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Transforming growth factor β1 genotype polymorphisms determine AV fistula patency in hemodialysis patients

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Background. In hemodialysis patients with an arteriovenous (AV) fistula, access failure is primarily due to fistula stenosis, which predisposes to thrombosis and subsequent access loss. The risk for access failure differs interindividually, an observation that is independent from vascular anatomy in a significant number of patients. Fistula stenosis is histologically characterized by intimal hyperplasia, which is induced by growth factors, among which transforming growth factor β1 (TGF-β1) is of major importance. The quantitative production of TGF-β1 interindividually differs due to polymorphisms in the gene region encoding the signal sequence of the cytokine. We hypothesized that the TGF-β1 genotype, by influencing the development of arteriovenous fistula stenosis, determines the risk for vascular access failure.

Methods. One hundred twenty patients who had undergone placement of an AV fistula for initiation of hemodialysis treatment were genotyped for the polymorphic bases at position +869 and +915 of the TGF-β1 gene. The primary end-point was time from fistula placement to access failure.

Results. AV fistula patency was significantly associated with the TGF-β1 genotype ($P = 0.0046$); patency was 62.4% and 81.2% after 12 months for TGF-β1 high and intermediate producers, respectively. In contrast, AV fistula patency neither differed between diabetic and nondiabetic patients, nor between patients with and without manifest cardiovascular disease.

Conclusion. Polymorphisms in the gene region encoding the signal sequence of TGF-β1 influence the risk for hemodialysis access failure. By inducing synthesis of extracellular matrix proteins, overproduction of TGF-β1 may accelerate the development of intimal hyperplasia, resulting in fistula stenosis and subsequent access failure.

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Vascular access complications substantially contribute to morbidity and hospitalization in hemodialysis patients. They account for 16 to 20% of dialysis patient hospitalizations in the United States [1] at a cost of $1 billion annually [2].

The major complications of permanent vascular access are arteriovenous (AV) fistula stenoses and vascular access infections. AV fistula stenoses account for over 80% of AV access thromboses [3, 4]; AV access thromboses are responsible for 80 to 85% of AV access failures. The surgical technique of fistula anastomosis and the regular care and puncture techniques are well-known factors that influence fistula patency. However, a large interindividual difference in the likelihood of developing stenoses remains unexplained. Thus, the prospective identification of patients who are prone to early stenosis would be of high clinical importance. These patients might benefit from a prophylactic medication inhibiting the development of AV fistula stenoses.

AV access stenosis is histologically characterized as intimal hyperplasia [5–8]; vascular smooth muscle cells (VSMC) initially proliferate in the media, and then migrate from the media to the intima, where they finally induce intimal expansion via exuberant synthesis of extracellular matrix [9]. VSMC proliferation, migration, and extracellular matrix synthesis are mediated by several growth factors, among which transforming growth factor β1 (TGF-β1) and platelet-derived growth factor (PDGF) are of major importance [9]. The synthesis of TGF-β1 interindividually differs due to polymorphisms in the gene region encoding the signal sequence of the cytokine [10]. We hypothesized that hemodialysis patients with a TGF-β1 high-producer genotype are prone to earlier fistula stenosis and subsequent access failure.

METHODS

Patients

We identified all patients from two institutions (Department of Nephrology, University Homburg, Germany,

and Zentrum für Heimdialyse, Homburg, Germany) who were treated for end-stage renal disease between January 1998 and December 2001, for whom complete clinical information could be recovered from the files and for whom blood samples for genotyping were available. Patients were included in our study if a wrist (radial-cephalic) or elbow (brachial-cephalic or brachial-basilic) primary AV fistula was created as first vascular access for hemodialysis treatment between January 1, 1984 and December 31, 2001, excluding patients with an AV graft of synthetic material. The primary end point was time from fistula placement to the first episode of access failure (unassisted patency). Access failure was determined on the basis of medical charts of our institution, in which all fistula interventions are recorded, as well as by repeated standardized interviews of the patients by a single investigator (G.H.H.). Access failure was defined as the need for any angioplastic or surgical intervention to correct or replace a poorly or nonfunctioning fistula, which occurred at least 8 weeks after fistula placement.

Any access failure within the first 8 weeks after fistula placement was considered related to insufficient fistula dilation rather than to intimal hyperplasia. Thus, in patients in whom access failure occurred within 8 weeks after fistula placement, and in whom a new AV fistula had to be created, this second AV fistula was subsequently analyzed, and unassisted patency was defined as time from placement of the second AV fistula to access failure, as defined above.

Patients were censored at the time of hemodialysis therapy discontinuation for recovery of renal function (more than 3 months without need for renal replacement therapy, $N = 2$), renal transplantation ($N = 13$), death due to a functioning access ($N = 4$), or loss to follow-up ($N = 3$). No study patient switched to peritoneal dialysis. The observation period was terminated on March 1, 2002.

Patients were excluded if the first episode of access failure was not due to stenosis-related events defined as angioplastic or surgical interventions because of AV fistula aneurysm, steal syndrome, or infection.

Comorbidity was assessed by chart review. Coronary artery disease was diagnosed in patients who had either had a myocardial infarction or who had undergone coronary artery bypass surgery or coronary artery angioplasty at any time before the first episode of AV fistula failure or before censoring. In patients who had had a stroke or had undergone carotic endarterectomy or carotic angioplasty, cerebrovascular disease was diagnosed. Finally, in patients who had undergone peripheral bypass surgery, nontraumatic lower extremity amputation, or lower limb artery angioplasty, peripheral artery disease was diagnosed. Patients were defined as having cardiovascular disease if they had coronary artery disease, cerebrovascular disease, or peripheral artery disease.

We recruited 64 healthy Caucasian men ($N = 34$) and women ($N = 30$) without kidney disease to be control patients. Informed consent was obtained from all patients and controls, and genotyping studies were approved by the local ethics committee.

TGF-β1 genotyping

We analyzed 2 single nucleotide polymorphisms in the DNA sequence encoding the signal sequence of the TGF-β1 protein, located at position +869 (codon 10, T>C, leucin>proline) and position +915 (codon 25, G>C, arginine>proline). Both single-base substitutions result in different levels of TGF-β1 production. According to Perrey et al [11], 3 different cytokine-producer types are distinguished: high-producer haplotypes are TC (codon 10)/GG (codon 25) and TT/GG; intermediate-producer haplotypes are CC/GG, TC/GC, and TT/GC; and low-producer haplotypes are CC/CC, CC/GC, TT/CC, and TC/CC, respectively. Genomic DNA was isolated from anticoagulated venous blood samples using the QIAamp DNA isolation kit (Qiagen, Hilden, Germany). TGF-β1 genotyping was performed using the amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) methodology, as described by Perrey et al [12].

Briefly, 50 to 100 ng DNA were amplified in a final volume of 25 μL containing 2.5 mmol $MgCl_2$ (Qiagen), 1× reaction buffer (Qiagen), 200 μmol each desoxynucleoside triphosphate (dNTP) (Roche Applied Science, Mannheim, Germany), 1× Solution Q (Qiagen), 5 μmol of each primer, and 1 U HotStar Taq polymerase (Qiagen). The protocol for the PCR Express Thermal Cycler (Hybaid, Heidelberg, Germany) was as follows: 15 minutes at 95°C, 10 cycles of 20 seconds at 95°C, 50 seconds at 65°C, 50 seconds at 72°C, 24 cycles of 20 seconds at 95°C, 50 seconds at 59°C, 50 seconds at 72°C, and 7 minutes at 72°C. The amplified products (PCR product size of TGF-β1 codon 10, 241 bp; codon 25, 233 bp) were fractionated electrophoretically on a 2% agarose gel and visualized by ethidium bromide staining (0.5 mg/mL) and ultraviolet light detection. All typing analysis contained negative and positive controls.

Statistics

Data management and statistical analysis were done using the Prism statistical software (version 3.03; Graphpad, San Diego, CA, USA). Frequency counts were compared by chi-square analysis. Continuous data are reported as mean ± standard deviation and compared using the Kruskal-Wallis test. The distribution of genotype frequencies in patients and control were compared using a chi-square analysis. Survival curves were calculated by the Kaplan-Meier method and compared by the log-rank test. In addition, for comparison of 2 survival curves, the hazard ratio and its 95% confidence interval were calculated.

Table 1. Transforming growth factor β1 (TGF-β1) signal sequence polymorphic allele frequencies

Genotype	Patients *N*	Controls *N*	Cytokine-producer type	Patients *N*	Controls *N*
CC/CC	1	0	TGF-β1 low producer	5	4
CC/GC	4	4			
CC/GG	20	7	TGF-β1 intermediate producer	34	13
TC/GC	11	5			
TT/GC	3	1			
TC/GG	34	18	TGF-β1 high producer	81	47
TT/GG	47	29			

The first two characters indicate the bases at position +869 (codon 10); the following two characters give the bases at position +915 (codon 25) of the signal sequence. The low producer genotypes TT/CC and TC/CC were found neither among the hemodialysis patients nor among control patients.

Table 2. Demographic characteristics, primary renal diseases, and comorbidity

		TGF-β_1 producer type			
	Total *N* = 120	High *N* = 81	Intermediate *N* = 34	Low *N* = 5	*P*
Mean age	57.1 ± 14.4	57.2 ± 14.8	56.1 ± 14.3	62.6 ± 7.2	0.70
Male/female	70/50	47/34	19/15	4/1	0.59
Elbow/wrist fistula	18/102	12/69	5/29	1/4	0.95
Primary renal disease					
Diabetic nephropathy	39 (32.5%)	24 (29.6%)	12 (35.3%)	3 (60%)	0.86
Glomerulonephritis	20 (16.7%)	15 (18.5%)	5 (14.7%)	0 (0%)	
Autosomal-dominant polycystic renal disease	14 (11.7%)	11 (13.6%)	2 (5.9%)	1 (20%)	
Pyelonephritis	7 (5.8%)	6 (7.4%)	1 (2.9%)	0 (0%)	
Nephrosclerosis	7 (5.8%)	5 (6.2%)	2 (5.9%)	0 (0%)	
Other/unknown primary renal disease	33 (27.5%)	20 (24.7%)	12 (35.5%)	1 (20.0%)	
Comorbidity					
Coronary artery disease	23 (19.2%)	17 (21.0%)	5 (14.7%)	1 (20.0%)	0.74
Cerebrovascular disease	9 (7.5%)	7 (8.6%)	2 (5.9%)	0 (0.0%)	0.71
Peripheral artery disease	12 (10.0%)	5 (6.2%)	6 (17.6%)	1 (20.0%)	0.13
Diabetes mellitus	46 (38.3%)	28 (34.6%)	15 (44.1%)	3 (60%)	0.38

RESULTS

Patient characteristics

One hundred twenty patients met the inclusion criteria and were enrolled in the study. Table 1 shows the frequencies of the two single-nucleotide polymorphisms in the DNA encoding the signal sequence of the TGF-β1 protein at positions +869 (codon 10) and +915 (codon 25). According to their genotype, 81 patients were classified as TGF-β1 high producers, 34 patients were intermediate producers, and 5 patients were low producers, respectively. The genotype distributions for the 2 polymorphisms did not differ between patients and healthy controls (Table 1).

TGF-β1 high producers, intermediate producers, and low producers were further characterized according to demographic data, primary renal diseases, and comorbidity (Table 2). The 3 groups did not significantly differ with respect to age, gender, location of AV fistula (wrist vs. elbow), primary renal disease, and the presence of coronary artery disease, cerebrovascular disease, or peripheral artery disease. All patients were of Caucasian ethnicity, with the exception of 1 patient in the TGF-β1 high producer group who was of African ethnicity.

Relationship between the TGF-β1 phenotype and AV fistula patency

Among the 81 patients who were TGF-β1 high producers, 50 patients developed AV fistula failure after a mean time of 13.6 ± 13.5 months. The remaining 31 patients who had no fistula failure until censoring were followed for 36.0 ± 25.8 months.

Within the 34 TGF-β1 intermediate producers, 9 patients had fistula failure that occurred after 10.8 ± 7.9 months. The 25 patients who had no fistula failure had a mean follow-up of 31.7 ± 24.8 months. Time of follow-up in patients without fistula failure did not significantly differ between TGF-β1 high and intermediate producers.

Of the 5 patients who were TGF-β1 low producers, fistula failure occurred in 2 patients after 5 and 18 months, respectively, whereas 3 patients were censored after 4, 14, and 23 months with functioning AV fistulas. Because the number of TGF-β1 low producers was too low to allow for a valid statistical comparison to intermediate and high TGF-β1 producers, these 5 patients were excluded from the analysis of fistula patency stratified from TGF-β1 producer type. However, inclusion of

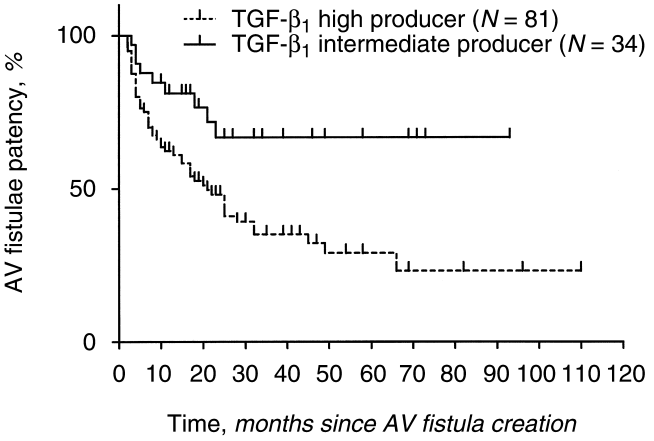

Fig. 1. Kaplan-Meier analysis of arteriovenous (AV) fistula patency stratified by transforming growth factor β1 (TGF-β1) producer type. By log-rank test, AV fistula patency was significantly better for TGF-β1 intermediate producers (solid line) compared to TGF-β1 high producers (dotted line); $P = 0.0046$. TGF-β1 low producers were too low in number to allow a valid statistical comparison to intermediate and high producers.

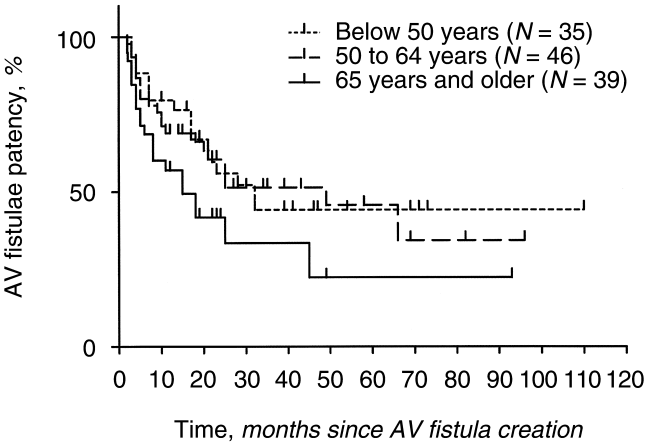

Fig. 2. Kaplan-Meier analysis of arteriovenous (AV) fistula patency stratified by age. By log-rank test, AV fistula patency did not differ significantly between patients who were younger than 50 years of age (dotted line), 50 to 64 years of age (dashed line), and 65 years of age and older (solid line), respectively. $P = 0.10$.

TGF-β1 low producers did not significantly change results of fistula survival analysis.

As depicted in Figure 1, AV fistula patency differed significantly when patients were classified according to their TGF-β1 phenotype. Fistula patency was 62.4% and 81.2% for TGF-β1 high and intermediate producers after 12 months, respectively; after 24 months, it was 48.1% and 66.7%, respectively ($P = 0.0046$; hazard ratio, 2.63 [1.28 to 3.90] for high producers compared to intermediate producers).

Relationship between demographic characteristics and AV fistula patency

There was no difference in AV fistula patency between male and female patients; the hazard ratio for access failure was 1.00 (0.60 to 1.69) for female patients compared to male patients. When classifying the study group according to their age, patients who were at least 65 years of age when the fistula was created tended to have earlier AV fistula failure, although differences were not significant ($P = 0.10$) (Fig. 2).

Relationship between comorbidity and AV fistula patency

AV fistula patency did not differ in the 39 patients who had end-stage renal disease due to diabetic nephropathy compared to the 81 patients who had any other primary renal disease ($P = 0.30$) (Fig. 3). Twelve months after fistula placement, patency was 62.6% and 70.9% for patients with and without diabetic nephropathy, respectively. The hazard ratio for access failure was 1.31 (0.77 to 2.35) for patients with diabetic nephropathy compared to those with any other primary renal disease.

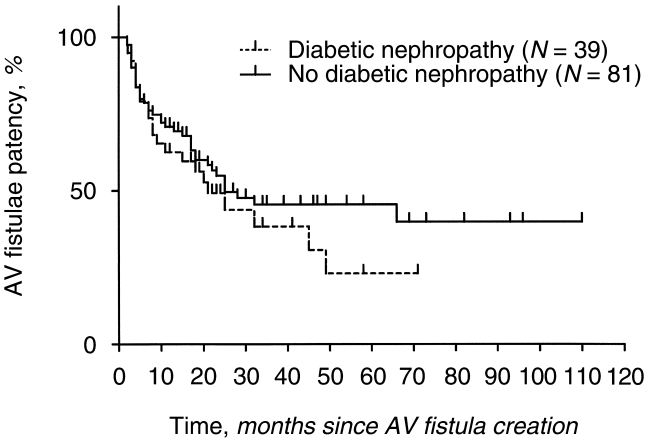

Fig. 3. Kaplan-Meier analysis of arteriovenous (AV) fistula patency stratified by primary renal disease. By log-rank test, AV fistula patency did not differ significantly between patients with diabetic nephropathy (dotted line) and patients with any other primary renal disease (solid line). $P = 0.30$.

When comparing all patients with diabetes mellitus (patients who had diabetic nephropathy and those who had any other primary renal disease, but who were diagnosed as having diabetes mellitus as a comorbidity; $N = 46$) to nondiabetic patients ($N = 74$), the hazard ratio for access failure was 1.46 (0.88 to 2.62) for diabetic patients.

Patients with cardiovascular disease did not develop fistula failure earlier than patients without cardiovascular disease [12 months' patency, 72.9% and 66.0%, respectively; hazard ratio 0.99 (0.88 to 2.17) for patients with cardiovascular disease] (Fig. 4).

Moreover, there was no significant difference in fistula patency between patients with an elbow fistula and those with a wrist fistula (data not shown).

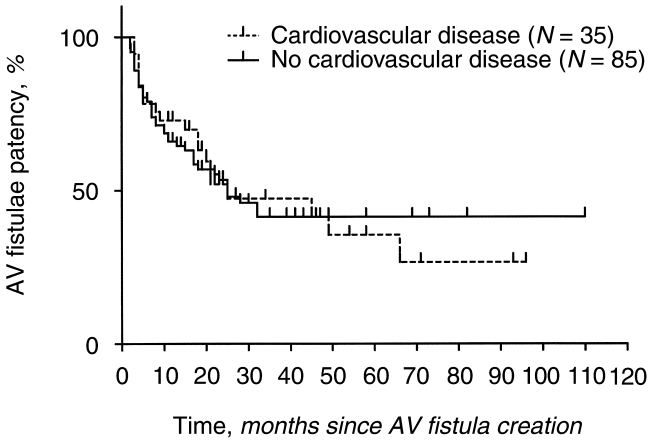

Fig. 4. Kaplan-Meier analysis of arteriovenous (AV) fistula patency stratified by comorbidity. By log-rank test, AV fistula patency did not differ significantly between patients with cardiovascular disease (dotted line) and patients without cardiovascular disease (solid line). $P = 1.00$.

DISCUSSION

Hemodialysis treatment requires a well-functioning vascular access. Current Disease Outcomes Quality Initiative (DOQI) guidelines recommend wrist (radial-cephalic) and elbow (brachial-cephalic) primary AV fistulas as the preferred type of access [13] because of lower complication rates and lower morbidity associated with their creation compared to other access options. In primary AV fistulas, stenosis is the most important functional defect, resulting from intimal hyperplasia [5–8] with VSMC proliferation, migration, and extracellular matrix synthesis [9]. AV fistula stenosis reduces dialysis quality via a decrease in fistula blood flow, and it predisposes to thrombosis and subsequent access failure. The likelihood of AV fistula stenosis differs interindividually; however, risk factors contributing to vascular access complications are only partly defined [14].

TGF-β1 is a multifunctional cytokine that is implicated in the regulation of proliferation and differentiation of many cell types [15]. It exerts its effect by binding to cell-surface receptors, three of which have been identified in a variety of cells, including VSMC.

Physiologically, TGF-β1 is among the key cytokines that play a role in the initiation and termination of wound healing after tissue injury [16]. Pathologically, increases or decreases in the expression of TGF-β1 have been linked to numerous diseases—in fibrotic disease of the kidney, liver, and lung, overproduction of TGF-β1 results in excessive deposition of extracellular matrix [15, 16]. In contrast, a decreased TGF-β1 expression has been linked to atherosclerosis [17], as TGF-β1 inhibits migration and proliferation of macrophages and protects endothelial function [18, 19].

Polymorphisms in the gene for TGF-β1 determine the production of TGF-β1 [10] and can predict the susceptibility to certain diseases. Polymorphisms that result in increased TGF-β1 production are linked to fibrotic lung disease [10] and arterial hypertension [20, 21]. Polymorphisms leading to decreased TGF-β1 predispose to myocardial infarction [20] and atopic dermatitis [22]. TGF-β1 expression is increased in the luminal and abluminal neointima of stenosed AV fistulas and correlates to neointimal cell number [8, 23–25]. TGF-β1 is thought to be produced locally by medial and neointimal smooth muscle cells, as well as by macrophages and lymphocytes within the stenotic lesion of AV fistulas [24]. TGF-β1 stimulates extracellular matrix protein production and inhibits the degradation of matrix proteins [16]. It has a bimodal effect on smooth muscle cell proliferation [19]. In addition, TGF-β1 induces the expression of PDGF [26] and fibroblast growth factor (FGF) [27]. PDGF and FGF are important mediators of the smooth muscle cell proliferation and/or their migration into the intima [28, 29], and of accumulation of extracellular matrix proteins [30], which characterizes neointimal hyperplasia. In accordance, PDGF and FGF are overexpressed in stenosed AV fistula [6].

In agreement with a prominent role of TGF-β1 in AV fistula stenosis, the injection of TGF-β1 was shown to induce a more pronounced intimal thickening than placebo in arterial balloon injury, an animal model of intimal hyperplasia in which histologic changes closely resemble those found in AV fistula stenosis [31]. Administration of neutralizing anti-TGF-β1 significantly reduces intimal hyperplasia after arterial balloon injury [32]. We suggest that our data may further confirm an important role of TGF-β1 in the development of AV fistula stenosis. Hemodialysis patients who are predisposed to increased TGF-β1 synthesis because of genetic polymorphisms were found to have a significantly shorter unassisted AV fistula patency.

It was recently reported that TGF-β1 polymorphisms do not result in different serum levels of TGF-β1 in hemodialysis patients [33]. These data do not contradict our findings, as overexpression of TGF-β1 in stenosed AV fistula should be regarded as a localized phenomenon occurring in tissue injury that does not result in a systemic increase in TGF-β1 serum levels. In accordance, it was recently shown that the local expression of TGF-β1 in stenosed AV fistulas does not correlate to TGF-β1 serum levels, and that no increase in TGF-β1 levels is found in patients with AV fistula stenosis compared to uremic controls [24].

Neither older age nor the presence of diabetes mellitus were significant risk factors for AV fistula failure, which is in accordance with most recent multicenter [34, 35] and large single-center [36] studies on AV fistula patency. Earlier single-center studies, which reported a significantly poorer fistula patency in elderly or diabetic patients, included smaller numbers of patients [37, 38]. In contrast, diabetes mellitus and/or older age may predis-

pose to access failure in patients with an AV graft of synthetic material [35, 39].

In addition, we found that patients with cardiovascular disease are not prone to earlier fistula failure, which is in accordance with recent studies reporting no correlation between failure of native AV fistula and a history of peripheral vascular disease [40, 41], coronary artery disease [34, 41], or cerebral vascular disease [34]. Intimal hyperplasia occurring in AV fistula stenosis, as well as in restenosis after coronary intervention, histologically and pathogenetically differs from atherosclerotic lesions, in which inflammation and endothelial dysfunction play a more prominent role than in AV fistula stenosis. This may explain the seeming contradiction as to why a high production of TGF-β1 is detrimental in AV fistula, in which TGF-β1 induces extracellular matrix production, but is protective in atherosclerotic lesions due to the immunosuppression and protection of endothelial function mediated by TGF-β1.

CONCLUSION

We suggest that our data may allow identifying prospectively those patients with chronic renal failure who are prone to early access failure. At present, a prophylactic medication lowering the risk of AV fistula thrombosis may be offered to these patients [42]. As first clinical trials on tranilast, which inhibits the release of TGF-β and other cytokines, showed a clinically significant reduction in restenosis after coronary angioplasty without severe systemic side effects [43], the selective pharmacologic blockade of signals for matrix production may be an attractive, albeit eager, future goal in improving AV access patency.

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