R = 0.6$) and was stronger for L/V. Linear correlation was found between sVCAM-1 and the membrane type after HD ($R = 0.5$).
Conclusions. The relationships between adhesion molecules and dialyzer type suggest that they may serve as markers of biocompatibility. The sL-selectin correlation, which suggests an impact of the membrane on leukocyte function, requires further investigation as one of the possible explanations for the increased incidence of infections in HD children (**Adv Clin Exp Med 2008, 17, 2, 183–189**).

Key words: sL-selectin, sVCAM-1, immune system, chronic kidney disease.

Streszczenie

Wprowadzenie. Zaburzenie odporności, obserwowane u pacjentów hemodializowanych, może wynikać m.in. z zastosowania bioniezgodnych błon dializacyjnych.
Cel pracy. Analiza wpływu pojedynczej sesji hemodializy i typu błony dializacyjnej na stężenia rozpuszczalnych (s) cząstek adhezyjnych i ocena przydatności tych molekuł jako markerów biozgodności zastosowanych dializatorów.
Materiał i metody. Stężenia sL-selektyny i sVCAM-1 w surowicy oznaczano metodą ELISA. Powyższe parametry i współczynnik sL-selektyna/sVCAM-1 (L/V) oceniano u dzieci i młodych dorosłych przewlekle hemodializowanych: 14 pacjentów – z użyciem dializatorów kuprofanowych (CU), 10 – błon celulozowych pokrytych witaminą E, 8 – dializatorów polisulfonowych i w grupie kontrolnej 15 osób. Wykonano również podstawowe badania laboratoryjne, w analizie regresji uwzględniono także wiek pacjentów, BMI i czas terapii.
Wyniki. Wykazano liniową zależność między sL-selektyną, L/V i typem dializatora, zarówno przed, jak i po zabiegu hemodializy. Współczynnik korelacji dla równania regresji liniowej po HD był wyższy niż przed zabiegiem (sL-selektyna – R = 0.6 vs. R = 0.5; L/V – R = 0.7 vs. R = 0.6) i silniejszy w przypadku L/V. Liniową zależność między sVCAM-1 i typem dializatora wykazano po sesji HD (R = 0.5).

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Wnioski. Zależności między cząstkami adhezyjnymi a typem dializatora wskazują, że parametry te mogą pełnić rolę markerów biozgodności. Korelacja obserwowana w przypadku sL-selektyny może sugerować wpływ błon dializacyjnych na funkcję leukocytów. Konieczne są dalsze badania tej molekuły w celu wyjaśnienia przyczyn zwiększonej podatności na infekcje pacjentów pediatrycznych leczonych hemodializami (**Adv Clin Exp Med 2008, 17, 2, 183–189**).

Słowa kluczowe: sL-selektyna, sVCAM-1, układ immunologiczny, przewlekła choroba nerek.

Hemodialysis (HD)-related complications are at least in part attributed to contact between the blood and the artificial surface of the dialyzer [1]. This leads to the activation of both cellular and plasmatic components. The intensity of these reactions is considered an index of biocompatibility. Along with its well-established markers, such as anaphylatoxins, monocyte-derived cytokines, and cell-associated adhesion molecules [1], recent investigations concentrate on the influence of HD on oxidative stress [2, 3] and apoptosis [4].

The membrane-bound forms of selectins (E-selectin, L-selectin, P-selectin) and molecules of the immunoglobulin superfamily (e.g. ICAM-1, VCAM-1) take part in a process of leukocyte migration called the adhesion cascade [5]. Their expressions change during hemodialysis sessions, thus serving as markers of biocompatibility [6, 7]. However, investigation of soluble adhesion molecules shed proteolytically from cells into the circulation has given contradictory results in HD patients [2, 6, 8–10]. Moreover, despite a vast literature on vitamin E-coated cellulose membranes, little is still known about soluble adhesion molecule interaction with this type of dialyzer [11–13].

In this study the sL-selectin and sVCAM-1 concentrations and the sL-selectin/sVCAM-1 ratio were evaluated in patients on maintenance hemodialysis treated using cuprophane, polysulfone, and vitamin E-modified cellulose membranes. The choice of these particular soluble adhesion molecules was conditioned by the fact that they both determine the adhesion cascade and appear in the circulation only after leukocyte (sL-selectin) and endothelial (sVCAM-1) activation triggered by the chronic inflammatory process characteristic of chronic kidney disease. The aim was to analyze whether the type of dialyzer used in a single dialysis session may influence adhesion molecule concentrations and whether the ratio combining the leukocyte and endothelium activation markers may serve to assess a dialyzer's biocompatibility.

Material and Methods

The children and young adults on maintenance HD enrolled in this study were divided into three groups. The CU group consisted of 14 patients (7 girls, 7 boys, mean age: 17.5 years, range: 11.5–22 years) on cuprophane (CU) membrane HD (mean time of therapy: 2 years, range: 1 month–7 years). The VE group included 10 patients (5 girls, 5 boys, mean age: 18 years, range: 13–20.5 years) on vitamin E-modified cellulose (VE) membrane HD (mean time of therapy: 7 years, range: 1.8–12 years). The PS group contained 8 patients (5 girls, 3 boys, mean age: 18 years, range: 16.5–20 years) on polysulfone (PS) membrane HD (mean time of therapy: 2.3 years, range: 1.5–3 years). BMI values ranged from 18 to 24 (mean: 21.3) kg/m^2. The original renal diseases were: chronic glomerulonephritis (12 patients), chronic pyelonephritis (11 patients), and urinary tract malformations (9 patients). The control group consisted of 15 patients (7 girls, 8 boys, mean age: 15 years, range: 12–18 years) diagnosed for urinary tract abnormalities or urolithiasis and with normal kidney function.

HD sessions (4–5 hours) were performed three times a week through a-v fistulas using bicarbonate dialysate. The membrane area was between 1.0 m^2 and 1.6 m^2; the dialyzers were not reused. All patients were on a stable anticoagulation regimen using low-molecular-weight heparin. All of them took erythropoietin twice a week. None of the patients had clinical or laboratory evidence of infection (normal WBC count) or malignancies, took antibiotics, NSAIDs, or corticosteroids, or were receiving immunosuppressive therapy. The study design has been approved by the local ethics committee and informed consent was obtained from the subjects and their parents, if necessary.

Blood samples were drawn from the efferent line of the first-use dialyzer before starting an uncomplicated HD session and at the end of HD. In the controls the blood was drawn from a peripheral vein. Samples were centrifuged at 4°C at 2000 × *g* for 10 minutes and then the serum samples were stored at –20°C until assay. Serum concentrations of sL-selectin and sVCAM-1 were evaluated by commercially available ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA) on a Statfax 2100 (Analco). Each sample was measured in duplicate and the arithmetical mean was considered the final result. The standards (recombinant human sL-selectin and sVCAM-1) and serum samples were transferred to 96-well microplates coated with murine monoclonal antibodies to

human sL-selectin and sVCAM-1, respectively. The wells were first incubated with a sheep polyclonal antibody to recombinant human antigen, then with the appropriate substrate (tetramethylbenzidine). The reaction was stopped with an acid solution and the absorbance was measured at 450 nm with the correction wavelength set at 620 nm. The measurements were performed according to the manufacturer's instructions and the results were calculated by reference to standard curves. The limits of detection and the intra- and interassay variations for the adhesion molecules were sL-selectin: 0.3 ng/ml, 4 and 8%, and sVCAM-1: 2 ng/ml, 3.5 and 7%.

Results are expressed as median values. Differences between all groups as well as between adhesion molecule values during HD sessions were evaluated using nonparametric tests (Kruskall-Wallis, Mann-Whitney *U*, Wilcoxon). Correlations between variables were evaluated by Spearman's correlation coefficient. Regression analysis was also performed. The Statistica 6.0 (StatSoft) package was used for statistical analysis. A p value < 0.05 was considered significant.

Results

Baseline laboratory test results are presented in Table 1.

Table 1. Baseline laboratory test results in all patients (n = 32) before and after a single dialysis session presented as the mean ± *SD*

Tabela 1. Wyniki podstawowych badań laboratoryjnych wykonanych u wszystkich pacjentów (n = 32) przed i po zabiegu HD, przedstawione jako średnia i odchylenie standardowe

Parameters (Parametry)	Before HD (Przed HD) n = 32	After HD (Po HD) n = 32
Hematocrit (Hematokryt) %	29.8 ± 3.8	32.8 ± 4.0
Erythrocytes (Erytrocyty) T/l	3.2 ± 0.4	3.6 ± 0.5
Leukocytes (Leukocyty) G/l	6.7 ± 2.2	6.4 ± 2.5
Neutrophils (Neutrofile) %	59.1 ± 12.9	62.0 ± 10.0
Lymphocytes (Limfocyty) %	33.4 ± 12.5	29.5 ± 9.2
Urea (Mocznik) mmol/l	22.5 ± 5.3	9.7 ± 3.8
Creatinine (Kreatynina) µmol/l	900.2 ± 224.4	436.0 ± 147.9

Serum sL-Selectin Levels

Median sL-selectin values in all HD patients were lower than in controls both before and after a single dialysis session ($p = 0.0001$). The levels of sL-selectin remained unchanged during HD sessions performed with all the dialyzers evaluated in the study (Table 2). There were also no differences between patients dialyzed with different membranes before HD (Table 2). Median values of sL-selectin after HD in the PS patients were higher than those in the CU and VE patients, but failed to differentiate between CU and VE individuals (Table 2). sL-selectin levels correlated with hematocrit and creatinine in CU patients before HD ($R = 0.57$, $p = 0.01$ and $R = –0.66$, $p = 0.001$, respectively) and with neutrophil count (CU: $R = –0.61$, $p = 0.02$), urea (VE: $R = –0.87$, $p = 0.001$), and hematocrit (PS: $R = 0.83$, $p = 0.01$) after HD.

There was linear correlation between sL-selectin and the type of dialyzer. The linear regression equation describing the relationship before HD was calculated as y = 193x + 920 (y being sL-selectin, x the type of dialyzer). Both the regression coefficient (193) and the constant term (920) were statistically significant ($p = 0.003$ and $p = 0.0001$, respectively). The correlation coefficient was $R = 0.5$. The equation describing the relationship after HD was calculated as y = 166x + 1027, with both the regression coefficient and the constant term being statistically significant ($p = 0.001$ and $p = 0.000001$, respectively). The correlation coefficient was $R = 0.6$.

Serum sVCAM-1 Levels

In all groups the sVCAM-1 concentrations were higher than in the controls both before and after HD ($p = 0.0001$ and $p = 0.00001$, respectively). There was a significant increase in sVCAM-1 levels during a single HD session with the cuprophane and vitamin E-coated membranes, whereas polysulfone membrane dialysis had no influence on sVCAM-1 levels (Table 2). The values before HD failed to differentiate between the dialyzers (Table 2). Median sVCAM-1 values after HD in the CU patients were higher than the values in the VE and PS patients, but no difference was seen between the VE and PS groups (Table 3). sVCAM-1 levels correlated with lymphocyte count (PS: $R = 0.71$, $p = 0.04$) and urea (VE: $R = –0.85$, $p = 0.001$)

before HD and with hematocrit (CU: $R = –0.67$, $p = 0.01$), neutrophil count (CU: $R = 0.68$, $p = 0.009$), urea (VE: $R = –0.84$, $p = 0.002$), and creatinine (VE: $R = –0.64$, $p = 0.04$) after HD.

Linear correlation was also found between sVCAM-1 and the dialyzer type after the HD session. The linear regression equation was calculated as y = –402x + 3025. Both the correlation coefficient and the constant term were statistically significant ($p = 0.004$ and $p = 0.000001$, respectively). The correlation coefficient was $R = 0.5$.

sL-Selectin/sVCAM-1 Ratio Before HD

In all groups the median values of the sL-selectin/sVCAM-1 ratio were decreased vs. controls ($p = 0.00001$) and the median values in the CU were lower than in the PS and in VE groups (Table 2). There was no significant difference between the ratios in the PS and VE patients (Table 2). L/V in the CU patients correlated with hematocrit ($R = 0.5$, $p = 0.02$), creatinine ($R = –0.56$, $p = 0.01$), and total protein ($R = –0.54$, $p = 0.01$). In the VE patients there was correlation with urea ($R = 0.77$, $p = 0.01$) and in PS patients with calcium ($R = –0.77$, $p = 0.44$).

There was also a linear correlation between L/V and the type of dialyzer. The linear regression equation describing the relationship before HD was calculated as y = 0.2x+0.5. Both the regression coefficient (0.2) and the constant term (0.5) were statistically significant ($p = 0.001$ and $p = 0.0001$, respectively). The correlation coefficient was $R = 0.6$.

sL-Selectin/sVCAM-1 Ratio After HD

There was a significant decrease in the sL-selectin/sVCAM-1 ratio in all groups when values after and before HD were compared (Table 2). The lowest sL-selectin/sVCAM-1 values were observed in CU patients (Table 2). There was no difference between PS and VE patients in the ratio values after HD ($p = 0.2$), in the same way as before HD. L/V correlated with neutrophil count (CU: $R = –0.75$, $p = 0.003$), hematocrit (VE: $R = –0.72$, $p = 0.02$), erythrocyte count (VE: $R = –0.68$, $p = 0.03$), and urea (VE: $R = 0.69$, $p = 0.02$).

There has been a linear correlation between L/V and the type of dialyzer. The linear regression equation describing the relationship after HD was calculated as y = 0.2x+0.3. Both the regression coefficient (0.2) and the constant term (0.3) were statistically significant ($p = 0.00006$ and $p = 0.002$, respectively). The correlation coefficient was $R = 0.7$.

Discussion

The contact of blood with a complement-activating dialyzer membrane triggers granulocytopenia and rebound granulocytosis, accompanied by changing expressions of such receptors as Mac-1 (β_2-integrin binding to VCAM-1) and CD62L (L-selectin) [6, 7]. In particular, the "high Mac-1, low CD62L" phenotype characteristic of granulocytes during an HD session has been considered the underlying cause of the above phenomena and the subsequent soluble (s) L-selectin shedding [6, 7, 14]. Additionally, investigations of synthetic membranes has not revealed such changes and, therefore, "high Mac-1, low CD62L" has long been regarded as a perfect biocompatibility index [6, 7]. However, simultaneous evaluation of sL-selectin, strictly connected with CD62L expression, during HD on different membranes has given contradictory results [6, 7, 15]. Moreover, the present assessment of sL-selectin levels failed to differentiate between cellulosic and non-cellulosic dialyzers [15]. The situation was similar when sVCAM-1 concentrations were taken into account [8–10, 15]. Therefore, in the present study it was decided to perform linear regression analysis and search for any relationship between the type of dialysis membrane and sL-selectin and sVCAM-1 concentrations. These two parameters characterizing leukocyte (sL-selectin) and vascular (sVCAM-1) activation were also combined and the usefulness of the sL-selectin/sVCAM-1 ratio (L/V) as a marker of biocompatibility was assessed.

The present investigation revealed a significant decrease in sL-selectin and L/V values as well as sVCAM-1 increase in all HD patients before a single dialysis session compared with controls. These results confirmed previous observations of decreased sL-selectin [7, 15] and enhanced sVCAM-1 [8, 10] concentrations in chronically hemodialyzed patients. There were also significant discrepancies in L/V values between examined dialyzers, whereas sL-selectin and sVCAM-1 values failed to differentiate between these groups. The L/V values in the CU group were the lowest both before and after a single HD session. Moreover, L/V in the VE group was lower than in the PS group, although the difference did not reach statistical significance. These results are concordant with data on differences between the biocompatibility of cellulosic and non-cellulosic membranes as well as discrepancies between vitamin E-coated and synthetic dialyzers [2, 16, 17]. However, in none of the above-mentioned studies were cuprophane, vitamin E-bonded, and polysulfone membranes analyzed together. Nonetheless, the data of the present study suggest that the sL-selectin/

Table 2. Median values of sL-selectin, sVCAM-1, and the sL-selectin/sVCAM-1 ratio (L/V) in the examined groups dialyzed with cuprophane (CU), vitamin E-coated (VE), and polysulfone (PS) membranes

Tabela 2. Mediany wartości stężeń sL-selektyny, sVCAM-1 i wskaźnika sL-selektyna/sVCAM-1 w badanych grupach dializowanych za pomocą błon kuprofanowych (CU), pokrytych witaminą E (VE) i polisulfonowych (PS)

Median values (Mediany)	sL-selectin concentration (Stężenie sL-selektyny) ng/ml			
	before HD (przed HD)	after HD (po HD)	Wilcoxon test before HD vs. after HD	control group (grupa kontrolna) n = 15
CU group, n = 14	1210.0	1285.0	p = 0.95	
VE group, n = 10	1280.0	1245.0 ‡	p = 0.89	6492.6
PS group, n = 8	1580.0	1530.0 §	p = 1.0	
Kruskall-Wallis test (CU, VE, PS)	p = 0.10	p = 0.03	–	–
Median values (Mediany)	sVCAM-1 concentration (stężenie sVCAM-1) ng/ml			
	before HD (przed HD)	after HD (po HD)	Wilcoxon test before HD vs. after HD	control group (grupa kontrolna) n = 15
CU group, n = 14	1782.5	2612.5 †	p = 0.02	
VE group, n = 10	1440.0	1667.5	p = 0.01	625.0
PS group, n = 8	1662.5	1725.0 ¶	p = 0.09	
Kruskall-Wallis test (CU, VE, PS)	p = 0.07	p = 0.00	–	–
Median values (Mediany)	sL-selectin/sVCAM-1 ratio (wskaźnik sL-selektyna/sVCAM-1)			
	before HD (przed HD)	after HD (po HD)	Wilcoxon test before HD vs. after HD	control group (grupa kontrolna) n = 15
CU group, n = 14	0.66*	0.39 †	p = 0.01	
VE group, n = 10	0.88	0.74	p = 0.01	9.62
PS group, n = 8	1.1 ¶	0.92 ¶	p = 0.02	
Kruskall-Wallis test (CU, VE, PS)	p = 0.01	p = 0.01	–	–

Mann-Whitney *U* test results before HD:
* $p = 0.04$ CU vs. VE,
¶ $p = 0.01$ CU vs. PS.

Mann-Whitney *U* test results after HD
† $p = 0.01$ CU vs. VE,
‡ $p = 0.02$ VE vs. PS,
§ $p = 0.02$ CU vs. PS,
¶ $p = 0.01$ CU vs. PS.

Wyniki testu *U* Manna-Whitneya przed HD:
* $p = 0{,}04$ CU vs VE,
¶ $p = 0{,}01$ CU vs PS.

Wyniki testu *U* Manna-Whitneya po HD:
† $p = 0.01$ CU vs VE,
‡ $p = 0.02$ VE vs PS,
§ $p = 0.02$ CU vs PS,
¶ $p = 0.01$ CU vs PS.

/sVCAM-1 ratio differentiates between various membranes more effectively than selected adhesion molecules alone and may be considered a useful marker of biocompatibility. The linear correlations observed between sL-selectin, sVCAM-1, L/V, and the type of dialyzer favor soluble adhesion molecules as possible markers of biocompatibility. In the case of sL-selectin and L/V, such correlations were found both before and after HD, which may suggest that the membrane's impact on sL-selectin extends into the interdialytic period. The discrepancies in L/V values observed before HD may also confirm the persistence of such influence until the next session. Moreover, in both cases the correlation after HD was stronger than before HD, which shows the concurrent dependence of sL-selectin and L/V on a single dialysis session. The fact that the correlation coefficient for the linear regression equation was higher in the case of L/V than in the case of sL-selectin also suggests the superiority of

the sL-selectin/sVCAM-1 ratio over sL-selectin alone in assessing membrane biocompatibility. The sVCAM-1 correlation with membrane type, observed only after HD, may suggest that the influence of a single session on this molecule is less pronounced and does not concern the interdialytic period. The above correlations of sL-selectin, sVCAM-1, and membrane type may also favor a hypothesis formulated by Grooteman et al. [18] that dialyzers influence circulating blood cells rather than endothelial cells. This can also be confirmed by correlations between adhesion molecule, L/V, and neutrophil counts observed only after HD. From a pediatric point of view, this may mean greater impact on leukocyte impairment in HD children and partly clarify why pediatric HD patients are prone to infections. However, the present authors are aware that an investigation on a larger group of patients is needed to confirm this hypothesis.

sL-selectin levels remained unchanged throughout the HD sessions with all the evaluated dialyzers, which is in contrast to reports by Kawabata et al. [6] and Dou et al. [7] on CU-related sL-selectin elevation. The finding of the present study, however, is concordant with the hypothesis of granulocyte hyporesponsiveness presented by Dou et al. [7]. The authors claim that frequent contact of blood with a bioincompatible cellulose membrane causes chronic stimulation and subsequent resistance of granulocytes to repetitive stimuli [7]. Moreover, median sL-selectin values in PS patients after HD were higher than in CU and VE children, thus confirming the weaker impact of polysulfones than other dialyzers on granulocyte impairment. The sVCAM-1 increase after CU and VE sessions, also reported by Mrowka et al. [8], showed the poor biocompatibility of these two membranes compared with PS. Rabb et al. [9] reported on sVCAM-1 decrease due to molecule adsorption on CU membrane after a three-hour session, while the dialyses in the present study lasted for at least four hours. Thus the detachment of bound sVCAM-1 is possible. Moreover, the surfaces of dialyzers used in children are smaller than those used in adults, so the adsorption could be less pronounced.

An interesting finding was that L/V after a single HD session was lower than before HD, irrespective of the dialyzer used. In the case of the CU and VE patients, such changes were partly predictable due to the relative bioincompatibility of thoee membranes. However, the L/V decrease in PS patients is in contrast with other reports in which polysulfone low-flux HD had no influence on evaluated soluble adhesion molecules [6, 15]. In the present material there were also no changes in sL-selectin or sVCAM-1 concentrations after polysulfone dialysis. Therefore, it is suggested that the sL-selectin/sVCAM-1 ratio may be more specific than single adhesion molecules in assessing leukocyte migration disturbances during HD.

The authors conclude that linear correlations observed between the examined molecules and membrane types as well as the significant differences in L/V values between the different dialyzers suggest the possible value of adhesion molecules in assessing membrane biocompatibility. The low L/V levels in CU patients probably show the bioincompatibility of this membrane in comparison with the vitamin E-coated and polysulfone dialyzers. However, the L/V decrease after single hemodialysis was observed in all groups and did not depend on the dialyzer type. These preliminary findings may mean that even an HD session with a biocompatible membrane influences the leukocyte migration process. Such an observation probably suggests the negative impact of hemodialysis on leukocyte function, which may be, at least in part, responsible for the increased incidence of infections in HD children. However, due to the small number of examined patients, the present authors can only speculate on such a hypothesis. Therefore, a large randomized study investigating the influence of membrane bioincompatibility on soluble adhesion molecules is required.

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Address for correspondence:

Danuta Zwolińska
Department of Pediatric Nephrology
Silesian Piasts University of Medicine
M. Skłodowskiej-Curie 50/52
50-369 Wrocław
Poland
Tel.: +48 71 733 13 50
E-mail: zwolin@nefped.am.wroc.pl

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