Serum metalloproteinases MMP-2, MMP-9, and metalloproteinase tissue inhibitors in patients are associated with arteriovenous fistula maturation

Eugene S. Lee, MD, PhD, Qiang Shen, MD, PhD, Robert L. Pitts, BS, Mingzhang Guo, DVM, PhD, Mack H. Wu, MD, Sue C. Sun, MD, and **Sarah Y. Yuan, MD, PhD,** *Sacramento, Calif*

Objective: **Many vascular surgeons construct arteriovenous fistulas (AVFs) for hemodialysis access as the primary choice access. A significant number of AVFs fail to mature, however, leading to patient frustration and repeated operations. Metalloproteinase (MMP) activity, particularly MMP-2 and MMP-9, may be important for AVF maturation. We therefore sought to identify whether serum MMP levels could serve as a biomarker for predicting future successful AVF maturation.**

Methods: **Blood was collected from patients with chronic renal insufficiency at the time of surgery for long-term hemodialysis access. Serum was separated from whole blood and ultracentrifuged at 1000*g* for 10 minutes. Serum aliquots were frozen at −80°C until used for analysis. Enzyme-linked immunosorbent assay was used to assay levels of MMP-2, MMP-9, and tissue inhibitor of metalloproteinase type 2 (TIMP-2), and TIMP type 4 (TIMP-4). Clinical end points were used to divide patients into failed and matured AVF groups. Successful maturation was considered in patients who had specific duplex findings or 1 month of successful two-needle cannulation hemodialysis. MMP/TIMP ratios were calculated as an index of the MMP axis activity because MMP activity parallels alterations in TIMP levels.**

Results: **Of 20 enrolled patients, AVF maturation was successful in 13 and failed in 7. Serum levels of MMP-2/TIMP-2 were significantly higher in patients with matured AVFs vs levels in those that failed ($P = .003$). Similarly, a trend toward increased serum levels of MMP-9/TIMP-4 was found in patients with successful AVF ($P = .06$).**

Conclusions: **MMP-2 and TIMP-2 levels were different among patients whose AVF matured vs those who did not. Further follow-up studies to determine the predictability of AVF maturation using relative patient serum levels of MMP-2 and TIMP-2 should be performed. (J Vasc Surg 2011;54:454-60.)**

Most patients with end-stage renal failure require hemodialysis, and the arteriovenous fistula (AVF) is the preferred method for access. AVFs are the most durable, are resistant to infection and thrombosis, and are preferred for lower mortality and cost profiles.[1] Recognizing the superiority of this access, the National Kidney Foundation recommends an aggressive approach to the creation of AVFs.[2] However, 20% to 60% of primary AVFs fail to develop into a functioning dialysis access.[3,4] Impaired vein remodeling, intimal hyperplasia, technical problems, unrecognized stenoses within the outflow vein, inflow problems, or steal syndromes can all lead to failure of achieving a mature AVF.

Vascular access failure is the most important cause for morbidity, repeat surgery, and hospitalization.[5] Given the challenges of successful access surgery, the biochemical and pathologic changes associated with AVF maturation and intimal hyperplasia should be sought. Recent studies have shown that matrix metalloproteinases (MMP) are important in the process of AVF maturation.[6,7] MMPs belong to a group of zinc-dependent proteases capable of degrading extracellular matrix (ECM) proteins.[8,9] In particular, MMP-2 is expressed by a variety of cell types and is activated by membrane-bound membrane type-1 (MT1-MMP) and is inhibited by tissue inhibitor metalloproteinases type 2 (TIMP-2).[10] MMP-9 is also expressed by a variety of cell types and is inhibited by TIMP type 4 (TIMP-4). Because increased expression of MMP-2 and MMP-9 has been found in the outflow vein tissue, after AVF construction,[7,11] MMP expression in patient serum at the time of the initial surgery may serve as an important biomarker of AVF maturation. In this study, we sought to identify whether patient serum MMP expression and inhibition are associated with successful AVF maturation.

METHODS

Human study methods. A prospective evaluation of patients undergoing AVF construction for chronic renal insufficiency was performed under Institutional Review Board approval at the Northern California Veterans Affairs Health Care System. All patients were enrolled and monitored at the Sacramento Veterans Affairs Medical Center, and provided written consent.

From the Department of Surgery, University of California, Davis.

Supported in part by the National Institutes of Health Grants: HL096640, HL061507, HL070752, the Peripheral Vascular Surgery Society Academic Award, and the Society for Vascular Surgery Seed Award.

Competition of interest: none.

Presented at the Twenty-fifth Annual Meeting Western Vascular Society, Sunriver, Ore, Sep 25-28, 2010.

Reprint requests: Eugene S. Lee, MD, PhD, 4860 Y St, Ste 3400, Vascular Center, Sacramento, CA 95817 (e-mail: eugenes.Lee@ucdmc.ucdavis.edu)

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a competition of interest.

0741-5214/$36.00

Published by Elsevier Inc. on behalf of the Society for Vascular Surgery.

doi:10.1016/j.jvs.2011.02.056

Table. Data for all patients within the study[a]

Pt	*Age ± SD*	*Procedure*	*DM*	*HTN*	*Tob*	*CAD*	*CVA*	*Statin use*
1	52	Wrist	Y	Y	N	N	N	N
2	48	Wrist	N	Y	N	N	N	Y
3	54	BB	N	Y	Y	N	N	Y
4	67	BB	Y	Y	N	N	N	Y
5	84	BB	N	Y	N	N	N	Y
6	67	Wrist	Y	Y	N	N	N	Y
7	81	BB	N	N	N	N	N	N
	65 ± 14	57%	43%	86%	14%	0%	0%	71%
8	52	BB	Y	Y	N	N	N	N
9	69	BB	Y	Y	Y	N	N	Y
10	68	BB	Y	Y	N	Y	N	Y
11	61	Wrist	Y	Y	N	Y	N	Y
12	62	BC	Y	Y	N	N	N	Y
13	64	BB	Y	Y	N	Y	N	Y
14	56	BB	Y	Y	N	N	N	N
15	58	BB	Y	Y	N	N	N	Y
16	46	BC	Y	Y	N	Y	N	Y
17	61	Wrist	Y	Y	Y	N	Y	Y
18	68	BC	Y	Y	N	N	N	Y
19	67	BC	N	Y	N	N	N	Y
20	59	BC	Y	Y	Y	N	N	N
	61 ± 7	15%	92%	100%	23%	31%	8%	77%
P[c]	.41	.17	.33	.82	.7	.94	.47	.92

AVF, Arteriovenous fistula; *BB,* brachiobasilic; *BC,* brachiocephalic; *CAD,* coronary artery disease; *CVA,* cerebrovascular accident (stroke); *DM,* diabetes mellitus; *ESRF DX,* diagnosis leading to end-stage renal failure; *F/U,* follow-up; *FSGS,* focal sclerosis glomerulosclerosis; *HTN,* hypertension; *NM,* nonmaturation; *Tob,* tobacco use.

[a]Continuous variables are reported with ± standard deviation.

[b]Access, at the time of the index AVF construction, whether another access site was achieved or this was an initial access site.

[c]*P* values comparing the two variables.

[d]Statistically significant.

A patient history and physical examination was performed and pertinent medical history documented. Preoperative vein mapping without a tourniquet was done using duplex ultrasound imaging to document vein diameter, patency, and arterial patency of both upper extremities. As specified in the 2006 Dialysis on Quality Initiative (DOQI) vascular access guidelines,[12] a radial artery diameter of >0.2 cm and a vein diameter of >0.2 cm at the wrist or >0.3 cm at the antecubital fossa was a requirement for primary AVF. The minimum diameter was also a determinant in the location in which the AVF would be constructed. Both arms were mapped in all patients, and the most distal vein segment in the nondominant arm was used if all diameter criteria were met.

Local anesthesia, with monitored anesthesia care, was used for the construction of the AVF. The arterial and venous segments were dissected according to routine surgical care. At the time of surgery, 20 mL of blood was obtained from the open artery via an 18-gauge angiocatheter. Blood samples were immediately placed in heparin-containing tubes (green-top tubes) and kept at room temperature for immediate transport to the laboratory.

Immediate centrifugation and preparation was performed ≤2 hours of sample collection. Once a serum pellet was collected, the sample was kept at −80°C for later enzyme-linked immunosorbent assay (ELISA analysis).

After surgical construction, the patients were seen in the vascular surgery clinic at 2 and 6 weeks to assess for surgical healing and AVF patency. Patients were monitored longitudinally until a mature AVF was achieved. If the AVF was abandoned for another site and a new AVF constructed, the follow-up ended for the abandoned access site. Intervention was recommended in AVFs with velocity findings consistent with stenosis. Patients with deep vein segments underwent secondary superficialization through a fistula elevation procedure or a basilic vein transposition.

Determination of AVF maturation. Patients were deemed to have a mature AVF when the first two of three criteria were met for patients not yet receiving dialysis or at least the third criterion was met if the patient required dialysis:

1. Vein diameter >0.6 mm.
2. Flow volumes >600 mL/min.
3. Complete two-needle cannulation for two-thirds or more of all dialysis runs for 1 month after initiating dialysis.

A duplex assessment of the AVF was performed 6 weeks after AVF creation, but no specific timeframe from AVF creation to the initial dialysis access was established, because early referral for AVF may lead to the diagnosis of "failed AVF," simply due to the nonuse of the dialysis

Table. Continued

ESRF DX	*Dialysis access*[b]	*Time on dialysis*	*Time to AVF use*	*F/U*	*Vein diameter, mm*[b]		*Flow volume (mL/min)*	*2nd proc*
					Before	*After*		
FSGS	Catheter	5	NM	3	0.26	0.33	162	N
FSGS	Catheter	2	Thromb	1	0.63	. . .	. . .	N
FSGS	Catheter	13	NM	5	0.36	0.6	609	N
DM	Initial	0	NM	10	0.22	0.38	248	Y
HTN	Initial	3	Thromb	3	0.38	. . .	. . .	N
DM	Initial	0	NM	2	0.23	0.37	300	N
HTN	Catheter	168	Thromb	2	0.41	. . .	. . .	N
	57%	27	. . .	3.7	0.36 ± 0.14	0.42 ± 0.12	329.8 ± 195	. . .
FSGS	Catheter	9	10	17	0.48	0.74	1435	Y
HTN	Catheter	1	5	18	0.29	0.8	1048	Y
DM	Initial	0	10	16	0.37	0.62	1300	Y
DM	Catheter	2	2	2	0.24	. . .	. . .	N
DM	Initial	0	4	15	0.29	0.85	972	Y
DM	Catheter	48	3	3	0.23	0.84	1009	Y
DM	Catheter	1	8	8	0.41	0.80	1000	Y
DM	Initial	0	9	22	0.56	1.3	4600	Y
DM	Catheter	1	3	3	0.67	1.0	1070	Y
DM	Catheter	4	4	26	0.22	. . .	. . .	N
DM	Catheter	5	4	18	0.42	0.79	1189	Y
HTN	Initial	0	50	50	0.3	0.99	1901	Y
DM	Catheter	13	4	30	0.34	0.84	800	Y
	69%	6.5	8.9	17.5	0.37 ± 0.14	0.87 ± 0.18	1484 ± 1075	
	.80	.25		.01[d]	.82	.0005[c]	.06	

access. If the hemodialysis access could not be accessed or duplex ultrasound imaging showed the vein segments were too small for hemodialysis access, the patients could undergo another hemodialysis access procedure. If the AVF was thrombosed, the access procedure was considered a failure. The patients were divided into a successful AVF group and a failed AVF group for protein analysis.

Serum analysis MMP methods. Patient serum levels of MMP-2, MMP-9, TIMP-2, and TIMP-4 were profiled using commercially available ELISA kits (R&D Systems, Minneapolis, Minn). Background activity in the negative control wells was subtracted from the experimental wells in reporting the data. The positive controls were the recombinant protein standards, and the negative controls were the calibrator diluents. The sensitivity of the assay for each protein was MMP-2, 0.0147 ng/mL; MMP-9, <0.156 ng/mL; TIMP-2, 0.011 ng/mL; and TIMP-4, 4.91 pg/mL.

Serum was obtained from peripheral blood collected from the patient by centrifugation at 400*g* for 30 minutes and was stored at −80°C until tested. Patient sera were divided according to the clinical outcome of failed vs matured AVF. For testing, serum was thawed and diluted with an appropriate concentration of dilution buffer. Samples and controls were added to 96-well plates that were coated with an antibody specific to the protein of interest. After 2 hours of incubation, plates were washed and then a secondary antibody to the protein of interest, which was conjugated to horseradish peroxidase, was added. After 2 hours of incubation, plates were washed and incubated with substrate solution for 30 minutes. An acid stop solution was added, and the plate was read on a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, Calif) at 450 nm, with wavelength correction at 540 nm. A ratio of MMP/TIMP was used as an indicator of MMP activity, with a higher ratio signifying higher activity.[13]

Statistical analysis. Results are reported as mean ± standard deviation. Excel software (Microsoft Inc, Redmond, Wash) was used to perform a two-tailed *t* test, which was used to compare groups with continuous variables, and a χ^2 analysis for proportions. A difference with a value of $P < .05$ was considered significant.

RESULTS

Of the 20 patients who were enrolled, AVF maturation was successful in 13 (age, 64.7 ± 14.2 years) and failed in 7 (age, 60.8 ± 7 years; $P = .41$). The preoperative vein diameters were similar in the matured group (0.37 ± 0.014 mm) and in the failed group (0.36 ± 0.14 mm; $P = .82$). There were 15% wrist fistulas in the mature group and 57% wrist fistulas in the failed group ($P = .17$). The groups had similar comorbidities (Table). The average follow-up for the matured AVF group was 18 months, and the average

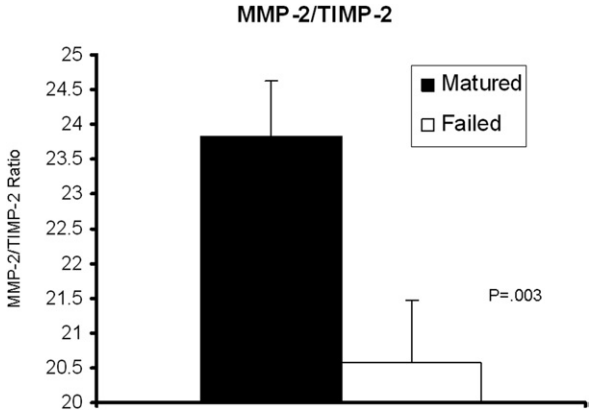

Fig 1. Significantly higher levels of matrix metalloproteinase-2 (*MMP-2*)/tissue inhibitor of metalloproteinase-2 (*TIMP-2*) ratios are identified in the serum of patients whose arteriovenous fistula successfully matured compared with the serum of patients whose arteriovenous fistula failed ($P = .003$). *Error bars* show the standard deviation.

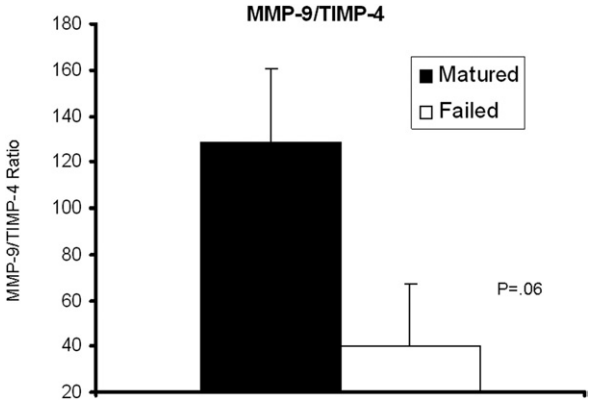

Fig 2. Higher levels of matrix metalloproteinase-9 (*MMP-9*) to tissue inhibitor of metalloproteinase-4 (*TIMP-4*) ratios are identified in serum of patients who had successful maturation of their arteriovenous fistula when compared to the serum of patients who had failure ($P = .06$). *Error bars* show the standard deviation.

time to abandonment for the failed AVF group was 4 months. Catheter placement was required in 13 of 20 patients (65%) during the follow-up period.

At 1 month after the index AVF construction, no AVF was deemed mature. The average time from AVF creation to actual dialysis access was 8.9 months. A secondary fistula elevation or transposition procedure was required in 11 of 13 patients who had matured AVF to allow for successful access of the index AVF.

Of the seven patients that had a failed AVF, four patients had a patent AVF but nonmaturation. One patient in the nonmaturation group had a maximum AVF vein diameter of 0.38 mm and a flow volume of 248 mL/min. This patient underwent fistulogram and balloon angioplasty, with marginal success in the attempt to accelerate maturation.

Significantly higher serum levels of MMP-2/TIMP-2 were found in patients who had AVFs that matured (23.8 ± 0.8) compared with those that failed (20.6 ± 0.9; $P = .003$). Similarly, a trend toward increased serum levels of MMP-9/TIMP-4 was found in patients with a successful AVF (128.6 ± 34.3) vs those with a failed AVF (40.2 ± 10.1; $P = .06$; Figs 1 and 2).

DISCUSSION

Since the National Kidney Foundation introduced the DOQI, guidelines and access management criteria are being used to judge vascular access programs.[2] In a recent review of the natural history of autogenous fistulas for first-time dialysis access, only 11% of primary AVFs matured without intervention, and 48% of the primary AVFs were ultimately used for hemodialysis access. The mean time to AVF maturation was 146 days, and the mean time for AVF abandonment was 162 days.[14]

Given these results, many patients undergo repetitive unsuccessful procedures. To improve this clinical problem, investigators have focused on vascular remodeling and vascular pathology. An animal model has been used to evaluate MMPs, which would be an obvious choice because these proteins have a well-established role in intimal hyperplasia[15] and vessel enlargement[6,7] when it comes to AVF maturation. The MMPs are a family of multidomain endopeptidases that regulate physiologic and pathologic vascular remodeling under conditions such as atherosclerosis, arterial aneurysm, graft, and wound healing. The major function of MMPs is degrading extracellular matrix components, thereby allowing circulating cells and smooth muscle cells to migrate into the vessel wall that undergoes outward remodeling. MMPs also target nonmatrix substrates such as cytokines (tumor necrosis factor-α, interleukin-1) and growth factors (vascular endothelial growth factor, transforming growth factor-β, basic fibroblast growth factor).[16]

Vascular cells (endothelium) and inflammatory cells (monocytes) can both produce MMPs. MMPs are often secreted as proenzymes (latent) and then are processed to active forms. Enzymatic activation requires removal of a prodomain secondary to conformational changes that reveal the catalytic site, and hence the basis for detection of both latent and activated MMPs by zymography.

The best recognized activity regulators are cell-associated MT-MMPs and TIMPs. Using MMP-2 as an example, activation occurs via formation of a tertiary complex containing MT1-MMP (activator), TIMP-2 (inhibitor), and MMP-2 on the endothelial cell surface. Equilibrium is maintained by balanced activities of these enzymes. Thus, the MMP/TIMP ratio is often used as an indicator of MMP activity, with a higher ratio indicating higher activity.[13,17]

The endothelium is an important source of MMPs in the vascular wall.[18] Endothelial injury can cause impaired MMP production and activity required for expansive remodeling.[19] In particular, MMP-2 and MMP-9 activity has

been established as a mechanistic step for the venous outflow segment of an AVF to mature in animal models.[6,7,15,20] However, intimal hyperplasia has also been associated with increased MMP-2[21] and MMP-9 activity.[15] The relative contribution of MMP activity to remodeling vs intimal hyperplasia is not clearly known.[4,22] Given the critical role of expansive remodeling in preserving vessel lumen and patency in fistula maturation, the MMP effect is considered more beneficial than detrimental. Indeed, a recent study demonstrates that MMPs are crucial in the expansive remodeling of AVF in rats.[7]

This study is an attempt to evaluate biologic serum markers to assess for the potential of AVF maturation in trying to predict initial AVF maturation. Biomarkers have demonstrated a great value in the diagnosis and treatment of many cardiovascular disease entities, such as troponin in the diagnosis of acute myocardial infarction[23] and C-reactive protein in predicting cardiac risk.[24] More recently, circulating biomarkers of fibrinogen, D-dimer, and interleukin-6 have been suggested as areas of interest in detecting the presence and progression of abdominal aortic aneurysms[25] and other types of vascular injury.[26] Circulating biomarkers offer diagnostic or prognostic value by reflecting the disease state and predilection based on plasma measurements of molecules or proteins.[24]

The clinical effect of using preoperative markers resides with their use as a potential adjunct to other known clinical parameters that best maximize AVF maturation, such as a thorough physical examination, arterial inflow assessment, and noninvasive vein mapping. The findings in this study, with a limited number of patients, point to the potential use of known enzymes and their inhibitors (MMP-2 and TIMP-2) that are necessary in vascular remodeling. The ELISA technique is readily available in many basic laboratories, and the potential application to clinical practice is possible.

However, the data presented in this study demonstrate relative value differences between patients with an AVF that matured vs those with an AVF did not. Patient sample size was small, and no significant differences were found in traditional clinical parameters in predicting success. A follow-up study designed to determine predictability is in progress so that an ELISA can be performed in a prospective manner and an estimate of successful maturation can be made.

Since the Fistula First Initiative, AVF prevalence has increased from 24% to 57.5% in the United States from 2004 to December 2010.[27] However, central vein catheter use for implementation of dialysis has risen 1.5- to 3.0-fold from 1996 to 2007 in the international community when evaluating trends of rising AVF prevalence and decreasing arteriovenous graft use.[28]

Others dispute the application of these numbers to the United States. Spergel[29] states that despite the rise in AVF prevalence rate over the past several years since the Fistula First Initiative in 2004, catheter use has remained flat, at 27% to 28%. This has left many difficulties in clinical care, such as the quandary of an AV graft vs an AVF and catheter in the face of marginal or unusable veins.[1] The use of MMPs in modulating practice patterns in deciding which treatment option is best is potentially promising and worth further research.

A severe limitation of this study has been the wide variation in the definition of AVF maturation.[4] Previous surgical studies have defined adequate maturation as deemed by the vascular surgeon and nephrologist according to thrill characteristics and AVF diameter.[30] Other studies have defined a mature AVF as multiple successful cannulation attempts for dialysis. Nephrologists have defined maturation in other ways. Lok et al[31] define AVF maturation as the continuous use of the AVF for 1 month within 6 months of AVF creation, regardless of secondary interventions required to mature the AVF. However, the Dialysis Access Consortium had the strictest definition of AVF maturation, where an AVF needs to have 8 of 12 successful dialysis sessions during a 30-day "suitability" period within the first 150 days of AVF creation.[3] The DOQI access group plans to publish guidelines for AVF maturation in 2011.

Serum MMP levels are markers of inflammation and are known to be increased in renal failure. Patients with renal failure, but who are not yet receiving dialysis, have the highest levels of serum MMP-2. When patients begin dialysis, the MMP levels decrease but do not fall to control levels.[17] The data presented in this study used patients in various stages of kidney failure and were predialysis or were receiving hemodialysis. These data were confounded by including nondialysis patients in various stages of renal insufficiency together with those patients receiving hemodialysis. MMP levels have been shown to decrease once a patient is receiving hemodialysis, but not quite to levels seen in control patients without any renal insufficiency. Ironically, MMP levels could be serially tested to identify a peak increase to when the timing of initial AVF should be constructed. Further data should be collected and standardized in patients who have not yet begun dialysis to assess the likelihood for AVF maturation.

CONCLUSIONS

MMP-2 and TIMP-2 levels were different among patients whose fistulas matured vs those whose fistulas did not mature. Further follow-up studies to determine the predictability of AVF maturation using relative patient serum levels of MMP-2 and TIMP-2 are ongoing.

AUTHOR CONTRIBUTIONS

Conception and design: EL, MW, SY
Analysis and interpretation: EL, QS, MG, SS
Data collection: QS, RP, MG,SS
Writing the article: EL, RP
Critical revision of the article: EL
Final approval of the article: EL
Statistical analysis: EL
Obtained funding: EL, MW, SY
Overall responsibility: EL

REFERENCES

1. James MT, Manns BJ, Hemmelgarn BR, Ravani P. What's next after fistula first: is an arteriovenous graft or central venous catheter preferable when an arteriovenous fistula is not possible? Semin Dial 2009;22:539-44.
2. Kopple JD. National kidney foundation K/DOQI clinical practice guidelines for nutrition in chronic renal failure. Am J Kidney Dis 2001;37(**1** Suppl 2):S137-81.
3. Dember LM, Beck GJ, Allon M, Delmez JA, Dixon BS, Greenberg A, et al. Effect of clopidogrel on early failure of arteriovenous fistulas for hemodialysis: a randomized controlled trial. JAMA 2008;299:2164-71.
4. Dixon BS. Why don't fistulas mature? Kidney Int 2006;70:1413-22.
5. Tonelli M. Randomized trials in hemodialysis patients: time to step up to the plate. JAMA 2008;299:2205-7.
6. Berceli SA, Jiang Z, Klingman NV, Schultz GS, Ozaki CK. Early differential MMP-2 and -9 dynamics during flow-induced arterial and vein graft adaptations. J Surg Res 2006;134:327-34.
7. Chan CY, Chen YS, Ma MC, Chen CF, Chen CF. Remodeling of experimental arteriovenous fistula with increased matrix metalloproteinase expression in rats. J Vasc Surg 2007;45:804-11.
8. Chase AJ, Newby AC. Regulation of matrix metalloproteinase (matrixin) genes in blood vessels: a multi-step recruitment model for pathological remodelling. J Vasc Res 2003;40:329-43.
9. Kargozaran H, Yuan SY, Breslin JW, Watson KD, Gaudreault N, Breen A, et al. A role for endothelial-derived matrix metalloproteinase-2 in breast cancer cell transmigration across the endothelial-basement membrane barrier. Clin Exp Metastasis 2007;24:495-502.
10. Goodall S, Crowther M, Hemingway DM, Bell PR, Thompson MM. Ubiquitous elevation of matrix metalloproteinase-2 expression in the vasculature of patients with abdominal aneurysms. Circulation 2001;104:304-9.
11. Lee ES, Shen Q, Pitts RL, Guo M, Wu MH, Yuan S. Vein tissue expression of matrix metalloproteinase as biomarker for hemodialysis arteriovenous fistula maturation. Vasc Endovasc Surg 2010;304:674-9.
12. Vascular Access 2006 Work Group. Clinical practice guidelines for vascular access. Am J Kid Dis 2006;48(Suppl):S176-247.
13. Raffetto JD, Khalil RA. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. Biochem Pharmacol 2008;75:346-59.
14. Biuckians A, Scott EC, Meier GH, Panneton JM, Glickman MH. The natural history of autologous fistulas as first-time dialysis access in the KDOQI era. J Vasc Surg 2008;47:415-21; discussion: 420-1.
15. Misra S, Fu AA, Anderson JL, Sethi S, Glockner JF, McKusick MA, et al. The rat femoral arteriovenous fistula model: increased expression of matrix metalloproteinase-2 and -9 at the venous stenosis. J Vasc Interv Radiol 2008;19:587-94.
16. Wang M, Zhao D, Spinetti G, Zhang J, Jiang LQ, Pintus G, et al. Matrix metalloproteinase 2 activation of transforming growth factor-beta1 (TGF-beta1) and TGF-beta1-type II receptor signaling within the aged arterial wall. Arterioscler Thromb Vasc Biol 2006;26:1503-9.
17. Preston GA, Barrett CV, Alcorta DA, Hogan SL, Dinwiddie L, Jennette JC, et al. Serum matrix metalloproteinases MMP-2 and MMP-3 levels in dialysis patients vary independently of CRP and IL-6 levels. Nephron 2002;92:817-23.
18. Dobaczewski M, Gonzalez-Quesada C, Frangogiannis NG. The extracellular matrix as a modulator of the inflammatory and reparative response following myocardial infarction. J Mol Cell Cardiol 2010;48:504-11.
19. Tronc F, Mallat Z, Lehoux S, Wassef M, Esposito B, Tedgui A, et al. Role of matrix metalloproteinases in blood flow-induced arterial enlargement: interaction with NO. Arterioscler Thromb Vasc Biol 2000;20:E120-6.
20. Karwowski JK, Markezich A, Whitson J, Abbruzzese TA, Zarins CK, Dalman RL. Dose-dependent limitation of arterial enlargement by the matrix metalloproteinase inhibitor RS-113;456. J Surg Res 1999;87:122-9.
21. Tummers AM, Mountain DJ, Mix JW, Kirkpatrick SS, Cassada DC, Stevens SL, et al. Serum levels of matrix metalloproteinase-2 as a marker of intimal hyperplasia. J Surg Res 2010;160:9-13.
22. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002;90:251-62.
23. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined–a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959-69.
24. Tsimikas S, Willerson JT, Ridker PM. C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. J Am Coll Cardiol 2006;47(8 Suppl):C19-31.
25. Golledge J, Tsao PS, Dalman RL, Norman PE. Circulating markers of abdominal aortic aneurysm presence and progression. Circulation 2008;118:2382-92.
26. Guo M, Daines D, Tang J, Shen Q, Perrin RM, Takada Y, et al. Fibrinogen-gamma C-terminal fragments induce endothelial barrier dysfunction and microvascular leak via integrin-mediated and RhoA-dependent mechanism. Arterioscler Thromb Vasc Biol 2009;29:394-400.
27. Arteriovenous Fistula First. http://www.fistulafirst.org. Accessed February 21, 2011.
28. Ethier J, Mendelssohn DC, Elder SJ, Hasegawa T, Akizawa T, Akiba T, et al. Vascular access use and outcomes: an international perspective from the Dialysis Outcomes and Practice Patterns Study. Nephrol Dial Transplant 2008;23:3219-26.
29. Spergel LM. Has the Fistula First Breakthrough Initiative caused an increase in catheter prevalence? Semin Dial 2008;21:550-2.
30. Patel ST, Hughes J, Mills JL Sr. Failure of arteriovenous fistula maturation: an unintended consequence of exceeding dialysis outcome quality initiative guidelines for hemodialysis access. J Vasc Surg 2003;38:439-45; discussion:445.
31. Lok CE, Allon M, Moist L, Oliver MJ, Shah H, Zimmerman D, et al. Risk equation determining unsuccessful cannulation events and failure to maturation in arteriovenous fistulas (REDUCE FTM I). J Am Soc Nephrol 2006;17:3204-12.

Submitted Nov 14, 2010; accepted Feb 22, 2011.

DISCUSSION

Dr Robert J. Hye (*San Diego, Calif*). The past decade has seen increasing recognition of the value of autogenous arteriovenous (AV) fistulas in the hemodialysis population. AV fistulas are associated with better patency, reduced risk of infection and less mortality as compared to AV grafts and catheters. The Centers of Medicare and Medicaid Services (CMS) Fistula First project and Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines have aggressively promoted the use of AV fistulas, and the prevalence in the U.S. has increased from 33% in January of 2004 to 56% in June of 2010. In my own organization, Southern California Kaiser Permanente, our fistula prevalence now exceeds 70% with some centers over 85%.

Efforts on the part of surgeons to provide arteriovenous fistulas for patients have been accompanied by a frustratingly high and actually, an increasing rate of maturation failure of between 25% and 45%. Anatomic and demographic variables such as blood vessel diameter, distensibility, age, gender, diabetes, and race have all been associated with an increased early failure rate. Biologic processes that either inhibit vasodilatation or result in neointimal hyperplasia have also been implicated as causing fistulas to fail to mature as expected.

Dr Lee and his colleagues have chosen to examine the biologic aspects of fistula failure and specifically the role of the metalloproteases (MMP)-2, MMP-9 and their tissue inhibitors in this process. A similar presentation from their group last year focused on tissue levels of these molecules in regard to fistula maturation while this report concentrates on serum levels, a potentially more clinically

useful tool. These proteases have been studied in a variety of vascular diseases, and their role is complex, involving variation in the expression of the proteases themselves as well as their regulatory inhibitors and other bioactive molecules.

Expression of metalloproteases has been studied in many vascular diseases and has been found to be increased in both patients with aneurysmal disease and chronic kidney disease. Patients with diabetic nephropathy have been reported to have both increased and decreased levels. Interestingly, one of the effects of statins is a reduction in expression and activity of MMP-9. The exact role of these proteins in vascular remodeling is not entirely defined as yet.

The ideal biophysiologic response to creation of an arteriovenous fistula that matures has been described as increased shear stress in both the inflow artery and outflow vein causing an increase in superoxide production. This combines with nitric oxide to form peroxynitrite which upregulates MMP production, enhancing vasodilatation by fragmenting the internal elastic lamina of the vessels. Occlusion of the fistula or maturation failure occurs when this process is interrupted or does not proceed as described. Thus, the authors' premise is that measuring MMP-2 and 9 activity and their regulatory inhibitors before, or at the time of fistula creation, may allow us to predict maturation. The results presented today, although in a very small sample size, show that measurement of metalloprotease activity expressed as a ratio of levels of the protease to its inhibitor, is increased at the time of surgery in patients who go on to develop usable arteriovenous fistulas.

While the results are appealing, a number of important issues are not addressed in the manuscript or presentation and are the basis of my questions. It is not clear to me when and from where the blood samples were obtained. Was it from the dissected artery or vein? Wouldn't it have been be more valid to obtain the sample preoperatively from a peripheral vein?

Most of the literature in this area documents increased MMP activity in response to fistula creation and not increased levels at baseline. Is their any evidence that patients with increased basal levels also have increased expression or activity in response to fistula creation?

Demographics of the patient population were not provided; specifically, how many women and diabetics were in each group as these are factors also associated with differing rates of maturation.

MMP expression varies with severity of renal failure, were the successful and failed fistula group comparable in the severity of their kidney disease?

Although a minimum criteria for vessel size is provided, there is no further description of the range or if the sizes were comparable between the successful and the failed group. Can you tell us about that?

Finally, the criteria for a successful fistula is fairly soft, three successful dialysis runs. Were these patients all dialyzed in the same unit? Did you examine flow rates or clearance in these patients? Do you have any longer-term data on outcome in these patients?

It would be very useful to have a biomarker such as MMP-2 or -9 to add to our armamentarium to be able to predict AV fistula maturation. I look forward to Dr Lee's further endeavors in this area. I would like to thank the society for the opportunity to discuss this interesting and well-presented paper.

Dr Eugene S. Lee. Thank you, Dr Hye, for discussing the manuscript and your insights. I will answer the questions in order presented. Blood samples were obtained from the dissected artery intraoperatively. In conducting the study, I was concerned that obtaining blood from the vein would unintentionally injure the vein and/or the endothelium, and I wanted to avoid this possibility. I am not sure whether blood from the peripheral vein vs the artery is more valid. In the next set of samples, I plan to draw both arterial and venous blood samples and will assay for the MMP levels to see a difference.

In terms of MMP activity in response to fistula creation is interesting, as the literature has confirmed that patients with worsening renal functioning have increased MMP activity until they go on to dialysis. To my knowledge, there is no information regarding serum MMP activity in response to fistula creation.

I plan to include demographic information in the final manuscript. However, the patient population is mainly a veteran or VA population and this group in the study is entirely men with a vast majority of patients who are diabetic. In fact, a large percentage of these patients have diabetes as the cause of their renal failure.

As for MMP expression, I will have to further evaluate the data with regards to the severity of renal failure. Many of the patients referred for AV access were in chronic kidney disease (CKD) stage 4, but at the time of surgery, there were a proportion of patients who had proceeded to CKD stage 5 (on hemodialysis) at the time of arteriovenous fistula (AVF) creation, making the analysis a mixed analysis of various levels of MMP expression. Clearly, this is a weakness in the purity of the data, but it does add further credence to the fact that varying renal dysfunction may not significantly impact the differences seen in patients who mature their fistulae vs those who do not.

In terms of vessel sizes, I do not have exact vessel sizes in each group for you today, but I recollect that the vessel sizes were comparable between groups. I hope to have this information in the final manuscript.

Finally, with respect to the rather "soft criteria" for successful fistula creation, I have been struggling with this definition. KDOQI has plans to publish a more uniform standard of a "successful" AVF in 2011. When one reviews the literature, the definition is quite varied; hopefully, soon we can come to a consensus. As for these patients being dialyzed in the same unit, the answer to this is no. In terms of flow rates, we do have information regarding flow volumes and vein diameters, which I will have to review.