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A Novel Method for Rapid Bedside Measurement of GFR

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ABSTRACT

Background Direct quantitative measurement of GFR (mGFR) remains a specialized task primarily performed in research settings. Multiple formulas for estimating GFR have been developed that use the readily available endogenous biomarkers creatinine and/or cystatin C. However, eGFR formulas have limitations, and an accurate mGFR is necessary in some clinical situations and for certain patient populations. We conducted a prospective, open-label study to evaluate a novel rapid technique for determining plasma volume and mGFR.

Methods We developed a new exogenous biomarker, visible fluorescent injectate (VFI), consisting of a large 150-kD rhodamine derivative and small 5-kD fluorescein carboxymethylated dextrans. After a single intravenous injection of VFI, plasma volume and mGFR can be determined on the basis of the plasma pharmacokinetics of the rhodamine derivative and fluorescein carboxymethylated dextrans, respectively. In this study involving 32 adults with normal kidney function (n=16), CKD stage 3 (n=8), or CKD stage 4 (n=8), we compared VFI-based mGFR values with values obtained by measuring iohexol plasma disappearance. VFI-based mGFR required three 0.5-ml blood draws over 3 hours; iohexol-based mGFR required five samples taken over 6 hours. Eight healthy participants received repeat VFI injections at 24 hours.

Results VFI-based mGFR values showed close linear correlation with the iohexol-based mGFR values in all participants. Injections were well tolerated, including when given on consecutive days. No serious adverse events were reported. VFI-based mGFR was highly reproducible.

Conclusions The VFI-based approach allows for the rapid determination of mGFR at the bedside while maintaining patient safety and measurement accuracy and reproducibility.

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The best index of renal function remains the GFR.[1] Its measurement relies on an ideal filtration marker that is neither absorbed nor secreted by the nephron and does not alter kidney function. To date, urinary clearance during a continuous infusion of inulin is considered the gold standard for such a measurement.[1] In clinical practice, inulin-based measurement of GFR (mGFR) is difficult to perform, expensive, and injectable inulin is presently not available in all countries, including the United States. Thus, clinicians rely primarily on eGFR formulas using levels of readily available endogenous markers such as creatinine[2,3] and cystatin C.[4] Despite their marked contribution to patient care, eGFR formulas have significant limitations. In this study, we present the results of a novel biomarker showing very promising results for the measurement of GFR and plasma volume at the bedside.

METHODS

Study Design

This was a phase 2b, prospective, open-label study (clinicaltrials.gov identifier NCT03095391) conducted at two sites: the University of Alabama at Birmingham

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and the Clinical Research Organization ICON. Four cohorts (of eight participants each) were enrolled between June 13, 2017 and August 30, 2017. All participants provided written informed consent and the study adhered to the Declaration of Helsinki. The study aimed to assess the safety and tolerability of visible fluorescent injectate (VFI) and to compare the GFRs determined by FAST mGFR technology (VFI mGFR) with iohexol clearance. VFI consisted of 12 mg of FD003 and 35 mg of FD001 (150 and 5 kD conjugated dextrans, respectively). VFI was infused intravenously over 30 seconds and blood samples were subsequently collected at 15, 60, and 170 minutes. Blood plasma was diluted at 250 μl of plasma to 2.0 ml of a fluorescence-enhancing reagent and analyzed on a Turner Trilogy filter fluorimeter to determine the concentrations of FD001 and FD003, respectively. Plasma volume was determined using the early time point, and the concentration of the small dextran GFR marker at time zero was calculated from the measured plasma volume. The four time points (0, 15, 60, and 170 minutes) were then fitted using a two-compartment model and the resulting area under the curve was calculated. The use of the time point 0 determination helped to better resolve the shape of the clearance curve. mGFR was calculated and adjusted to body surface area for comparison with iohexol clearance. For iohexol clearance determination, 5 ml of Omnipaque 300 was infused over 2 minutes and blood samples were taken at 120, 150, 180, 210, and 320 minutes.

Table 1. Baseline characteristics of participants in all four cohorts

Cohort	Participant ID	Race	Ethnicity	Sex	Age, yr	Weight, kg	Height, cm
Cohort 1	1009	White	Hispanic or Latino	F	19	59	158
	1010	White	Hispanic or Latino	M	64	92	171
	1011	White	Hispanic or Latino	M	58	92	176
	1012	Black	Not Hispanic or Latino	F	47	91	176
	1013	White	Not Hispanic or Latino	F	69	83	163
	1014	White	Hispanic or Latino	F	51	82	160
	1015	Black	Not Hispanic or Latino	F	30	74	171
	1016	White	Hispanic or Latino	F	48	65	153
Cohort 2	1001	White	Not Hispanic or Latino	M	24	74.6	171.5
	1002	White	Hispanic or Latino	F	69	59.2	152.1
	1003	White	Not Hispanic or Latino	M	62	76.8	168.5
	1004	White	Not Hispanic or Latino	M	34	79.5	175.4
	1005	White	Hispanic or Latino	F	75	63.6	152
	1006	White	Not Hispanic or Latino	F	50	85.7	169.8
	1007	White	Not Hispanic or Latino	F	61	57.1	164
	1008	White	Hispanic or Latino	M	68	70	160
Cohort 3	2001	White	Not Hispanic or Latino	M	68	110.3	192.2
	2004	White	Not Hispanic or Latino	F	52	68.1	161.6
	2005	White	Not Hispanic or Latino	M	74	102.4	177.5
	2006	White	Not Hispanic or Latino	F	70	76.4	156.5
	2007	White	Not Hispanic or Latino	M	73	103.5	179.8
	1017	White	Hispanic or Latino	M	70	105.6	169
	1018	Black	Not Hispanic or Latino	M	53	114.8	173.9
	2016	Black	Not Hispanic or Latino	F	62	76.6	165.6
Cohort 4	2003	Black	Not Hispanic or Latino	M	59	84.3	184.4
	2009	Black	Not Hispanic or Latino	M	69	147	194.5
	2010	White	Not Hispanic or Latino	F	74	88.7	165.7
	2011	Black	Not Hispanic or Latino	F	43	92.4	160.8
	2012	Black	Not Hispanic or Latino	F	49	90.1	161
	2014	White	Not Hispanic or Latino	M	49	86.4	179.2
	2015	Black	Not Hispanic or Latino	F	57	98.9	161.2
	2017	Black	Not Hispanic or Latino	F	46	88.9	177.8

F, female; M, male.

Significance Statement

Measurement of GFR (mGFR) in the clinical setting remains a highly useful but specialized parameter in the management of patients. Thus, physicians rely primarily on eGFR derived from formulas using endogenous biomarkers. Despite their significant contributions to clinical practice and research, these surrogate values have many limitations. This article describes a novel two-marker dextran based injectate known as visible fluorescent injectate, used to measure simultaneously plasma volume and GFR. The technique proved to be safe, rapid, accurate and reproducible at measuring GFR across a wide range of kidney function. In the future, visible fluorescent injectate will allow mGFR determination at the bedside and in outpatient clinical settings.

The University of Minnesota Advanced Research and Diagnostic Laboratory analyzed the samples. This iohexol method uses the Brochner–Mortensen method of calculation, and has proven to be an accurate comparator.[5]

Cohorts one and two consisted of healthy volunteers, and cohorts three and four included patients with CKD stage 3 and 4, respectively, with variable degrees of proteinuria. Eligible participants had to be 18–75 years old, with a body mass index $\geq$18 and $\leq$40 kg/m^2. Men and women agreed to use medically acceptable methods of contraception (except for participants who were confirmed sterile or postmenopausal females).

All participants underwent VFI mGFR determination within 21 days of screening. Cohort two received an additional VFI dose 24 hours after the first injection. Cohorts two, three, and four had iohexol-based GFR determination as well. Participants were followed for 21($\pm$1) days from their last VFI injection. All visits were conducted in the clinical research unit.

Study Variables

Demographics, medical and surgical history (including concomitant medication use), height, weight, vital signs, and physical examinations were documented. Laboratory tests included chemistry,

hematology, hepatic function panel, follicular stimulating hormone (women only), creatinine phosphokinase, HIV, and hepatitis C and B serologies, as well as urine pregnancy tests and drug screens. Twelve-lead electrocardiograms were obtained. eGFR for eligibility were determined using the CKD Epidemiology Collaboration equation.[3] Participants were assessed for adverse and serious adverse events at each encounter.

Statistical Analyses

A descriptive analysis of study results was reported. Correlation between FAST mGFR and iohexol GFR was determined using Pearson correlation. Bland–Altman analysis determined the limits of agreement.[6]

RESULTS

Thirty three participants were screened and consented. One participant from cohort three had to be withdrawn as no intravenous access could be secured to conduct the study, leaving 32 participants enrolled and included in the current analysis. Baseline characteristics of all participants are shown in Table 1. The mean age was 56.1 years (range 19–75), and 56% were women. There was diverse racial and ethnic representation, with 41% white, 31% black, and 9% Hispanic participants.

VFI administration was well tolerated across all ranges of kidney function and no serious adverse events were reported. The 24-hour repeat VFI mGFR assessment in eight healthy participants (cohort two) showed reliable reproducibility within 5% of baseline GFR values (Figure 1, Table 2). VFI mGFR required three 0.5-ml blood draws over 2.5 hours and were compared with iohexol mGFR on the basis of plasma disappearance studies, using samples taken over 6 hours. Across all cohorts, the VFI mGFR showed near perfect linear correlation when compared with iohexol mGFR, with a coefficient correlation value of 0.996 (Figure 2). Next, a Bland–Altman analysis was performed (Supplemental Figure 1) and confirmed agreement between the two measures of GFR, with a mean difference of −0.49 ml/min (95% confidence interval, −3.65 to +2.68). A representative normalized comparison of the GFR curves for a normal and CKD stage 4 participants is shown in Supplemental Figure 2.

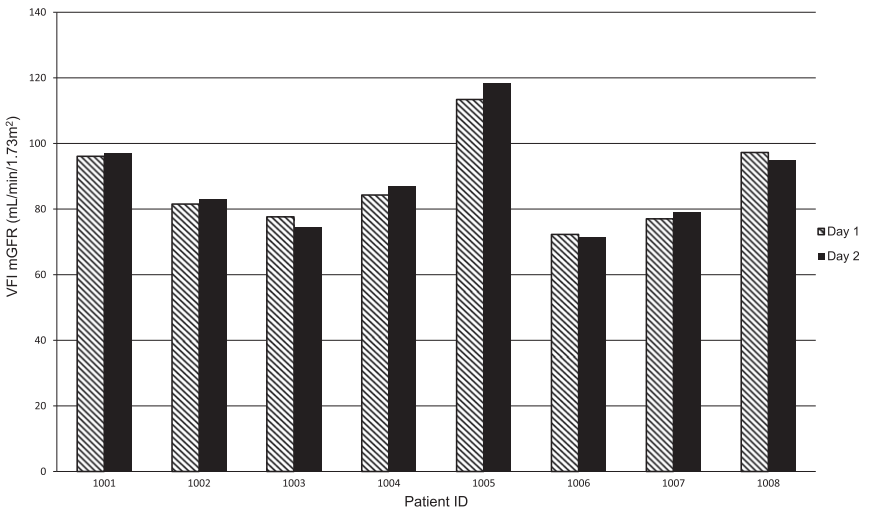

Figure 1. Repeat measurements of VFI mGFR in cohort two participants show very good reproducibility. The dashed line represents VFI mGFR values adjusted for body surface area, obtained on day 1. The solid black line represents VFI mGFR values adjusted for body surface area obtained on day 2, after the second VFI injection at 24 hours. The results show reliable reproducibility within 5% of baseline mGFR values. The VFI mGFR numerical values are provided in Table 2.

DISCUSSION

The gold standard for measuring GFR is inulin clearance; however, the need for a continuous infusion and multiple blood and urine collections limit its use even in research settings. Other methods using chromium 51-EDTA, iothalamate, and iohexol are acceptable alternatives but remain cumbersome, as they require specialized laboratory determinations and their assays can be expensive to perform.[1] In clinical practice, physicians have turned to endogenous biomarkers, such as creatinine, which are readily available. Creatinine-based eGFR formulas are widely used and represent the basis for many diagnostic and management guidelines.[2–4,7] Despite its widespread use, eGFR has limitations in special populations (those with abnormal muscle mass or body surface area), during changes in metabolism (like pregnancy), and when GFR is not steady (during growth, AKI, or after consumption of a high-protein diet).[8] Additionally, eGFR is least accurate when creatinine is normal and does not allow for the measurement of renal reserve.[9,10] Since the initial introduction of the Modification of Diet in Renal Disease eGFR equation,[2] many reiterations of the creatinine-based formula have been published, addressing some of the equation imprecisions across the GFR range, full-age spectrum, and different racial and ethnic backgrounds.[3,11] Other endogenous biomarkers have been explored as alternatives. Of particular interest is cystatin C (which does not share the inherent limitations of creatinine), especially after the standardization of its assay.[11,12] Estimating equations are practical but do not supersede the need for a direct measurement of GFR.[13] The search for a practical and safe exogenous biomarker that will allow a rapid assessment of GFR has been long in the making. Technical advances allowed the measurement of fluorescence intensity decay after a bolus injection of a fluorescence-labeled marker into rodents.[14] These measurements correlated well with kidney function. Subsequently,

Table 2. Comparison of VFI mGFR with iohexol mGFR, and eGFR by CKD-EPI and MDRD formulas in all four cohorts

Cohort	Participant ID	Iohexol mGFR, ml/min per 1.73 m^2	FAST mGFR, ml/min per 1.73 m^2	Creatinine, mg/dl	CKD-EPI eGFR, ml/min per 1.73 m^2	MDRD eGFR, ml/min per 1.73 m^2	FAST PV, ml
Cohort 1: healthy	1009	N/A	111	0.6	133	129	2115
participants	1010		73	1.1	71	67	3050
	1011		87	1.0	83	77	3187
	1012		112	0.7	120	109	3443
	1013		77	0.7	89	83	2775
	1014		89	0.7	101	88	3057
	1015		113	0.7	135	119	2649
	1016		102	0.6	108	107	2667
Cohort 2: healthy	1001	97	96	0.9	119	104	2632
participants;			97		119	104	
repeat VFI dose	1002	82	82	0.7	89	83	2294
			83		89	83	
	1003	79	78	0.7	101	114	3226
			74		101	114	
	1004	82	84	1.1	87	77	2487
			87		87	77	
	1005	114	113	0.5	95	120	2457
			119		95	120	
	1006	72	72	0.7	101	89	2462
			71		101	89	
	1007	77	77	0.8	80	73	2335
			79		80	73	
	1008	99	97	0.6	103	134	2457
			95		103	134	
Cohort 3:	2001	58	56	1.4	51	50	3609
30≤eGFR<60 ml/min	2004	36	37	1.1	58	52	2247
per 1.73 m^2	2005	52	51	1.2	59	59	3288
	2006	56	58	1.0	57	55	2224
	2007	55	54	1.4	49	69	4181
	2016	37	37	1.8	34	35	2505
	1017	39	42	1.5	46	46	3347
	1018	49	50	1.9	46	45	3458
Cohort 4:	2003	32	33	2.9	26	39	3631
15≤eGFR<30 ml/min	2009	17	18	3.4	20	22	6234
per 1.73 m^2	2010	29	31	1.7	29	29	3042
	2011	24	28	3.2	20	19	3204
	2012	23	25	2.7	23	23	3057
	2014	24	22	3.3	21	20	3556
	2015	31	32	2.2	28	28	2899
	2017	26	28	2.6	25	24	3409

CKD EPI, CKD Epidemiology Collaboration Equation; MDRD, Modification of Diet in Renal Disease equation; FAST PV, visible fluorescent injectate–based plasma volume measurement; N/A, not applicable.

using a single bolus of two distinct fluorescence-labeled conjugates (one rapidly filtered by the kidneys and another confined to the vascular space) into rats markedly improved the accuracy of these measurements,[14,15] and they proved reproducible in larger animal models.[16] The results of our phase 2b study show VFI to be a safe biomarker that allows the accurate, rapid, and reproducible measurement of GFR at the bedside in healthy volunteers and across a wide range of CKD. Determining the time point 0 concentration (using the large dextran molecule) improves the measurement accuracy and reduces the time and number of blood draws needed. Additionally, our technique uses a two-compartment model instead of a one-compartment model (as for iohexol and iothalamate), allowing us to measure vascular and not extracellular clearance of the marker. Therefore, less time is needed to generate an mGFR value even at more advanced stages of CKD. Further confirmatory testing in patients with CKD stage 5 is needed. Patients with very large body weights may require a longer time for the VFI to reach steady state, and future studies will determine whether mGFR generation will need an additional time point in that patient population. The fluorescent dyes in VFI allow a rapid read-out, whereas measuring GFR with iohexol or iothalamate

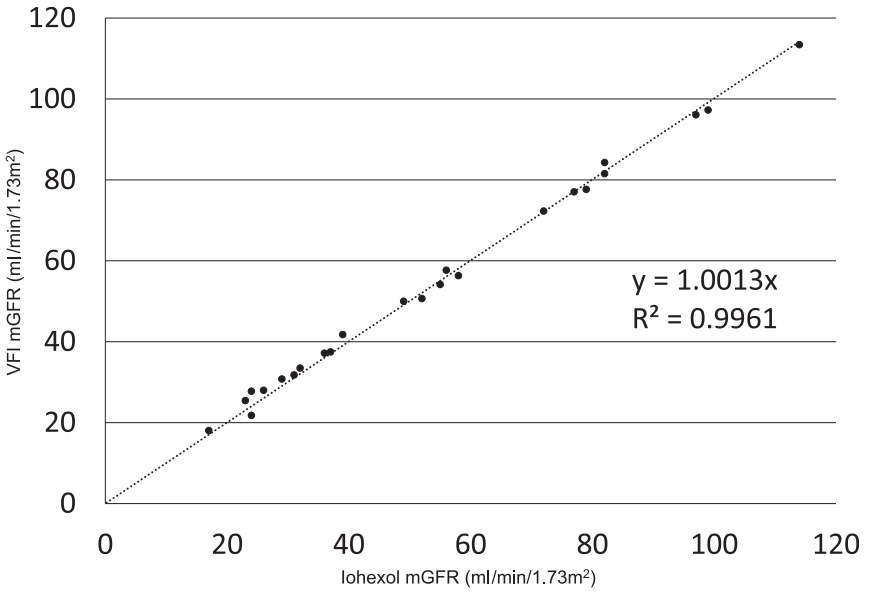

Figure 2. Linear correlation of VFI mGFR (ml/min per 1.73 m^2) and iohexol mGFR (ml/min per 1.73 m^2) show a coefficient of determination of R^2=0.996. VFI mGFR adjusted for body surface area correlated linearly with iohexol mGFR adjusted for body surface area.

requires time-consuming assay analysis using HPLC or mass spectrometry. The two-marker injectate is a promising biomarker for measurement of GFR. This novel technique will potentially allow clinicians to detect early renal function and reserve loss across a wider spectrum of patients, hence introducing earlier treatments to prevent renal loss. It will also allow for identification of hyperfiltration and earlier mitigating therapy. The ability to measure GFR may also change participant selection in research studies and allow for more accurate and timely outcome measures.

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DISCLOSURES

D.V.R. received research funding from FAST BioMedical. D.M. is an employee of FAST BioMedical. R.M.S. is a paid consultant for FAST BioMedical and TdB Consultancy, which collaborates with FAST BioMedical on dextran development. E.S.R. is an employee of FAST BioMedical. J.C.S. received research funding from FAST BioMedical to perform assays. E.D. received research funding from FAST BioMedical. J.S.S. is a cofounder, President, Director, and stockholder in FAST BioMedical. J.M. is the Chief Executive Officer and stock owner in FAST BioMedical. T.C. received research funding from FAST BioMedical. B.A.M. is a cofounder, partial owner, and Medical Director of FAST BioMedical.

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