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Do biologic markers predict cardiovascular end points in diabetic end-stage renal disease? A prospective longitudinal study

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Abstract

Background. Diabetic patients on hemodialysis are at high risk of death from cardiovascular disease, and research has suggested that various biologic markers of inflammation, oxidative stress and hemostasis may give added value to clinical information for predicting cardiovascular event (CVE)-free survival. This information could be particularly important in evaluating this population for renal transplant, given the scarcity of organs. We hypothesized that in diabetic patients undergoing renal replacement therapy (RRT) these biologic markers would prove useful in predicting event-free follow-up in a prospective study.

Methods. One hundred and fifty diabetic (76 type 1, 74 type 2) and 27 non-diabetic stable RRT patients were followed for 0.04–13.69 years for CVE (myocardial infarction, coronary arterial intervention, peripheral arterial bypass or amputation, cerebrovascular accident or carotid artery intervention), cardiac and all-cause mortality. Measured biologic markers of inflammation included the following: Il-6, C reactive protein, fibrinogen; of hemostasis: fibrinogen, plasminogen activator inhibitor (PAI), fibrinolytic activity, von Willebrand factor VII (vWF), platelet-selectin, viscosity and of oxidative stress: advanced glycated end products and antibody to oxidized low-density lipoprotein. For each, upper versus lower tertiles were compared for duration of event-free follow-up.

Results. Cardiovascular events prior to study entry occurred in 51.3% of DM1, 54.0% of DM2 and 25.9% of DM0 patients. Subsequent cardiovascular events were noted in 31.6% of DM1, 45.9% of DM2 and 11.1% of DM0 patients. All mean levels of biologic markers at baseline were abnormal ($P < 0.05$).

Conclusions. In this RRT population, all biologic marker levels except PAI did not improve clinical prediction of events.

Keywords: biologic markers; cardiovascular events; diabetes; plasminogen activator inhibitor; renal replacement therapy

Introduction

The rate of death on dialysis for diabetic patients remains higher than if those patients received a renal transplant. But clinicians lack good markers to help predict such events and risk assess patients ahead of transplantation. Recent trials designed to assess atherosclerosis risk in the community have suggested that evaluation of multiple biologic markers may not only risk stratify diabetic patients, but also give information about the pathogenesis of cardiovascular events (CVEs). In an effort to risk assess patients who were considered stable while undergoing some form of RRT, we endeavored to determine whether biomarker risk assessment in this population could supplement information already available to the clinician.

In a prior study of diabetic and non-diabetic individuals on RRT, we evaluated relationships between prior cardiovascular events (PCVE) and plasma markers for accelerated hemostasis, heightened inflammatory response as well as pathologic degrees of oxidative stress [1]. In the current presentation, we focused on the relationships between levels of biologic markers and subsequent cardiovascular event (SCVE)-free follow-up in clinically stable diabetic patients already initiated on RRT. We hypothesized that measurement of these biologic markers would supplement clinical evaluation in stratifying the likelihood of CVE-free survival in the run up to renal transplantation. At the time that this study was begun, usefulness for each biomarker selected in predicting cardiovascular outcomes in several other patient populations had been reported.

Patients and methods

Enrollment

The protocol and informed consent were approved by the appropriate Institutional Review Boards. Clinically stable

patients in the participating hospital and freestanding dialysis units were requested to donate baseline blood samples prior to a routine dialysis treatment. The investigative biologic markers were tested only once. History of myocardial infarction, cerebrovascular accident or intervention for coronary, carotid or peripheral arterial obstruction was recorded. Requirement for, and class of antihypertensive drugs was recorded for a subset of patients.

Stable hemodialysis (HD, $n = 128$), peritoneal dialysis (PD, $n = 22$) and renal transplant (RT, $n = 27$) patients were enrolled in this prospective longitudinal study. The prime focus of this analysis was the 150 diabetic patients, including 76 with type 1 diabetes (DM1) and 74 with type 2 diabetes (DM2). Another 27 patients without diabetes (DM0) are listed separately in the tables for comparison. Patients were enrolled from March 1996 until March 2000. Follow-up continued until 15 July 2010 for prespecified end points that included myocardial infarction, coronary artery intervention, peripheral ischemia with bypass or amputation surgery, cerebrovascular accident or carotid artery intervention, cardiac and all-cause mortality. End point event adjudication was based on review of medical records by JAD and LAW and included time to initial thromboembolic event or death (cardiovascular and non-cardiovascular). For analysis of time to initial event, subjects lost to follow-up were censored at the time of their last documented clinical visit. Follow-up of patients who received a kidney transplant during the study was concluded at that time. Thus, by design, CVE-free follow-up is understated. Information retrieved from the Social Security Death Index was considered valid for end point with default cause of death listed as 'unknown'.

Laboratory methods

Fibrinogen levels were determined by measuring clotting times [2]. High-molecular weight fibrinogen was measured using ethylenediamine tetraacetic acid (EDTA) plasma. Low-molecular weight (LMW) fibrinogen required the addition of thrombin, calcium and magnesium [3]. Factor VII antigen was determined from citrated plasma by immunoassay [enzyme-linked immunosorbent assay (ELISA)] using a commercially available kit (Asserchrom VII: AG Diagnostica STAGO, 5 Century Drive Parsippany, NJ 07054). Antigen levels of tissue plasminogen activator inhibitor (PAI-I) were determined from citrated plasma by immunoassay (ELISA) using kits (Biopool International, 6025 Nicolle St., Ventura, CA 93003). Von Willebrand factor (vWf) was measured in EDTA plasma by an immunoassay (ELISA) as described by Penny *et al.* [4]. Fibrinolytic activity was quantified from citrated plasma in euglobulins (fibrin plate method) [5]. C-reactive protein antigen (CRP) was determined by enzyme immunoassay using kits [UPI-Magiwell (United Biotech, Inc.) 110 Pioneer Way, Mountain View, CA 94041-1517]. Plasma viscosity was measured using a Brookfield digital viscometer (Cone/Plate Model DV-11). Levels of advanced glycated end products (AGEs) were measured in plasma by immunoassay (ELISA) using polyclonal antibodies to AGE-modified proteins (Picower Institute, Manhasset, NY) [6]. Antibodies to oxidized low-density lipoprotein (LDL) were measured by enzyme immunoassay [ELISA (ALPCO Diagnostic PO Box 451, Wingham, NH 03087)]. Platelet (P)-selectin was determined by immunoassay (ELISA) using kits (Aymed Lab, Inc., 458 Carlton Court, San Francisco, CA 94080). Interleukin-6 (IL-6) was measured in EDTA plasma by immunoassay (ELISA) using kits (Quantikine HS; R&D Systems, Minneapolis, MN).

Statistical methods

Comparisons of group data included analysis of individual types of RRT individually within the presence, type or absence of diabetes. Dialysis patients were considered under entry mode of treatment throughout the entire course of study.

The Kaplan–Meier function was utilized to estimate years of event-free follow-up. Since the median of such functions is considered the preferred measure for censored data, the medians and 95% confidence limits are reported. Comparisons of median follow-up times from function curves between the first and third tertiles were tested for significance using the log-rank test [7].

Frequency data were tested for significance using the Fisher's exact test (two tailed) since some expected frequencies were five or less. Study group means were compared using the general linear models procedure followed by Duncan's multiple range test. Differences between biomarker means for DM1 + 2 versus DM0 and PCVE no versus PCVE yes were tested for significance using unpaired *t*-tests. Measurement data are expressed as means with standard error of the mean as measure of dispersion. An alpha level less than or equal to 0.05 was considered statistically significant. Analyses were done using SAS software, Version 8·2 (SAS Institute, Inc., Cary, NC).

Role of the funding source

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Results

Table 1 lists CVEs recorded prior to enrollment (PCVE) as well as subsequent to enrollment (SCVE) in the study. When classified according to type of DM, 31.6% of DM1, 45.9% of DM2 and 11.1% of DM0 subjects experienced an SCVE. With respect to SCVE when classified according to

Table 1. Baseline demographics, PCVE and SCVEs for 177 RRT patients by diabetic patient type defined as type 1 (DM1), type 2 (DM2) or non-diabetic (DM0)

	DM0	DM1	DM2	Total DM
Baseline (*n*)	27	76	74	150
Age (years)[a]	59 ± 3.4	43.3 ± 1.0	64.6 ± 1.1	
Gender (f/m)	10/17	33/43	40/34	73/77
PCVE (+/0)	7/20 (26%)	39/37 (51%)	40/34 (54%)	79/71 (53%)
During follow-up:				
No event	12	24	21	45
Transplant	7	26	5	31
CV event (+/0)	3/24 (11%)	24/52 (32%)	34/40 (46%)	58/92 (37%)
Deaths				
CV	2[b]	2[b]	2[b]	4[b]
Non-CV	5	1	1	2
Unknown	0	1	1	2
Lost to follow-up (%)	8 (29.6)	14 (18.4)	18 (24.3)	40 (26.7)

[a]DM2 > DM0 > DM1, $P < 0.05$.
[b]Included in CV events.

mode of RRT at baseline, 33.6% of HD, 18.2% of PD and 51.9% of RT study subjects experienced an SCVE. Duration of follow-up was longest in the RT subset.

Table 2 demonstrates that the mean levels of factors for hemostasis, inflammation and oxidative stress for all renal failure patients were outside of the normal range (all $P < 0.05$).

Table 3 incorporates PCVE (yes versus no) into a summary of RRT and diabetes categories. HD patients with diabetes demonstrated no differences in the level of biologic markers for accelerated hemostasis, heightened inflammatory response or pathologic degrees of oxidative stress. Further analysis of the PAI-1 results is demonstrated in Table 4. In the type 1 diabetic group, normal-range levels of PAI-1 were associated with a 14-fold longer event-free follow-up than low-range levels. In the type 2 diabetic group however, depressed levels of PAI-1 were associated with a 10-fold longer event-free follow-up.

In those for whom data were available, serum albumin, cholesterol, age, body mass index and body surface area did not demonstrate a significant relationship with event-free follow-up.

Discussion

We hypothesized that the chosen biologic markers would supplement clinical evaluation in stratifying diabetic patient likelihood of CVE-free follow-up in the run-up to renal transplantation. A prior history of CVEs in the patients in this study has recently been shown to be a poor predictor for the likelihood of future CVEs in diabetic patients who remain stable while undergoing RRT [8]. Identification of increased risk for the diabetic patient requiring RRT might enhance or direct therapy, much as the identification of asymptomatic severe coronary artery disease has led to directed intervention and improved survival [9, 10]. We had anticipated that, in this prospective longitudinal study, knowledge of biologic markers in study patients identified by prior medical history [1] would permit us to assess future risk. Our pilot study demonstrated that virtually all biologic markers of inflammation, hemostasis and oxidative stress did not predict future CVEs in a high-risk population without a previous history of CVEs and are apparently of no use in directing therapy. We found contradictory results for PAI-1, which may be related to the presence or absence of insulin resistance. Further studies on larger numbers of both type 1 and type 2 diabetic renal failure patients with objective hard cardiovascular end points are needed to confirm our observations. As adipose tissue is able to synthesize PAI-1, it is likely that type 2 diabetic patients will have high levels. We suspect that very low levels of PAI-1 may be associated with a different form of vascular pathology within atherosclerotic plaque [11].

In addressing this question, the Bypass Angioplasty Revascularization Investigation type 2 Diabetes trial found that patients treated with an insulin-sensitizing regimen (thiazolidinediones or metformin) had a 15% lower concentration of PAI-1 than those treated with an insulin-stimulating regimen (sulfonylurea or meglitinide). Higher levels of PAI-1 and hence insulin resistance were associated with a greater need for revascularization [12]. The current finding of significantly shorter median event-free follow-up times, when PAI levels are elevated in the type 2 diabetic group without PCVE potentially confirms and extends this observation, meriting further study.

Concentrations of PAI-1 appeared to have statistically significant relationships with event-free follow-up only in DM1 subjects with PCVE and in DM2 subjects without PCVE. For DM1 the best (longest) median event-free follow-up times occurred in the highest tertile of PAI-1 results (approximately normal range) and the worst (shortest) in the lowest tertile (Table 4). For DM2, the best (longest) median event-free follow-up times occurred in the lowest tertile (approximately normal range) and the worst (shortest) in the highest tertile (Table 4). This finding is hypothesis generating and further study will be needed to understand why higher levels of a prothrombotic marker in Type 1 diabetic patients and lower levels in Type 2 diabetics were similarly associated with better event-free outcomes.

Our prior studies of DM1 early nephropathy subjects demonstrate that PAI levels rise from very low toward the normal range with improved glycemic control using intensive insulin therapy (plus ACE inhibition) [13]. These results demonstrate stabilized renal function [14] associated with less aggressive inflammatory response [15] and more efficient carbohydrate oxidation [16].

Advanced glycation end products are excreted by functioning native kidneys. Initiation of dialysis in the non-diabetic population is usually due to pure renal dysfunction and thus AGE levels may be high. Initiation of dialysis in

Table 2. Baseline biologic marker values (means ± SEM) for the HD, PD or RT RRT patients by diabetes mellitus type

Patients	Normals	DM0 27	DM1 76	DM2 74	Total DM 150
Viscosity (plasma)	1.24 ± 0.003 centipoise	1.26 ± 0.01	1.27 ± 0.01	1.26 ± 0.01	1.28 ± 0.01
Fibrinogen[a]	276 ± 30 mg/dL	363 ± 22	399 ± 12	416 ± 14	407 ± 9
LMW[a]	48 ± 10	85 ± 11	99 ± 5	108 ± 7	103 ± 4
% degrade		23.0 ± 2.2	24.6 ± 1.1	25.5 ± 1.0	25.0 ± 0.8
Fibrinolytic activity[b]	120.0 ± 0.9 mm^2	54.4 ± 4.3	76.5 ± 5.3	67.2 ± 7.3	71.9 ± 4.5
PAI-1	25.8 ± 0.3 ng/mL	19.1 ± 1.9	17.6 ± 1.5	18.5 ± 1.3	18.0 ± 1
Factor VII	93.4 ± 0.9%	112.0 ± 3.6	113.0 ± 1.8	114.1 ± 2.3	113.5 ± 1.4
vWf	102 ± 6%	167 ± 11	191 ± 7	176 ± 7	183 ± 5
P-selectin[b]	<100 ng/mL	157 ± 14	207 ± 13	184 ± 13	196 ± 9
IL-6	<5.6 pg/mL	17.9 ± 4.2	19.3 ± 3.4	23.9 ± 4.5	21.6 ± 2.8
CRP[c]	<2000 ng/mL	5852 ± 928	5327 ± 609	7809 ± 752	6533 ± 491
AGE	<10 IU	18.4 ± 1.7	15.9 ± 1.5	16.2 ± 1.0	16.1 ± 0.8
Ox LDL (antibody)[b]	275 ± 25 mU/mL	413 ± 92	1041 ± 176	788 ± 153	914 ± 117

[a]DM2>DM0, $P < 0.05$.
[b]DM1>DM0, $P < 0.05$.
[c]DM2>DM1, $P < 0.05$.

Table 3. Biologic markers and PCVE: relationship to SCVE

	Tertile 1					Tertile 3				
Variable	PCVE	*n*	Level	Median	95%CL	*n*	Level	Median	95%CL	P-value
Viscosity	0	20	<1.23	2.3	1.5–6.0	19	>1.31	1.5	1.1–5.2	0.3868
	+	21	<1.23	1.7	0.9–2.2	19	>1·33	2.0	0.6–3.6	0.3961
Fibrinogen	0	23	<330	2.4	1.7–5.2	24	>438	1.5	1.1–3.7	0.1237
	+	26	<372	2.2	0.8–3.3	27	>446	2.0	1.5–3.1	0.4567
LMW fibrinogen	0	24	<69	2.3	1.3–6.4	24	>110	1.6	1.4–4.2	0.3667
	+	25	<80	2.2	1.4–3.3	27	>120	3.1	1.5–3.6	0.6451
Fibrinogen degradation	0	25	<19.88	1.5	1.0–3.7	25	>27.51	2.0	1.4–4.2	0.8641
	+	26	<20.4	2.0	1.4–3.3	28	>28.62	2.2	0.9–3.4	0.8057
Fibrinolytic activity	0	22	<42	1.8	1.3–3.7	23	>72	2.3	1.4–4.3	0.7790
	+	25	<42	2.2	0.8–4.0	25	>90	1.9	0.8–2.3	0.5265
PAI	0	21	<10.7	4.3	1.4–6.0	21	>17.3	2.0	0.4–4.2	0.4263
	+	25	<12.3	1.5	0.6–2.2	25	>21·6	3.0	1.2–8.4	0.0134
Factor VII	0	21	<108.2	3.6	0.7–6.0	22	>118.2	2.3	1.5–4.3	0.8806
	+	25	<104.4	1.9	1.0–3.6	26	>119.7	2.2	1.2–4.0	0.6861
vWf	0	22	<138.9	3.7	1.5–6.0	22	>170.6	2.2	1.1–5.0	0.2573
	+	25	<158.8	2.2	0.8–5.6	26	>222.2	2.0	0.8–3.6	0.5326
P-selectin	0	21	<130	1.9	1.1–4.3	23	>205	2.6	1.4–5.5	0.3034
	+	25	<142	2.2	1.2–3.3	27	>186	3.4	1.5–6.7	0.5666
IL-6	0	24	<6.1	2.4	1.1–5.2	24	>12·6	2.2	1.3–3.7	0.3346
	+	26	<16.5	1.9	1.4–3.3	26	>16·5	1.9	1.4–3.3	0.6357
CRP	0	21	<1533	4.3	1.8–6.4	22	>4113	1.4	1.1–3.7	0.3039
	+	25	<3664	1.9	1.0–3.6	26	>10 482	2.3	1.7–3.6	0.7294
AGE units	0	14	<12.5	1.4	0.3–2.2	16	>21.25	2.6	1.5–4.2	0.0888
	+	18	<12.5	1.9	0.3–2.2	20	>16.25	2.7	1.5–5.6	0.0463
Ox LDL	0	21	<330	2.0	1.1–3.7	23	>605	2.3	1.5–5.2	0.3515
	+	22	<305	1.5	0.8–2.2	23	>540	2.2	0.9–3.3	0.5717

Data for all RRT-treated diabetic patients, ($n = 150$). Comparison of median event-free follow-up times for tertiles 1 and 3 and 95% confidence limits.

Table 4. Event-free survivals for the HD, PD and RT combined group for tertile comparison of PAI

Biologic marker	DM	PCVE	Tertile	*n*	Value	Median	95% CI	P-value
PAI-1	1	0	1	11	<9.1	2.3	1.0–6.4	
			3	12	>13.7	2.6	1.4–9.0	0.2595
		+	1	12	<10.9	0.6	0.2–3.6	
			3	12	>22.2	8.9	7.8–10.7	0.0020
PAI-1	2	0	1	9	<11.9	4.3	2.4–8.7	
			3	10	>19.7	0.4	0.1–3.6	0.0307
		+	1	12	<13.6	1.9	1.5–2.2	
			3	13	>20.9	2.0	0.6–3.3	0.4971

the diabetic population may be for cardiorenal dysfunction. Thus in the diabetic population, a low AGE may indicate that there is some residual renal function with associated decrease in cardiac function. These findings are consistent with those of Schwedler *et al*. [17].

Twenty years ago, in a small study of diabetic hemodialysis patients ($n = 100$), we reported a significant difference in all-cause mortality for calculated LDL cholesterol above versus <2 mmol/L [18]. This year, in a large study ($n = 800\,000$), Tonetti *et al*. found direct measurement of LDL-C to be progressively less reliable as an indicator of acute myocardial infarction as renal function diminished to a level requiring dialysis [19]. Since cardiac events are more common than fatal events, it is not likely that LDL cholesterol would have a statistically significant impact upon a composite of SCVEs.

Many newer biologic markers have been suggested [20] for populations at risk for cardiovascular events, including highly sensitive troponins, natriuretic peptides, apolipoproteins, homoarginenine, homocysteine, adrenomedullin, carboxymethyl lysine, asymmetric dimethylarginine, fibroblast growth factors and fetuin assays. The challenge is to identify biologic markers that add to what is known clinically and provide insight into mechanisms of risk. Investigators analyzing data from the German Diabetes and Dialysis Study (4D), for example, recently reported a correlation between low levels of homoargenine and sudden cardiac death and heart failure in diabetic patients on dialysis [21]. Other recent investigations [22] serve, however, to remind us that biologic marker manipulations (as surrogate end points) are expensive, and not necessarily predictive of beneficial healthcare outcomes.

Limitations

We focused upon patients who had been on RRT for varied amounts of time and simulated the population that one sees in dialysis units. This does not reflect a population that is initiating RRT or a population with renal dysfunction at the time of AV fistula creation. Our study design required hard end points and thus our results understate the incidence of peripheral vascular disease in the diabetic RRT community. We did not include claudication, bruits, ankle brachial index, color change or surgeries related to hemodialysis vascular access. Our results also understate the prevalence and impact of angina and congestive heart failure or transient ischemic episodes in this population. Truncating event-free survival at the date of last follow-up and at transplantation by design underestimated event-free survival in our study population. Among 150 diabetic renal failure patients, no hard end point occurred in 45 and the censoring event in 31 patients was a new kidney transplant. Since a large percentage of patients underwent transplantation during the course of the study, we repeated the analysis without censoring these patients to other prespecified end points. Although this analysis increased the mean duration of follow-up,

the results were quite similar in that the biologic markers that we had chosen were not helpful.

Conclusion

Our study demonstrates that biologic markers alone are not sufficient for stratifying the risk of CVEs in a stable diabetic population undergoing renal replacement therapy (RRT). This further illustrates the difficulties of decision-making for physician treating diabetic end-stage renal failure patients. The prevalence of cardiovascular disease is high, and neither patient history [8] nor biologic markers found helpful in lower risk populations are sufficiently predictive of risk in this population. Our results should point clinicians away from the use of many expensive tests for diabetic patients on dialysis to guide preoperative evaluations. Our findings will require confirmation in larger studies. PAI-1 may hold out promise as a predictive biologic marker and inhibition of PAI-1 a target for therapy [23], but its relationship to insulin resistance must be further investigated and understood before it has any use as a predictor of future CVEs in a diabetic population on dialysis.

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Conflict of interest statement. None declared.

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