ConfirmCKD™ Chronic Kidney Disease Biomarker

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INTRODUCTION

Chronic kidney disease is a progressive yet silent irreversible condition affecting nearly 850 million people worldwide. The majority of those affected remain undiagnosed and untreated.

The nonspecific nature of serum creatinine its low sensitivity for CKD requires repeat testing to establish a diagnosis at least >90 days apart, still, ~60% of patients do not return for timely follow-up even after an abnormal result is detected[2].

Results recently published by REVEAL-CKD estimates that between 61.6% - 95.5% of those with Stage 3 in the U.S. have not been diagnosed[3].

Although <1% of Medicare beneficiaries are ESRD, >8% of the CMS annual budget is attributed to ESRD-related costs, or more than $45 Billion a year[1].

Recent approval of new medications for CKD will help reduce progression to ESRD and dialysis, yet, the failure of eGFR to diagnose patients readily and accurately still results in <5% of affected individuals being treated.

The large financial burden ESRD places on healthcare and CMS has prompted an urgent "call to action" to diagnose patients earlier in the course of disease. A novel biomarker that efficiently identifies patients for immediate medical treatment will heavily reduce patient progression.

MATERIALS & METHODS

Study protocol – The study protocol was approved by WCG/WIRB IRB review panel. Serum was prepared from venipuncture blood collection and frozen at -80ºC.

Patient inclusion and exclusion criteria – subjects <18 years old, actively septic, or diagnosed with Stage 1 or 2 CKD were excluded from the study. 384 total patients were the subject of this study. CKD – patients who had a reported eGFR baseline and associated ICD10 diagnosis code for Stage 3a, 3b, 4, or 5 were included. Controls – renal function was considered within normal limits if eGFR was calculated to be >60 ml/min/1.73m2, (CKD-EPI Creatinine, 2021) and had no history of CKD. Case Controls Controls – high-risk patient populations with and without adjudicated/diagnosed CKD were evaluated as a side-arm of this study (AKI, CHF, Type 2 Diabetes, and Hypertension).

Statistical analysis – the optimal cut-off to distinguish CKD from controls was calculated using univariate ROC analysis. All reported p-values are 2-sided, with significance level of 0.01.

Measurement of serum hkNTGuca2b – circulating hkNTGuca2b was measured via proprietary mAb sandwich ELISA (Ethos Biosciences; Logan Township, NJ).

RESULTS

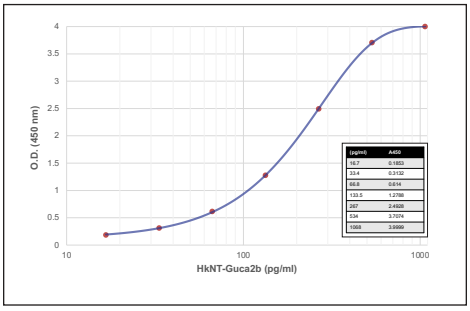

Figure 1: *Four parameter standard curve (R2=0.999) generated by measuring the absorbance of standard hkNT-Guca2b calibrator concentrations at 450 nanometers.*

Known calibrator concentrations (pg/ml) and associated absorbances pictured in table, left.

Equation: y = 4.242276 + (0.2083633 - 4.242276)/(1 + (x/225.301)^1.943628)

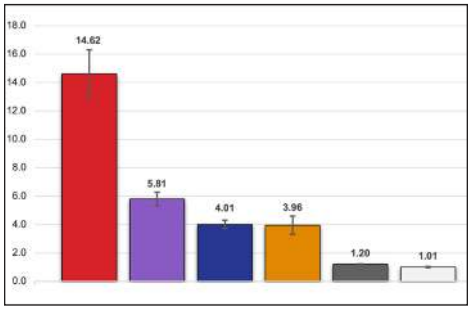

Figure 2: *Histogram above illustrates average hkNT-Guca2b levels in CKD stage 5 (14.62 ng/ml, n= 14), stage 4 (5.81 ng/ml, n=31), stage 3b (4.01 ng/ml, n=66), stage 3a (3.96 ng/ml, n=26), and controls, or No CKD, males (1.20 ng/ml, n=122), and females (1.01 ng/ml, n=125). Subjects in stage 5 were significantly elevated compared to stage 4 (p<0.0001), stage 4 levels were significantly higher than stages 3a and 3b (p<0.0001), which were significantly higher than controls regardless of gender (p<0.0001). The male control group expressed higher levels than females (p<0.01).*

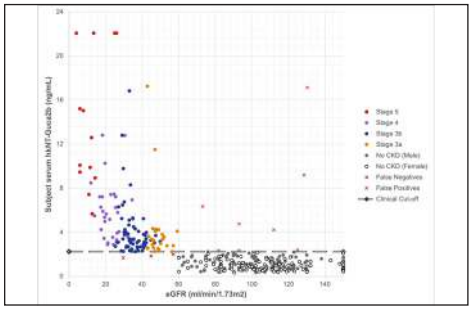

Figure 3: *Every subject was individually plotted according to their measured serum hkNT-Guca2b level and eGFR determined by CKD-EPI Creatinine 2021 and compared against a clinical cut-off value (dashed horizontal grey line). True positives (n=134) are expressed as colored dots including Stage 5 (red), Stage 4 (indigo), Stage 3b (dark blue), and Stage 3a (yellow). True negatives (n=239) are reflected as either male (grey) or female (white) dots. Two false negatives were classed as 3a and one as 3b (dark red X, n=3); and false positives (light red X, n=8).*

RESULTS (continued)

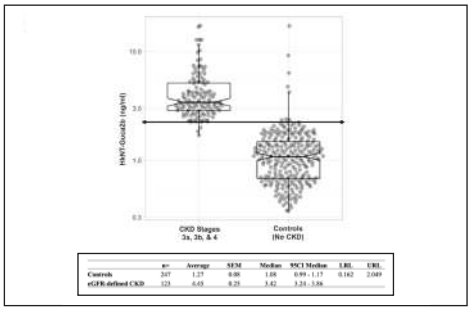

Figure 4: *Patient groups were stratified using the method of classification characterized by the National Kidney Foundation (NKF) as eGFR-defined CKD, having a baseline eGFR (at least two consecutive values at least three months apart) less than 60 ml/min/1.73m2 and greater than 15 ml/min/1.73m2 (n=123) and compared against a control population with healthy renal function defined by an eGFR equal to or greater than 60 ml/min/1.73m2 (n=247) estimated by the CKD-EPI Creatinine 2021 equation (p<0.0001).*

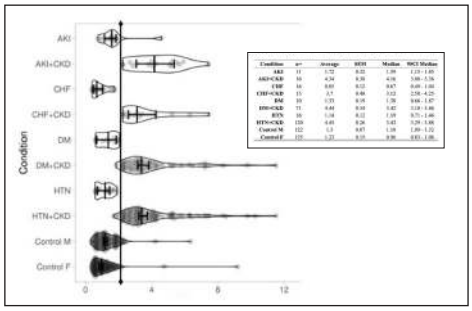

Figure 5: *Human subjects were stratified into populations based on the presence of high-risk comorbid target conditions for developing CKD including: history of acute kidney injury (AKI), congestive heart failure (CHF), diabetes mellitus (DM), and hypertension (HTN); and further classified into subpopulations based on whether the subject has been previously established by a practicing physician to have eGFR-defined CKD.*

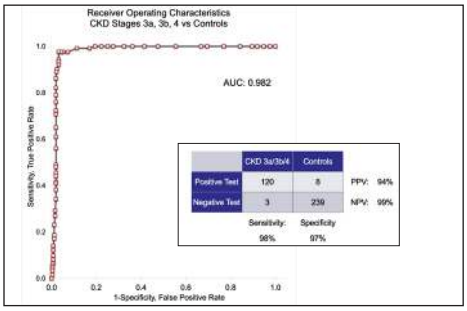

Figure 6: *Receiver operating curve analysis demonstrated an area under the curve (AUC) = 0.982; single test assessment discrimination between subjects clinically diagnosed with CKD (stage 3 or 4) and normal controls was 97.8% sensitive, 96.8% specific, 93.8% PPV, and 98.9% NPV.*

CONCLUSIONS

We present a novel, non-filtration dependent biomarker for identifying and diagnosing early CKD on a single test assessment ELISA with high sensitivity employing a specific circulating form of the Guca2b-gene product: hkNT-Guca2b. ROC analysis was conducted using a total of 370 subjects (not including 100% accuracy for ESRD, n=14) for the hkNT-Guca2b biomarker, which demonstrated remarkable sensitivity (98%), specificity (97%), PPV (94%), and NPV (99%) for clinically diagnosed (and adjudicated) Stage 3a, 3b, and 4 patients (n=123) against controls (n=247) with a single-assessment determination.

INTERPRETATION AND NEXT STEPS

PrognostX Health has spent the last 11 years and $11MM of grant funding to discover and develop a novel biomarker test for Chronic Kidney Disease.

Circulating hkNT-Guca2b is intrinsic to the kidney and non-filtration dependent, opposed to serum creatinine and cystatin C, which are non-renal and clinically utilized by estimating speed of filtration. Rising levels are a strong reflection of ongoing renal pathology rather than an estimate of filtration rate, and thus, repeat testing to prove chronicity is unnecessary.

Single test assessment accuracy for discerning CKD from control populations was validated in a 384-patient study4. Results from a smaller, independent cohort of CKD subjects Stage 1 and 2 demonstrated feasibility to predict rapid renal function decline or more aggressive CKD progression (studies ongoing).

The final goal is development of a screening test for those with Diabetes and Hypertension; Q2 Solutions (subsidiary of Quest) is set to launch the first FDA clinical trial to longitudinally monitor renal function post-Acute Kidney Injury in early 2024.

Adaption of the current format to a point-of-care lateral flow device for mobile and home health testing, specifically to aid in the mission of value-based kidney care companies to help stop progression to ESRD and dialysis by screening all those at high risk.

Strategic implementation and corporate alignment for development as a clinical chemistry platform test.

ACKNOWLEDGMENTS

1 https://www.kidney.org/ - NKF calls on USPSTF to prioritize CKD screening recommendations (2022, May 24) https://www.kidney.org/news/nkf-calls-uspstf-to-prioritize-screening-recommendations-for-CKD.
2 Danforth, K. N. et al. (2019) Follow-up of abnormal eGFR results within a large integrated health care delivery system: A Mixed-Methods Study. Am J Kid Dis, 74(5), 589–600.
3 Tangri, N. et al. (2022) REVEAL-CKD: Management and monitoring of patients with CKD Stage 3 in France, Germany, Italy, Japan, and the USA. and Estimated eGFR decline before and after a CKD diagnosis among patients with CKD Stage 3. ASN Kidney Week 2022.
4 Carrithers, A.L. et al. (2023) A new test to identify chronic kidney disease - A costly and silent killer. https://doi.org/10.33548/SCIENTIA949.