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The Clinicopathological Determinants of Native Arteriovenous Fistula Failure in Patients on Maintenance Hemodialysis*

Uwarunkowania kliniczne i histopatologiczne niewydolności przetoki z naczyń własnych pacjenta u chorych przewlekle hemodializowanych

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Progressive narrowing of the venous part of dialysis fistulae is caused by hemodynamic and inflammatory factors.
Objectives. The pathogenic and clinical determinants of deterioration of the functioning of arteriovenous fistulae in chronically hemodialyzed patients were evaluated.
Material and Methods. The hemodynamic parameters and the activity of inflammatory growth factors in the vessel wall of newly implanted fistulae were assessed and correlated with the clinical course of 34 hemodialyzed patients. Measurements taken at the time of implanting the fistulae included blood flow in the venous part of the anastomosis and its widest diameter by ultrasound Power Doppler, a histopathologic examination of fistula wall samples and measurements of mRNA expression for growth factors PDGFβ1 and TGFβ in the fistula wall. The results were correlated with clinical data from 36 months' observation: duration of fistula maturation, adequacy of dialysis treatment (eKt/V), the patient's survival, morbidity linked with vascular access problems and general cardiovascular morbidity.
Results. The mean duration of fistula maturation was 44.9 days (N = 43, SD = 38.6), whereas the average duration of fistula usage as dialysis access was 795.9 ± 480.6 days. Fistula blood flow at the time of implantation, averaged 1782.2 ± 1735.3 ml/min. The mean number of hospitalization days due to vascular access morbidity was 9.9 ± 15.6 days and it correlated positively with the fistula blood flow (R = 0.596, P = 0.004). There was a negative correlation between the expression of PDGFβ1 mRNA and fistula blood flow (R = –0.673, P = 0.011), as well as between TGFβ expression and patient survival (R = –0.722, P = 0.002).
Conclusions. Inflammatory activity of the vessel wall growth factors PDGFβ1 and TGFβ implies impairment of fistula function and the patient's cardiovascular morbidity (**Adv Clin Exp Med 2013, 22, 4, 495–500**).

Key words: maintenance hemodialysis, dialysis fistula, hemodynamic parameters, growth factors, morbidity, patient's survival.

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Streszczenie

Wprowadzenie. Postępujące zwężenie żylnej części przetoki dializacyjnej jest spowodowane czynnikami hemodynamicznymi i zapalnymi.
Cel pracy. Ocena wyznaczników patogenetycznych i klinicznych dysfunkcji przetoki tętniczo-żylnej z naczyń własnych pacjenta u chorych przewlekle hemodializowanych.
Materiał i metody. Oceniano wskaźniki hemodynamiczne i aktywność prozapalnych czynników wzrostu w ścianie naczynia zespolenia tętniczo-żylnego po jego implantacji, po czym badano istotność korelacji tych czynników z parametrami klinicznymi u 34 hemodializowanych pacjentów obserwowanych w ciągu 36 miesięcy. Bezpośrednio po implantacji zespolenia badano szybkość przepływu krwi w jego żylnej części oraz jej największą średnicę za pomocą ultradźwiękowej techniki dopplerowskiej, oceniano histopatologicznie preparaty wycinków ściany naczynia, mierząc przy tym ekspresję mRNA dla czynników wzrostu PDGFβ1 i TGFβ. Wyniki korelowano z danymi klinicznymi zebranymi w czasie 36-miesięcznej obserwacji tych pacjentów: czasem „dojrzewania" zespolenia, adekwatnością dializoterapii (eKt/V), czasem przeżycia pacjentów, ich chorobowością związaną z dostępem naczyniowym i ogólną chorobowością z przyczyn sercowo-naczyniowych.
Wyniki. Średnia liczba dni hospitalizacji z powodu powikłań dostępu naczyniowego w czasie obserwacji wyniosła 9,9 ± 15,6 dnia i była dodatnio skorelowana z przepływem krwi w żylnej części przetoki (R = 0,596; p = 0,004). Ekspresja mRNA dla PDGFβ1 wykazywała znamienną negatywną korelację z szybkością przepływu krwi przez przetokę (R = –0,673; p = 0,011), a ekspresja mRNA dla TGFβ korelowała negatywnie z okresem przeżycia pacjentów w ciągu 36-miesięcznej obserwacji (R = –0,722; p = 0,002).
Wnioski. Wzmożona aktywność genów dla prozapalnych czynników wzrostu PDGFβ1 i TGFβ w ścianie zespolenia zapowiada dysfunkcję przetoki i skrócone przeżycie pacjentów hemodializowanych (**Adv Clin Exp Med 2013, 22, 4, 495–500**).

Słowa kluczowe: przetoka dializacyjna, czynniki wzrostu, parametry hemodynamiczne, przewlekła hemodializoterapia.

Vascular access problems limit dialysis delivery, compromising the efficacy of treatment and resulting in increased patent morbidity and mortality [1]. The most frequent abnormality is thrombosis caused by local stenosis with subsequent cessation of fistula blood flow [2]. The condition of the vascular endothelium lining the inner surface of arteriovenous anastomoses is influenced by many factors, such as the hemodynamic characteristics of the fistula blood flow and the local inflammatory environment [3].

In this study, the hemodynamic and histopathological determinants of failing native fistulae in chronically hemodialyzed patients were analyzed, and possible correlations between these parameters and the clinical course in these patients were investigated. The main positive correlates of patents' vascular access morbidity were the fistula hemodynamic characteristics, while fistula wall expression of genes for the inflammatory growth factors PDGFβ1 and TGFβ showed a significant negative correlation with fistula blood flow and patient survival during the three-years follow-up. The authors infer from these results that inflammatory conditions that already affect vascular walls in the predialysis period may have a negative impact on patients' survival in the early course of maintenance dialysis therapy.

Material and Methods

Design of the Study

Patients were included in study group upon the creation of an arteriovenous fistula as vascular access for chronic hemodialysis treatment. At the time of enrollment a sample of the venous part of anastomosis was obtained to assess its histopathological characteristics and the expression of mRNA for PDGFβ1 and TGFβ. Doppler Ultrasound (DU) examinations analyzing the fistula blood flow and anastomosis diameter were performed four weeks after the first dialysis and then at three month intervals or whenever fistula failure was suspected during physical examination and observation of the dialysis parameters. Patients were observed prospectively for the 36 subsequent months, noting the following clinical parameters every three months: the adequacy of dialysis (eKT/V); the number of dialysis days with inadequate, reduced blood flow; the number of days with the use of a temporary catheter to substitute for a failing fistula; the patient's survival time and morbidity (number of hospitalization days) due to vascular access problems; and overall cardiovascular morbidity. The results of the histopathological assessments of the fistulae and the measurements of mRNA for growth factors that were done at the start of the study were then correlated with the clinical parameters collected during the observation period.

Study Subjects

The patients enrolled were treated with maintenance hemodialysis for end-stage renal disease at the Fresenius Nephrocare Dialysis Center and the Dialysis Center of the Regional Specialist Hospital in Wroclaw, Poland. The study group consisted of 34 participants (17 males and 17 females), mean age 66.4 years (27–86 years). The reasons for renal failure in these patients included diabetic nephropathy (29.4%), hypertensive nephropathy (23.5%), glomerulonephritis (17.6%), chronic pyelonephritis (5.9%) and systemic vasculitis (5.9%). The causes in the remaining 17.7% were not determined. The control group for the real-time polymerase chain reaction (RT-PCR) studies included 10 samples of vein wall collected during reparative surgery for traumatic hand injury.

Each study subject gave his or her informed consent for participation. The protocol of the study was prepared in accordance with the Helsinki Declaration and was approved by the Bioethical Committee of Wroclaw Medical University.

Histopathological Study

Slices of the venous part of an anastomosis (1.5 mm thick) were collected during the creation of a dialysis fistula, fixed in Methacarn solution (Sigma), dehydrated and embedded in paraffin. Subsequently the paraffin blocks were cut into slices, rehydrated, stained with hematoxylin-eosin and inspected under light microscope magnification (x400). Each time the thickness of the vessel wall was measured and the mean value of the shortest and longest diameter was calculated (ten preparations per individual). The results were expressed as mean ± SD for 10 preparations.

Expression of mRNA for PDGFβ1 and TGFβ

Assessment of mRNA activity for growth factors PDGFβ1 and TGFβ in the venous part of the fistula walls was performed on slices (1.5 mm) collected during creation of anastomosis and preserved in RNAlater solution (Sigma Aldrich). Isolation of the total RNA was accomplished with the EZNA Total RNA Kit (Omega Bio-Tek), following the protocol provided by the manufacturer. After isolation, the concentration of tRNA in each sample was checked with a NanoDrop spectrophotometer (Thermo Scientific) at the 260 nm wavelength. The RT-PCR reaction was then performed using a High Capacity cDNA Reverse Transcription Kit with RNAse Inhibitor (Applied Biosystems). Expression of the following genes was assessed: PDGFβ1, PDGFβ, TGFβ and GADPH as a "housekeeping gene" (positive control). An ABI PRISM 7900 HT Fast RT-PCR System (Applied Biosystems) thermocycler was used with 45 cycles at 95°C. Gene expression was calculated from the standard curve of fluorescence of the reporter dye versus time (cycle number).

Duplex Doppler Ultrasound Examination

A BK Pro Focus device (BK Medical) with a 12 MHz vascular head was used for the DU, examining a segment of the venous part of fistula 20 cm from the anastomosis. Each time the widest diameter of the vessel and blood flow were measured. The latter was calculated from the formula: $F_b = \pi (D/2)^2 V$, where F_b= blood flow, D = vessel diameter and V = mean velocity of blood flow. Measurements were taken routinely every three months, or at any clinical symptom of a failing fistula.

Clinical Data

For the purposes of the study the medical histories and dialysis reports of all the participants were analyzed. The patients were enrolled between November 1, 2006, and November 30, 2010. In each case the following data were noted: the duration of fistula maturation (the time from creation to first dialysis), the number of inadequate dialyses (reduced blood flow due to fistula insufficiency, single needle dialyses), the number of days using a temporary catheter due to a failing fistula, patient morbidity (the number of hospitalization days) caused by vascular access problems, general cardiovascular morbidity and patient mortality (the number of days from fistula creation to death).

Statistical Analysis

The distribution of variables was checked using the Shapiro-Wilk test; the significance of differences between the mean values of normally distributed variables was assessed with Student's t-test; and the Mann-Whitney U test was used for skewed variables. Correlations between parameters were calculated using the Spearman test. All the differences and correlations were regarded significant at $P < 0.05$. Multiple regression analysis using the Cox method was employed to select and rank the parameters of the study predicting fistula failure. All statistical analyses were done using the STATISTICA v. 10 software package (StaSoft Polska, Kraków, Poland).

Results

During the study period (2006–2010), 451 native arteriovenous anastomoses were created as vascular access for the maintenance dialysis programs of patients at the Region Specialist Hospital Department of Nephrology and the Fresenius Dialysis Center, both in Wroclaw, Poland. Out of this number 34 patients were enrolled in the study after having Cimino Brescia fistulae created on their wrists (21 patients) or cubital fossa (13 patients).

The mean thickness of the venous part of fistula walls measured in the histopathological preparations (N = 34) collected during fistula creation averaged 8.03 ± 4.05 mm.

The average velocity of blood flow in the fistula at 24 months after creation of the anastomosis was 1782.18 ± 1735.26 ml/min; at 48 months it was 2825.36 ± 3968.64 ml/min. Similarly, the longest fistula diameter increased from 7.87 ± 3.37 mm at 24 months to 9.57 ± 4.54 mm at 48 months.

Measurements of mRNA expression for growth factors in the vascular wall (RT-PCR, samples collected during the creation of the anastomosis) yielded the following results: PDGFβ1 3.57 ± 0.7 (N = 34). The mean value of the study group was significantly higher than that of control group (3.20 ± 0.16, N = 10, U Mann Whitney's test Z = 2.157, P = 0.031, Figure 1).

The mean values of mRNA for PDGFβ2 and TGFβ in the study group did not significantly differ from controls (PDGFβ2: 4.51 ± 1.14, N = 34, controls: 4.39 ± 1.13, N = 10; TGFβ: 8.72 ± 0.96, N = 34, controls: 8.49 ± 0.21, N = 10).

The most relevant correlations between study parameters (Spearman test) are listed in Table 2.

Table 1. Principal clinical data on fistula maturation and use during 36 months of observation

Tabela 1. Najistotniejsze dane kliniczne dotyczące dojrzewania i użycia przetoki w ciągu 36-miesięcznej obserwacji

Duration of maturation – days from implantation to first use (Czas dojrzewania – dni od implantacji do pierwszego użycia)	44.94 (N = 34, 4–154, SD = 38.6)
Number of dialyses with reduced blood flow due to fistula insufficiency (Liczba zabiegów ze zmniejszonym przepływem krwi z powodu niewydolności przetoki)	18.71 ± 28.98
Number of hospitalization days due to fistula malfunction (Liczba dni hospitalizacji z powodu dysfunkcji przetoki)	9.95 ± 15.57
General cardiovascular morbidity – number of hospitalization days for cardiovascular reasons (Ogólna chorobowość sercowo-naczyniowa – liczba dni hospitalizacji)	16.4 ± 31.97
Number of days with temporary catheter due to fistula malfunction (Liczba dni z użyciem cewnika tymczasowego z powodu dysfunkcji przetoki)	48.76 ± 162.06
Duration of fistula use – days from implantation to patient death (Czas używania przetoki – liczba dni od implantacji przetoki do śmierci pacjenta)	795.89 ± 480.6

Data are presented as mean ± standard deviation.

Dane przedstawiono jako średnią ± odchylenie standardowe.

Table 2. Selected significant correlations between study parameters (Spearman test)

Tabela 2. Wybrane znamienne statystycznie korelacje między parametrami badania (test Spearmana)

Correlations (Korelacje)	Spearman R	P value
TGFβ mRNA and patient survival after fistula implantation (TGFβ mRNA i przeżycie pacjentów)	–0.722	0.0002
PDGFβ1 mRNA and fistula blood flow (PDGFβ1 mRNA i przepływ krwi przez przetokę)	–0.674	0.0011
Longest diameter of fistula and cardiovascular morbidity (Największa średnica przetoki i chorobowość sercowo-naczyniowa)	–0.504	0.0199
Mean thickness of fistula wall and cardiovascular morbidity (Średnia grubość ściany przetoki i chorobowość sercowo-naczyniowa)	0.449	0.0059
Longest diameter of fistula and morbidity due to fistula insufficiency (Największa średnica przetoki i chorobowość związana z niewydolnością przetoki)	0.578	0.0060

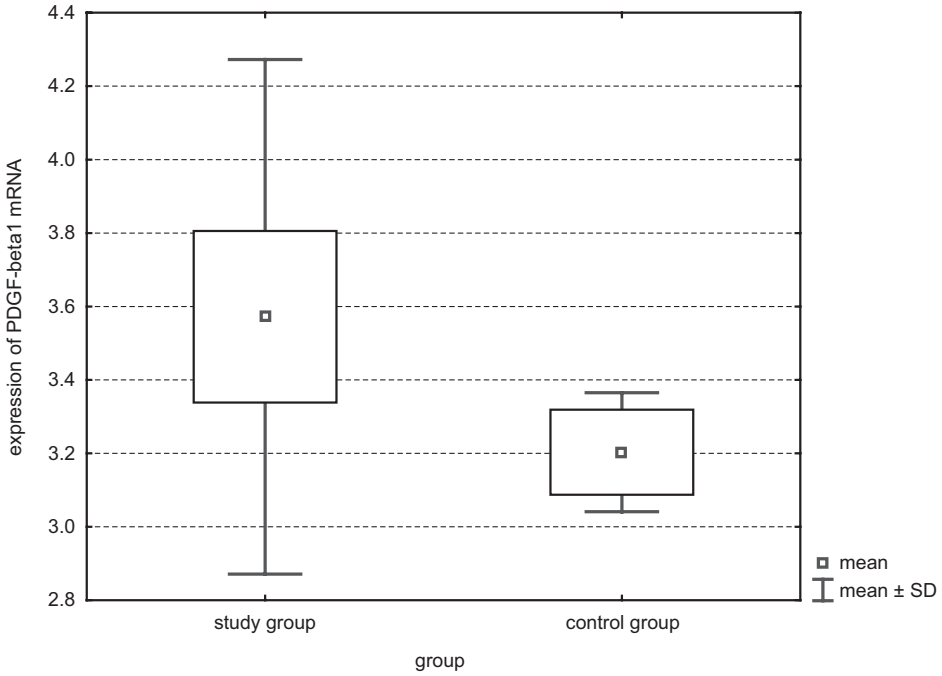

Fig. 1. Expression of mRNA for PDGFβ1 in vascular walls (mean values, standard errors and standard deviations) assessed in patients during the creation of anastomosis (study group) and in persons subjected to reparative vascular surgery due to trauma (control group). The mean value in the study group significantly exceeded that of the control subjects (P = 0.031)

Ryc. 1. Ekspresja mRNA dla PDGFβ1 w ścianie naczyń (wartości średnie, błąd standardowy oraz odchylenia standardowe) oceniano u pacjentów podczas tworzenia zespolenia (grupa badana) oraz u osób poddanych reparacyjnej chirurgii naczyniowej z powodu urazu (grupa kontrolna). Średnie wartości w grupie badanej były istotnie większe niż te z grupy kontrolnej (p = 0,031)

Discussion

Functional abnormalities of dialysis fistulae account for 15–24% of hospitalizations in chronically hemodialyzed patients, contributing to their mortality [1] and high healthcare costs. Failure of arteriovenous anastomoses may be caused by either abnormal maturation of newly planted fistulae or their progressive narrowing, initiated by thrombosis. Although strict definitions of maturation vary among vascular societies, there is a general consensus that fistula maturation time is the period between its implantation and first use for routine hemodialysis [4]. For the patients in this study, the mean maturation time was 44.9 ± 38.6 days, which did not considerably differ from those reported by Ng et al. in a cohort of 4929 patients [5]. In the present study the duration of fistula maturation correlated with its longest diameter as assessed by Doppler ultrasound examination: Wider fistulas were ready for first use earlier than narrower ones (R = –0.59, P = 0.022). Surveillance of fistula maturation and subsequent use for hemodialysis was based on frequent physical examinations by medical personnel [2] backed by color Doppler ultrasound assessment of fistula flow and possible recirculation [6]. In the current study (N = 34), the blood flow in fistulae 24 months after creation averaged 1782 ± 1735 ml/min and was comparable to reference values [7]. Nevertheless, the reliability of this comparison is questionable for two reasons: firstly because of high standard deviations in the measurements in the current study, and secondly because the majority of the fistulae in this group were implanted at the wrist, as opposed to the cubital fossa in the cited study [7]. The mean duration of fistula use (the number of days from implantation to patient death) was also insignificantly different from data reported by other authors for comparable fistulas [8]. The observations in the current study indicated considerable morbidity in hemodialyzed patients caused by impaired fistula patency [1]: The mean number of hospitalization days due to vascular access failure (9.95 ± 15.57) within the study period was only insignificantly lower than the number of hospitalization days caused by cardiovascular complications (16.39 ± 31.97), which is a well-recognized reason for morbidity and mortality of patients receiving hemodialysis [9].

The main aim of the study was to assess possible clinicopathological correlates of fistula failure. Reduced fistula blood flow measured by Doppler ultrasound examination, a well-established

method of access flow surveillance [10], was been found to be significantly negatively correlated only with the expression of PDGFβ1 in the vascular wall (R = –0.674, P = 0.0011). No other parameter of the study significantly correlated with fistula blood flow, whether assessed by the Spearman test or by multiple regression analysis. The importance of growth factor expression in the vascular wall in determining fistula patency is hardly surprising, since immune/inflammatory mechanisms, along with the suppression of nitric oxide generation by the endothelium, mediate neointimal hyperplasia leading to fistula failure [3].

In addition to demonstrating a negative correlation between fistula blood flow and expression of mRNA for PDGFβ1 in the fistula wall, the current study has shown that after the creation of an anastomosis the local activity of this gene significantly exceeded respective values in the control group. The exact cellular source of PDGF in this setting is unknown, since the gene expression in vascular wall was determined quantitatively, without histological PCR *in situ* studies, but it is conceivable that local inflammatory mechanisms mediated by infiltrating macrophages could by implicated [3]. In fact, activity of proinflammatory cytokine IL-6 and growth factor IGF have been found in neointimal hypertrophy of stenotic fistulae [11, 12]. The current study demonstrated that the expression of gene for TGFβin fistula wall is predictive of patients' survival during three years of maintenance hemodialysis treatment. Since samples of fistula walls for assessment of PDGFβ1 and TGFβ expression were collected during the creation of anastomoses in predialysis period, these results indicate that inflammatory conditions in patients being prepared for hemodialysis therapy could influence both the patency of vascular access and patient survival during renal replacement treatment.

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