The effect of renal replacement therapy on the concentration of selected arachidonic acid derivatives

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Abstract
Background: Chronic kidney disease (CKD) is one of the most frequently occurring diseases. A side effect of dialysis is, among other things, platelet activation. Arachidonic acid (AA) derivatives are released from activated platelets. In addition to being involved in many physical processes, they are also involved in the development of inflammation, asthma, cancer, diabetes, hypertension, and the pathogenesis of kidney disease. The aim of the study was to determine the concentrations of arachidonic acid (AA) derivatives (TXB 2, 5-HETE, 12-HETE, 15-HETE), in patients with CKD.
Methods: 117 patients were qualified to the study group. Based on the type of renal replacement therapy, patients are divided into the following groups: hemodialysis, peritoneal dialysis, kidney transplant patients and conservative treatment (30; 30; 27; 30 patients). The control group consisted of 30 healthy volunteers. The concentrations of TXB 2, 5-HETE, 12-HETE, 15-HETE, in the blood serum was measured by ELISA methods.
Results: It was shown that renal replacement therapy significantly influences the concentration of TXB 2 p=0.010 5-HETE p<0.001 and 15-HETE p<0.001). There was a relationship between the type of renal replacement therapy and the duration of dialysis, and the concentration of TXB 2, 12-HETE acid, and 15-HETE (p<0.001; p<0.001;p<0.001).
Conclusions: The type of renal replacement therapy used had an effect on the concentration of arachidonic acid derivatives (TXB2, 5-HETE and 15-HETE). Apart from the type of therapy used in CKD patients, the factors significantly affecting the release of arachidonic acid derivatives were the age of patients, the duration of dialysis, the cause of CKD, and the stage of its advancement.
Background
Chronic kidney disease (CKD) is one of the most frequently occurring diseases and affects 6-15% of the world's population [1,2]. Progression of renal failure aggravates ongoing inflammation and increases oxidative stress [3]. Chronic inflammation leads to activation of the endothelium, increased synthesis of adhesion molecules, penetration of monocytes into the intima of the vessels, as well as stimulation of thrombotic processes [4].
Conservative treatment is used in the initial stages of CKD, and dialysis or renal transplantation is
necessary at the end stage. One side effect of dialysis is the synthesis of pro-inflammatory factors and increased oxidative stress, as a result of the blood coming into contact with artificial materials of dialyzers, and the creation of vascular access which is necessary for dialysis. Consequences of this includes transient leukopenia, activation of platelets and cells of the immune and complement systems, and an increase in interleukin 1 concentration [5].

Platelets also play a very important role during organ transplantation. Their activation is regulated by many cell-derived blood-borne factors, and to a large extent depends on the pro-oxidative-antioxidant balance. Healthy endothelium synthesizes prostacyclin (PGI₂) and nitric oxide to prevent adhesion and activation of platelets. During ischemia and reperfusion of organs undergoing transplantation, there is excessive adhesion of platelets and leukocytes, leading to inflammation and tissue damage. As a result, it can strongly stimulate the immune response, as well as cause an increase in organ alloreactivity [6].

Arachidonic acid (AA) derivatives are released from activated platelets. In addition to being involved in many physical processes, they are also involved in the development of inflammation, asthma, cancer, diabetes, hypertension, and the pathogenesis of kidney disease. Thromboxane A₂ (TXA₂) is mainly synthesized by activated platelets in response to platelet aggregation and vasoconstriction. In solutions, TXA₂ is unstable and rapidly degraded to an inactive but more stable form of TXB₂ [7,8,9]. Although TXA₂ appears to be of minor importance in maintaining renal function under physiological conditions, increased TXA₂ biosynthesis in the kidney is confirmed in various animal models of kidney disease. One of the most important events that occurs in a transplanted organ is ischemia-reperfusion injury (I/R), which unfortunately is an inherent aspect of organ transplantation. [10]. Several authors have already reported that during I/R injury and allograft rejection, there is increased production of thromboxane synthase and consequently increased Thromboxane B₂ (TXB₂) concentration. Inhibition of TXA₂ synthesis during reperfusion significantly improves graft function in animal models of kidney transplantation [11,12].

The 5-, 12-, and 15- hydroxyeicosatetraenoic (HETE) acids are formed from arachidonic acid by the
lipoxygenase pathway [13]. 12-HETE activity is found in platelets as a result of platelet activation by agonists such as thrombin or collagen. The role of the 12-HETE isoform in platelets is not entirely clear, because we know that it does not play a significant role in the direct regulation of platelet function. The relationship between HETE acids, chronic kidney disease, platelet activation, and the type of renal replacement therapy used is not yet fully understood. Studies show that lipoxygenases are involved in kidney damage in the course of diabetic nephropathy, and it has been demonstrated that the urine concentration of 12-HETE significantly increases in this group of patients [14,15]. In addition, 12-HETE, together with 15-HETE, induces the synthesis of TGF-β1(transforming growth factor β1) in mesangial cells, where its action stimulates the synthesis of extracellular matrix proteins that lead to kidney fibrosis.

Knowledge of the relationship between the type of renal replacement therapy used and the level of circulating arachidonic acid derivatives can be extremely important. It has been shown, for example, that during peritoneal dialysis, increased eicosanoids are synthesized by macrophages and peritoneal mesenchymal cells due to the properties of dialysis fluids, which are generally not biocompatible [16]. According to literature data, understanding the relationship between the level of arachidonic acid derivatives and the type of renal replacement therapy used may inform us on the chances of patient survival post-kidney transplantation, whether dialysis is still providing effective treatment for a patient, or which type of renal replacement therapy is appropriate for a given patient.

Methods

1. Study group
There were 147 participants: 117 patients were qualified to the study group. Based on the type of renal replacement therapy, patients are divided into the following groups: before (HD A) and after (HD B) hemodialysis (blood was collected from patients immediately before and after dialysis); peritoneal dialysis (PD), patients before (TE) and after (TE A) kidney transplantations (5-7 days after surgery) and conservative treatment (CT) (CKD stage 2-5) (30; 30; 27; 30 patients). The control group consisted of 30 healthy volunteers [17]. Detailed information regarding the study and control can be found in Tables 1 and 2.
2. Samples

Blood samples (K$_2$EDTA) (7.5 ml) and serum (7.5 ml) were drawn from all study participants. Blood was drawn from hemodialysis patients via their arteriovenous fistula, and peripheral venipuncture was used for all other participants (from hemodialysis patients immediately before and after the dialysis and from transplant patient before transplantation and 5-7 days after surgery. K$_2$EDTA samples were centrifuged at 2600 rpm for 10 minutes at 20°C to obtain plasma respectively [17]. Serum samples were centrifuged at 6000 rpm for 10 minutes at 20°C to obtain serum respectively.

2. Concentrations arachidonic acid derivatives

The concentrations of TXB$_2$, 5-HETE, 12-HETE, 15-HETE were each determined by an ELISA (Quantikine® Colorimetric Sandwich ELISAs, R&D Systems, USA, Quantikine® Colorimetric Sandwich ELISAs, My BioSource, USA; Quantikine® Colorimetric Sandwich ELISAs, Cayman Chemical Company, USA).

3. Statistical analysis

To assess data distributions, Schapiro Wilka test was used, which in the case of some variables (5-HETE concentrations) showed a non-parametric distribution. To analyze quantitative data Exact Fisher and Chi-square tests were used. A T-test and ANOVA were used for univariate systems, the differences between associated (paired) and unrelated (unpaired) (parametric distribution). The Kruskal-Wallis ANOVA was used to evaluate differences, as well as the U - Test for unpaired data or the Wilcoxon test for paired data (non-parametric distributions). A linear multiple regression model was used to determine the multifactor evaluation of relationships between the parameters studied. Statistical analysis of the results was carried out using Statistica PL 12 Trial (StatSoft)[17].

Results

We found a statistically significant relationship between the concentration of TXB$_2$ and the studied groups (p=0.010) (Table 3). The lowest TXB$_2$ concentration was found in the control group and the highest in patients before kidney transplantation (Figure 1). Statistically significant differences in the concentration of thromboxane were also demonstrated in the serum of patients before hemodialysis.
(HD A) and before kidney transplantation (TE), as well as the control group (NK). An association was also found between serum TXB₂ concentrations in group HD B and TE A and HD B and NK. The relationship between the concentration of thromboxane in the serum of patients treated conservatively, before kidney transplantation, and in the control group, as well as between the peritoneal dialysis and control groups, was also demonstrated. Statistically significant differences were also observed between the concentration of TXB₂ in the TE and NK groups, and TE A and NK (Table 4).

There were statistically significant differences between the concentrations of 5-HETE acid in the blood serum of patients in different study groups (p<0.001) (Table 3). The lowest concentration of 5-HETE was obtained in patients before hemodialysis and the highest was in the control group (Figure 2). Differences in the concentration of 5-HETE acid between HD A and PD groups, and TE and NK groups were observed, as well as between HD B and PD groups, TE and TE A groups, and between HD B and NK groups. There was also a relationship between the concentration of 5-HETE acid in the blood serum of patients with peritoneal dialysis and control group, and before and after kidney transplantation with the control group, as well as between patients treated conservatively (CT) and before kidney transplantation (TE) and the control group (NK) (Table 4).

There was no relationship between serum 12-HETE concentrations between the study groups (Table 3). Statistically significant differences occurred between the concentration of 12-HETE in blood serum of patients treated conservatively (CT) and after renal transplantation (TE A) (Table 4), and between the group of patients after transplantation and control group (Table 4).

We found a statistically significant differences between the concentrations of 15-HETE acid in the blood serum of patients in different study groups (p<0.001) (Table 3). The lowest concentrations of 15-HETE acid were observed in patients before hemodialysis and in the control group, and the highest concentration was in the group of patients after hemodialysis (Figure 3). There were also statistically significant differences between the concentration of 15-HETE in the serum of patients before hemodialysis (HD A) and peritoneal dialysis (PD), treated conservatively, and after renal
transplantation (TE A). The analysis shows a relationship between 15-HETE acid concentration in HD B and CT, HD B and TE, and HD B and NK groups. Significant differences in the concentration of 15-HETE acid occurred in patients on peritoneal dialysis, before kidney transplantation, and in the control group, as well as between transplant patients and the control group. There were significant differences in the concentration of 15-HETE acid in the blood serum of patients before and after hemodialysis, and before and after renal transplantation.

Concentration of arachidonic acid derivatives is influenced by sex, duration of dialysis, and the cause and severity of CKD.

We found a correlation between TXB$_2$, 12-HETE acid, and 15-HETE acid concentrations, the type of renal replacement therapy used, and the duration of dialysis. There were also statistically significant differences between the concentration of the examined parameters, the type of renal replacement therapy, and the age of patient (Table 5). We also observed a relationship between the concentration of 5-HETE acid in the blood serum of patients and the stage of chronic kidney disease (Table 5). 5-HETE concentration was highest in patients in the second stage of CKD, and lowest in patients in the fifth stage.

Based on multivariate regression analysis, it was found that parameters such as the type of renal replacement therapy, age of patients, duration of dialysis, causes and stage of CKD had an effect on thromboxane concentration at approximately 37% (Table 6).

Discussion

In patients suffering from chronic kidney disease, there is an increased activation of platelets [5] as a result of dialysis, increased inflammation, and oxidative stress, and hence an increased production of thromboxane and HETE acids, amongst other factors. It has also been shown that peritoneal dialysis leads to an increased synthesis of eicosanoids by peritoneal macrophages and mesenchymal cells due to the properties of the dialysis fluid, which are generally not biocompatible [16]. Therefore, knowledge of the relationship between the type of renal replacement therapy and the level of arachidonic acid derivatives is extremely important. According to the literature, it is possible to use this knowledge to gain insights into the chances of survival of a patient following kidney transplantation.
transplantation, whether dialysis is still providing effective treatment, or which type of renal replacement therapy is suitable for a given patient.

Thromboxane A\textsubscript{2} (TXA\textsubscript{2}) is mainly synthesized by activated platelets, in response to platelet aggregation and vasoconstriction. It has a very short half-life (about 30 seconds). In aqueous solutions, TXA\textsubscript{2} is unstable and rapidly degraded to an inactive but more stable form of TXB\textsubscript{2}.

Although TXA\textsubscript{2} appears to be of minor importance in maintaining renal function under physiological conditions, increased TXA\textsubscript{2} biosynthesis in the kidney is confirmed in various animal models of kidney disease. One of the most important events that occurs in a transplanted organ is ischemia-reperfusion injury (I/R), which unfortunately is an inherent aspect of transplantation. The mechanism of I/R damage includes activation of the inflammatory response, formation of reactive oxygen species, and microcirculation disorders. In addition, several mediators such as TNF-α, endothelin, and arachidonic acid eicosanoid metabolites contribute to such mechanisms, including hydroxyeicosatetraenoic (HETE) acids and thromboxane [10]. Several authors have already reported that during I/R injury and allograft rejection, there is increased production of thromboxane synthase and consequently increased TXB\textsubscript{2} concentration. Inhibition of TXA\textsubscript{2} synthesis during reperfusion significantly improves graft function in animal models of kidney transplantation [10,18].

Kidney mesangial cells and podocytes produce TXA\textsubscript{2}. Glomerulonephritis, cyclosporine overdose, or rejection of kidney transplants reduce renal blood flow to the kidney afferent and efferent arterioles, supplying the glomerulus, reduce mesangial cell count, increase plasminogen activator inhibitor-1 (PAI-1) activity, decrease tissue plasminogen activator (t-PA) activity, and increase TGF-β levels. This leads to the deposition of fibrin and matrix proteins in the glomeruli and mesangium, and leads to the worsening of renal failure [19,20].

In our study, a statistically significant relationship was found between the concentration of thromboxane B\textsubscript{2} and the groups studied. The lowest concentration of TXB\textsubscript{2} was found in the control group and the highest in patients before renal transplantation. Statistically significant differences were also observed between the concentration of TXB\textsubscript{2} in the TE and NK groups and TE A and NK. In
patients after kidney transplantation, a decrease in thromboxane concentration was observed, however, it was not statistically significant. There was no statistically significant difference between TXB$_2$ concentration in patients before or after hemodialysis. On the other hand, there were significant differences between the concentration of thromboxane in hemodialysis patients and peritoneal dialysis, with a lower concentration of TBX$_2$ in the PD group.

In the literature, there are many reports on the concentration of thromboxane in patients with different types of renal replacement therapy. Dołęgowska et al. showed that kidney transplantation is associated with changes in TXB$_2$ concentration, and that thromboxane alone may be a marker of organ function. In addition, after kidney transplant patients were divided into three groups: early graft function (EGF), slow graft function (SGF), and delayed graft function (DGF), the authors showed that the concentration of thromboxane increased within the first five minutes after transplantation in each of these groups [10]. However, our own studies showed a decrease in TXB$_2$ concentration within a few days after kidney transplantation. Averna et al. showed that the administration of drugs that reduce or eliminate thromboxane-dependent activation of platelets in vivo may reduce the risk of cardiovascular events but may also prevent the long-term survival of patients with kidney transplantation [21]. Considering that an increase in thromboxane concentration may be indicative of transplant rejection and may lead to an increase in TGF-β concentration, which is also a sign of poor functioning of the transplanted kidney [19,20], together with the results obtained in our own studies, it can be suggested that the decrease in TXB$_2$ and TGF-β after kidney transplantation may be a sign of good prognosis for this group of patients. The relationship between the concentration of thromboxane and TGF-β is also confirmed by correlations obtained in our own studies. A positive correlation was found between the concentrations of thromboxane and TGF-β and platelet-derived growth factor-B (PDGF-B) in patients treated conservatively, as well as the positive correlation between PDGF-B and TXB$_2$ concentrations after renal transplantation, and a strong positive correlation between the concentration of thromboxane and TGF-β in the control group. Orlińska et al. showed that both exogenous and endogenous transforming growth factor (TGF) regulates the
production of thromboxane, and elevated levels of TGF-β lead to the increased production of TXB$_2$ [22].

The data also supports the hypothesis that a lower concentration of TXB$_2$ and TGF-β after transplantation is a good prognosis for these patients, because no significant correlation was found between TXB$_2$ and TGF-β after kidney transplantation. Stępniewska et al. observed a significantly lower concentration of thromboxane in hemodialysis patients than in peritoneal dialyses patients and in those treated conservatively (stage 3–5). They also showed that the type of renal replacement therapy affects the concentration of arachidonic acid metabolites, and the concentrations of thromboxane, 20-HETE acid and 15-HETE acid can be indicators of kidney damage and possible cardiovascular diseases [19]. Zhao et al. in turn found that in patients on peritoneal dialysis, there was an increased synthesis of eicosanoids by macrophages and peritoneal mesenchymal cells due to the properties of dialysis fluids, which are generally not biocompatible [16].

In other studies, however, the platelets of patients undergoing regular hemodialysis have been shown to be exposed to increased oxidative stress due to endothelial damage and carbohydrate and lipid metabolism disorders. They are activated excessively because their function is weakened due to ineffective antioxidant activity [23,24]. Platelets are the main source of TXB$_2$, and so the excessive activation of platelets may lead to an increased release of TXB$_2$.

In our own studies, thromboxane concentrations were low in the PD group, and significantly higher in the groups before and after hemodialysis, as well as in patients treated conservatively. This may support the thesis that platelets are excessively activated during hemodialysis and that these patients are more exposed to oxidative stress than PD patients.

The 5-, 12-, and 15-HETE acids are formed from arachidonic acid on the lipoxygenase pathway [153]. The relationship between HETE acids, chronic kidney disease, and platelet activation and the type of renal replacement therapy is not yet fully understood. Studies show that lipoxygenases are involved in kidney damage in the course of diabetic nephropathy, and the concentration of 12-HETE acid in urine significantly increases in this group of patients. Higher expression of 12/15 LOX (12/15
lipoxygenase) is associated with an increase in fibronectin and other mediators of diabetic nephropathy [25,26].

According to the latest research, HETE acids can strongly influence the intensity of the inflammatory process. Namely, 5-HETE levels promote the production of T lymphocytes, whereas 12-HETE and 15-HETE stimulate the overexpression of pro-inflammatory genes in macrophages. In addition, 12-HETE, together with 15-HETE, induces the synthesis of TGF-β1 in mesangial cells, where it stimulates the synthesis of extracellular matrix proteins that lead to kidney fibrosis. Interestingly, these HETE activities are exerted only in an autocrine or paracrine manner due to the unstable nature of HETE and its very short half-life [27, 28, 29, 30,31].

It has also been shown that AA lipoxygenase derivatives are involved in the regulation of blood pressure. Increased urinary excretion of 12-HETE was found in patients with primary hypertension [11]. HETE acids may also affect early kidney transplantation, as evidenced by significant changes in 5-HETE, 12-HETE, and 15-HETE concentrations after renal transplantation [27,32]. Matsuyama et al. reported that the activity of AA derivatives formed on the cyclooxygenase and lipoxygenase pathway correlates with the intensity of I/R [27,33]. In addition, several other authors report that elevated HETE levels in animals were detected during allograft rejection. These observations clearly justify the need to study this pathway of AA metabolism during human kidney transplantation [16, 27, 34, 35].

Wang et al. have shown that free fatty acids (FFA) are best removed during low flow hemodialysis. As much as 60% of FFA is removed from the plasma after 4 hours of hemodialysis. Lipids with a higher molecular weight such as triglycerides and sphingomyelin are not effectively removed. The concentration of FFA and SFA (saturated fatty acids) is increased between successive hemodialysis procedures, which is crucial to prevent the risk of cardiovascular events [36]. During peritoneal dialysis, however, there is an increased synthesis of eicosanoids by macrophages and peritoneal mesenchymal cells due to the properties of dialysis fluids, which are generally not biocompatible. The volume and nutritional status of peritoneal dialysis patients also affect the plasma lipid profile and are associated with inflammatory biomarkers (e.g., isoprostanes) and oxidative stress [23]. In our own studies, confirmation was obtained in the form of significantly higher concentrations of 5-HETE and
15-HETE in the group PD than in the group before hemodialysis, and also after hemodialysis in the case of 5-HETE.

Stępniewska et al. did not demonstrate a relationship between the concentration of 5- and 15-HETE acids and the type of renal replacement therapy used [19]. In the studies described in this work, however, this relationship was found. The lowest concentration of 5-HETE was observed in patients before hemodialysis and the highest in the control group. In the case of 15-HETE acid, the lowest concentration was observed in patients before hemodialysis and in the control group, and the highest concentration in the group of patients after hemodialysis. Reinhold et al. studied the relationship between concentrations 12- and 15-HETE and the function of a transplanted kidney. In this study, they observed a correlation between the concentration of HETE acids and kidney function two weeks post-transplantation but did not find a relationship between 12- and 15-HETE concentrations and acute transplant rejection [37].

Dołęgowska et al. showed that kidney transplants in humans are accompanied by significant perioperative changes in the metabolism of AA derivatives arising on the LOX pathway, expressed by changes in 5-, 12- and 15-HETE concentrations. These changes concern early kidney function after transplantation. In addition, after division of kidney transplant patients into three graft function groups, early, slow, and delayed, 5-, 12- and 15-HETE concentrations decreased in the first 5 minutes after transplantation in both the slow and delayed graft function groups, but not the early graft function group [16]. HETE acids may in the future serve as new perioperative predictor of early organ function after transplantation. Dołęgowska et al., however, confirmed the results obtained by Reinhard et al. showing no relationship between HETE concentration and acute rejection of the transplant. This study also updates the hypothesis previously suggested by other scientists, which is that knowledge of AA metabolism in the early phase of allograft reperfusion may offer a completely new way to attenuate reperfusion injury during organ transplantation in humans [16].

In our study, there was a significant relationship between 5-HETE concentration before and after kidney transplantation and the control group. The concentration of 5-HETE was highest in the control group, and lowest after kidney transplantation. There was also a significant difference between 15-
HETE concentration before and after kidney transplantation, with an increase in the concentration of 15-HETE acid after kidney transplantation. There was no relationship between serum 12-HETE concentrations between the groups, however, a significantly lower concentration of 12-HETE acid after renal transplantation was demonstrated compared to the control group and patients who were treated conservatively. Considering the results obtained by other scientists, and taking into account the high importance of these acids in the body's inflammatory response, an increase in the concentration of HETE acids after transplantation may indicate poor functioning of a transplanted kidney.

On the basis of our own results, it is difficult to state clearly whether the concentration of HETE acids can indicate the possibility of graft rejection or not, due to the increase in 15-HETE acid concentration and decrease in 5-HETE acid concentration after kidney transplantation, and the lower concentration of 12-HETE compared, for example, compared with patients treated conservatively or the control group. Long-term observation of patients after kidney transplantation would be necessary to fully understand the importance of HETE acids in predicting graft rejection.

It should also be pointed out that eicosanoids are mediators of inflammation, and can also influence the metabolism of fibroblasts in wound healing processes and the reorganization of connective tissue [38]. The creation of vascular access in hemodialysis patients would also explain the increase in systemic compounds whose function is the chemotaxis of fibroblasts and the stimulation of healing processes.

In our study, there was a relationship between the concentration of thromboxane, 12-HETE and 15-HETE, and the duration of dialysis and the type of therapy used. It has been shown that the longer the dialysis takes, the lower the concentration of TXB₂ and 15-HETE acid. In the case of 12-HETE acid, its highest concentration occurred in patients before kidney transplantation, who, on average, had the longest duration of dialysis. In addition, based on a multivariate regression analysis, it was found that parameters such as the type of renal replacement therapy, age of patients, duration of dialysis, cause and stage of CKD had an effect on thromboxane concentration in 37% of. The type of renal replacement therapy (p = 0.042) and duration dialysis (p = 0.004) both had a significant effect on the
concentration of thromboxane.

The results obtained in this study do not confirm those obtained by other scientists, except the concentration of 12-HETE, which indicates increased activation of platelets in patients undergoing long-term dialysis. Because increased production of thromboxane leads to the deposition of fibrin and matrix proteins in the glomeruli and mesangium, and subsequently leads to the worsening of renal failure [19,20], a lower thromboxane concentration is an indicator of good prognosis for patients on long-term dialysis. A decreasing concentration of 15-HETE may in turn indicate a lower chance of graft rejection after transplant.

There are no reports on the dependence of 5-HETE on the subject of chronic disease and the stage of chronic kidney disease. It has been shown, however, that supplementation with polyunsaturated fatty acids (PUFA) over a period of 8 weeks results in a decrease in the pro-inflammatory leukotriene-B4 (LTB4) and 5-HETE, and increases the synthesis of less inflammatory leukotriene, LTB5 and 5-hydroperoxyeicosatetraenoic (5-HPETE) in patients with CKD stages 2-5 [39]. In our own studies, the concentration of 5-HETE decreased as kidney disease progressed. This may indicate less pro-inflammatory processes and a good prognosis for patients in the fifth stage of CKD.

Conclusions
1. The type of renal replacement therapy used significantly affects the level of arachidonic acid derivatives (TXB2, 5-HETE and 15-HETE).

2. Apart from the type of therapy used in CKD patients, the factors significantly affecting the release of arachidonic acid derivatives were the age of patients, the duration of dialysis, the cause of CKD, and the stage of its advancement.

3. Concentrations of arachidonic acid derivatives in patients with CKD may help us on the selection of appropriate renal replacement therapy, prognosis of patients after kidney transplantation or long-term dialysis.

Abbreviations
12/15 LOX -12/15 lipoxygenase

12-HETE - 12- hydroxyeicosatetraenoic acid
15-HETE – 15-hydroxyeicosatetraenoic acid
5-HETE – 5-hydroxyeicosatetraenoic acid
AA – arachidonic acid
CKD – chronic kidney disease
CT – conservative treatment
DGF - delayed graft function
EGF - early graft function
FFA - fatty acids
HD A – before hemodialysis group
HD B – after hemodialysis group
I/R – ischemia-reperfusion injury
LOX – lipoxygenase
LTB4 - pro-inflammatory leukotriene-B4
NK – control group
PAI-1 – plasminogen activator inhibitor-1
PD – peritoneal dialysis
PGI2 – prostacyclin I2
PUFA - polyunsaturated fatty acids
SGF- slow graft function
TE – before transplantation group
TE A after transplantation group
TGF-β1 - transforming growth factor β1
t-PA tissue plasminogen activator
TXA2Thromboxane A2
TXB2 – Tromboxane B2
Declarations
Ethics approval and consent to participate:
The Bioethical Commission at the Pomeranian Medical University in Szczecin approved the research carried out (no KB-0012/36/11). All participants, including the healthy volunteers in the control group, were informed about the purpose and scope of the study and gave their consent to donate samples and for the resulting data to be published.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication:
Written informed consent was obtained from the patient for publication of this study.

Availability of data and materials
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interest:
The authors declare that they have no competing interests.

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Authors' contributions
ECH - writing an article, project originator
collecting material
RH - statistical analysis
MW - collecting material
NS - translation, literature analysis
BD - substantive correction of the article
All authors have read and approved the manuscript.

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Tables
Table 1. General characteristics of hemodialyzed patients (HD), peritoneal dialysis (PD) treated conservatively (CT), kidney transplantation (TE) and control group (NK) participating in the study (mean ± OS)
| Parameters                      | HD    | PD    | CT    | TE    | NK    |
|--------------------------------|-------|-------|-------|-------|-------|
| Gender                         | M-18  | M-16  | M-17  | M-14  | M-18  |
| [M- male; F - female]          | K-12  | K-14  | K-13  | K-13  | K-12  |
| Age [years]                    | 63±16 | 55±15 | 66±15 | 57±11 | 50±8  |
| Dialysis duration [months]     | 25±16 | 26±22 | -     | 54±34 | -     |
| Causes of CKD                  |       |       |       |       |       |
| 1 - DM                         | 5 (17%)| 5 (17%)| 4 (13%)| 1 (4%)|       |
| 2 - HA                         | 15 (50%)| 3 (10%)| 6 (20%)| 0 (0%)|       |
| 3 - GID                        | 2 (7%) | 9 (30%)| 6 (20%)| 3 (11%)|       |
| 4 - ADPKD                      | 0 (0%) | 0 (0 %)| 4 (13%)| 2 (7%) |       |
| 5 - other                      | 5 (17%)| 10 (33%)| 4 (13%)| 6 (22%)|       |
| 6 - unknown                    | 3 (10 %)| 3 (10%)| 6 (20%)| 15 (56%)|       |

*P* - statistical significance for differences between HD, PD and CT groups, TE and NK exact Fisher test for qualitative variables; for quantitative variables - one-way ANOVA and; 

*P** - statistical significance for differences between HD, PD and CT groups and TE exact Fisher test for qualitative variables for quantitative variables - one-way ANOVA or; DM - diabetic nephropathy; HA - hypertension; GID- glomerular inflammation kidney; ADPKD - polycystic kidney disease inherited autosomal dominant; NS - no statistically significant differences.

Table 2. General characteristics of hemodialysis patients (A - before, B - after HD), peritoneal dialysis (PD) treated conservatively (CT)before and after kidney transplantation (TE and TE A) and control group (NK) taking partin the study (mean ± OS).
### Parameters

| Parameters       | HD A       | HD B       | PD         | CT         | TE         | TE A       | NK         |
|------------------|------------|------------|------------|------------|------------|------------|------------|
| Kt/V             | 1,28± 0,21 | -          | 2,77± 1,06 | -          | -          | -          | -          |

### Concentration of creatinine [mg/dl]

|                  |            |            |            |            |            |            |            |
|------------------|------------|------------|------------|------------|------------|------------|------------|
| HD A             | 7,9± 2,4   | 3,5 ± 1,3  | 4,4± 2,2   | 2,5 ± 1,1  | 3,4±2,9    | 0,8± (     |
| HD B             |            |            |            |            |            |            |            |
| PD               |            |            |            |            |            |            |            |
| CT               |            |            |            |            |            |            |            |
| TE               |            |            |            |            |            |            |            |
| TE A             |            |            |            |            |            |            |            |
| NK               |            |            |            |            |            |            |            |

### Stage of CKD:

| Stage | HD A | HD B | PD | CT | TE | TE A | NK |
|-------|------|------|----|----|----|------|----|
| 1     |      |      |    |    |    |      |    |
| 2     |      |      |    |    |    |      |    |
| 3     |      |      |    |    |    |      |    |
| 4     |      |      |    |    |    |      |    |
| 5     |      |      |    |    |    |      |    |

### Table 3 Concentration of platelet-derived growth factors of patients with chronic renal hemodialyzed disease (before and after HD A, HD B), peritoneal dialysis (PD), conservative treatment (CT), before and after kidney transplantation (TE, TE A) and in the control group (NK) (mean ± OS, median – lower and upper quartile).

| Concentration of arachidonic acid derivatives | TXB$_2$ [ng/mL] | 5-HETE [ng/mL] | 12-HETE [ng/mL] | 15-HETE [ng/mL] |
|-----------------------------------------------|------------------|----------------|------------------|-----------------|
| Groups                                       |                  |                |                  |                 |
| HD A                                         | 34,6 ± 9         | 284,2 ± 428,4  | 3,0 ± 0,4        |                 |
|       | mean | min | max | median | min | max | median |
|-------|------|-----|-----|--------|-----|-----|--------|
| HD B  | 34.3 ± 10.5 | 304.8 ± 516.2 | 3.1 ± 0.6 | 38.5 (30.7; 40.9) | 65.92 (1.11; 1580) | 3.0 (1.3; 4.9) |
| PD    | 28.3 ± 15.2 | 530.0 ± 553.3 | 3.1 ± 2.1 | 29.8 (17.8; 37.4) | 199.4 (11.6; 1511) | 3.0 (0.1; 13.2) |
| CT    | 34.2 ± 8.0 | 318.7 ± 366.0 | 3.0 ± 0.8 | 35.6 (26.9; 42.3) | 151.4 (8.4; 1333.3) | 3.2 (0.1; 4.1) |
| TE    | 36.7 ± 42.9 | 525.6 ± 358.0 | 3.7 ± 1.9 | 23.16 (14.18; 38.7) | 622.6 (25.7; 1219.0) | 2.8 (1.8; 8.8) |
| TE A  | 27.9 ± 8.8 | 409.8 ± 377.1 | 2.8 ± 0.8 | 26.7 (20.9; 34.7) | 265.4 (13.6; 1157) | 2.7 (0.1; 4.8) |
| NK    | 19.6 ± 15 | 838.1 ± 497.8 | 3.3 ± 1.0 | 18.3 (10.0; 25.5) | 792.0 (21.9; 1933.8) | 3.1 (1.5; 6.9) |
Table 4. Statistical differences in the concentration of arachidonic acid derivatives, between the studied groups (p-value)

|       | Groups  | TXB<sub>2</sub> |     |     |     |     |     |
|-------|---------|----------------|-----|-----|-----|-----|-----|
|       |         | HD A | HD B | PD  | CT  | TE  | TE A | NK |
| HD A  | -       | NS   | NS   | NS  | NS  | NS  | 0.006| <0.001|
| HD B  | NS      | -    | NS   | NS  | NS  | NS  | 0.016| <0.001|
| PD    | NS      | NS   | -    | NS  | NS  | NS  | NS  | NS |
| CT    | NS      | NS   | NS   | -   | NS  | NS  | 0.007| <0.001|
| TE    | NS      | NS   | NS   | NS  | -   | NS  | 0.016| <0.001|
| TE A  | 0.006   | 0.016| NS   | 0.007| NS | -   | 0.01|
| NK    | <0.001  | <0.001| NS   | <0.001| 0.048| 0.015| -  |

|       | Groups  | 5-HETE |     |     |     |     |     |
|-------|---------|---------|-----|-----|-----|-----|-----|
|       |         | HD A | HD B | PD  | CT  | TE  | TE A | NK |
| HD A  | -       | NS   | 0.028| NS  | 0.004| NS  | NS  | <0.001|
| HD B  | NS      | 0.008| -    | NS  | NS  | NS  | NS  | NS |
| PD    | 0.028   | 0.008| -    | NS  | NS  | NS  | NS  | 0.01|
| CT    | NS      | NS   | NS   | NS  | -   | 0.034| NS  | <0.001|
| TE    | 0.004   | 0.002| NS   | 0.034| -  | NS  | NS  | NS |
| TE A  | NS      | 0.017| NS   | NS  | NS  | NS  | -   | NS |
| NK    | <0.001  | <0.001| 0.013| <0.001| NS | NS  | NS  | -  |

|       | Groups  | 12-HETE |     |     |     |     |     |
|-------|---------|---------|-----|-----|-----|-----|-----|
|       |         | HD A | HD B | PD  | CT  | TE  | TE A | NK |
| HD A  | -       | NS   | NS   | NS  | NS  | NS  | NS  | NS |
| HD B  | NS      | NS   | -    | NS  | NS  | NS  | NS  | NS |
| PD    | NS      | NS   | NS   | NS  | NS  | NS  | NS  | NS |
| CT    | NS      | NS   | NS   | -   | NS  | 0.013| NS  | NS |
| TE    | NS      | NS   | NS   | NS  | -   | NS  | 0.04 | NS |
| TE A  | NS      | NS   | NS   | NS  | 0.013| NS  | -   | 0.04|
| NK    | NS      | NS   | NS   | NS  | 0.048| NS  | -   | NS |

|       | Groups  | 15-HETE |     |     |     |     |     |
|-------|---------|---------|-----|-----|-----|-----|-----|
|       |         | HD A | HD B | PD  | CT  | TE  | TE A | NK |
| HD A  | -       | <0.001| <0.001| <0.001| NS  | <0.001| NS  | NS |
| HD B  | <0.001  | -    | NS   | 0.028| <0.001| NS  | <0.001| NS |
| PD    | <0.001  | NS   | -    | NS  | <0.001| NS  | 0.00 | NS |
| CT    | <0.001  | 0.028| NS   | -   | NS  | 0.013| NS  | NS |
| TE    | NS      | <0.001| <0.001| NS  | -   | <0.001| NS  | NS |
| TE A  | <0.001  | NS   | NS   | 0.013| <0.001| -   | 0.01 | NS |
| NK    | NS      | <0.001| 0.001| NS  | NS  | 0.010| -   | NS |

NS- No significant

Table 5. The influence of particular parameters on concentrations of arachidonic acid derivatives.
The table presents p values defining statistical significance. The relationship between gender, duration of dialysis, age, stage of chronic kidney disease and the causes of chronic kidney disease and the concentrations of arachidonic acid derivatives was assessed using one-way ANOVA.

HD and duration of dialysis - the relationship between the type of therapy (hemodialysis, peritoneal dialysis, patients before kidney transplantation), duration of dialysis and the concentrations of arachidonic acid derivatives

HD and age - dependence between the studied groups (hemodialysis, peritoneal dialysis, conservative treatment, patients before kidney transplantation and control group), patients' age and concentrations of arachidonic acid derivatives

The stage of chronic kidney disease - the relationship between the severity of chronic kidney disease based on eGFR and the activity of concentrations of arachidonic acid derivatives

The causes of chronic kidney disease - the relationship between selected causes of chronic disease and the concentrations of arachidonic acid derivatives

NS - no statistically significant relationship was found.

Table 6. Analysis of the impact of the tested parameters on the concentration and activity values of the tested parameters - multifactorial regression analysis.
| Dependent variable | Independent variable          | β    | R^2  | p    | p  for the model |
|-------------------|-------------------------------|------|------|------|------------------|
| TXB₂              | Renal replacement therapy     | -4.65| 0.37 | 0.042| <0.001           |
|                   | Age                           | -11.75|      |      | 0.07             |
|                   | Duration of dialysis          | -64.57|      |      | 0.004            |
|                   | Stage of CKD                  | 0.28 |      |      | 0.056            |
|                   | Causes of CKD                | -18.36|      |      | 0.14             |

β – standardized coefficient in the regression equation, R^2 – coefficient of determination, p – p-value, TXB₂ – tromboxan

Figures
ANOVA analysis of the relationship between the type of renal replacement therapy and the concentration of TXB2. TXB2 concentration - differences between NK groups, CT, HD A, HD B, PD, TE, TE A (p = 0.010). NK- control group CT - treated conservatively; HD A - before hemodialysis; HD B - after hemodialysis; PD - peritoneal dialysis; TE- before kidney transplantation: TE A - after kidney transplantation.
Kruskal Wallis's ANOVA analysis of the relationship between the type renal replacement therapy and the concentration of 5-HETE. 5-HETE concentration - differences between CT, HD A, HD B, PD, TE, TE A, NK groups (p < 0.001). NK - control group CT - treated conservatively; HD A - before hemodialysis; HD B - after hemodialysis; PD - peritoneal dialysis; TE - before kidney transplantation; TE A - after kidney transplantation.
ANOVA analysis of the relationship between the type of renal replacement therapy and the concentration of 15-HETE. 15-HETE concentration - differences between NK groups, CT, HD A, HD B, PD, TE, TE A (p <0.001). NK- control group CT - treated conservatively; HD A - before hemodialysis; HD B - after hemodialysis; PD - peritoneal dialysis; TE - before kidney transplantation: TE A - after kidney transplantation.