Plasma Kidney Injury Molecule-1 for Preoperative Prediction of Renal Cell Carcinoma Versus Benign Renal Masses, and Association With Clinical Outcomes

Wenxin Xu, MD[1]; Valerie Gaborieau, DUT[2]; Samuel M. Niman, MS[1]; Anush Mukeria, MD, DSc[3]; Xiaowen Liu, PhD[4]; Krishna P. Maremanda, PhD[5]; Ayumi Takakura, PhD[5]; David Zaridze, MD, DSc[3]; Matthew L. Freedman, MD[1]; Wanling Xie, MS[1]; David F. McDermott, MD[4]; Toni K. Choueiri, MD[1]; Paul J. Catalano, ScD[1]; Venkata Sabbisetti, PhD[5]; Joseph V. Bonventre, MD, PhD[5]; Phillip M. Pierorazio, MD[6]; Nirmish Singla, MD, MSc[7]; Paul Brennan, PhD[2]; and Rupal S. Bhatt, MD, PhD[4]

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ABSTRACT

PURPOSE Both clear cell and papillary renal cell carcinomas (RCCs) overexpress kidney injury molecule-1 (KIM-1). We investigated whether plasma KIM-1 (pKIM-1) may be a useful risk stratification tool among patients with suspicious renal masses.

METHODS Prenephrectomy pKIM-1 was measured in two independent cohorts of patients with renal masses. Cohort 1, from the prospective K2 trial, included 162 patients found to have clear cell RCC (cases) and 162 patients with benign renal masses (controls). Cohort 2 included 247 patients with small (cT1a) renal masses from an academic biorepository, of whom 184 had RCC. We assessed the relationship between pKIM-1, surgical pathology, and clinical outcomes.

RESULTS In Cohort 1, pKIM-1 distinguished RCC versus benign masses with area under the receiver operating curve (AUC-ROC, 0.81 [95% CI, 0.76 to 0.86]). In Cohort 2 (cT1a only), pKIM-1 distinguished RCC versus benign masses (AUC-ROC, 0.74 [95% CI, 0.67 to 0.80]) and the addition of pKIM-1 to an established nomogram for predicting malignancy improved the model AUC-ROC (0.65 [95% CI, 0.57 to 0.74] *v* 0.78 [95% CI, 0.72 to 0.85]). A pKIM-1 cutpoint identified using Cohort 2 demonstrated sensitivity of 92.5% and specificity of 60% for identifying RCC in Cohort 1. In long-term follow-up of RCC cases (Cohort 1), higher prenephrectomy pKIM-1 was associated with worse metastasis-free survival (multivariable MFS hazard ratio [HR] 1.29 per unit increase in log pKIM-1, 95% CI, 1.10 to 1.53) and overall survival (multivariable OS HR 1.31 per unit increase in log pKIM-1, 95% CI, 1.10 to 1.54). In long-term follow-up of Cohort 2, no metastatic events occurred, consistent with the favorable prognosis of resected cT1a RCC.

CONCLUSION Among patients with renal masses, pKIM-1 is associated with malignant pathology, worse MFS, and risk of death. pKIM-1 may be useful for selecting patients with renal masses for intervention versus surveillance.

ACCOMPANYING CONTENT

Appendix

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INTRODUCTION

There are over 76,000 cases of renal cell carcinoma (RCC) in the United States per year, and RCC causes over 13,000 deaths annually.[1] Clear cell RCC (ccRCC) and papillary RCC are the most common RCC subtypes and together account for approximately 90% of all cases.[2,3] Suspicious renal masses, representing possible RCC, are often found incidentally on imaging studies and represent a common clinical conundrum. Although renal masses are often managed by surgical resection,[4] up to one third of partial nephrectomies for renal masses result in benign findings only.[5,6] Percutaneous renal mass biopsy is invasive and has only modest negative predictive value, limiting its routine use.[4] There is significant overtreatment of benign renal masses and clinically indolent RCCs, and better risk stratification is needed to select appropriate patients for active surveillance.[7,8]

Blood-based biomarkers for kidney cancer could fulfill this unmet need. Kidney injury molecule-1 (KIM-1) is a

CONTEXT

Key Objective

Does plasma kidney injury molecule-1 (KIM-1) predict higher chance of malignancy and worse cancer outcomes among patients with suspicious renal masses?

Knowledge Generated

In two independent international cohorts, prenephrectomy plasma KIM-1 distinguishes benign masses from renal cell carcinoma (RCC) and improves performance when added to an established clinical nomogram. Among patients with RCC, those with higher prenephrectomy KIM-1 have worse risk of metastasis and risk of death.

Relevance Statement *(M.A. Carducci)*

This evaluation of KIM-1 in individuals with renal masses is an important proof of concept that blood-based risk stratification of kidney cancer is feasible even for patients with early-stage disease. Patients with renal cell cancer had higher values of KIM-1 when compared to those with benign tumors or cysts—intriguing enough yet requires subsequent validation and clinical trial testing.*

*Relevance section written by *JCO* Associate Editor Michael A. Carducci, MD, FACP, FASCO.

transmembrane glycoprotein that is shed from kidney cancer cells and is a candidate circulating biomarker for clear cell and papillary RCC.[9-12] We previously found that elevated plasma KIM-1 (pKIM-1) after nephrectomy for RCC predicts worse disease-free survival and overall survival,[13] and that patients with new diagnosis of RCC have elevated KIM-1 in banked plasma samples up to 5 years before diagnosis.[9] No previous studies, to our knowledge, have addressed the question of whether pKIM-1 can help to risk stratify patients before surgery. We therefore investigated whether prenephrectomy pKIM-1 can distinguish RCC versus benign renal masses, and whether higher prenephrectomy pKIM-1 is associated with worse cancer-related outcomes.

METHODS

Patient Population

We analyzed prenephrectomy plasma and clinical data from two independent cohorts. Cohort 1 consists of adults with renal masses from the WHO/International Agency for Research on Cancer (IARC) K2 multinational prospective study,[14] who presented to the N.N. Blokhin Russian Medical Research Center for Oncology in Moscow between 2007 and 2012. Within this cohort, 162 patients were subsequently found to have benign renal masses (controls), and the WHO/IARC study statistical team selected an equal number of 162 patients found to have ccRCC (cases). Cases and controls were matched for age (within 5 years) and sex. Sample size was calculated using estimated population means of 59 pg/mL in patients without RCC and 122 pg/mL among patients with T1a RCC, which would give >80% power to distinguish nonmalignant versus malignant histologies (SE of the mean 15.6 pg/mL and two-tailed α rate 5%).

Clinical data were collected for all patients, including baseline patient demographics, surgery type, and tumor histology determined after resection. Prospective follow-up for clinical outcomes was performed as part of the IARC K2 study protocol. Patients received annual follow-up visits by phone to evaluate for clinical outcomes including survival and metastasis; these data were verified with medical records from the treating cancer center and with the Moscow Cancer Registry. Additional clinical data including serum creatinine levels and radiographic measurements of tumor size before nephrectomy were abstracted from the patients' medical records.

Cohort 2 is from the Johns Hopkins Brady Urological Institute Biorepository.[15] We obtained plasma from 247 patients who were diagnosed with renal masses ≤4 cm and had plasma collected as part of the biorepository banking protocol before surgical resection. Clinical characteristics including baseline patient demographics, surgical characteristics, and tumor histology were collected by prospective follow-up and retrospective chart review.[7,15]

To establish baseline pKIM-1 levels among patients without renal masses, we additionally obtained plasma from 48 healthy volunteers from the Brigham and Women's Hospital biorepository. Participants in all cohorts provided informed consent before participation, and consent for exploratory biomarker analysis was embedded within the respective consent forms for the IARC K2 trial and the Johns Hopkins Biorepository. Approval was granted by the institutional review board at each participating study center. This study was conducted in accordance with the Declaration of Helsinki.

Measurement of pKIM-1

We measured pKIM-1 levels using a microbead-based assay in Cohort 1 and among healthy volunteers,[12] and a custom MSD assay in Cohort 2.[16,17] Both assays were described and validated in previous studies.[12,16-18] Additionally, a subset of 80 samples from Cohort 1 were remeasured for pKIM-1 using the MSD assay to confirm assay concordance. Plasma KIM-1 measurements were conducted in duplicate, and investigators were blinded to the identity of individual plasma samples during pKIM-1 analysis. For the microbead-based assay, each sample was diluted 10-fold in sample diluent buffer (0.1 M HEPES, 0.1 M NaCl, 0.1% Tween-20, and 1% BS; pH 7.4; filter sterilized). Thirty microliters (30 μL) of diluted sample, recombinant standards, and internal controls were incubated with approximately 6,000 microbeads coupled with KIM-1 capture antibody for 1 hour (R&D Systems, Minneapolis, MN, Cat # AF1750). Beads were then washed 3× with PBST and incubated with detection antibody for 45 minutes (R&D Systems, Cat # BAF1750). Beads were washed 3× with PBS-Tween and incubated with Streptavidin-PE (Invitrogen, Waltham, MA) for 15 min. The signal from the fluorochrome was captured using Bio-Plex 200 system (Bio-Rad, Hercules, CA). Data were generated and interpreted using a five-parametric logistic regression analysis.

For the MSD assay, pKIM-1 was assessed with a custom sandwich immunoassay using the R-PLEX platform from Meso Scale Discovery (MSD, Rockville, MD). Human TIM-1/KIM-1/HAVCR Antibody (R&D Systems), EZ-Link Sulfo-NHS-LC-Biotin (Thermo Fisher, Waltham, MA), and MSD GOLD SULFO-TAG NHS-ESTER (MSD) were used to prepare the biotin-conjugated antibody and detection antibody. Human KIM-1 recombinant protein (R&D Systems) was used to prepare the calibration curve (lowest calibration point, 4.88 pg/mL). KIM-1 assays were performed by Metabolism and Mitochondrial Research Core (Beth Israel Deaconess Medical Center, Boston, MA). The assay plates were read using a MESO QUICKPLEX SQ 120 instrument and data were analyzed using Discovery Workbench 4.0 software.

Statistical Analysis

Classification of Renal Masses

Area under the receiver operating curve (AUC-ROC) analyses and univariable logistic models were used to characterize the value of pKIM-1 in identifying benign masses versus ccRCC. To evaluate for potential confounders, we assessed the relationships between pKIM-1 and tumor size, and between pKIM-1 and serum creatinine. Multivariable logistic regression models were used to assess the added value of pKIM-1 to a historical nomogram that featured age, sex, smoking status, and tumor size to identify benign versus malignant masses.[8] Correlation statistics between continuous variables were calculated using the Spearman method, and Wilcoxon tests were used to evaluate continuous variables between groups.

Time-to-Event Analysis

In Cohort 1, patients with ccRCC were followed prospectively as part of the K2 trial. Univariable and multivariable Cox survival models were used to determine whether preneph-rectomy pKIM-1 was prognostic for metastasis-free survival (MFS) or overall survival (OS). Patients who had known metastasis at the time of surgery (n = 28) were excluded from the both the univariable MFS and OS analyses. MFS was defined as months elapsed from time of surgery to first distant metastatic event or death. Overall survival time was defined as months elapsed from time of surgery to death. Patients without events were censored at the date of last follow-up. Multivariable survival models were adjusted for age, sex, cancer stage, and surgery type (partial *v* radical nephrectomy). Patients with metastases (n = 28) at baseline were included in the multivariable models. Time-to-event analysis was not performed in Cohort 2 as there were no disease recurrence or metastasis events detected during long-term follow-up, consistent with the expected low recurrence rate among patients with surgically resected cT1a RCC.

RESULTS

Patient Characteristics

Baseline characteristics of our patient cohorts are shown in Tables 1 and 2. Cohort 1 (K2 study) consisted of 162 patients with ccRCC (cases) and 162 patients with benign renal masses (controls). Demographics including age and gender distribution were similar among patients with malignant versus benign renal masses. The proportion of males (39%) was lower than expected, which was a result of gender matching since the benign renal mass samples with plasma available were predominantly female. The majority of patients underwent radical nephrectomy (78%), and the remainder underwent partial nephrectomy or surgery type was unknown. Among patients with radiographic tumor measurements, the majority (67%) had renal masses ≤7 cm in maximal diameter and 39% had masses ≤4 cm. Among patients with benign masses, the majority had oncocytomas (63) or angiolipomas (59), while the remainder had renal cysts (29), angiomyolipomas (eight), or angioleiomyomas (three). During the follow-up period (median follow-up 119 months), 83 patients died. Among the patients who died, 82 (99%) had ccRCC, and the cause of death was attributed to ccRCC in 66 (80%) of those patients.

Cohort 2 (Hopkins) included 247 patients, all of whom had renal masses ≤4 cm. The majority of patients (79%) underwent partial nephrectomy, while others had radical nephrectomy (12%) or surgery type was unknown (10%). One hundred eighty-four patients were found to have RCC, including ccRCC (112), papillary RCC (44), and chromophobe RCC (28). Among benign masses, the most common were oncocytomas (34), followed by angiomyolipomas/angiolipomas (18) and renal cysts (11). During the follow-

TABLE 1. Baseline Patient Characteristics

	No. of Patients (%) or Median (IQR)			
	Cohort 1 (K2 Study)		Cohort 2 (Hopkins)	
Variable	Benign	Malignant	Benign	Malignant
Histology				
Oncocytoma	63 (39)		34 (54)	
Renal cyst	29 (18)		11 (17)	
Angiomyolipoma/angiolipoma	67 (41)		18 (29)	
Angioleiomyoma	3 (2)			
Clear cell RCC		162 (100)		112 (61)
Papillary RCC				44 (24)
Chromophobe RCC				28 (15)
Tumor size (cm)				
0-4	80 (49)	47 (29)	63 (100)	184 (100)
4-7	46 (28)	43 (27)		
7-10	16 (10)	33 (20)		
>10	16 (10)	35 (22)		
Unknown	4 (2)	4 (2)		
Age, years	55.2 (43.4-62.4)	57.9 (49.7-64.2)	62.6 (53.2-70.0)	61.9 (53.3-69.2)
Sex				
Male	56 (35)	69 (43)	37 (59)	114 (62)
	162 (50)	162 (50)	63 (26)	184 (74)
Total	324 (100)		247 (100)	

NOTE. Tumor size represents maximal dimension measured on presurgical imaging. Histology was determined from pathologic analysis of resected tumor.
Abbreviation: RCC, renal cell carcinoma.

up period (median follow-up 28 months), four patients died and no deaths were attributed to RCC.

Assay Characteristics

We compared the results of pKIM-1 measurement using the microbead- and MSD-based assays among 80 samples from Cohort 1 (40 cases and 40 controls) in which additional plasma was available. The two assays were closely correlated (Pearson correlation coefficient 0.95, Appendix Fig A1, online only).

Since KIM-1 is shed directly from the ccRCC cell membrane, we investigated whether tumor size and pKIM-1 are correlated. pKIM-1 was correlated with tumor size among patients with ccRCC but not among patients with benign masses (Appendix Fig A2).

Serum creatinine data were available from patients in Cohort 1. We investigated whether impaired renal function is a confounder for pKIM-1. No association was seen between serum creatinine and pKIM-1 among patients with either benign or malignant masses (Appendix Fig A3A). The discriminatory value of pKIM-1 as a biomarker for RCC was similar among patients with high versus low baseline creatinine (Appendix Fig A3B).

TABLE 2. Characteristics of Patients Found to Have Renal Cell Carcinoma

	No. of Patients (%)	
Variable	Cohort 1	Cohort 2
Total	162 (100)	184 (100)
Nodal status		
N0	138 (85)	184 (100)
N1	24 (15)	
Metastases at presentation		
No	134 (83)	184 (100)
Yes	28 (17)	
Overall cancer stage		
I	76 (47)	184 (100)
II	16 (10)	
III	39 (24)	
IV	31 (19)	

Prenephrectomy pKIM-1 in Patients with RCC Versus Benign Masses

We investigated pKIM-1 levels among patients with benign versus malignant renal masses. The distribution of pKIM-1 among patients in each cohort is shown in Figure 1A. In both Cohort 1 and Cohort 2, univariable logistic regression models

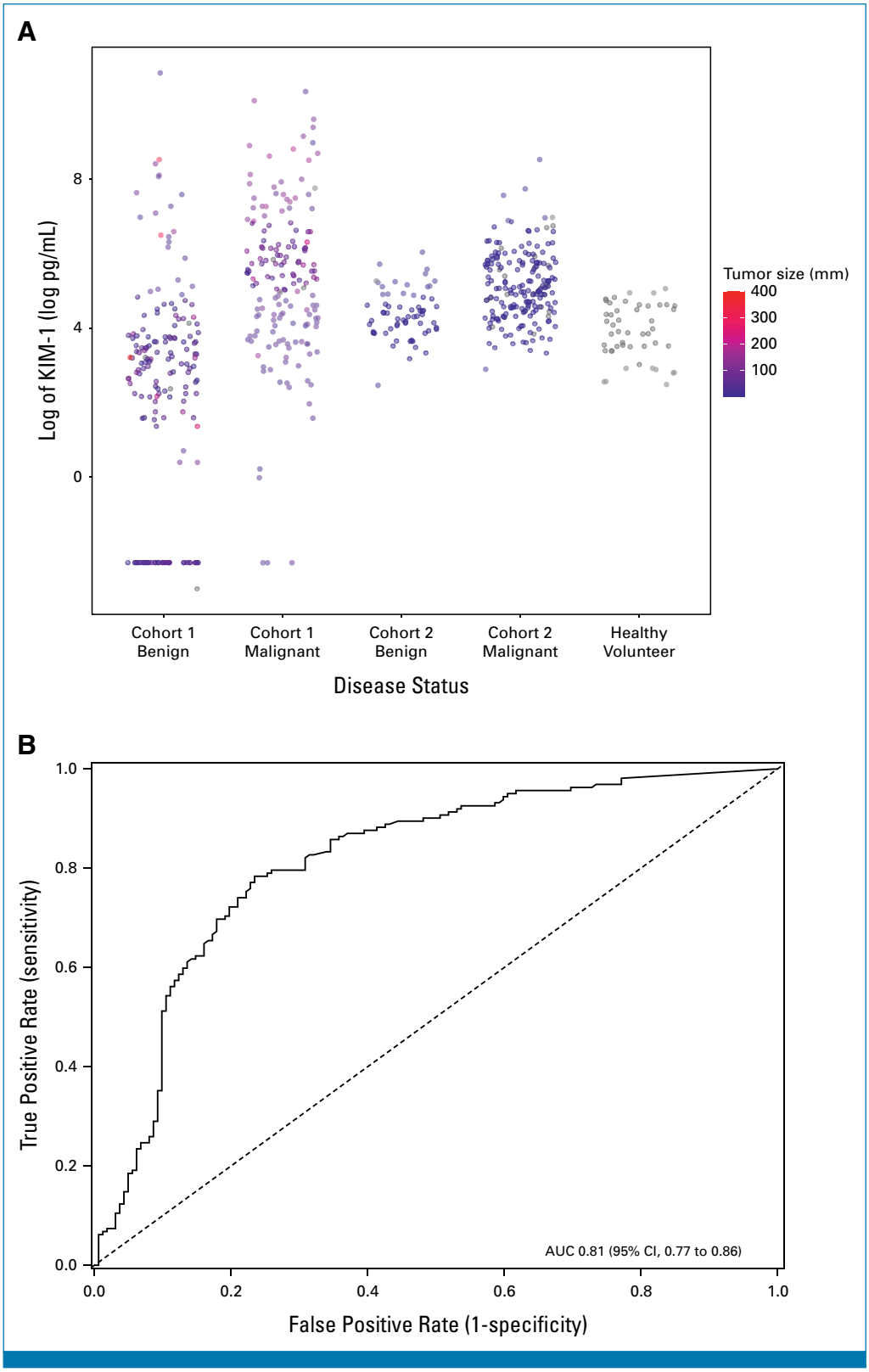

FIG 1. Plasma KIM-1 in patients with clear cell RCC versus benign renal masses. (A) Plasma KIM-1 values among patients with renal masses (Cohort 1 and Cohort 2) and healthy volunteers. Healthy volunteer plasma samples were taken from the Brigham and Women's Hospital biorepository. The lower limit of detection is 1.0 pg/mL for the microbead-based assay (Cohort 1 and healthy volunteers) and 11.7 pg/mL for the MSD assay (Cohort 2). (B) Receiver operating curve analysis of plasma KIM-1 to distinguish clear cell RCC versus benign renal masses. Cohort 1, all patients. (C) Receiver operating curve analysis, Cohort 1, patients stratified by tumor size. (D) Receiver operating curve analysis, Cohort 2 (cT1a renal (continued on following page)

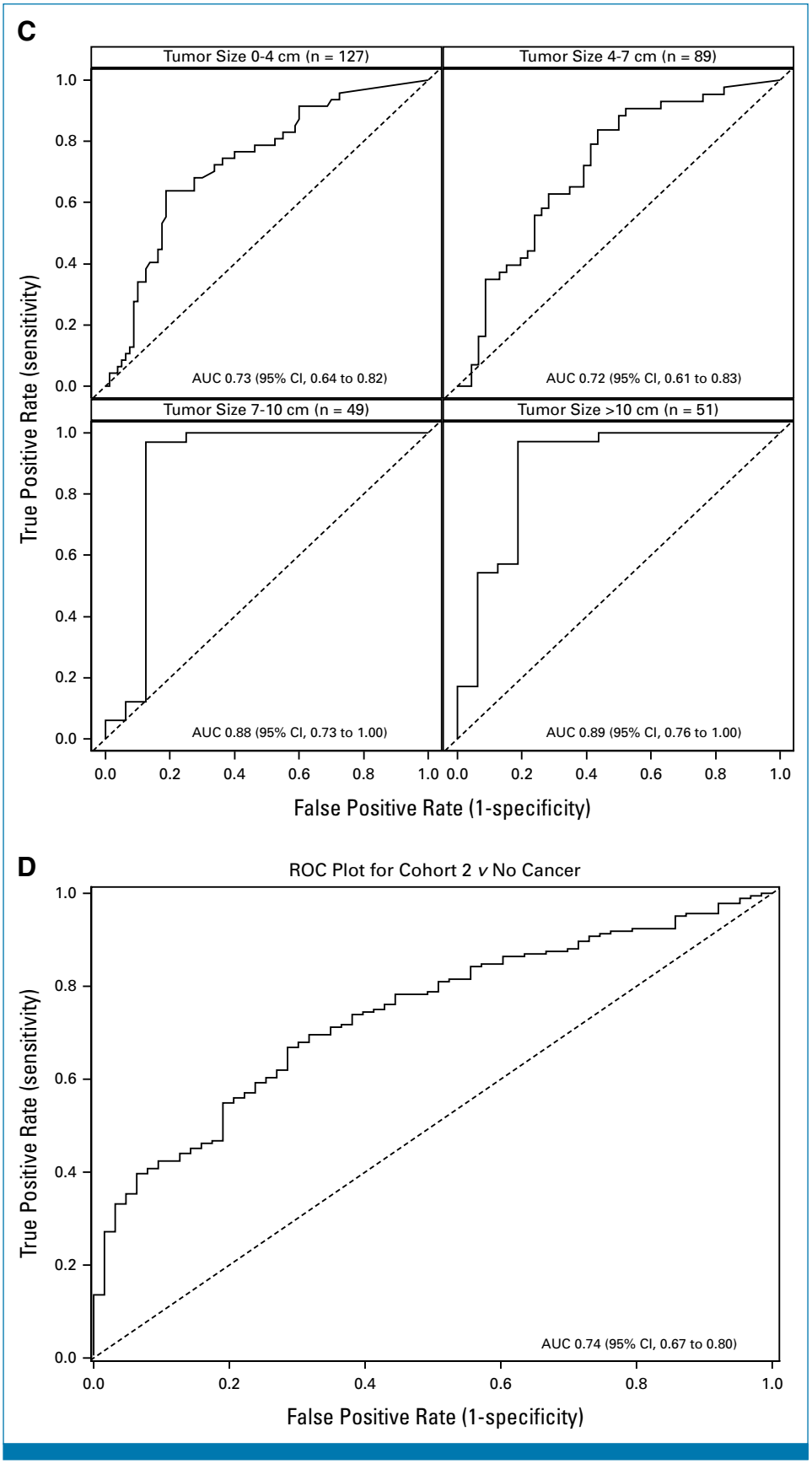

FIG 1. (Continued). masses). (E) Scatter plot of Lane nomogram score versus plasma KIM-1. The dashed lines represent median values of the Lane score and plasma KIM-1 value, 0.79 and 4.81 pg/mL, respectively. KIM-1, kidney injury molecule-1; RCC, renal cell carcinoma; ROC, receiver operating curve.

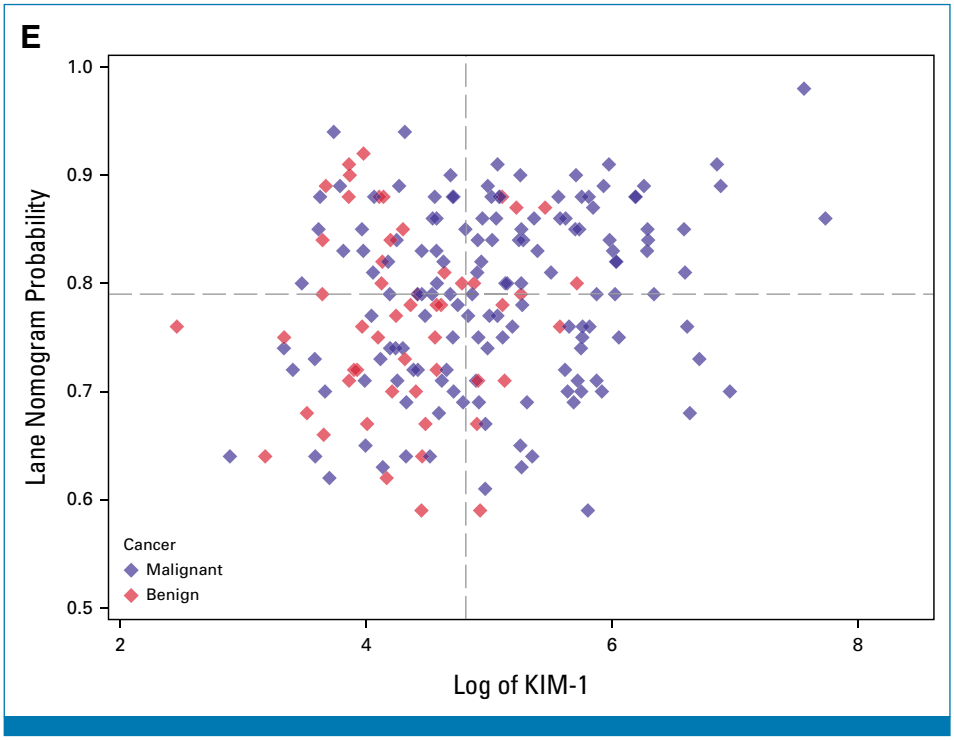

FIG 1. (Continued).

showed that pKIM-1 levels were higher in patients with RCC compared with those with benign renal masses (Cohort 1, odds ratio [OR], 1.63 per log increase in pKIM-1, 95% CI, 1.43 to 1.85; $P < .001$; Cohort 2, OR, 3.11 per log increase in pKIM-1, 95% CI, 2.02 to 4.79; $P < .001$).

We evaluated the performance of pKIM-1 as a diagnostic test using receiver operating curve analysis (Fig 1B). In Cohort 1, pKIM-1 was able to distinguish ccRCC versus benign masses with AUC-ROC 0.81 (95% CI, 0.76 to 0.86). Plasma KIM-1 was found to be elevated in both large and small ccRCCs, but discrimination was improved among patients with T2 tumors (>7 cm) compared with T1 tumors (≤7 cm; Fig 1C). Among small (cT1a) renal masses 0-4 cm, pKIM-1 was associated with AUC-ROC 0.73 (95% CI, 0.64 to 0.82) for distinguishing benign versus malignant tumors. As an exploratory analysis, excluding patients with known metastases in Cohort 1 resulted in a similar AUC-ROC of 0.79 (95% CI, 0.73 to 0.84, Appendix Fig A4).

We next validated the performance of pKIM-1 in Cohort 2. In these patients with cT1a renal masses, pKIM-1 had AUC-ROC 0.74 (95% CI, 0.67 to 0.80, Fig 1D) for distinguishing all RCC subtypes versus benign tumors. This improved slightly (AUC-ROC 0.75, 95% CI, 0.68 to 0.82) when limiting the comparison to papillary and clear cell RCC versus benign tumors. The overall prevalence of RCC was 97% among patients with the highest quartile of pKIM-1 and 55% among patients with the lowest quartile of pKIM-1. Using the Lane clinical nomogram, which predicts the likelihood of malignancy in renal masses and includes age, gender, tumor size, and smoking history, we found no evidence of correlation between pKIM-1 values and nomogram probability estimates (Fig 1E). The addition of pKIM-1 to the Lane nomogram was able to better identify patients who had malignant masses despite a low Lane score and improved the model Akaike information criterion (AIC) and AUC-ROC (AIC 227 *v* 202, AUC-ROC 0.65 [95% CI, 0.57 to 0.74] *v* 0.78 [95% CI, 0.72 to 0.85]).[8]

As an exploratory analysis, we evaluated the performance of pKIM-1 among the 44 patients in Cohort 2 who had papillary RCC. Patients with papillary RCC had higher pKIM-1 compared with those with benign masses ($P < .001$). Among patients with cT1a renal masses, pKIM-1 distinguished patients with papillary RCC versus benign histologies with AUC-ROC 0.77 (95% CI, 0.68 to 0.82).

We performed a cutoff analysis using only samples tested with the MSD assay. This included 247 patients from Cohort 2 (development set) and 80 patients from Cohort 1 (test set). In the development set, an optimal cutpoint (pKIM-1 ≥ 105 pg/mL) was chosen on the basis of shortest distance to the upper left corner of the receiver operating curve. When this cutoff was evaluated in the test set, this resulted in a sensitivity of 92.5% and a specificity of 60% for identifying RCC (Appendix Fig A5).

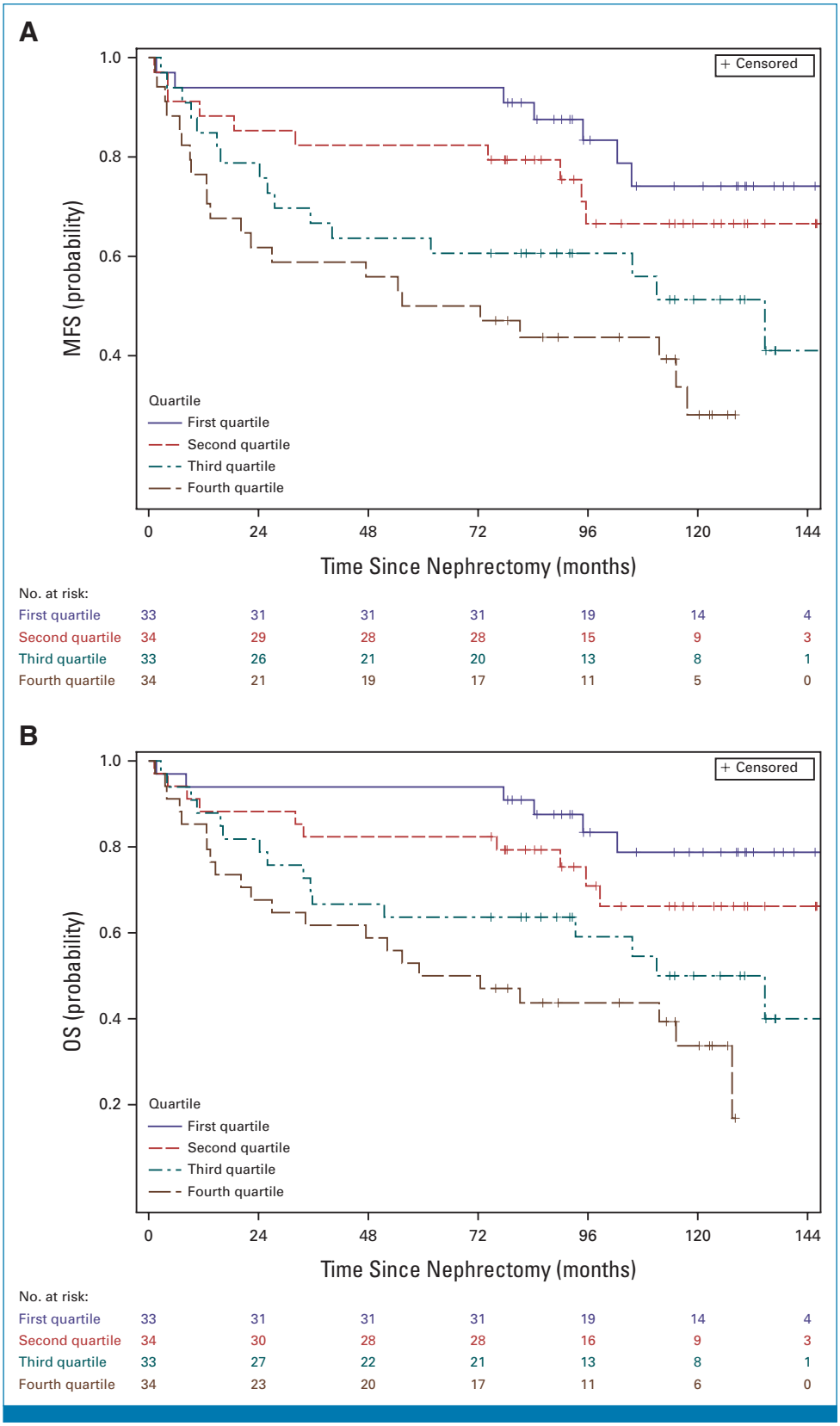

FIG 2. (A) Kaplan-Meier curves for metastasis-free survival (MFS) by prenephrectomy plasma KIM-1 quartile, Cohort 1. (B) Kaplan-Meier curves for overall survival (OS) by prenephrectomy plasma KIM-1 quartile, Cohort 1. KIM-1, kidney injury molecule-1.

TABLE 3. Multivariable HR Estimates for Plasma KIM-1 and Metastasis-Free Survival, Cohort 1

Variable	HR	95% CI	*P*
Log pKIM-1	1.29	1.10 to 1.53	.0025
Age	1.01	0.98 to 1.03	.6551
Male sex	1.26	0.71 to 2.26	.4317
Radical nephrectomy	1.34	0.60 to 2.97	.4716
Cancer stage			
II	1.17	0.47 to 2.91	.7300
III	2.03	1.03 to 3.98	.0400
IV	4.15	1.15 to 15.02	.0299

NOTE. HRs are calculated relative to a reference group with female sex, partial nephrectomy, and stage I renal cell carcinoma.
Abbreviations: HR, hazard ratio; pKIM-1, plasma kidney injury molecule-1.

TABLE 4. Multivariable HR Estimates for Plasma KIM-1 and Overall Survival, Cohort 1

Variable	HR	95% CI	*P*
Log pKIM-1	1.31	1.10 to 1.54	.0019
Age	1.01	0.98 to 1.03	.6672
Male sex	1.21	0.67 to 2.18	.5285
Radical nephrectomy	1.32	0.60 to 2.93	.4908
Cancer stage			
II	1.17	0.47 to 2.92	.7313
III	2.18	1.10 to 4.31	.0259
IV	4.49	1.22 to 16.51	.0237

NOTE. HRs are calculated relative to a reference group with female sex, partial nephrectomy, and stage I renal cell carcinoma.
Abbreviations: HR, hazard ratio; pKIM-1, plasma kidney injury molecule-1.

pKIM-1 as a Predictor of MFS Among Patients With RCC

In Cohort 1, we assessed whether prenephrectomy pKIM-1 is associated with MFS among patients with localized RCC. Each increase in pKIM-1 quartile was associated with progressively higher risk of subsequent metastatic event or death (Fig 2A). A univariable Cox proportional hazards model for MFS showed a hazard ratio (HR) of 1.36 (95% CI, 1.19 to 1.56; $P < .001$) per log increase in pKIM-1. This association remained significant after multivariable adjustment for age, sex, surgery type, and cancer stage (HR, 1.29 per log increase in pKIM-1, 95% CI, 1.10 to 1.53; $P = .003$, Table 3).

Time-to-event analyses were not performed in Cohort 2, since no metastasis events occurred during the follow-up period, which reflects the favorable prognosis of resected pT1a RCC.

pKIM-1 as a Predictor of OS Among Patients With RCC

In Cohort 1, we assessed whether pKIM-1 before nephrectomy was associated with subsequent risk of death among patients with localized RCC. Higher pKIM-1 quartiles were associated with progressively worse overall survival (Fig 2B). Among the 66 patients who had death attributed to RCC, all except one (98.5%) had prenephrectomy pKIM-1 higher than the median.

In a univariable Cox model, pKIM-1 was associated with increased risk for death (HR, 1.37 per log increase in log pKIM-1, 95% CI, 1.20 to 1.58; $P < .001$) and this remained significant after multivariable adjustment for age, sex, surgery type, and tumor stage (HR, 1.31 per log increase in log pKIM-1, 95% CI, 1.10 to 1.54; $P = .002$; Table 4).

As a sensitivity analysis, further adjustment of our multivariable models for tumor size did not substantively alter the predictive value of pKIM-1 and MFS or OS (Appendix Table A1).

DISCUSSION

In this study, we analyzed pKIM-1 levels among patients presenting with a renal mass suspicious for malignancy. We demonstrate in two independent cohorts that elevated prenephrectomy pKIM-1 is associated with increased risk of finding RCC at the time of surgery. Among patients with RCC, those with higher presurgery pKIM-1 have worse MFS and worse overall survival. We find that pKIM-1 is, to our knowledge, the only known circulating biomarker that is specific for both clear cell and papillary RCC, the two most common histologies of kidney cancer. These data support a potential role for pKIM-1 as a diagnostic and prognostic biomarker in patients with suspicious renal masses.

There are currently no circulating biomarkers in clinical use for RCC. Although previous studies have investigated RCC detection using several different platforms to identify circulating cytokines, tumor-associated proteins, circulating tumor cells, cell-free tumor DNA and RNA, and cell-free DNA methylation patterns, none of these techniques are currently used in routine practice.[19-25] Recently, a novel imaging biomarker ^{89}Zr-DFO-girentuximab positron emission tomography demonstrated sensitivity of 86% and specificity of 87% for detecting ccRCC among patients with renal masses, but is not designed to detect nonclear cell RCCs, which comprise 30% of all renal malignancies.[26] ^{99m}Tc-sestamibi single-photon emission computed tomography/computed tomography has demonstrated 87.5% sensitivity and 95% specificity for distinguishing benign oncocytic tumors from other renal masses, but its diagnostic utility is limited by identifying only a subset of benign masses.[27] Compared with these other approaches, pKIM-1 has several properties that make it an attractive

candidate biomarker for RCC. The assays for measuring pKIM-1 are inexpensive, easily scalable, and require only small volumes of plasma (30 μL).[12] pKIM-1 is stable to multiple freeze/thaw cycles and can therefore be measured in banked plasma.[18,28] KIM-1 is already qualified by the US Food and Drug Administration as a biomarker for nephrotoxicity, which would simplify its implementation if found to have clinical value in kidney cancer.[29]

The identification of circulating biomarkers is especially challenging for patients with early-stage disease. Tumor products must be released into the systemic circulation to be detectable in blood samples. On the one hand, this is a clinical challenge since many early-stage tumors may have limited access to the systemic circulation, and there may be an inherent sensitivity limit for detection of such tumors from peripheral blood tests. On the other hand, tumors that have access to the circulation might also have greater metastatic potential, which is supported by our finding that patients with low pKIM-1 have better MFS and OS even when RCC is present.

In a previous study, we showed that elevated pKIM-1 after nephrectomy is associated with recurrence risk in RCC.[13] The current manuscript investigates a different clinical question, for example, the use of pKIM-1 to elucidate cancer risk before surgery. Our results represent an important proof of concept that blood-based risk stratification of kidney cancer is feasible even for patients with early-stage disease.

Strengths of this study include its large sample size and the use of two independent cohorts, including a large prospective study (K2) and a mixed prospective/retrospective study (Hopkins). The majority of our patients had small renal masses, which reflects a real-world clinical conundrum where there is often equipoise as to whether surgery is necessary. Our cohorts include a wide spectrum of benign and malignant renal mass histologies, which also reflects real-world practice. Currently, the management of renal masses relies on clinical risk stratification, with some patients proceeding to surgery or other interventions, while others are managed with imaging surveillance.[8,30] In the DISSRM registry, among 224 patients undergoing active surveillance for small renal masses, zero metastatic events occurred, suggesting that current surveillance protocols might be too conservative.[31] In the future, pKIM-1 could be a helpful tool to identify more candidates for active surveillance.

There are several limitations to this study. We did not have data on the presence of necrosis on preoperative imaging, limiting our ability to evaluate an alternative preoperative nomogram.[32,33] Cohort 1 was a matched case-control study, while Cohort 2 was a retrospective cohort, therefore the baseline characteristics of our cohorts do not represent the global population of individuals with renal masses. There is overlap in pKIM-1 between benign and malignant masses, making this biomarker less specific among the lower end of the pKIM-1 distribution and among patients with smaller renal masses (Figs 1A and 1E). pKIM-1 is also a known biomarker for renal injury, including medication-related toxicity, and we were not able to assess the effects of concurrent medications on pKIM-1 levels within our cohorts.[12]

Our study design did not directly evaluate active surveillance as an alternative strategy to resection. Since all patients in our study underwent excision of their kidney masses, we do not know how patients with low pKIM-1 would have fared on surveillance alone. Among patients with renal masses, high pKIM-1 strongly suggested the presence of cancer and higher metastatic risk. Although we found that a subset of small RCCs do not release KIM-1 into the circulation, they also appear to have low metastatic potential. These findings, in our opinion, support future prospective studies of active surveillance in patients with small renal masses and low pKIM-1. Future studies could determine whether serial measurement of pKIM-1 is a useful adjunct for surveillance of a renal mass (similar to the use of serial PSA in active surveillance for prostate cancer),[34] and whether combining pKIM-1 with other biomarkers may further improve assay performance.

In conclusion, among patients with renal masses, those with elevated prenephrectomy pKIM-1 have higher risk of malignant pathology, worse MFS, and higher risk of death. Plasma KIM-1 may be a useful minimally invasive biomarker for risk stratification of patients with suspicious renal masses.

AFFILIATIONS

[1]Dana-Farber Cancer Institute, Boston, MA
[2]International Agency for Research on Cancer, Lyon, France
[3]N.N. Blokhin National Medical Research Centre of Oncology, Moscow, Russia
[4]Beth Israel Deaconess Medical Center, Boston, MA
[5]Brigham and Women's Hospital, Boston, MA
[6]Penn Presbyterian Medical Center, Philadelphia, PA
[7]Brady Urological Institute, Johns Hopkins University, Baltimore, MD

CORRESPONDING AUTHOR

Wenxin Xu, MD; Twitter: @VincentWenxinXu; e-mail: wenxin_xu1@dfci.harvard.edu.

DISCLAIMER

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/World Health Organization.

EQUAL CONTRIBUTION

V.G., S.M.N., and A.M. contributed equally to this work.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: Wenxin Xu, Valerie Gaborieau, Ayumi Takakura, Venkata Sabbisetti, Joseph V. Bonventre, Rupal S. Bhatt
Financial support: Wenxin Xu, Toni K. Choueiri
Administrative support: Wenxin Xu, Anush Mukeria, Toni K. Choueiri, Paul Brennan
Provision of study materials or patients: Wenxin Xu, Valerie Gaborieau, Anush Mukeria, David Zaridze, David F. McDermott, Toni K. Choueiri, Phillip M. Pierorazio, Nirmish Singla, Paul Brennan
Collection and assembly of data: Wenxin Xu, Valerie Gaborieau, Anush Mukeria, Xiaowen Liu, Krishna P. Maremanda, Ayumi Takakura, David Zaridze, Venkata Sabbisetti, Phillip M. Pierorazio, Nirmish Singla, Paul Brennan, Rupal S. Bhatt
Data analysis and interpretation: Wenxin Xu, Valerie Gaborieau, Samuel M. Niman, Matthew L. Freedman, Wanling Xie, David F. McDermott, Toni K. Choueiri, Paul J. Catalano, Venkata Sabbisetti, Joseph V. Bonventre, Phillip M. Pierorazio, Paul Brennan, Rupal S. Bhatt
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

REFERENCES

1. SEER: Cancer of the Kidney and Renal Pelvis—Cancer Stat Facts. 2023. https://seer.cancer.gov/statfacts/html/kidrp.html
2. Störkel S, van den Berg E: Morphological classification of renal cancer. World J Urol 13:153-158, 1995
3. Patard J-J, Leray E, Rioux-Leclercq N, et al: Prognostic value of histologic subtypes in renal cell carcinoma: A multicenter experience. J Clin Oncol 23:2763-2771, 2005
4. Campbell S, Uzzo RG, Allaf ME, et al: Renal mass and localized renal cancer: AUA guideline. J Urol 198:520-529, 2017
5. Frank I, Blute ML, Cheville JC, et al: Solid renal tumors: An analysis of pathological features related to tumor size. J Urol 170:2217-2220, 2003
6. Kim JH, Li S, Khandwala Y, et al: Association of prevalence of benign pathologic findings after partial nephrectomy with preoperative imaging patterns in the United States from 2007 to 2014. JAMA Surg 154:225-231, 2019
7. Pierorazio PM, Johnson MH, Ball MW, et al: Five-year analysis of a multi-institutional prospective clinical trial of delayed intervention and surveillance for small renal masses: The DISSRM registry. Eur Urol 68:408-415, 2015
8. Lane BR, Babineau D, Kattan MW, et al: A preoperative prognostic nomogram for solid enhancing renal tumors 7 cm or less amenable to partial nephrectomy. J Urol 178:429-434, 2007
9. Scelo G, Muller DC, Riboli E, et al: KIM-1 as a blood-based marker for early detection of kidney cancer: A prospective nested case-control study. Clin Cancer Res 24:5594-5601, 2018
10. Han WK, Alinani A, Wu C-L, et al: Human kidney injury molecule-1 is a tissue and urinary tumor marker of renal cell carcinoma. J Am Soc Nephrol 16:1126-1134, 2005
11. Zhang PL, Mashni JW, Sabbisetti VS, et al: Urine kidney injury molecule-1: A potential non-invasive biomarker for patients with renal cell carcinoma. Int Urol Nephrol 46:379-388, 2014
12. Sabbisetti VS, Waikar SS, Antoine DJ, et al: Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. J Am Soc Nephrol 25:2177-2186, 2014
13. Xu W, Puligandla M, Halbert B, et al: Plasma KIM-1 is associated with recurrence risk after nephrectomy for localized renal cell carcinoma: A trial of the ECOG-ACRIN research group (E2805). Clin Cancer Res 27:3397-3403, 2021
14. Scelo G, Purdue MP, Brown KM, et al: Genome-wide association study identifies multiple risk loci for renal cell carcinoma. Nat Commun 8:15724, 2017
15. Chalfin HJ, Fabian E, Mangold L, et al: Role of biobanking in urology: A review. BJU Int 118:864-868, 2016
16. McWilliam SJ, Antoine DJ, Sabbisetti V, et al: Reference intervals for urinary renal injury biomarkers KIM-1 and NGAL in healthy children. Biomarkers Med 8:1189-1197, 2014
17. Schmidt IM, Colona MR Srivastava A, et al: Plasma kidney injury molecule-1 in systemic lupus erythematosus: Discordance between ELISA and proximity extension assay. Kidney Med 4:100496, 2022
18. Sabbisetti VS, Ito K, Wang C, et al: Novel assays for detection of urinary KIM-1 in mouse models of kidney injury. Toxicol Sci 131:13-25, 2013
19. Zurita AJ, Jonasch E, Wang X, et al: A cytokine and angiogenic factor (CAF) analysis in plasma for selection of sorafenib therapy in patients with metastatic renal cell carcinoma. Ann Oncol 23: 46-52, 2012
20. Rini B: Dissecting responsive phenotypes through cytokine and angiogenic factor analysis. Ann Oncol 23:6-7, 2012
21. Kim TH, Kang Y-T, Cho Y-H, et al: Detection of circulating tumour cells and their potential use as a biomarker for advanced renal cell carcinoma. Can Urol Assoc J 13:E285-E291, 2019
22. Nuzzo PV, Berchuck JE, Korthauer K, et al: Detection of renal cell carcinoma using plasma and urine cell-free DNA methylomes. Nat Med 26:1041-1043, 2020
23. Farber NJ, Kim CJ, Modi PK, et al: Renal cell carcinoma: The search for a reliable biomarker. Transl Cancer Res 6:620-632, 2017
24. Ball MW, Gorin MA, Guner G, et al: Circulating tumor DNA as a marker of therapeutic response in patients with renal cell carcinoma: A pilot study. Clin Genitourin Cancer 14:e515-e520, 2016
25. Morrissey JJ, Mellnick VM, Luo J, et al: Evaluation of urine aquaporin-1 and perilipin-2 concentrations as biomarkers to screen for renal cell carcinoma: A prospective cohort study. JAMA Oncol 1: 204-212, 2015
26. Shuch BM, Pantuck AJ, Bernhard J-C, et al: Results from phase 3 study of ^{89}Zr-DFO-girentuximab for PET/CT imaging of clear cell renal cell carcinoma (ZIRCON). J Clin Oncol 41, 2023 (6_suppl; abstr LBA602)
27. Gorin MA, Rowe SP, Baras AS, et al: Prospective evaluation of ^{99m}Tc-sestamibi SPECT/CT for the diagnosis of renal oncocytomas and hybrid oncocytic/chromophobe tumors. Eur Urol 69:413-416, 2016
28. Schuh MP, Nehus E, Ma Q, et al: Long-term stability of urinary biomarkers of acute kidney injury in children. Am J Kidney Dis 67:56-61, 2016
29. United States Food and Drug Administration: Research C for DE and List of Qualified Biomarkers. 2021
30. Daugherty M, Sedaghatpour D, Shapiro O, et al: The metastatic potential of renal tumors: Influence of histologic subtypes on definition of small renal masses, risk stratification, and future active surveillance protocols. Urol Oncol 35:153.e15-153.e20, 2017
31. Metcalf MR, Cheaib JG, Biles MJ, et al: Outcomes of active surveillance for young patients with small renal masses: Prospective data from the DISSRM registry. J Urol 205:1286-1293, 2021
32. Raj GV, Thompson RH, Leibovich BC, et al: Preoperative nomogram predicting 12-year probability of metastatic renal cancer. J Urol 179:2146-2151, 2008; discussion 2151
33. Mano R, Duzgol C, Ganat M, et al: Preoperative nomogram predicting 12-year probability of metastatic renal cancer—Evaluation in a contemporary cohort. Urol Oncol 38:853.e1-853.e7, 2020
34. Hamdy FC, Donovan JL, Lane JA, et al: Fifteen-year outcomes after monitoring, surgery, or radiotherapy for prostate cancer. N Engl J Med 388:1547-1558, 2023

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Plasma Kidney Injury Molecule-1 for Preoperative Prediction of Renal Cell Carcinoma Versus Benign Renal Masses, and Association With Clinical Outcomes

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Wenxin Xu
Consulting or Advisory Role: Exelixis, Jazz Pharmaceuticals, Xencor, Merck, Aveo
Research Funding: OncoHost (Inst)

Matthew L. Freedman
Stock and Other Ownership Interests: Precede Bio
Consulting or Advisory Role: Precede Bio
Patents, Royalties, Other Intellectual Property: A pending patent has been filed for detecting neuroendocrine prostate cancer using DNA methylation

Wanling Xie
Consulting or Advisory Role: Convergent Therapeutics

David F. McDermott
Consulting or Advisory Role: Bristol Myers Squibb, Merck, Genentech/Roche, Pfizer, Exelixis, Novartis, Array BioPharma, Peloton Therapeutics, EMD Serono, Jounce Therapeutics, Alkermes, Lilly, Eisai, Calithera Biosciences, Iovance Biotherapeutics, Werewolf Therapeutics, Synthekine, AVEO, Xilio Therapeutics, Cullinan Oncology
Research Funding: Bristol Myers Squibb (Inst), Merck (Inst), Genentech (Inst), Novartis (Inst), Alkermes (Inst)
Other Relationship: Beth Israel Deaconess Medical Center

Toni K. Choueiri
Stock and Other Ownership Interests: Precede Bio, Osel, Tempest Therapeutics, Pionyr, Curesponse, InnDura, Primium
Honoraria: HiberCell, Pfizer, Bayer, Novartis, GlaxoSmithKline, Merck, Bristol Myers Squibb, Roche/Genentech, Eisai, Foundation Medicine, AstraZeneca, Exelixis, Prometheus, Ipsen, Sanofi/Aventis, Peloton Therapeutics, UpToDate, NCCN, Michael J. Hennessy Associates, Analysis Group, Clinical Care Options, PlatformQ Health, Navinata Health, Harborside Press, ASCO, The New England Journal of Medicine, Lancet Oncology, EMD Serono, Lilly, Tempest Therapeutics, Arcus Biosciences, Arcus Biosciences, Alkermes, Gilead Sciences, Scholar Rock, Janssen Oncology, Precede Bio, Aravive, Infinity Pharmaceuticals, ESMO, NiKang Therapeutics, Kanaph Therapeutics, Gilead Sciences
Consulting or Advisory Role: Pfizer, Bayer, Novartis, GlaxoSmithKline, Merck, Bristol Myers Squibb, Roche/Genentech, Eisai, Foundation Medicine, AstraZeneca, Exelixis, Prometheus, Ipsen, Sanofi/Aventis, Peloton Therapeutics, UpToDate, NCCN, Michael J. Hennessy Associates, Analysis Group, Clinical Care Options, PlatformQ Health, Navinata Health, Harborside Press, ASCO, The New England Journal of Medicine, Lancet Oncology, EMD Serono, Lilly, Tempest Therapeutics, Arcus Biosciences, alkermes, Gilead Sciences, Scholar Rock, Janssen Oncology, Precede Bio, Aravive, Infinity Pharmaceuticals, ESMO, NiKang Therapeutics, Kanaph Therapeutics, Gilead Sciences, Neomorph
Research Funding: Pfizer (Inst), Novartis (Inst), Merck (Inst), Exelixis (Inst), TRACON Pharma (Inst), GlaxoSmithKline (Inst), Bristol Myers Squibb (Inst), AstraZeneca (Inst), Peloton Therapeutics (Inst), Roche/Genentech (Inst), Agensys (Inst), Eisai (Inst), Takeda (Inst), Ipsen (Inst), Seattle Genetics/Astellas (Inst), Bayer (Inst), Roche (Inst), Calithera Biosciences (Inst), NiKang Therapeutics (Inst), Arcus Biosciences (Inst), AVEO (Inst)
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Other Relationship: Medical writing and editorial assistance support may have been funded by Communications companies funded by pharmaceutical companies such as ClinicalThinking, Health Interactions, Envision Pharma Group, Fishawack Group of Companies, Parexel

Paul J. Catalano
Research Funding: Regeneron (Inst), Astellas Pharma (Inst)

Venkata Sabbisetti
Employment: Regeneron, Regeneron
Research Funding: National Cancer Institute
Patents, Royalties, Other Intellectual Property: VS holds patent on using KIM-1 as a biomarker for kidney and ovarian cancer

Joseph V. Bonventre
Stock and Other Ownership Interests: MediBeacon, Autonomous Medical Devices, Innoviva, DxNow, Oisin, Pacific Biosciences Research Center, Renalytix AI PLC
Honoraria: University of Washington
Consulting or Advisory Role: Sarepta Therapeutics, Praxis Therapeutics, Praxis Therapeutics, Motric Bio, Mitsubishi Tanabe Pharma
Patents, Royalties, Other Intellectual Property: KIM-1 Patents assigned to MassGeneralBrigham, Kidney Organoid Patents assigned to MassGeneralBrigham

Phillip M. Pierorazio
Patents, Royalties, Other Intellectual Property: UpToDate Chapter on Renal Cell Carcinoma
Uncompensated Relationships: Testicular Cancer Awareness Foundation

Rupal S. Bhatt
Employment: Bristol Myers Squibb
Stock and Other Ownership Interests: Bristol Myers Squibb
Patents, Royalties, Other Intellectual Property: Patent Filed for novel RCC treatments (Inst)

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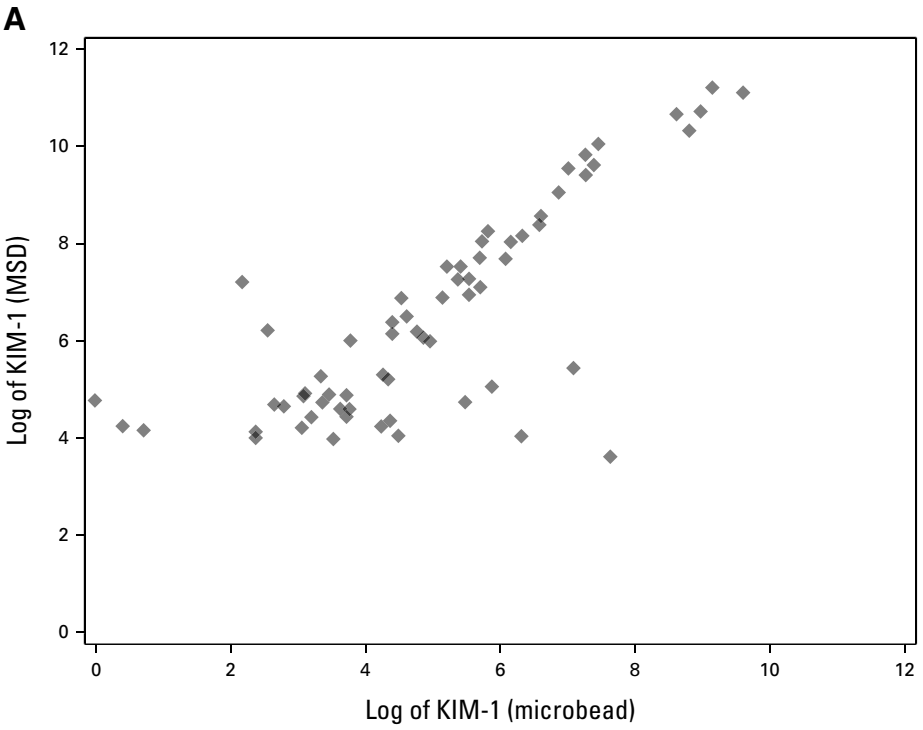
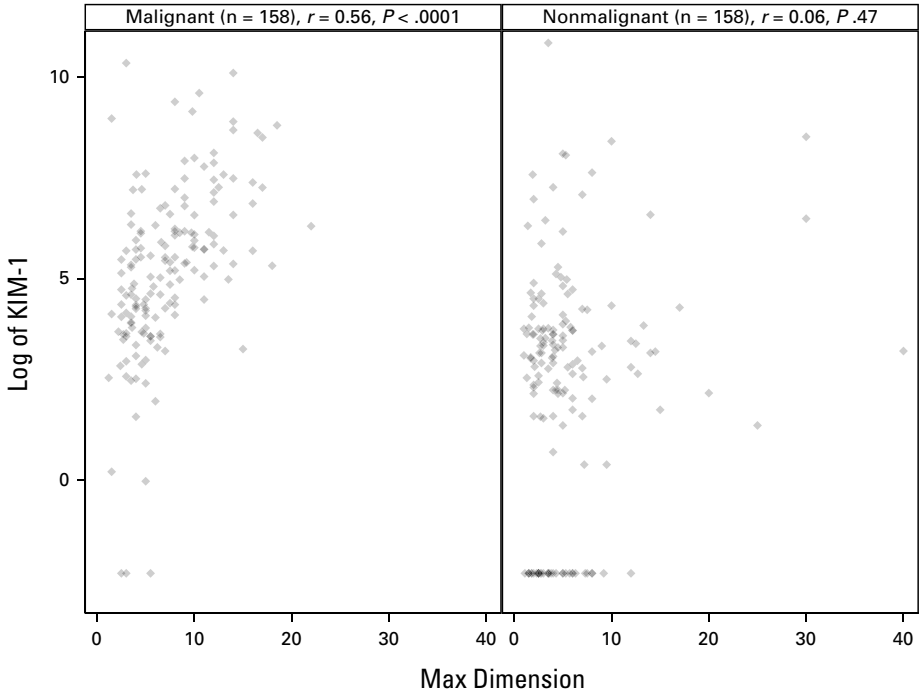
APPENDIX



B

Variable	Median	Mean	SD	Coefficient of Variation
KIM-1 MSD	178.28	4984.45	13496.70	270.78
KIM-1 microbead	77.30	800.21	2285.78	285.65

FIG A1. Comparison of microbead and MSD assays for plasma KIM-1. (A) Scatterplot of MSD versus microbead assay measurements. (B) Summary statistics for the two KIM-1 assays among the 80 samples assessed using both assays. KIM-1, kidney injury molecule-1.



B

Spearman Correlation Coefficients Prob > |r| Under H0: Rho = 0 No. of Observations			
	KIM-1	Tumor Size	Grade
KIM-1	1.00000 80	0.55542 <.0001 78	0.33921 0.0323 40
Tumor size	0.55542 <.0001 78	1.00000 316	0.27249 0.0005 158
Grade	0.33921 0.0323 40	0.27249 0.0005 158	1.00000 162

FIG A2. Association between plasma KIM-1 and baseline characteristics. (A) Scatter plot of tumor size (mm) versus plasma KIM-1. (B) Correlations between of tumor size, tumor grade, and plasma KIM-1. KIM-1, kidney injury molecule-1.

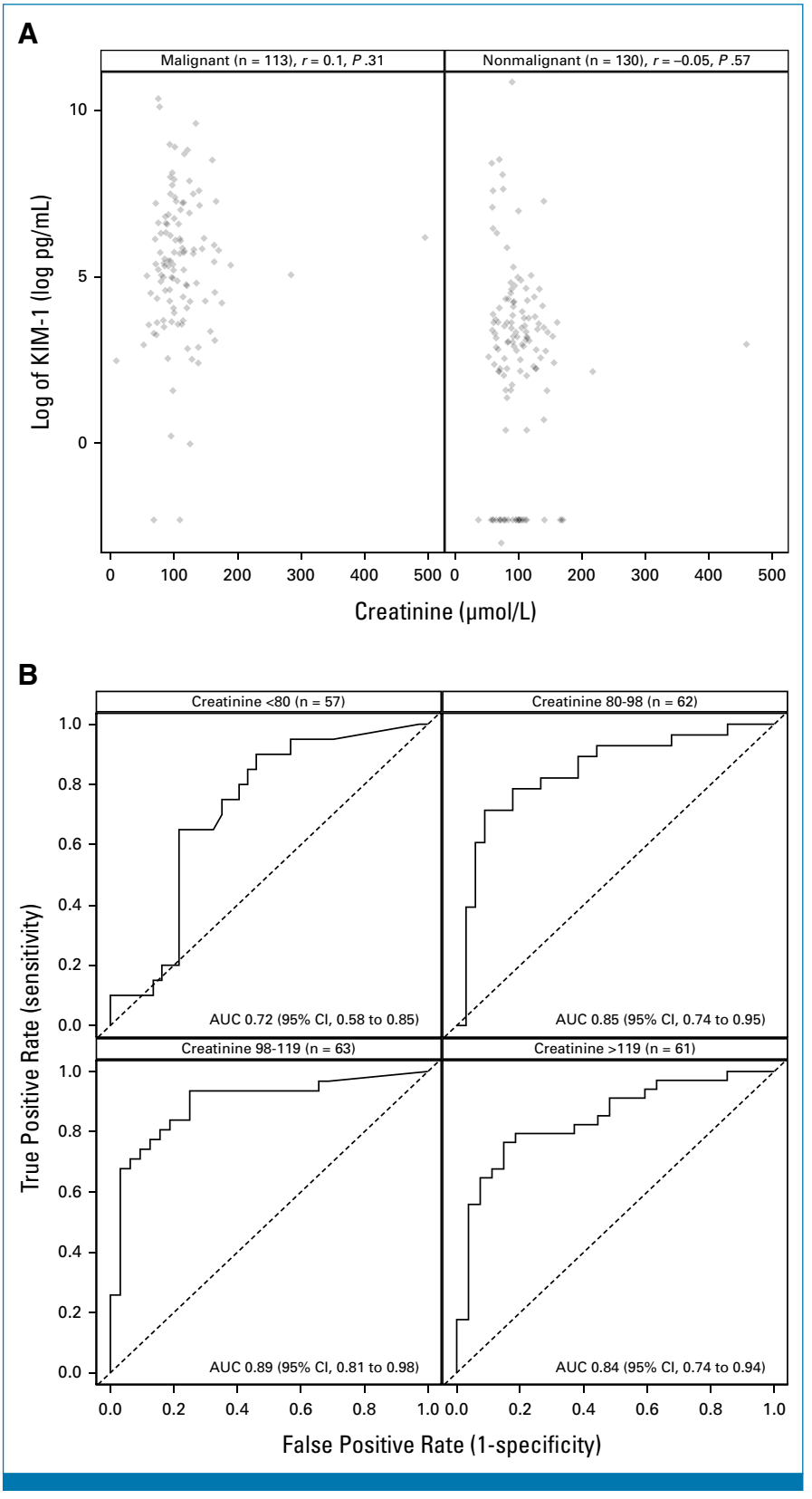


FIG A3. Association between plasma KIM-1 and serum creatinine. (A) Scatter plot of serum creatinine (μmol/L) versus plasma KIM-1. (B) Receiver operating curve analysis of plasma KIM-1 to distinguish clear cell renal cell carcinoma versus benign renal masses, stratified by baseline creatinine (μmol/L). KIM-1, kidney injury molecule-1.

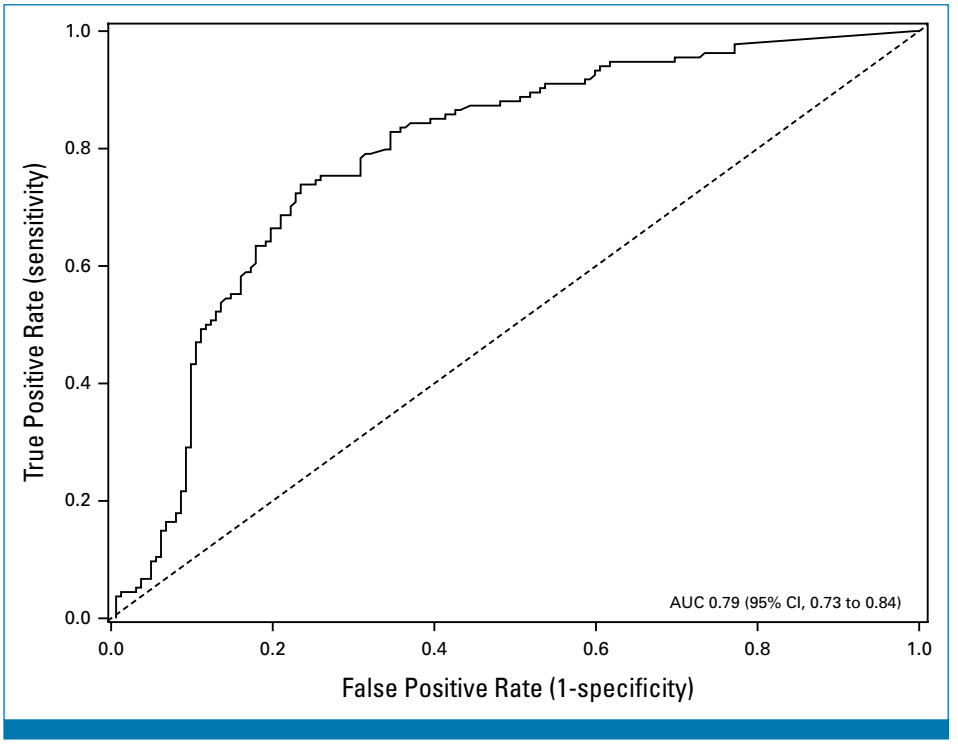


FIG A4. Receiver operating curve in Cohort 1, excluding patients with metastasis.

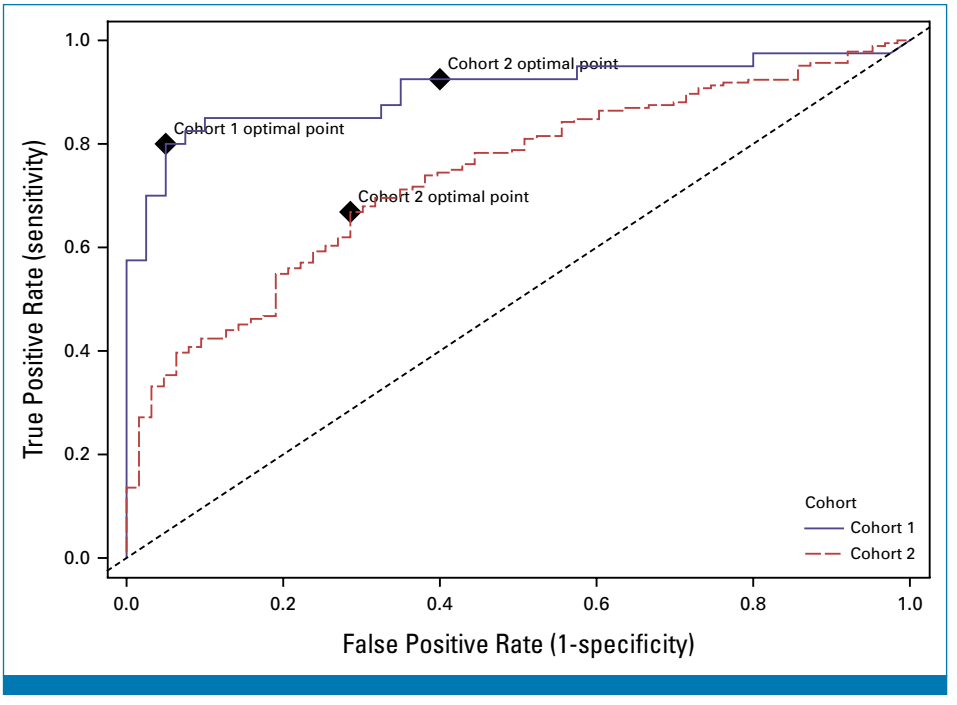


FIG A5. Exploratory cutoff validation analysis of plasma KIM-1. KIM-1, kidney injury molecule-1.

TABLE A1. MFS and OS Multivariable Models Adjusted for Tumor Size

Description	HR	95% LCL	95% UCL
MFS model with tumor size (continuous)			
Log KIM-1	1.260	1.057	1.502
Age	1.010	0.982	1.039
Sex, male *v* female	1.291	0.711	2.345
Surgery type, radical *v* partial/unknown	1.289	0.533	3.121
Stage II *v* I	1.018	0.369	2.807
Stage III *v* I	1.808	0.807	4.049
Stage IV *v* I	3.941	1.066	14.569
Tumor max dimension	1.049	0.955	1.153
OS model with tumor size (continuous)			
Log KIM-1	1.266	1.061	1.509
Surgery type, radical *v* partial/unknown	1.233	0.508	2.995
Age	1.011	0.982	1.040
Sex, male *v* female	1.245	0.678	2.286
Stage II *v* I	0.976	0.349	2.729
Stage III *v* I	1.878	0.826	4.270
Stage IV *v* I	4.178	1.110	15.722
Tumor max dimension	1.061	0.964	1.167

Abbreviations: HR, hazard ratio; LCL, lower confidence limit; KIM-1, kidney injury molecule-1; MFS, metastasis-free survival; OS, overall survival; UCL, upper confidence limit.