EARLY DETECTION OF PROGRESSIVE CHRONIC KIDNEY DISEASE BY MONITORING CHANGE IN eGFR IN CKD STAGES 1, 2 & 3

by

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ABSTRACT

RATIONALE: Early detection and effective treatment of chronic kidney disease (CKD) is reported to halt or slow progression (pCKD) to end-stage renal disease (ESRD) in many patients. Current guidelines recommend an eGFR upper reporting limit of > 60 mL/min/1.73m^2. However, this severely limits the detection of pCKD as the first time a patient is diagnosed with CKD, they are already in Stage 3.

OBJECTIVE: To determine if the rate of change in eGFR during early stages of CKD (*i.e.* 1 - 3) is different in those who progress to ESRD compared to those who are currently not anticipated to progress.

METHODS: This retrospective case-control (1:2) study used 5 years of hospital laboratory data (2008 – 2013). All subjects had a maximum eGFR-EPI > 90 mL/min/1.73m^2. Cases had a minimum eGFR-EPI < 15 mL/min/1.73m^2, while age- and sex-matched controls (± 5 years) had a minimum eGFR-EPI > 45 mL/min/1.73m^2. JOINPOINT (JP) regression software was used to identify and estimate the declining "linear" slope for eGFR most reflective of early pCKD. Multi-level modelling (MLM) was used for statistical analysis.

RESULTS: There were 30 cases (13 women, 17 men), and 60 controls (26 women, 34 men), for a total of 3,217 observations in 90 subjects. The mean eGFR-EPI slope by MLM was -2.9 mL/min/1.73m^2/year (95%CI: -3.3 to -2.4) for controls, and -13.0 mL/min/1.73m^2/year (95%CI: -16.6 to -9.4) for cases. The median intra-individual variation for eGFR-EPI was 9.5% (95%CI: 4 - 17%) for controls and 24% (95%CI: 6 - 45%) for cases. The average "reference change value" (RCV) needed between two serial values to detect a significant decrease was -25%.

CONCLUSIONS: Although eGFR declined in a linear fashion in some subjects, it may be more accurate to describe pCKD as an event-to-event process overlying progressive deterioration. Thus, analysis assuming linear decline should be undertaken cautiously in CKD. In order to detect pCKD earlier, regression with visual review of eGFR time profiles is optimal. Individuals at high risk need to be monitored at a useful frequency to take full advantage of the testing performed and the potential to significantly modify patient outcomes.

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ACR — albumin to creatinine ratio – random urine samples

AKI — Acute kidney injury, as evidenced by a wide range in eGFR results within a short period of time (*e.g*. days or weeks). Note that this is "presumptive" AKI as it is based on lab results rather than clinical information; it could also be due to another "critical clinical event".

BV — Biological variation

CV_i is intra-individual variation; the day-to-day variation within a person, also known as CV_w.

CV_g is inter- individual variation; the variation between people, also known as group variation.

CV_a is between-day analytical variation. It may be concentration dependent.

$CV_{a+i} = sqrt(CV_a^2 + CV_i^2)$ thus, $CV_i = sqrt(CV_{a+i}^2 - CV_a^2)$

$CV_{total} = sqrt(CV_a^2 + CV_i^2 + CV_g^2)$ or, $CV_{total} = sqrt(CV_{a+i}^2 + CV_g^2)$

NOTE:

Average $CV_{a+i} = sqrt(1/n \times sum(CV_{a+i}^2$ for each individual))

CKD — Chronic Kidney Disease is commonly defined as an eGFR < 60 mL/min/1.73m^2 for over 3 months, and is classified into 5 stages:

CKD Stage 1: eGFR ≥ 90 mL/min/1.73m^2, with evidence of structural damage

CKD Stage 2: eGFR between 60 - 89 mL/min/1.73m^2

CKD Stage 3: eGFR between 30 - 59 mL/min/1.73m^2

CKD Stage 4: eGFR between 15 - 29 mL/min/1.73m^2

CKD Stage 5: eGFR < 15 mL/min/1.73m^2

NOTE: Stage 3 may be subdivided to Stage 3a and 3b at 45 mL/min/1.73 m^2.

pCKD — "progressive" CKD (versus stable)

CI — Confidence interval (*e.g*. 95%CI)

CREA — creatinine = serum concentration (sCREA)

CV	Coefficient of variation (expressed as %), is also described as "relative SD" as it is the standard deviation normalized by the mean ($CV = 100 \times SD / mean$). Note that a 95%CI is equal to ±2 CVs, and thus represents a 4 CV range.
CV_i and CV_g	Coefficient of variation for intra-individual (CV_i) and inter-individual variation (CV_g) (see BV).
ESRD	end stage renal disease (Stage 5 CKD: eGFR < 15 mL/min/1.73m^2)
eGFR	"estimated" glomerular filtration rate
eGFR-EPI	estimated glomerular filtration rate, calculated from the EPI equation: $141 \times \min(CREA/\kappa, 1)^{\alpha} \times \max(CREA/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ if female x 1.159 if African descent where $\kappa = 0.7$ if female or 0.9 if male, and $\alpha = -0.329$ if female or $\alpha = -0.411$ if male
eGFR-MDRD	estimated glomerular filtration rate, calculated from the MDRD equation (automatically reported by most labs when serum CREA is ordered): $175 \times (CREA/88.4)^{-1.154} \times (Age)^{-0.203} \times (0.742$ if female$) \times (1.212$ if African descent$)$ (SI units)
GFR	glomerular filtration rate, expressed as mL/min or mL/min/1.73 m^2
Group	group 1 = cases minimum eGFR-EPI < 15 mL/min/1.73 m^2 group 0 = controls minimum eGFR-EPI > 45 mL/min/1.73 m^2 Both cases and controls had a maximum eGFR-EPI > 90 mL/min/1.73 m^2 There are two age- and sex-matched controls for each case.
GRP or casectrl	Case = 1, Control = 0
HbA1c	Hemoglobin, A1c fraction
IDMS	Isotope Dilution Mass Spectrometry – gold standard calibration for CREA.
II	Index of Individuality: $II = \sqrt{(CV_a^2 + CV_i^2)} / CV_g$ which simplifies to CV_i/CV_g if $CV_a << CV_i$

JP — JOINPOINT Statistical Software from the National Cancer Institute was used to identify the best CKD slope (J_SLOPE).

(https://surveillance.cancer.gov/joinpoint/download)

J_SLOPE — See Slope.

LIS — Laboratory information system

RCV — Reference change value (RCV)[13]: $2^{½} \times Z \times \text{sqrt}(CV_a^2 + CV_i^2) = 2^{½} \times Z \times \text{sqrt}(CV_{a+i}^2)$

CREA	**CV_a**	**CV_i**	CV_{a+i}	**Z**	**1.65**	**2.33**	**1.96**	**2.58**
				probability	0.05	0.01	0.05	0.01
				x sided	1	1	2	2
				$2^{½} \times Z$	2.33	3.30	2.77	3.65
46 µmol/L	10%	6%[12]	*11.7%†*		27%	38%	32%	43%
90 µmol/L	*8.7%†*	6%[12]	10.6%*		25%	35%	29%	39%
90 µmol/L	5%	9.3%†	10.6%*		25%	35%	29%	39%
≥170 µmol/L	2%	6%[12]	*6.3%†*		15%	21%	18%	23%

(*) observed in this study for controls. Thus, a decrease in results of 25% is significant at $p<0.05$.

† estimated given the other two CV variables.

SD — standard deviation

SEX and SEXg — SEX (F or M); SEXg (M = 0 F = 1)

SLOPE — Overall eGFR-EPI slope calculated in SAS using all data from each subject.

J_SLOPE — Slope chosen from JP analysis to represent the most appropriate estimate of the slope during the early stages of CKD (*i.e.* Stage 1 and 2). This is the same as the overall SLOPE for many subjects.

STAGE — See CKD.

time_yr — Time of sample compared to first sample from that subject in terms of years (*e.g*. 2.4 years). Baseline time is the first sample in the study database.

Time Profiles	eGFR-EPI "time profiles" are the visual graphs of eGFR-EPI (y axis) versus time_yr (x axis), which were reviewed using JOINPOINT.
TRIAD	There were 30 TRIADs of 1 case and 2 age/sex-matched controls (90 subjects).
WEIGHT	WEIGHT = 1/(E_StdErr_slope * E_StdErr_slope)
"_0"	This is the code added to any variable or file name to indicate baseline data. Summary data (*e.g*. max *etc*.) or slope may be included in the final "_0" file.

CHAPTER 1: INTRODUCTION

Early detection and effective treatment of chronic kidney disease (CKD), and of its risk factors and predisposing diseases, is reported to halt or slow disease progression to end-stage renal disease (ESRD) in many patients[1]. In 1999, Levey *et al.* introduced a new calculation to estimate the glomerular filtration rate (eGFR) from the routine measurement of serum creatinine (CREA)[2]; and, in 2002 the K/DOQI guidelines recommended that eGFR be used as a screening test in high risk diseases such as diabetes or cardiovascular disease[3] using a new 5 stage classification system for CKD based on eGFR results[2,4]. The Stage 3 upper limit of 60 mL/min/1.73m^2 was chosen as the cutoff for CKD diagnosis, and > 60 mL/min/1.73m^2 was recommended[1,3,4] as the eGFR upper reporting limit for several reasons, including: 1) the calculation was developed using data from only CKD patients and was not validated for extrapolation to higher eGFRs in the normal range or early CKD stages; 2) the performance characteristics of CREA methods are not all optimized at the lower concentrations encountered in the early CKD stages; and, 3) there are no current treatment guidelines above an eGFR of 60 mL/min/1.73m^2. Unfortunately, this severely limits early detection of progressive CKD (pCKD), as the first time a patient is diagnosed with CKD, they will already be in Stage 3.

CHAPTER 2: LITERATURE REVIEW

BACKGROUND OF RENAL FUNCTION and CHRONIC KIDNEY DISEASE

Kidneys remove the body's metabolic wastes, while ensuring homeostasis and regulation of fluid, electrolyte and acid-base balance. In addition, they produce essential hormones for red blood cell production, blood pressure regulation, and calcium and bone metabolism. Adult kidneys weigh approximately 135 grams each, and receive about 20% of the cardiac output, filtering it to produce approximately 180 L of filtrate a day. Most of the filtrate is reabsorbed, resulting in an excretion dependent on homeostatic (and other) input parameters, of only 2 L of urine per day.

Despite the remarkable reserve capacity of the kidneys, CKD is not uncommon. CKD is defined as either kidney damage (*e.g.* pathological abnormalities or abnormalities in blood or urine tests or in imaging studies), or a consistent GFR < 60 mL/min/1.73m^2 for three or more months[3]. The five stages of CKD have been defined as follows[3,4]: 1) kidney damage with normal or increased eGFR (*i.e.* hyperfiltration), where eGFR is ≥ 90 mL/min/1.73m^2; 2) kidney damage with a mild decrease in eGFR, where eGFR is 60 - 89 mL/min/1.73m^2; 3) a moderate decrease in eGFR, where eGFR is 30 - 59 mL/min/1.73m^2; 4) a severe decrease in eGFR, where eGFR is 15 - 29 mL/min/1.73m^2; and, 5) kidney failure, also known as "end stage renal disease" (ESRD), where eGFR is < 15 mL/min/1.73m^2 or the patient is on dialysis. Furthermore, it has been recommended that Stage 3 be subdivided into Stages 3a and 3b at 45 mL/min/1.73m^2 for screening and treatment purposes[5].

Although the current overall prevalence of CKD in adults in North America is approximately 10%, it is expected to increase significantly as the average age of the population increases because the major risk factors for CKD also increase with age[1,6,7]. Diabetes is the main cause of CKD and ESRD, accounting for over 30 - 40% of CKD cases, followed by hypertension which accounts for 20 - 30% of CKD

cases[1,6,7]. Conversely, over 35% of adults with diabetes and over 20% of adults with hypertension, have CKD[7]. Other common risk factors for CKD include glomerulonephritis, infection (pyelonephritis), drugs and toxins, all of which may be associated with acute kidney injury (AKI), as well as genetic predisposition and ethnicity[1,4,6,7]. CKD occurs more frequently in women than men, however, more men progress to ESRD[6]. The prevalence of CKD in people over 65 is estimated at 30%, while the overall prevalence of ESRD is relatively rare at 0.1 – 0.2%[1,4].

Reducing the burden of CKD and ESRD is important for both the individual and the population. As health care systems are already challenged, it is recognized that an unchecked epidemic of CKD would be unmanageable. People with CKD are more likely to die of cardiovascular disease or other co-morbidities than to reach ESRD[4]. While significant improvements have been made in the length and quality of life achieved for patients with ESRD, avoiding this final stage of kidney failure is preferable. In 2010, there were approximately 39,000 people living with ESRD in Canada, which was triple the prevalence in 1991[7].

Only a small percentage of people reach ESRD and thus require dialysis or renal transplantation. Once a patient does reach ESRD, the process is irreversible. A significant reduction in the development and progression of CKD may be achievable early in the disease by addressing modifiable risk factors and optimizing management of predisposing factors[1,4]. Investigations on population health interventions are well underway to address the modifiable lifestyle risk factors for obesity, hypertension and cardiovascular disease such as exercising, smoking cessation, optimal nutrient and food consumption, hyperlipidemia, stress reduction and adequate sleep.

As kidney disease usually evolves and progresses without notable signs or symptoms, periodic testing is common practice for high risk groups[1]. Fortunately, the two main tests used to detect and

monitor CKD are inexpensive and routinely available in most developed countries. Serum creatinine (CREA), one of the 7 most common blood tests ordered, reflects *kidney functioning* as it is eliminated from the body by kidney filtration and secretion. CREA can easily be measured whenever blood is drawn (*i.e.* glucose or HbA1c measurement for diabetes screening or monitoring). Since 2006, most laboratories in Canada automatically calculate and report eGFR whenever CREA is ordered. The second useful test assesses *kidney damage* by determining the amount of protein, as the albumin to creatinine ratio (ACR), excreted in a random urine sample. Normally the body tries to conserve protein, so only minimal amounts should be detectable. The high-risk groups who should be screened for CKD include: people over 50 years or who have a family history of CKD, and people with diabetes, hypertension, or cardiovascular disease[1].

MORE ABOUT SERUM CREATININE AND ESTIMATED GLOMERULAR FILTRATION RATE:

GFR is a more intuitive result than CREA as it reflects the percent of kidney function remaining. For example, an eGFR of 60 mL/min/1.73m^2 suggests that approximately 50 - 60% of kidney function remains. The eGFR-MDRD calculation derived by Levey *et al.* in the 1999 Modification of Diet in Renal Disease (MDRD) study is commonly used in its abbreviated form: 175 x [CREA/88.4]$^{-1.154}$ x age$^{-0.203}$ X 0.742 for women X 1.21 if African American[2,8]. By normalizing eGFR for age and sex, a single set of CKD criteria can be used for both men and women. This is considered more effective in identifying CKD than CREA and its population reference intervals.

In 2006, clinical chemists in Ontario and British Columbia decided to use an upper reporting limit of >120 mL/min/1.73m^2 to emphasize the importance of serial monitoring in individual patients as they implemented eGFR reporting[9]. Analysis of 19,333 CREA results by Gamma-Dynacare Laboratories during the first three days of eGFR implementation in Ontario demonstrated that, depending on age, 24% to 63% of people tested had an eGFR in Stage 2 (60 – 89 mL/min/1.73m^2)[10]. There are several causes of

transiently increased CREA concentration including drugs (*e.g.* which may complete for tubular secretion, directly reduce GFR, or cause AKI), rapid body water shifts (*e.g.* dehydration, which is commonly associated with various illnesses), and high protein ingestion or concentration[9]. In addition, compounds such as Vitamin C, bilirubin and ketoacids may cross react in some CREA methods[9].

In 2009, Levey *et al.* reported the development and validation of the eGFR-EPI equation[12]. It is purported to have less negative bias in patients with higher GFRs (*e.g.* ≥ 60 mL/min/1.73m^2), reducing the number of patients classified with CKD (*i.e.* reducing the false positive rate).

INTRA-INDIVIDUAL BIOLOGICAL VARIATION AND REFERENCE CHANGE VALUES

An important variable in result interpretation is consideration of the inherent day-to-day biological variation expected in an individual[13,14]. Repeat sampling over time (*e.g.* days, weeks, *etc.*), demonstrates that the average intra-individual biological variation (%CV_i; *e.g*. 1SD) of CREA is approximately ± 6.0% in healthy individuals[14,15]. Westgard's biological variation repository cites the average between-individual variation (CV_g) for CREA as 14.7%[14]. Thus, CREA's average biological variation (BV) parameters indicate "marked individuality" for this test (Index of individuality (II) = 0.41 = CV_i/CV_g)[13], which means that a subject's CREA may double and still be considered normal if it is within the population reference interval (*e.g.* normal range). When monitoring test results from a particular subject, population reference intervals should be used with caution for tests with an II < 0.6 as they are not a sensitive indicator of significant change[13].

Taking analytical variation (CV_a) into account, where CV_a = 5 - 6% at 90 µmol/L CREA (assay imprecision at 1SD), a persistent change of 18 - 20% in CREA (or eGFR) would be significant at $p<0.05$, while a 26 - 28% change would be highly significant at $p<0.01$. This significant or "critical" change, also known as the "reference change value" (RCV), is calculated as $2^{½} \times Z \times \text{sqrt}(CV_a^2 + CV_i^2)$ [13]. By using a

patient as their own reference, the emphasis in result interpretation may be shifted from population-based decision limits to personalized result interpretation and care[15,16].

CURRENT LITERATURE ON RATE OF CHANGE OF SERUM CREATININE AND eGFR

Creatinine clearance typically decreases by 50% as people age from 25 to 85 years. This represents a decline in GFR of approximately 1 mL/min/1.73m^2/year from 120 mL/min/1.73m^2 to 60 mL/min/1.73m^2, respectively. In contrast, eGFR in CKD patients is reported to decline by 1 to 4 or 5 mL/min/1.73m^2/year, while patients with diabetes with overt nephropathy and untreated hypertension may decline by up to 10 to 12 mL/min/1.73m^2/year[17-25].

In 2006, Erikson and Ingebretsen reported on their 10 year longitudinal observational study in Norway which included 3074 people with an eGFR between 30 and 59 mL/min/1.73m^2 (CKD Stage 3) for more than 3 months[22]. The median number of results for each patient was 9 (4 - 15 for women; 6 – 22 for men), and the median observation period was 44 months. The mean overall eGFR change was -1.03 mL/min/1.73m^2/year. This changed with age for both men (-0.94, -1.29, -2.07 mL/min/1.73m^2/year) and women (-0.33, -0.91, -1.42 mL/min/1.73m^2/year) by decade (aged <69, 70 - 79, >79 years respectively). Although, only 6% of participants (8% of men, 4% of women) had a rapid eGFR change of more than -5 mL/min/1.73m^2/year, 31% of these people died while 2% progressed to ESRD. Approximately 73% of patients demonstrated a decline in eGFR, with women tending to have a slower decline; leaving 27% of patients who did not experience a decline (the original 2 year MDRD study[2] found 19% of patients did not decline, however, for those that did, the mean eGFR change was -4 mL/min/1.73m^2/year). The authors pointed out that the current staging system is limited by its focus on the level of eGFR, rather than on its rate of progression.

Erikson and Ingebretsen[22] used “multilevel multivariate linear regression analysis” (MLM) to estimate the change in eGFR for each patient, and the effect of age and sex on this estimate. eGFR was

assumed to follow a "linear trend on time" for each patient at the first level of the MLM, and SAS PROC MIXED was used for the analysis. Linear decline is a common representation for CKD, and thus MLM has been considered a reasonable statistical approach to CKD data analysis. However, similar to other studies, it was not apparent that the assumption of linearity was validated in their study, making this a potential limitation of their study.

In 2010, Barbour *et al.* reported on an eight year study from British Columbia which investigated the differences in progression of CKD to ESRD and/or death amongst a cohort of 3444 Caucasian, Oriental Asian and South Asian CKD patients[23]. The annual rate of eGFR decline was calculated by linear regression and they reported the mean, median and range of the overall data for each ethnic group. The number of results available for each patient over each year was not reported. They concluded that "Oriental Asian and South Asian patients have a shorter time to ESRD (faster rates of renal decline) and yet better survival compared to Caucasians" based on their median eGFR rates of change of -2.93, -3.56 and -2.11 mL/min/1.73m^2/year respectively (p = 0.027). However, the IQR of eGFR progression varied from -6.26 to -0.76, -6.95 to -1.15, and -4.91 to +0.07 mL/min/1.73m^2/year respectively for each ethnic group, and the groups included patients with significant outcomes (ESRD and morbidity) and those without (*i.e.* what would be both cases and controls in our study).

A recent Ontario study in 2011 by Clark *et al.* looked at the ability of dipstick proteinuria assessment to identify "rapid kidney eGFR function decline"[25] (RKFD = pCKD). This community-based prospective cohort study of 2,574 participants had a median follow-up of 7 years. Progressive CKD, defined as >5% decline in annual eGFR from baseline, was observed in 8.5% of participants. The authors decided *not* to use a pCKD definition of -3 mL/min/1.73m^2 (observed in 15.6% of participants) because this mostly occurred in participants with eGFR > 90 mL/min/1.73m^2. The median annual eGFR rate of change in participants without pCKD was -0.57 mL/min/1.73m^2 (IQR: -1.7 to 0.7 mL/min/1.73m^2), which was equivalent to an annual percent change of -0.70% (IQR: -2.1% to 0.8%). Participants with pCKD had

a median annual eGFR rate of change of -5.66 mL/min/1.73m^2 (IQR: -7.7 to -4.4 mL/min/1.73m^2), which was equivalent to a median annual percent change of -6.9% (IQR: -9.2% to -5.6%). This study suggested that percent change was a more reliable indicator of pCKD and of incident CKD than absolute change, especially for participants with a baseline eGFR > 90 mL/min/1.73m^2. As more than 80% of their pCKD participants had an eGFR > 60 mL/min/1.73m^2, the authors emphasized the value of "changing the focus from static eGFR assessment among those with an eGFR < 60 mL/min/1.73m^2 to dynamic assessment of those with an eGFR both above and below 60 mL/min/1.73m^2 "to enable early identification of patients who would benefit from potential interventional strategies to delay the progress of their disease".

Although Rutherford *et al.* reported on the "need to quantitate rate" of progression in 1977[17], follow-up on this approach has only gained more interest recently, with several studies reporting change in eGFR as annual or overall, rates or % change[17-25]. Rates have been calculated as slopes by direct linear regression, or by multiple regression models. Although it is clear that most people do not progress and that a significant number of people actually "improve", studies tend to calculate and report overall statistics. It is unclear if or how the challenges of variable progression patterns across individuals (*e.g.* monophasic vs multiphasic), and the inherent analytical and biological variation of CREA (eGFR) is commonly addressed. In addition, although not confirmed in many studies, external quality assurance monitoring demonstrates that it is important to use the same laboratory or at least the same methods whenever possible, and to have an adequate number of samples per individual to be able to discern a true change in eGFR from the inherent day-to-day biological variation experienced by an individual.

HYPOTHESIS AND OBJECTIVE:

The hypothesis of this study is that progressive chronic kidney disease (pCKD) can be identified early by assessing the change in eGFR compared to using a single threshold approach (e.g. eGFR < 60 mL/min/1.73m^2). The primary objective is to determine if the changes in eGFR for subjects who progressed to ESRD are different from those who are currently not anticipated to progress, when they were in the early CKD stages (1, 2, 3).

CHAPTER 3: METHODS

STUDY DESIGN

A retrospective case-control (1:2) design was used for this study. All subjects had a maximum eGFR-EPI above 90 mL/min/1.73m^2. Cases had a minimum eGFR-EPI result of less than 15 mL/min/1.73m^2, while controls were age- and sex-matched (± 5 years) and had a minimum eGFR-EPI result above 45 mL/min/1.73m^2.

ETHICS APPROVAL

Ethics approval was granted by the Queen's University and Affiliated Teaching Hospitals Health Sciences Research Ethics Board for use of Kingston General Hospital's (KGH) laboratory information system (LIS) data on April 26th 2013 (APPENDIX i).

DATA

A database was obtained from KGH's LIS which included the following data for the 5 year period from June 2008 to May 2013:

Subject demographics included: patient ID number (CR), date of birth (DOB), sex (SEX)

Sample information included: sample ID number, date/time of sample collection, result, ordering physician, ordering location

Results included: serum creatinine (CREA), and calculated eGFR-MDRD

PRIMARY OUTCOME: eGFR-EPI rate of decline over time in years (*i.e.* eGFR-EPI J_SLOPE)

A second database for the same time period (2008 to 2013) was obtained from the NephroCare® information system at KGH. This database was developed to identify and monitor all patients on dialysis or registered in the CKD clinic, as well as patients referred for nephrology consult for

any kidney related illness or disease. Therefore, there may be patients with CKD and pCKD in the Kingston area who have either not been diagnosed or referred, including the patients who "present late" with Stage 4 or 5 CKD. It includes the patient hospital number and one of the following diagnostic codes for each patient: 1) conventional hemodialysis, 2) transplant, 3) CKD, 4) discontinued therapy, 5) deceased, 6) recovered, 7) general nephrology consult, 8) discharged, or 9) lost to follow-up. This information was obtained to confirm case (1, 2) and control (3) status.

Following basic data clean-up of the KGH LIS database, calculations were performed in SAS to produce further variables (*e.g.* eGFR-EPI, time from first sample, *etc.*).

SAMPLE SIZE CALCULATION

Since the study was a matched case with multiple control design, the sample size and power calculation was chosen to reflect the matched design, *i.e.* taking into account the correlation (non-negative) between cases and controls, and the correlation among controls.

For a 1:1 matched case-control study with continuous variables, the sample size, n (number of matched pairs) for comparing means is:

$$n = \frac{\sigma_d^2}{d^2}(z_\beta + z_{\alpha/2})^2$$

where, d is the mean difference between case and control to be detected (*e.g.* effect size); z_β is the desired power (*i.e.* "0.84" for 80% power or 20% chance of a Type II (false negative) error), and $z_{\alpha/2}$ is the level of statistical significance (*i.e.* "1.96" for α = 0.05), and σ_d is the standard deviation of the pair differences, which can be expressed as:

$$\sigma_d^2 = \sigma_1^2 + \sigma_0^2 - 2\rho\sigma_1\sigma_0$$

where, σ_1 and σ_0 are the corresponding standard deviations for cases and controls, and p is the correlation between case and control within pairs. Sample size calculation will be most conservative when p = *0*, which corresponds to two independent samples (*i.e.* unmatched case-control study).

For a general *1:r* matched case control study:

$$\sigma_d^2 = \sigma_1^2 + \frac{\sigma_0^2}{r} - 2\rho\sigma_1\sigma_0$$

When standard deviations for cases and controls are the same, *i.e.* $\sigma_1 = \sigma_0 = \sigma$, and there is no correlation, p *= 0*, then:

$$\sigma_d^2 = \left(\frac{r+1}{r}\right)\sigma^2$$

and the total number of cases required is:

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2}{d^2}(z_\beta + z_{\alpha/2})^2$$

The number of controls required will be *nr.*

In this study, 1:2 case to controls (*r = 2*) matching was used. Using a SD for the pair differences of 15 mL/min/1.73m^2 and assuming zero correlation (*i.e.* p *= 0*), 27 cases and 54 controls would be needed (total 81) for an 80% power to detect a mean difference of 10 mL/min/1.73m^2 between the cases and controls at the 0.05 level of significance[26].

If the matching is effective making age and sex significant covariates for slope, then there may be correlation between cases and controls, and/or among controls (*i.e.* p > *0*), and:

$$\sigma_d^2 < \left(\frac{r+1}{r}\right)\sigma^2$$

making the actual sample size required smaller. For example, if p *= 0.5*, and the SD for both the cases and controls was 15 mL/min/1.73m^2, then 14 cases would be required. However, if the SDs for the cases and controls are different (*i.e.* 15 and 1.8 mL/min/1.73m^2), then for p *= 0, 0.25, 0.5*, 0.75 or 0.9, the number of cases required would be 27, 26, 24, 22 and 21 respectively. In this case, the use of p *= 0* would be a conservative estimate that would provide a power of more than 80% (*i.e.* 87%).

INCLUSION and EXCLUSION CRITERIA:

Cases and controls were randomly selected for eGFR-EPI time profile review, and included if: their eGFR-EPI declined in a chronic fashion over at least a year; they had more than 12 observations; their observations spanned more than 3.8 years; and, if their maximum eGFR-EPI was greater than 90 mL/min/1.73m^2 (Figure 1). The necessary observation time span of 3.8 years provided 100 potential subjects for review as cases. A case and its 2 controls were called a TRIAD.

Cases and controls were excluded if their eGFR-EPI was essentially stable or increasing; if their first or last eGFR-EPI measurement appeared to be an outlier compared with the remaining measurements, potentially influencing the slope significantly; or if presumptive AKI (acute kidney injury) was their dominant clinical pattern as opposed to pCKD.

NOTE: AKI may be a contributing factor to pCKD as well as a specific disease entity. While an absolute increase in CREA of 26 µmol/L has been recommended as an initial screen for AKI[27], in this study wide fluctuations in CREA (varying across several CKD stages) that resolved within a week or two were considered presumptive of AKI. A subject was excluded even if their minimum eGFR-EPI was less than 15 mL/min/1.73m^2 if there was no overall decline in their eGFR-EPI, and thus "presumptive" AKI (based on lab results rather than clinical information) was more likely than pCKD.

JOINPOINT REGRESSION ANALYSIS

JOINPOINT (JP) regression analysis (JOINPOINT Statistical Software, Version 4.0.4 May 6, 2013; National Institutes of Health, National Cancer Institute, Surveillance Research Program, Bethesda, MD 20892) was performed for each subject to objectively: (i) identify the JPs (inflection points) where the slopes changed; and, (ii) estimate the associated the slopes. The best "linear" eGFR-EPI J_SLOPE which was most reflective of early pCKD (*i.e*. above approximately 45 mL/min/1.73m^2), was identified individually for each subject. For simplicity and the purpose of this study, the maximum number of JPs was set to 3 (*i.e.* 0, 1, 2, and 3 JPs; where, JP = 0 = straight line through all data with no inflection points). The JP program settings and an illustration of the JP outputs and a time profile are illustrated in APPENDIX ii.

FIVE YEAR DATABASE versus eGFR-EPI J_SLOPE SUBSET DATABASE

After the JP time profiles were visually reviewed to identify the best "linear" slope and its associated data which was most reflective of early pCKD, the "eGFR-EPI J_SLOPE subset" database was derived by including only the observations from these selected time intervals. It should be noted that some eGFR-EPI results for the controls were below 45 mL/min/1.73m^2 (*i.e.* CKD Stages 3b, 4 and 5) as the end time was defined by when the apparent mean eGFR-EPI fell below 45 mL/min/1.73m^2. The results and discussion are based on the eGFR-EPI J_SLOPE subset data unless otherwise indicated.

MULTILEVEL MODELING: "eGFR-EPI J_SLOPES" (subset) database

With eGFR-EPI J_SLOPE selected (from the JP analysis) as the "best" indicator of the rate of early CKD progression with linearity confirmed, a multilevel (2-level) model (MLM)[28,29] was applied on the eGFR-EPI J_SLOPEs as the outcome variable to determine if cases had a higher (faster) rate of decline than controls (*i.e.* a more negative mean eGFR-EPI J_SLOPE). This MLM is a 2-level model, with level 1

(for individuals) variables including age (as a continuous variable) and sex of individuals, while the matching variable TRIAD is considered as a level 2 variable. Because of sex matching, TRIAD can be grouped into two groups according to two sexes, thus sex can also be considered as a level 2 variable. As a result, the proposed 2-level MLM includes case-control group, age and sex as fixed effects, while TRIAD is considered as a random effect which is nested within sex. The final MLM analysis was weighted based on the variance of the J_SLOPE estimate.

The intra-class correlation coefficient (ICC) was used to estimate the proportions of total variance. Since the variation between TRIADs for cases is very different from that observed for controls, TRIAD treated as a random effect was set to allow cases and controls to have different variances. Based on the 2-level MLM, we can define ICC as:

$$ICC = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_i^2} = CV_g/(CV_g + CV_i)$$

Note that σ_g^2 and CV_g for cases and controls are different.

SAS Statistical Software 9.3 (SAS Institute, Cary, NC, USA) was used for the MLM analysis, descriptive statistics and correlations. Statistical significance was set at an alpha of 0.05. APPENDIX iii highlights the data preparation and the final programs performed in SAS.

BIOCHEMICAL METHODS

Biochemical analysis was performed in the Core Laboratory at Kingston General Hospital (KGH), using one of two Beckman Coulter UniCel® DxC 800 instruments (Beckman Coulter Inc., Fullerton CA) using Beckman reagents. The serum creatinine (CREA) Jaffe method was IDMS standardized, and performed with a between-day imprecision (%CV_a) of 10% at 46 µmol/L, and 2% at 170 µmol/L and 590 µmol/L. eGFR was automatically calculated and reported with any CREA ordered on adults using the MDRD equation.

CALCULATIONS FOR BIOLOGICAL VARIATION

Analytical imprecision, CV_a, may be determined by analyzing samples in duplicate or by estimation from laboratory internal quality control between-day imprecision data. Use of internal quality control data assumes that the quality control material performs similarly to subject samples, and that precision estimates are available for concentrations similar to that observed for the subject data. As the clinical samples in this study were not run in duplicate, internal quality control data was used to estimate the CV_a. At 90 µmol/L (*e.g.* Stage 1 CKD), the CV_a is approximately 5%.

When routine results are reported, their measurement of uncertainty is a combination of analytical and intra-individual biological variation (*i.e.* CV_a and CV_i), which can be written as CV_{a+i}. The reference change value[13] calculation is thus:

$$RCV = 2^{½} \times Z \times \text{sqrt}(CV_a^2 + CV_i^2) = 2^{½} \times Z \times \text{sqrt}(CV_{a+i}^2) = 2^{½} \times Z \times CV_{a+i} .$$

Note that as CVs cannot be directly added or subtracted, calculations (*e.g.* for average CV_{a+i}) were performed similar to variance calculations using squares and square roots (*see* List of Abbreviations).

CHAPTER 4: RESULTS

DESCRIPTIVE STATISTICS

The 5 year database (2008 – 2013) provided from the KGH LIS included 775,580 observations for CREA and eGFR-MDRD in 105,453 adults, 18 years or older. The database was culled to retain only subjects with more than 12 observations and having a maximum eGFR-EPI greater than 90 mL/min/1.73m^2 (263,009 observations in 8,187 subjects), and then culled further to retain only subjects with observation times (*e.g.* max_time_yr, maximum observation time in years) greater than 3.8 years (66,807 observations in 1,702 subjects) (Figure 1). All subjects with a minimum eGFR-EPI less than 15 mL/min/1.73m^2 were included in a subgroup for case selection (8,604 observations in 100 subjects), while all subjects with a minimum eGFR-EPI greater than 45 mL/min/1.73m^2 were included in a second subgroup for control selection (39,086 observations in 1,256 subjects). Potential cases were randomly selected for eGFR-EPI time profile review: 83 JP time profiles were reviewed to identify **30 cases** with a decline in renal function (screening ratio of 2.77 to 1). Age- and sex-matched controls were then also randomly selected: 121 JP time profiles were reviewed to identify **60 controls** with a decline in renal function (screening ratio of 2 to 1). In the final 90 subjects, there were 13 women and 17 men in the case group, and 26 women and 34 men in the control group, for a total of **39** (43%) women and **51** (57%) men. Thus, 30 TRIADS of 1 case, and 2 age- and sex-matched controls were collected: 13 TRIADs of women, 17 TRIADs of men.

Of the 4,845 observations in the final 90 subjects, 60% were from the cases and 40% were from the controls, illustrating that cases were investigated more frequently. The mean number of observations for cases was 97 (117 for women, 82 for men) and for controls was 32 (28 for women, 36 for men). The mean observation time was 4.5 years (cases 4.4 years, controls 4.5 years). Neither number of observations nor mean observation time were significant in the MLM.

In order to address the study objective, the final "eGFR-EPI J_SLOPE subset" database included only the observation data associated with a declining slope for all 90 subjects. Of the 3,217 observations, 42.5% were from cases and 57.5% were from controls. The mean number of observations for cases was 46 (women 56; men 37) and for controls was 31 (women 26; men 34) (Table 1). Ten subjects had less than 12 observations: 7 cases (4, 5, 7, 9, 10, 11 and 11 observations) and 3 controls (7, 9, 11 observations). The highest number of observations in a subject was reduced from 513 to 166 observations. The mean observation time was 3.9 years: 2.9 years for cases and 4.4 years for controls. Twenty five subjects had observation times less than 3.8 years (20 cases, 5 controls); 9 cases and 1 control had maximum observation times of less than 2 years.

Twenty four (24) of the 90 subjects had eGFR-EPI J_SLOPEs that were different from the SLOPEs determined from their overall data: 20 of these were cases (*i.e.* 20 of the 30 cases), and 4 were controls.

Descriptive statistics for the eGFR-EPI J_SLOPE subset are presented in Table 1 and Figures 2 - 5. The mean (SD) age of the subjects was 54.7 (9.8) years, with a range of 33 to 72 years. The age difference between the cases and controls within each TRIAD ranged from -4.7 to 5.2 years. The median maximum_eGFR-EPI was 107 mL/min/1.73m^2; while, the median minimum_eGFR-EPI was 72 mL/min/1.73m^2 for controls, and 10 - 30 mL/min/1.73m^2 for cases (women *vs.* men).

The median eGFR-EPI J_SLOPE was -3.0 mL/min/1.73m^2/year (95%CI: -6.7 to -0.9) for the controls and -11.5 mL/min/1.73m^2/year (95%CI: -40 to -2.8) for the cases (Table 1 and Figure 5). The median intra-individual variation for eGFR-EPI (eGFR-EPI-CV_{a+i}) was 9.5% for the controls (95%CI: 4 - 17%) and 24% for the cases (95%CI: 6 - 45%) (Table 1 and Figure 4). Table 2 summarizes the biological variation parameters observed for various subgroups of our subjects (*i.e.* by sex, by case/control).

There were 1047 subjects in the NephroCare® database with five classifications: conventional hemodialysis, transplant, CKD, stopped therapy and deceased (original clinical diagnosis unknown). Although an initial strategy was to use this database to identify cases, many subjects in the NephroCare®

database did not demonstrate a declining eGFR time profile in the current 5 year period. Of the final 90 subjects, 6 patients from the NephroCare® database were included in the 30 cases. Thus, only 20% were "known" to nephrology. No controls were identified as "false negatives" through this analysis.

JOINPOINT TIME PROFILES

An important aspect of the study protocol was to individually assess the eGFR time profiles to visually confirm CKD, and to identify the time frames associated with early eGFR decline. In this study population from a tertiary care hospital with specialist clinic testing, the eGFR time profiles were exceptionally heterogeneous (APPENDIX iv). Episodes suggestive of AKI were common, and in some subjects occurred multiple times. On the other hand, it was not unusual for kidney function to recover significantly. In addition, there were good examples of subjects with stable eGFRs and minimal variability over the 5 year time window. Some subjects demonstrated significant intra-individual variation (eGFR-EPI-CV_{a+i}), with maximum results twice as high as their minimum results. Examples of JP time profiles are presented in APPENDIX iv, with cases at the top, and their age- and sex-matched controls below. These JP time profiles include all observations in the 5 year database (n = 4845), not just those associated with the eGFR-EPI J_SLOPE subset (n = 3217).

CORRELATIONS

eGFR-MDRD and eGFR-EPI are purported to provide similar estimates of eGFR below 60 mL/min/1.73m^2 (CKD Stages 3 to 5), while eGFR-EPI is recommended for estimating results above 60 mL/min/1.73m^2 (CKD Stages 1 and 2). There was a significant overall correlation for these two estimates at baseline (Figure 6: Spearman correlation coefficient = 0.941 (95%CI: 0.911 - 0.961), n=90), especially for eGFRs less than 80 mL/min/1.73m^2. However using the eGFR-EPI J_SLOPE subset (n = 3217), at eGFRs greater than 80 mL/min/1.73m^2, the eGFR-MDRD tended to be greater than eGFR-EPI, with an age and sex dependency (Figure 7). This data supports the use eGFR-EPI for this study.

There was a significant inverse relationship between eGFR-EPI J_SLOPE and eGFR-EPI-CV_{a+i} for controls (Figure 8: Spearman correlation = -0.571 (95%CI: -0.718 to -0.367)), but not for cases (p = 0.113). The variability in individual eGFR-EPI results observed in controls is illustrated in Figure 9.

MULTILEVEL MODELING: "eGFR-EPI J_SLOPES"

The intra-class correlation coefficient (ICC) for eGFR-EPI J_SLOPES was 96% for cases (57.2725/(57.2725 + 2.3389),p = 0.007) and 5% for controls (0.1236/(0.1236 + 2.3389), p = 0.383). Thus, virtually all of the variation in eGFR-EPI J_SLOPE is associated with the variation across cases, while the variation across controls was small in comparison, as demonstrated in Figure 5 b.

Based on the MLM, the average eGFR-EPI J_SLOPE for a 55.5 year old control was -2.9 mL/min/1.73m^2/year (95%CI: -3.3 to -2.4 mL/min/1.73m^2/year), while that for a case was -13.0 mL/min/1.73m^2/year (95%CI: -16.6 to -9.4 mL/min/1.73m^2/year), for an overall difference of -10.2 mL/min/1.73m^2/year (95%CI: -13.8 to -6.6 mL/min/1.73m^2/year; T = -5.64, p < 0.0001). Figure 10 illustrates the differences in eGFR-EPI J_SLOPEs between cases and controls in a boxplot diagram. While the %CVs (1SD) for the eGFR-EPI J_SLOPEs for the cases (77%) and controls (56%) were relatively wide (Table 1), the separation between the groups in this study is clearly evident. Sex did not affect eGFR-EPI J_SLOPE (p = 0.507), and similarly, age at baseline was also not significant (p = 0.201). However, the trend of eGFR-EPI J_SLOPE to decrease with age at baseline may at times be useful to consider given the wide range of ages often encountered in CKD (Figure 11). The MLM equation was:

J_SLOPE = -1.07 -10.2 (if a case) -0.03 (age) -0.32 (if female)

Figure 12 demonstrates the average eGFR-EPI decline that would be expected in a 55.5 year old control or a case over a follow-up period of 11 years.

FIGURE 1: Distribution of subjects and observations.

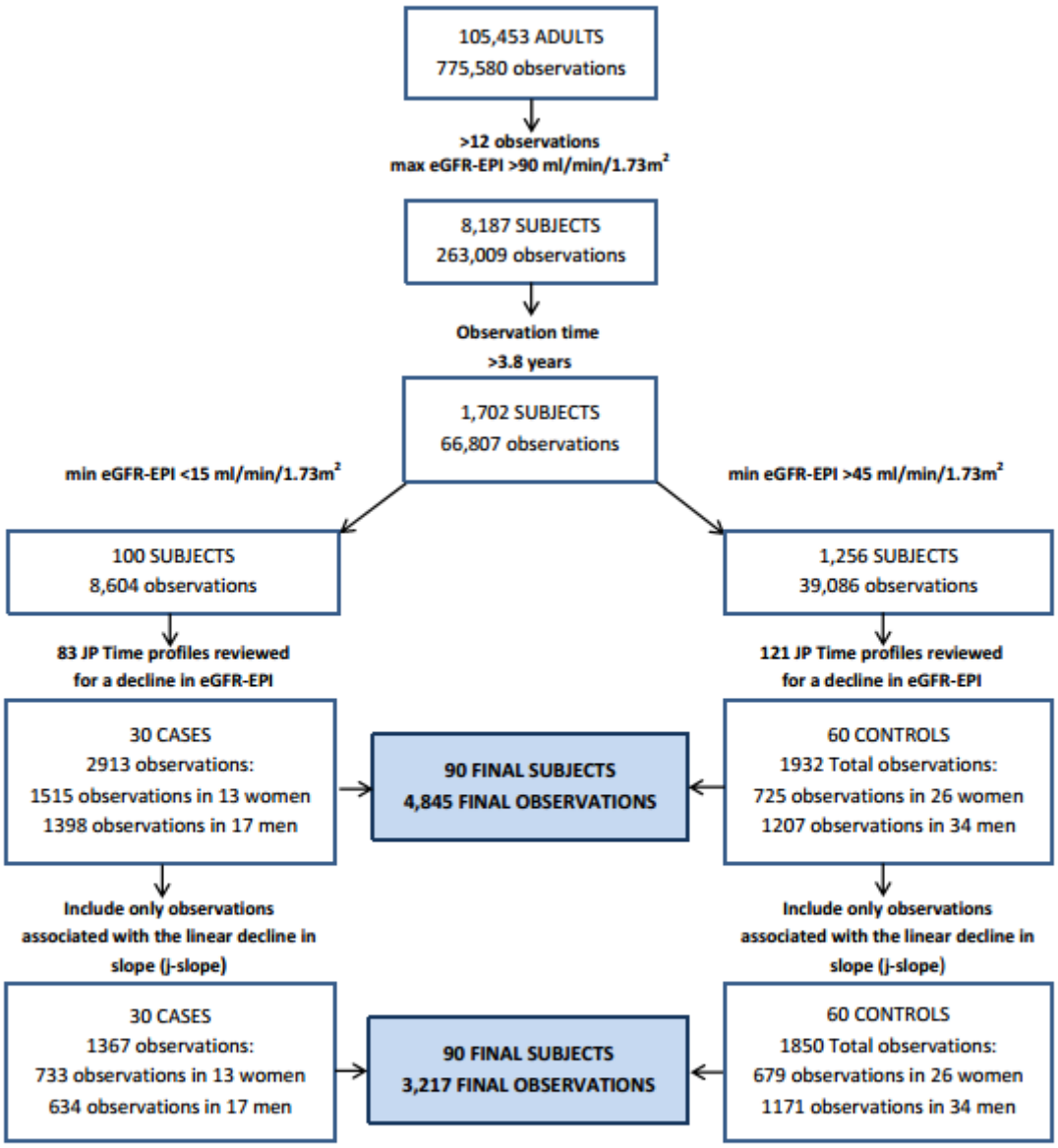
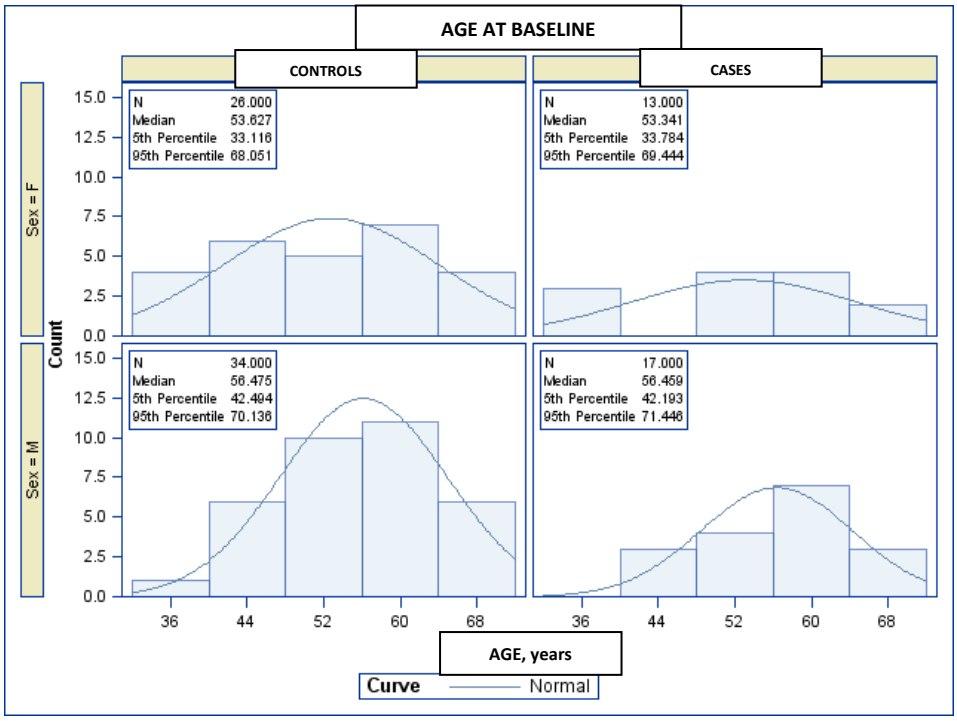


TABLE 1: Descriptive Statistics: eGFR-EPI J_SLOPE subset (n = 3217). (* from sum of squares)

Sex	Case Control	VARIABLE	NOBS	MEAN	STD	%CV	SKEW-NESS	KURT-OSIS	MIN	MAX	P5	MEDIAN	P95
ALL	ALL	age_02	90	54.7	9.8	17.9	-0.36	-0.58	33.0	71.9	36.0	55.7	69.4
ALL	ctrl	age_02	60	54.7	9.9	18.2	-0.30	-0.69	33.0	71.9	37.2	55.4	69.3
ALL	case	age_02	30	54.9	9.8	17.8	-0.52	-0.17	33.8	71.4	34.5	56.4	69.4
F	ctrl	age_02	26	52.8	11.2	21.3	-0.20	-1.01	33.0	70.2	33.1	53.6	68.1
F	case	age_02	13	53.0	11.8	22.3	-0.41	-0.89	33.8	69.4	33.8	53.3	69.4
M	ctrl	age_02	34	56.1	8.7	15.5	-0.14	-0.76	38.7	71.9	42.5	56.5	70.1
M	case	age_02	17	56.4	7.9	14.0	-0.11	-0.18	42.2	71.4	42.2	56.5	71.4
ALL	ALL	nobs2	90	35.7	39.6	110.8	3.28	13.04	4.0	259.0	9.0	21.5	116.0
ALL	ctrl	nobs2	60	31	25.8	83.8	2.88	10.12	7.0	156.0	12.0	22.0	77.5
ALL	case	nobs2	30	46	57.5	126.2	2.45	6.32	4.0	259.0	5.0	20.5	166.0
F	ctrl	nobs2	26	26.1	20.4	78.2	3.67	15.82	11.0	116.0	14.0	19.5	46.0
F	case	nobs2	13	56.4	67.3	119.3	2.58	7.55	9.0	259.0	9.0	35.0	259.0
M	ctrl	nobs2	34	34.4	29.1	84.5	2.56	8.44	7.0	156.0	9.0	23.5	85.0
M	case	nobs2	17	37.3	49.3	132.3	2.28	4.24	4.0	166.0	4.0	19.0	166.0
ALL	ALL	J_slope2	90	-7.0	8.5	-120.8	-2.59	6.86	-40.5	-0.5	-24.6	-3.8	-1.0
ALL	ctrl	J_slope2	60	-3.2	1.8	-56.0	-0.72	-0.35	-7.5	-0.5	-6.7	-3.0	-0.9
ALL	case	J_slope2	30	-14.5	11.2	-76.7	-1.24	0.69	-40.5	-2.2	-40.1	-11.5	-2.8
F	ctrl	J_slope2	26	-3.2	1.8	-55.8	-0.66	-0.61	-7.0	-0.8	-6.4	-2.8	-1.0
F	case	J_slope2	13	-14.8	11.1	-75.0	-1.39	1.41	-40.5	-2.8	-40.5	-12.3	-2.8
M	ctrl	J_slope2	34	-3.2	1.8	-57.0	-0.79	-0.05	-7.5	-0.5	-6.8	-3.0	-0.8
M	case	J_slope2	17	-14.4	11.5	-80.4	-1.27	0.89	-40.1	-2.2	-40.1	-9.8	-2.2
ALL	ALL	min_eEPI2	90	62.7	24.5	39.1	-0.36	-0.01	5.0	114.8	9.7	65.9	102.0
ALL	ctrl	min_eEPI2	60	73.1	16.3	22.3	0.60	-0.13	46.5	114.8	50.9	71.8	106.0
ALL	case	min_eEPI2	30	41.8	25.1	60.0	0.57	0.05	5.0	102.0	8.9	43.4	94.2
F	ctrl	min_eEPI2	26	73.9	19.9	26.9	0.62	-0.57	48.8	114.8	49.8	71.5	109.2
F	case	min_eEPI2	13	40.5	29.5	72.7	0.74	-0.16	8.9	102.0	8.9	35.9	102.0
M	ctrl	min_eEPI2	34	72.6	13.3	18.3	0.34	-0.40	46.5	98.5	52.0	71.8	97.0
M	case	min_eEPI2	17	42.8	22.0	51.6	0.45	0.77	5.0	94.2	5.0	44.1	94.2
ALL	ALL	max_eEPI2	90	109.2	14.1	12.9	0.84	0.38	87.0	149.9	90.8	107.5	138.2
ALL	ctrl	max_eEPI2	60	109.9	12.8	11.6	0.88	0.35	90.8	145.1	93.1	107.5	135.4
ALL	case	max_eEPI2	30	107.8	16.6	15.4	0.92	0.42	87.0	149.9	90.2	107.4	144.1
F	ctrl	max_eEPI2	26	110.7	12.7	11.5	0.84	0.20	91.0	141.1	96.3	108.0	134.4
F	case	max_eEPI2	13	109.8	15.9	14.5	0.55	0.29	90.2	144.1	90.2	109.3	144.1
M	ctrl	max_eEPI2	34	109.2	13.0	11.9	0.96	0.72	90.8	145.1	93.0	107.3	136.5
M	case	max_eEPI2	17	106.3	17.5	16.4	1.28	1.30	87.0	149.9	87.0	101.8	149.9
ALL	ALL	CV_i_eEPI2	90	18.0*			1.53	2.18	3.0	52.3	4.7	12.3	35.7
ALL	ctrl	CV_i_eEPI2	60	10.6*			0.39	-0.35	3.0	19.4	4.4	9.5	17.2
ALL	case	CV_i_eEPI2	30	27.3*			0.38	0.01	4.7	52.3	5.8	23.9	44.6
F	ctrl	CV_i_eEPI2	26	11.6*			0.31	-1.03	4.5	19.4	5.3	10.1	18.0
F	case	CV_i_eEPI2	13	30.6*			-0.04	-1.01	5.8	52.3	5.8	32.8	52.3
M	ctrl	CV_i_eEPI2	34	9.8*			0.10	-0.03	3.0	17.3	3.5	9.3	13.4
M	case	CV_i_eEPI2	17	24.4*			0.49	2.66	4.7	44.6	4.7	22.4	44.6
ALL	ALL	max_time_yr2	90	3.9	1.1	29.1	-1.31	0.54	0.8	5.0	1.3	4.3	4.9
ALL	ctrl	max_time_yr2	60	4.4	0.5	11.7	-2.44	10.79	1.7	5.0	3.7	4.5	4.9
ALL	case	max_time_yr2	30	2.9	1.3	46.6	0.18	-1.38	0.8	5.0	1.1	2.5	4.9
F	ctrl	max_time_yr2	26	4.4	0.4	8.3	-0.61	0.63	3.4	5.0	3.8	4.4	4.9
F	case	max_time_yr2	13	2.7	1.4	52.4	0.65	-1.33	1.2	4.9	1.2	2.2	4.9
M	ctrl	max_time_yr2	34	4.4	0.6	13.9	-2.57	9.88	1.7	4.9	3.7	4.5	4.9
M	case	max_time_yr2	17	3.0	1.3	43.3	-0.16	-1.07	0.8	5.0	0.8	3.0	5.0

FIGURE 2: Descriptive Statistics for a) age at baseline and b) number of observations per subject.



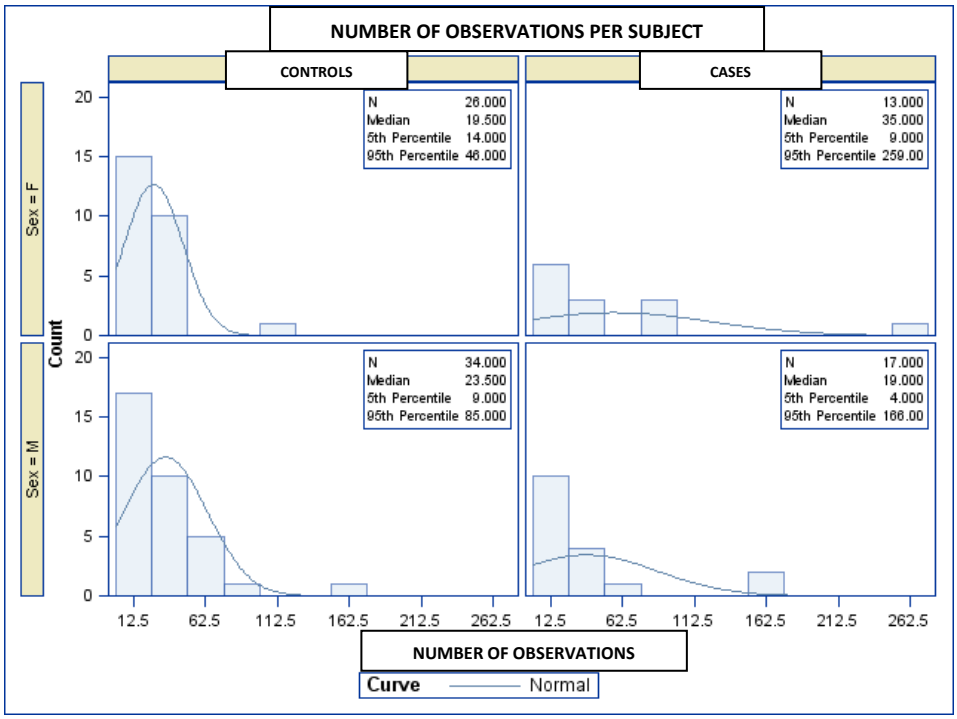
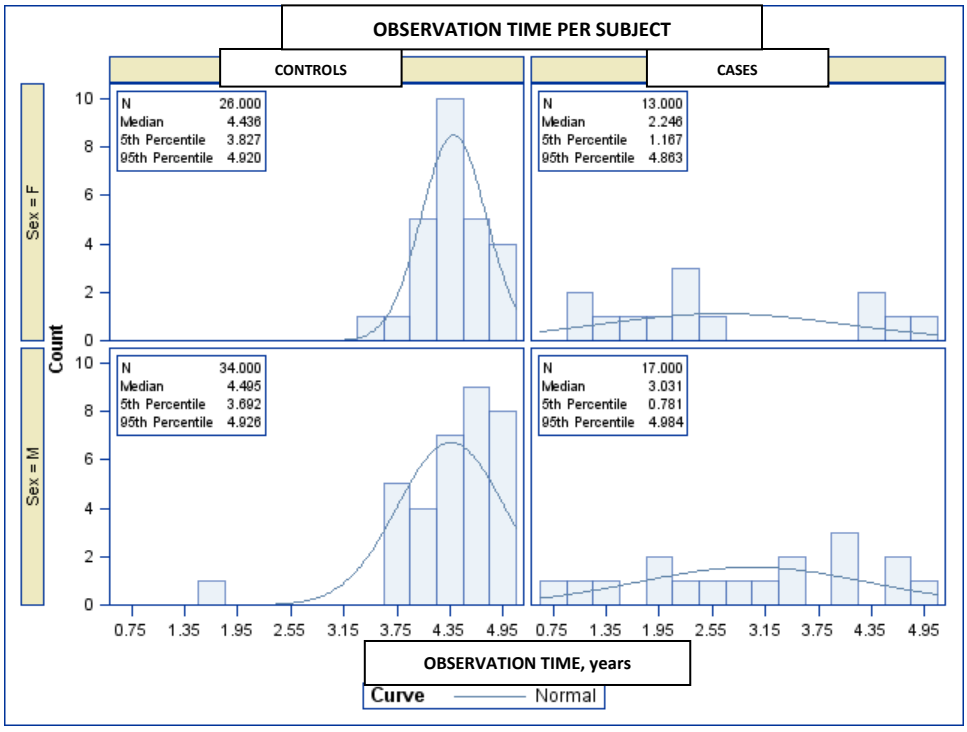


FIGURE 2: Descriptive Statistics for c) observation time per subject, and d) CKD stage for each observation.



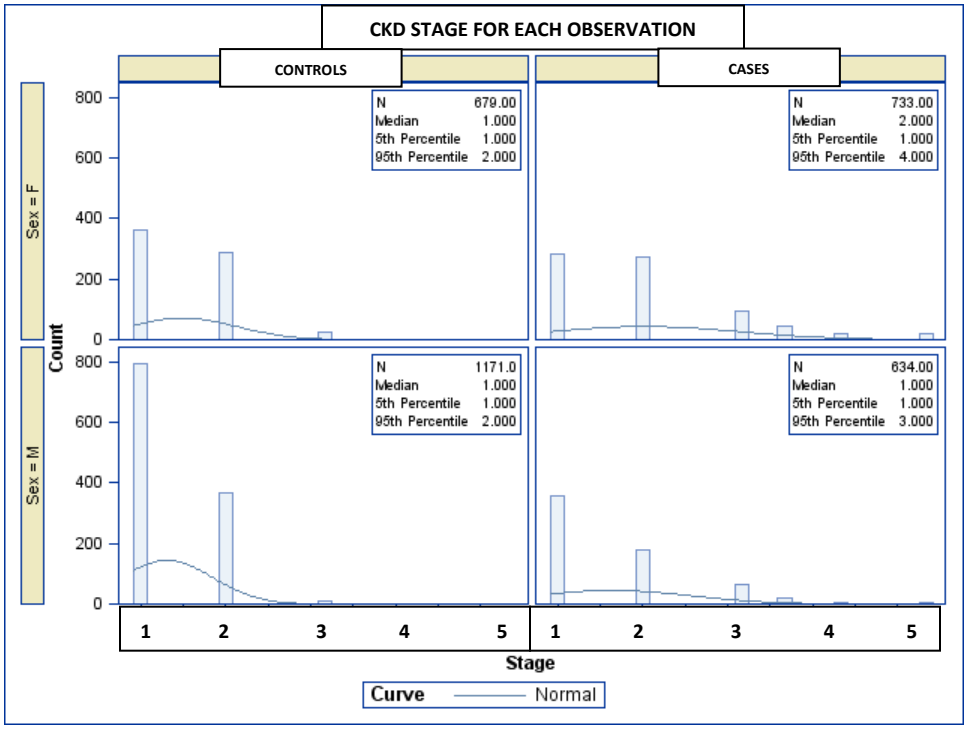
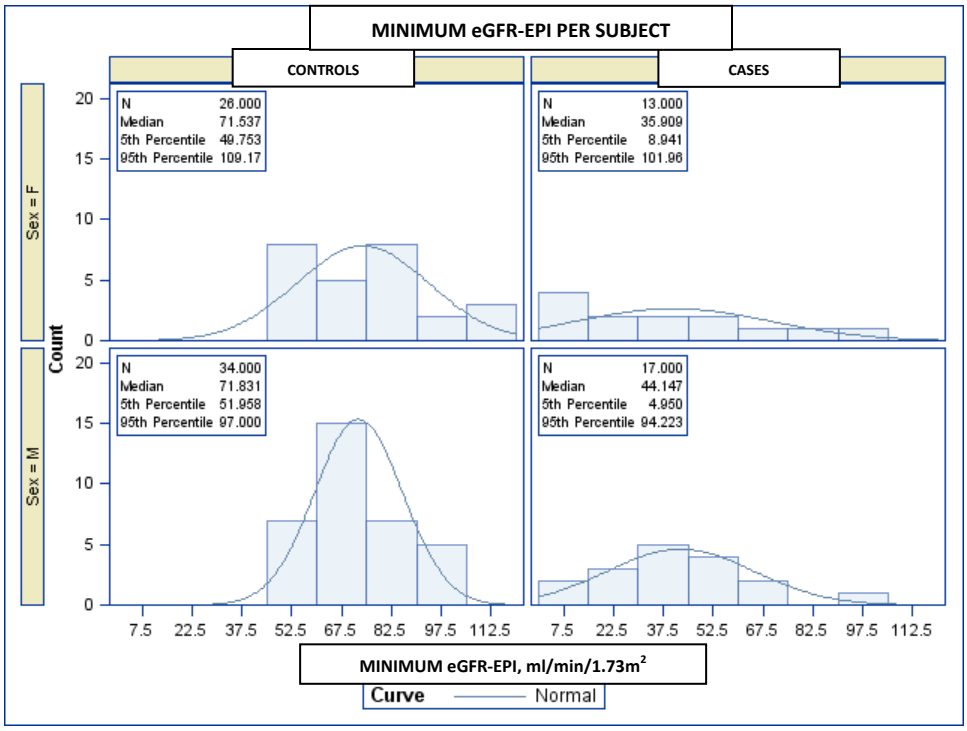


FIGURE 3: Descriptive Statistics for a) minimum and b) maximum eGFR-EPIs per subject.



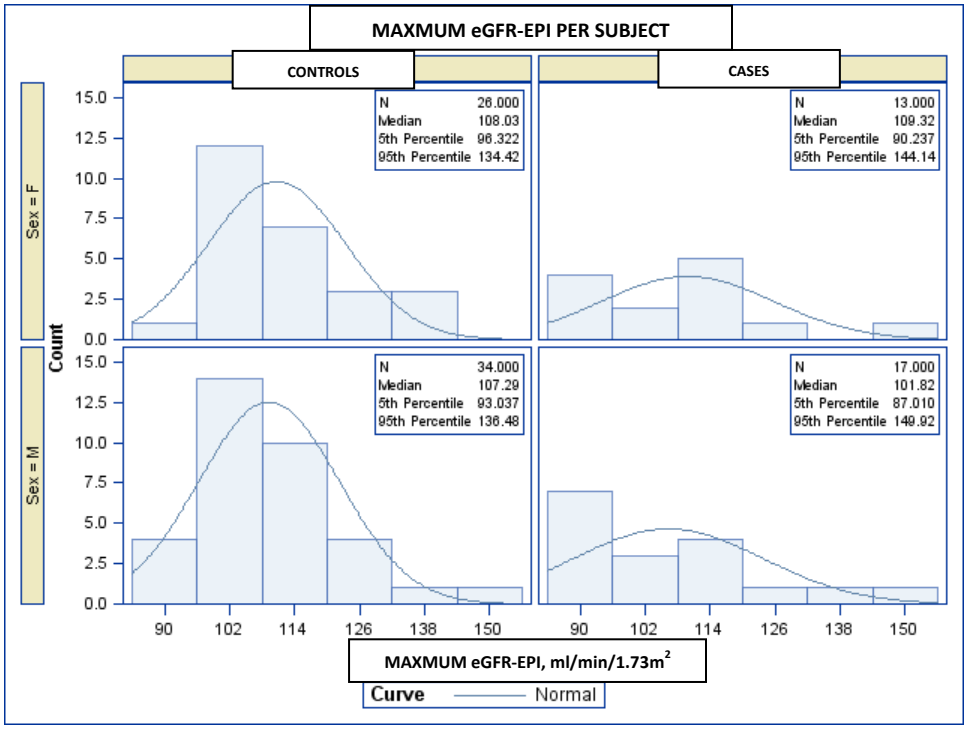
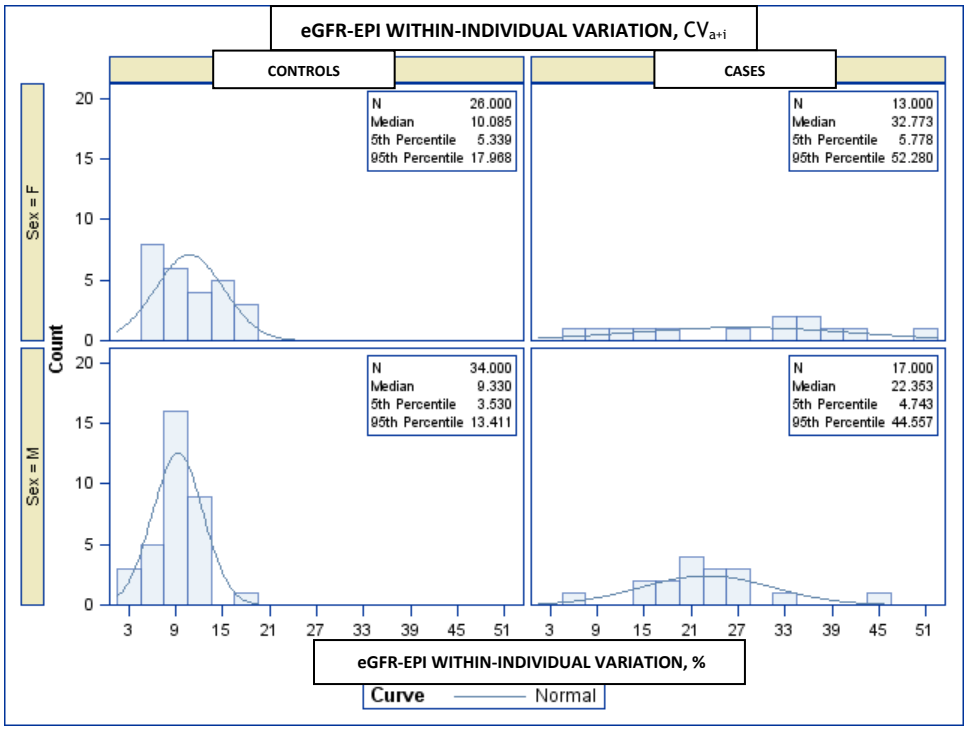


FIGURE 4: Descriptive Statistics for a) eGFR-EPI within-individual variation, % (CV_{a+i}), and b) reference change value, % (RCV).



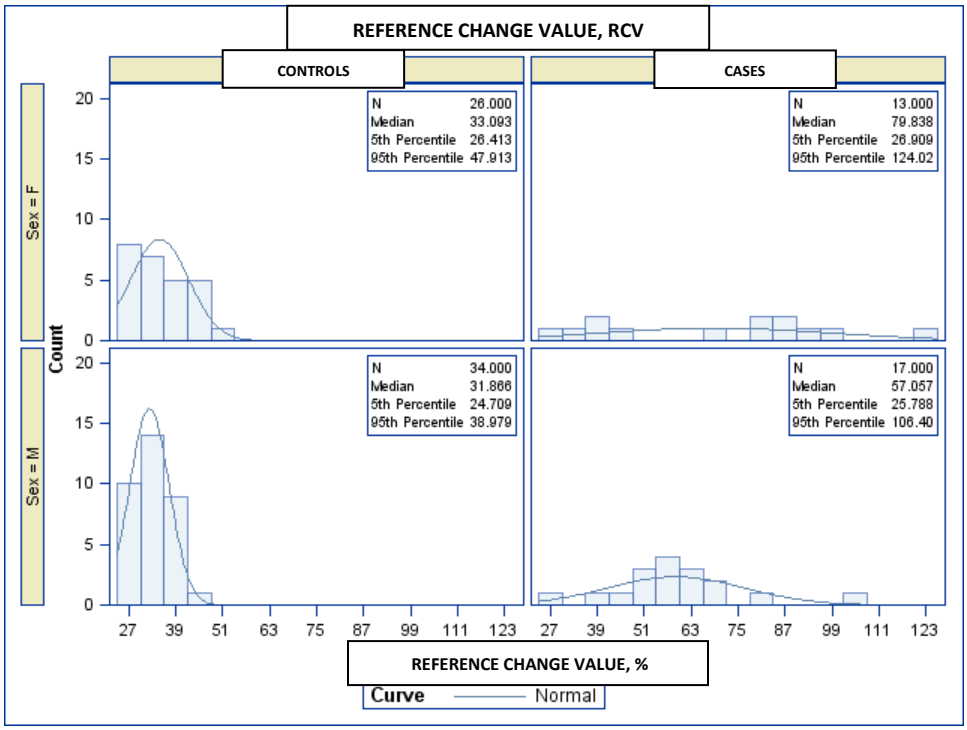


FIGURE 4: Descriptive Statistics for c) years to detection using RCV (absolute change from baseline eGFR-EPI / J-SLOPE).

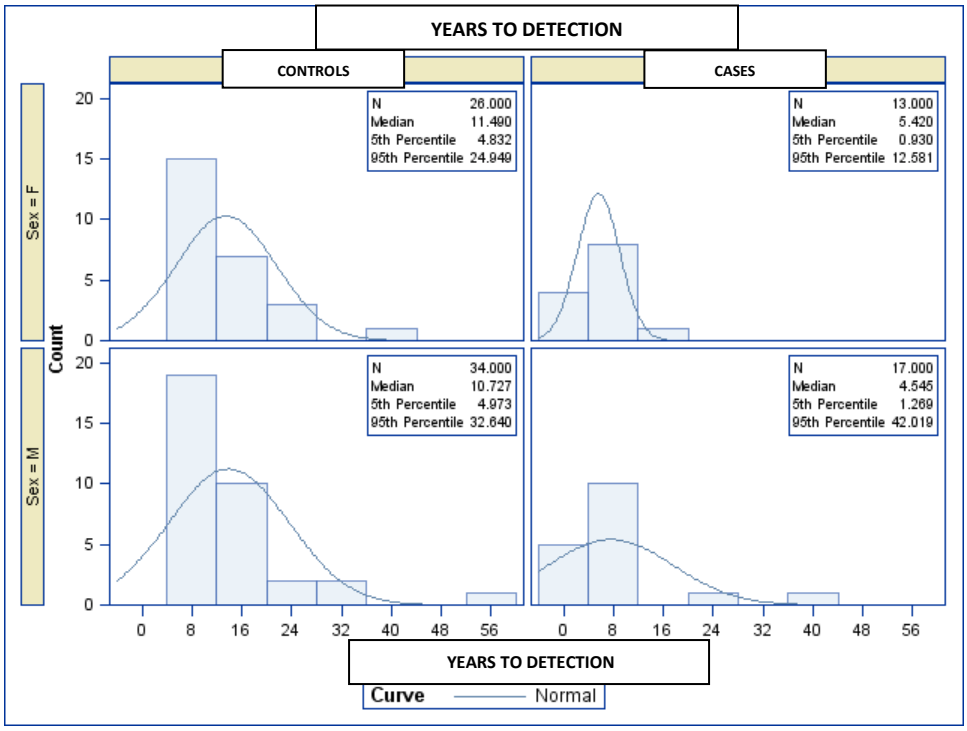
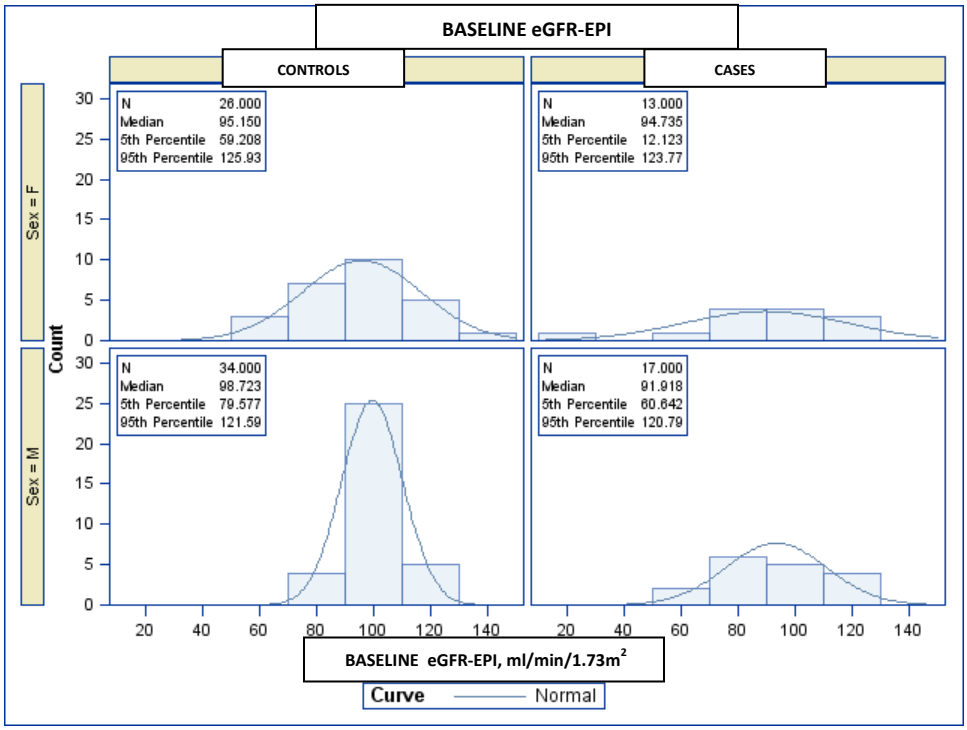


FIGURE 5: Descriptive Statistics for a) baseline eGFR-EPI and b) J_SLOPE.



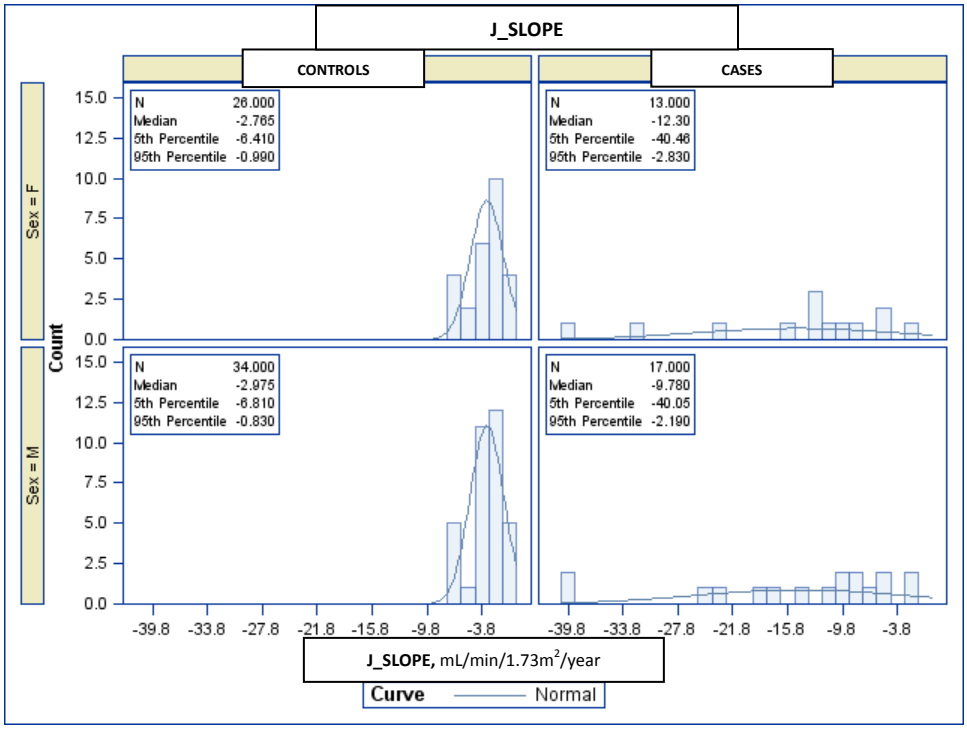
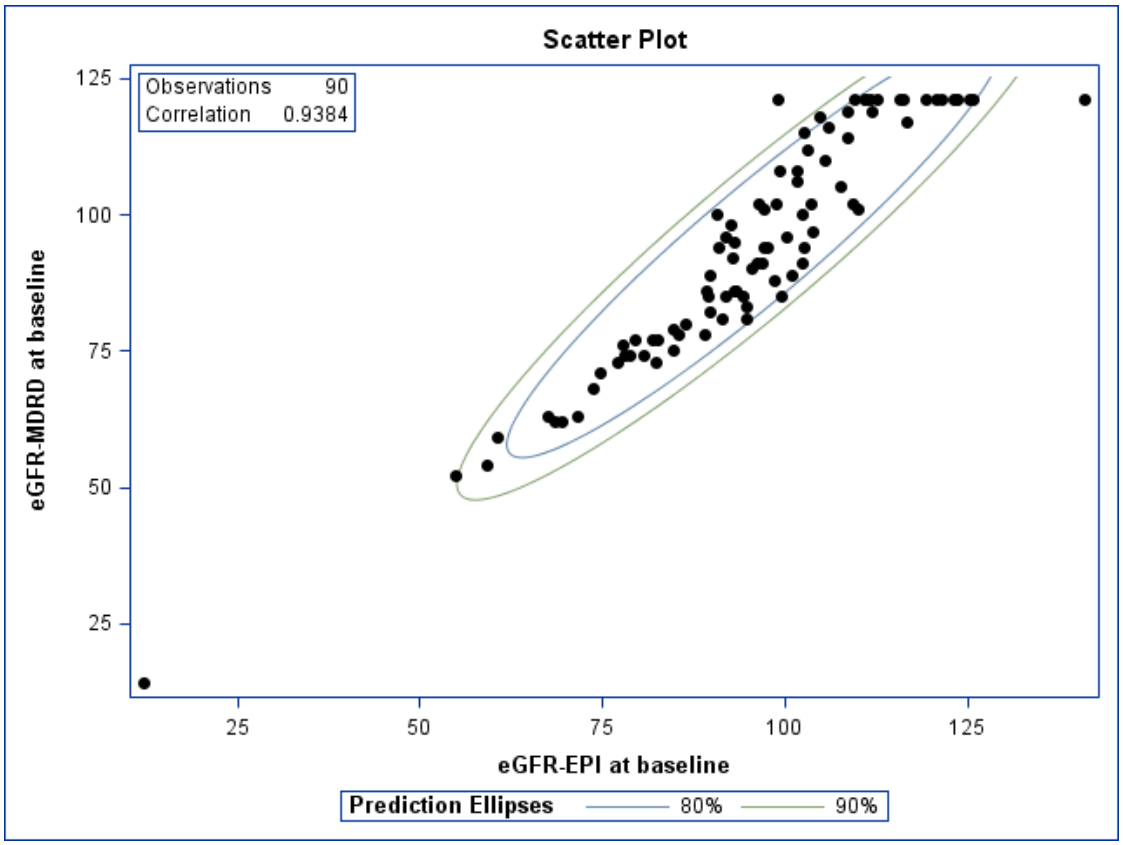


FIGURE 6: Correlation between eGFR-EPI and eGFR-MDRD at baseline

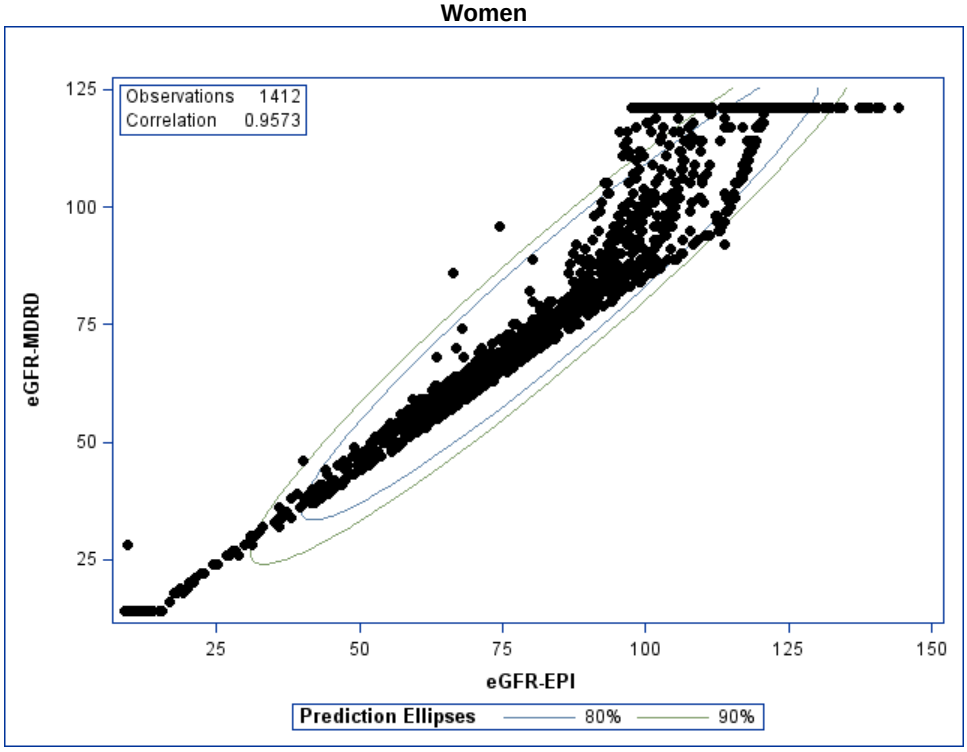
(J_SLOPE subset, n = 90).



Spearman Correlation Coefficient: 0.941 (95%CI: 0.911 – 0.961) $p < 0.0001$

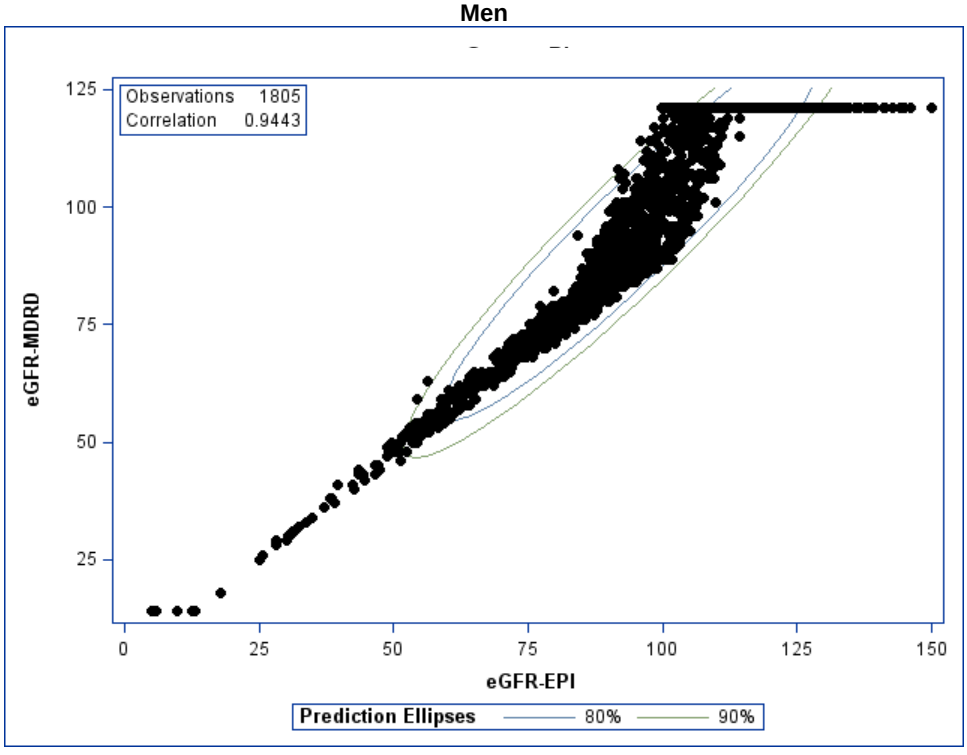
FIGURE 7: Correlation between eGFR-EPI and eGFR-MDRD for a) women and b) men.

Women



Spearman Correlation Coefficient: 0.971 (95%CI: 0.968 – 0.974) p < 0.0001

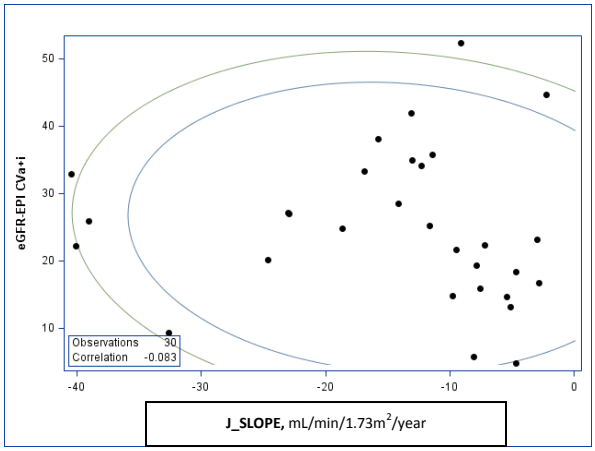
Men



Spearman Correlation Coefficient: 0.959 (95%CI: 0.955 – 0.963) p < 0.0001

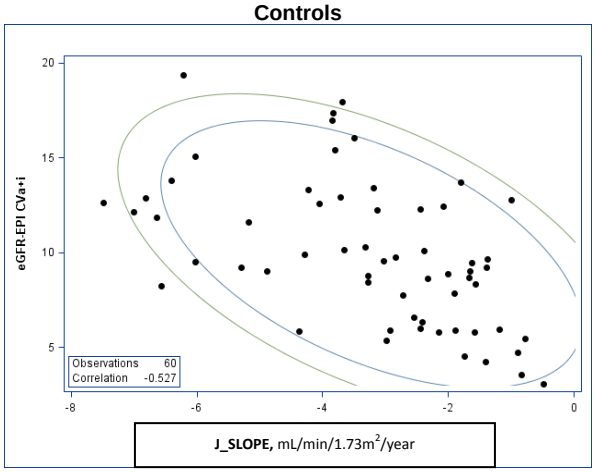
FIGURE 8: Correlation between J_Slope and eGFR-EPI within-individual variation, % (CV_{a+i}) for a) cases b) controls, and c) all subjects. (*80% and 90% prediction ellipses*).

Cases



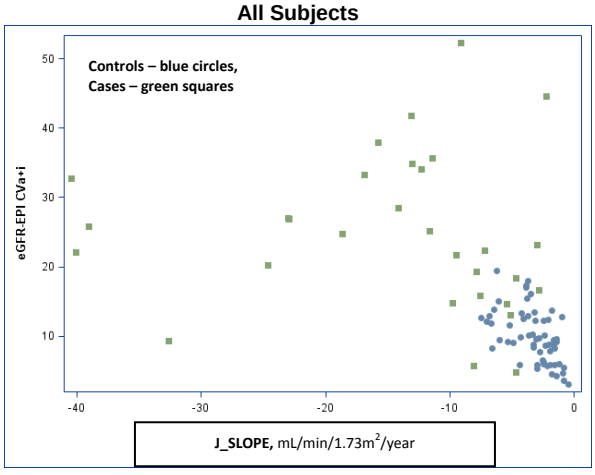
Spearman Correlation Coefficient: -0.296 (95%CI: -0.590 to 0.077) p = 0.113

Controls



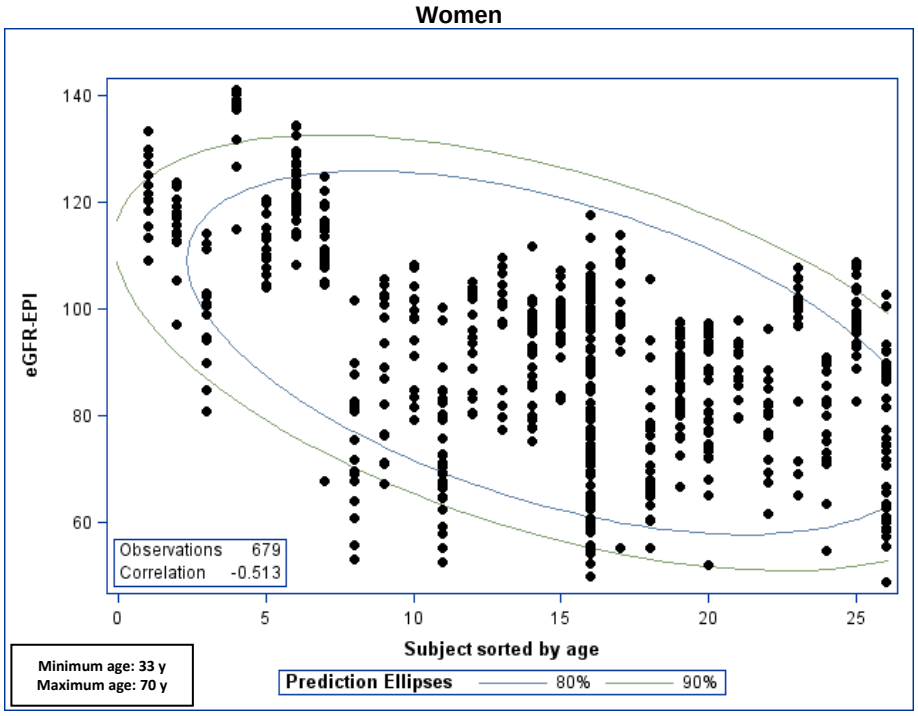
Spearman Correlation Coefficient: -0.571 (95%CI: -0.718 to -0.367) p < 0.0001

All Subjects

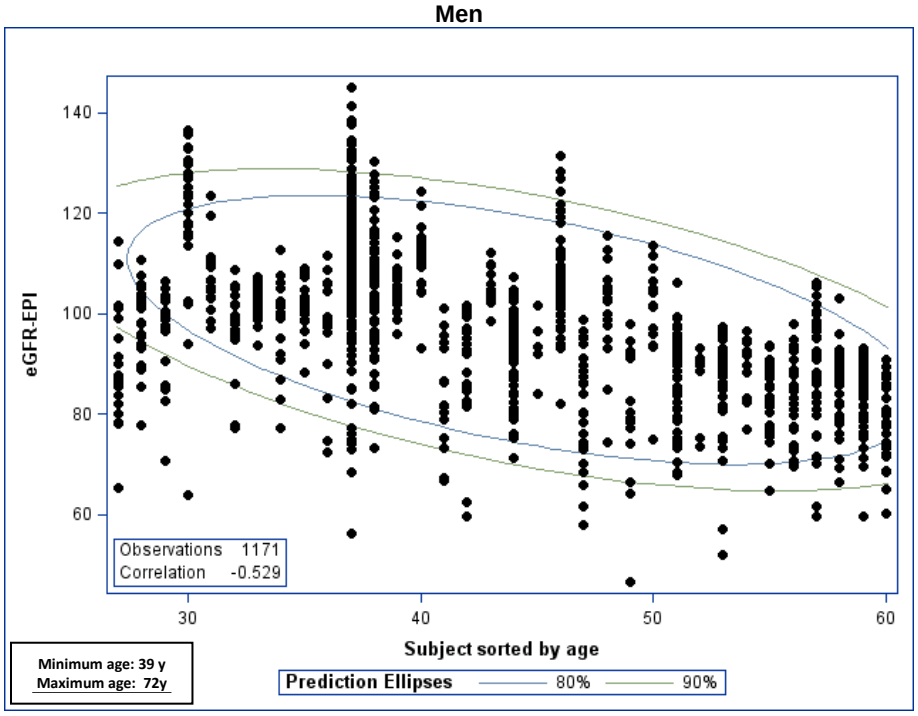


Spearman Correlation Coefficient: -0.689 (95%CI: -0.783 to -0.560) p < 0.0001

FIGURE 9: eGFR-EPI result variability in **controls** for a) 26 women and b) 34 men.

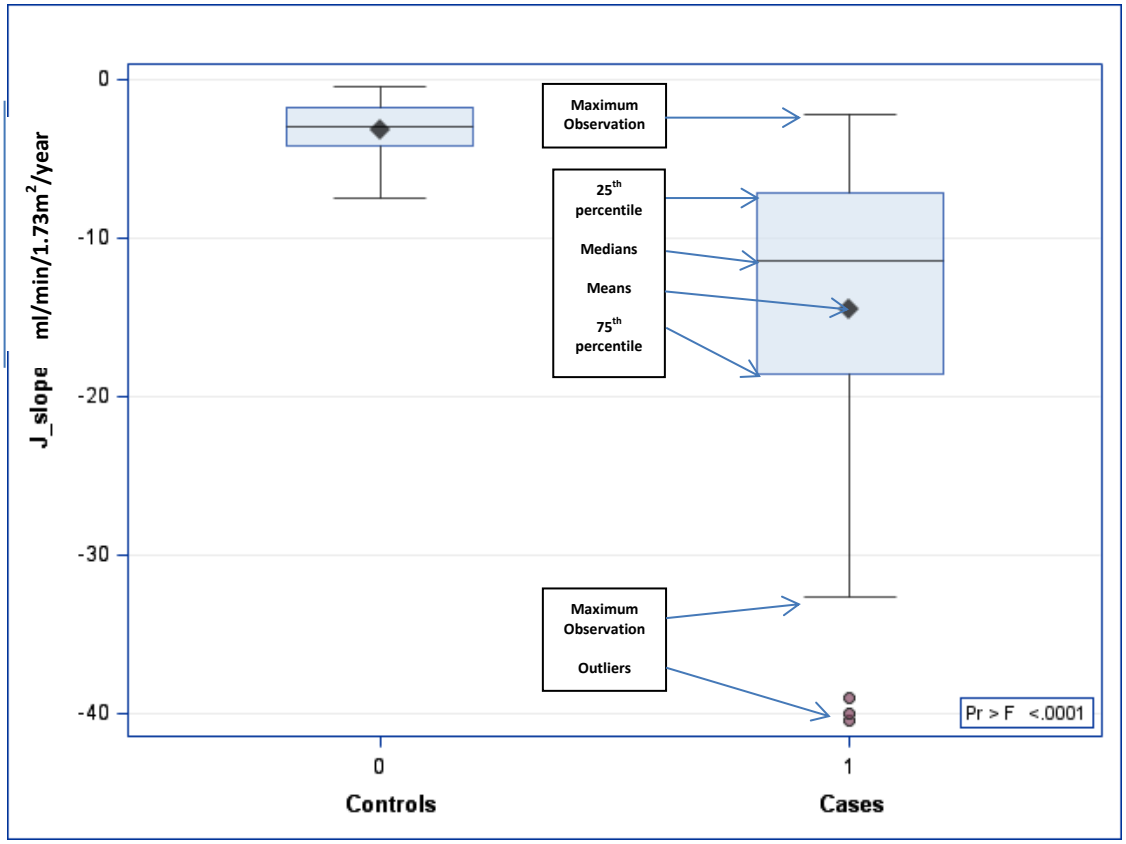


Spearman Correlation Coefficient: -0.473 (95%CI: -0.529 to -0.412) p < 0.0001
(NOTE: First 7 women on the left are less than 45 years old)



Spearman Correlation Coefficient: -0.573 (95%CI: -0.610 to -0.533) p < 0.0001
(NOTE: First 5 men on the left are less than 45 years old)

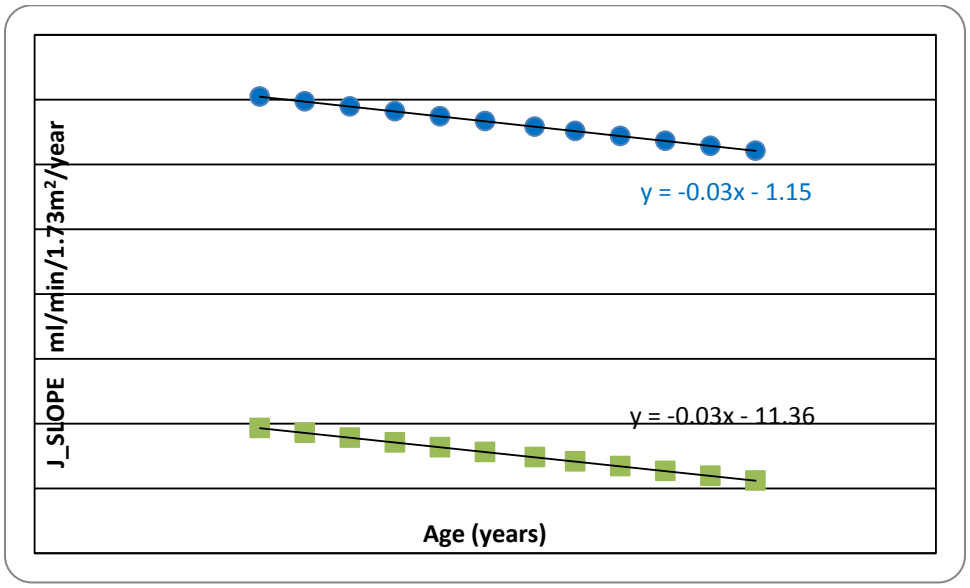
FIGURE 10: Comparison of controls and cases for J_SLOPE (n = 90).



J_SLOPE Mean for controls = -3.2 and for cases = -14.5 (mL/min/1.73m^2/year). F Value: 59.22, $p < 0.0001$

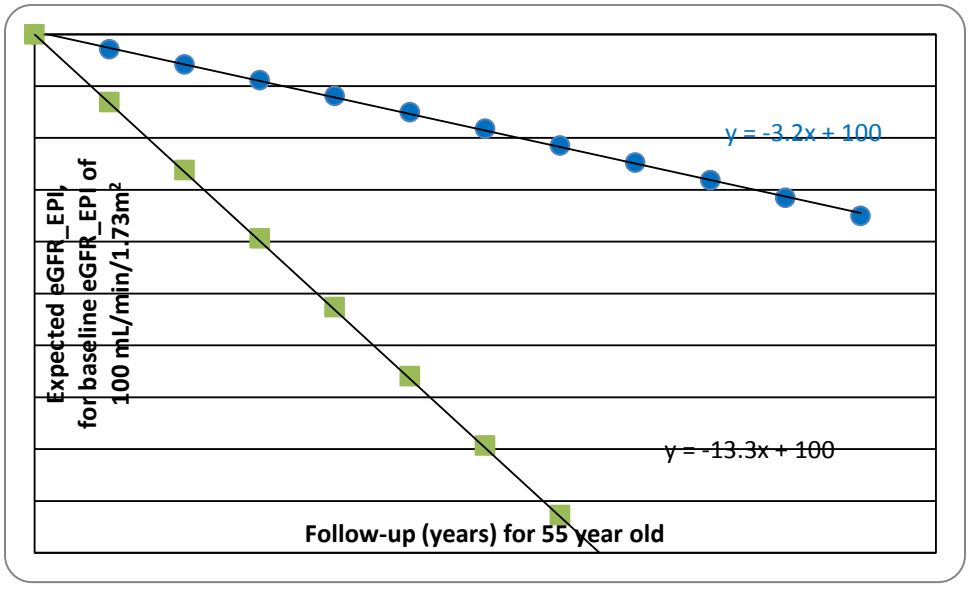
Wilcoxon Two-Sample Test Statistic: 610.5 Normal Approximation Z: -6.454, $p < 0.0001$

FIGURE 11: J_SLOPE demonstrated an insignificant trend to change with age (p = 0.201).



J_SLOPE = - 10.18 (if case) - 0.03 (age) – 1.23
(controls - blue circles; cases - green squares)

FIGURE 12: Average expected decline in eGFR-EPI over time for a 55 year old subject.



J_SLOPE = - 10.18 (if case) - 0.03 (age) – 1.23 mL/min/1.73m^2/year
(controls - blue circles; cases - green squares)

Intercept (-1.07, p=0.421) and sex (-0.317 if female, p=0.507) are not significant.
A mean factor was used for sex (-0.16 = -0.317 / 2).
Case/control status was significant (p<0.0001).

TABLE 2: Biological Variation.

	CONTROLS			*Literature*	CASES			TOTAL
	Female	Male	**All**	All	Female	Male	**All**	
Total CV (%)	20.7	15.4	**17.6**	16.6†	36.2	29.1	**33.4**	25.3
CV_{a+i} (%)	11.6	9.8	**10.6**	7.8†	30.6	24.4	**27.3**	18.0
CV_g (%)	17.1	11.9	**14.0**	***14.7***	19.3	15.9	**19.3**	17.9
II	0.67	0.82	**0.75**	**0.53†**				
CV_i (%)	10.4	8.4	**9.3**	***6.0***	30.2	23.9	**26.8**	17.2
CV_i / CV_{a+i}	*0.90*	*0.86*	***0.88***	***0.76†***	*0.99*	*0.98*	***0.98***	*0.96*
RCV (%)	27	23	**25**	***18†***	71	57	64	42

Total CV = 100 *x* SD / mean	Raw eGFR-EPI results across groups (*e.g.* female, male, all).
CV_{a+i}	Raw eGFR-EPI results across subjects. Mean from sum of squares.
$CV_g = \sqrt{(\text{Total } CV^2 - CV_{a+i}^2)}$	Using the Total CV and CV_{a+i} in this table.
$II = CV_{a+i} / CV_g$	Using the CV_{a+i} and CV_g in this table.
$CV_i = \sqrt{(CV_{a+i}^2 - CV_a^2)}$	Using the CV_{a+i} in this table, and assuming* CV_a = 5%.
RCV = 2.77 *x* CV_{a+i}	One-sided, $p \leq 0.05$.
Literature[12]	Based on 28 papers; † Estimated from literature CV_i and CV_g
TOTAL	Across controls and cases.

CHAPTER 5: DISCUSSION

Current CKD guidelines3 are based on a diagnostic approach using a universal threshold of eGFR < 60 mL/min/1.73m^2. This upper cut-off for Stage 3 CKD represents a 50% reduction in kidney function from that of a healthy 25 year old, a level expected in most 85 year olds following natural aging of the kidneys. Unfortunately, this approach may not be equally sensitive for men and women, for different ages, or even for different individuals. It certainly does not provide the time actually available at more favourable periods for establishing supportive or effective preventive measures. The main objective of this study was to determine if change in eGFR data in the early CKD stages (1, 2, 3) can provide a more discriminating approach for subjects who presumptively progressed to ESRD (*i.e.* pCKD) compared to CKD subjects who were currently not anticipated to progress. We observed significant differences in the early slopes of cases versus controls, and we also observed that cases were more likely to have more than one slope or phase. It was apparent that while biological variation, CV_{a+i}, may be useful for screening and potentially for ruling out pCKD, RCV was not a particularly sensitive approach for the detection of early progressive disease.

Review of each subject's time profile demonstrated that while eGFR *declined in a linear fashion* in some subjects, there was a significant degree of *heterogeneity* in most subjects. Furthermore, as observed in our study, Perkins *et al.* also reported AKI to be a common occurrence in their three study groups with declining, stable and increasing eGFR, and they speculated on the influence of AKI data on the overall slope calculations[30]. Progressive CKD may be more accurately described as a step-wise or event-to-event process overlying progressive deterioration rather than as a simple rate of decline. And, it may be even more complicated if there are several underlying processes progressing at different rates and affected to different degrees by different events. Thus, analysis assuming linear decline should be undertaken cautiously in people with CKD.

Specific eGFR-EPI J_SLOPEs in the early stages of CKD (*i.e.* for eGFR-EPIs above 45 mL/min/1.73m^2) were different from the overall slopes using all 5 year data in 67% of cases and 7% of controls, emphasizing the importance of visual review time profiles in select subjects. MLM demonstrated that cases had a mean slope of -13.0 mL/min/1.73m^2/year, while controls had a mean slope of -2.9 mL/min/1.73m^2/year, for a significant average difference of -10.2 mL/min/1.73m^2/year.

Previous research has shown "average" rates of change of -1 to -5 mL/min/1.73m^2/year for whole study populations[17-25,30-32], which usually include 20% to 30% of subjects who have an increasing rate[32], and a similar proportion of subjects who are stable[22,30,32]. These average rates are usually associated with relatively wide ranges of -17 to +13[32] mL/min/1.73m^2/year, IQRs spanning -8.2 to 6.7 mL/min/1.73m^2/year[30] or -4.91 to -0.07 mL/min/1.73m^2/year (range -92.12, 89.45)[23], or 95%CIs approximately equal to the mean itself[18]. Although, the significant rates of decline observed in the controls in our study may be related to the inclusion criteria of declining slope, to the relatively short time frame of the study (5 years), and/or to the hospital-based subject population, Turin *et al.* found similar results in 529,312 community-dwelling subjects who had a median of 3 CREA results over a 4 year period[32]. During their 2.5 year follow-up period they demonstrated a graded increase in risk of death for both declining and increasing eGFR-EPIs with changes greater than 5 mL/min/1.73m^2/year or 7% change/year[30,32]. And, similar to Rifkin *et al*. in 2008, they noted that a change in eGFR-EPI independent of baseline eGFR results (*e.g.* even within Stage 2) was associated with an increased risk of death, "suggesting that even with preserved kidney function the rate of change has prognostic information for future mortality risk" [32,33].

The variation in the individual eGFR-EPI homeostatic set points and in the spread of results for each control subject is clearly evident in Figure 9. The median biological variation eGFR-EPI-CV_{a+i} for controls of 9.5% was less than half that observed in cases of 24%, with all controls having an eGFR-EPI-CV_{a+i} less than 18%. Figure 8 demonstrates that eGFR-EPI-CV_{a+i}'s < 20% were associated with eGFR-EPI

J_SLOPES of -10 to 0 mL/min/1.73m^2/year, while eGFR-EPI-CV_{a+i}'s > 20% had relatively unpredictable eGFR-EPI J_SLOPES of -40.5 to 0 mL/min/1.73m^2/year. This potentially biphasic relationship might provide the rationale for using eGFR-EPI-CV_{a+i} as an initial screen of cumulative results. For example, if the variation across results is minimal, then it should be unlikely that the rate of change is significant (Figure 13).

The mean eGFR-EPI-CV_{a+i} of 10.6% for controls observed in this study would result in a mean CV_i of 9.3% if the CV_a is 5%. A CV_i of 9.3% is fifty percent higher than the CREA CV_i of 6% reported in the biological variation database[14]. While this may be associated with the hospital data source of our results, or possibly due to a wider age range than usually tested in healthy biological variation studies, the possibility that it is associated with the CKD process in our controls cannot be ruled out. It is currently believed that homeostatic set points may change with disease, but that the biological variation around these set points is usually the same in health or disease[13,14]. If confirmed, increased biological variation in cases compared to controls will be an important and useful finding.

RCVs, "reference change values", also known as significant changes, are useful in result interpretation to address the question, "Are two serial results statistically different?"; or stated another way, "Is the difference more than could be accounted for by pre-analytical, analytical and biological variation"?[16] In this study, controls demonstrated a median baseline eGFR-EPI of 97 mL/min/1.73m^2. Given an average eGFR-EPI-CV_{a+i} of 10.6% and a CV_a of 5%, the average RCV for a significant decrease in results would be 24.6% (*i.e.* 24 mL/min/1.73m^2; one-sided Z=1.65 and p≤0.05). At their median rate of change of -2.9 mL/min/1.73m^2/year, it would take 8 years to decrease by 24 mL/min/1.73m^2! Obviously, this change would be picked up earlier by mere visual review of their time profiles. The sensitivity of an RCV can be improved by reducing either the analytical imprecision (CV_a) or by using a subject's own CV_i if it happens to be less than the average CV_i. If the CV_a and the CV_i were both 6%, for a

CV_{a+i} of 8.5%, the RCV would be reduced to 20% (or 19.4 mL/min/1.73m^2). Unfortunately, this decline would still take almost 7 years to identify.

In an initial report on their first year accrual data of 2 CREA results from each subject, Turin *et al.* defined a "certain drop" as a drop in CKD category with ≥ 25% decrease in eGFR-EPI[34], which is similar to the RCV in our study. As the association with ESRD risk did not hold up after adjustment for the eGFR or covariates at the subjects' last visit, they suggested that "eGFR trajectories based on more than two CREA measurments over a period longer than 1 year are required to determine ESRD risk and allow more reliable risk prediction." An RCV interpretation of their data would suggest that 7% of serial results demonstrated a significant change (3.3% declined and 3.7% increased) and would thus require confirmatory testing. Levey and Coresh concluded in their 2012 Lancet review, that "serial measurements…can be used to monitor disease progression and guide therapy. However, variability can occur over time because of fluctuations in disease activity and treatment; therefore, a long period of observation might be needed to assess the rate of progression."[31] While reports have considered the potential of confounding due to "regression to the mean"[30], Lely *et al.* emphasized that "when using slope analysis, it is important to calculate slopes with as many measurements as possible"[18]. Consideration of the individualistic nature of the event-to-event process underlying pCKD, along with the advantages of early detection of pCKD (as opposed to early detection of progression to ESRD) may be useful in determining the frequency of repetitive testing in high risk subjects.

eGFR result interpretation needs to evolve from a point-to-point interpretation with mental estimation of a potential significant difference or slope to a dynamic approach that has a better ability for early detection of a significant change in slope, and that is interpreted in the context of a patient's past and current medical history. When a series of three or more points are analyzed by regression, statistical power is enhanced by taking advantage of the cumulative, progressive and inter-related nature of the data such that a significant change may be detected considerably earlier. By calculating

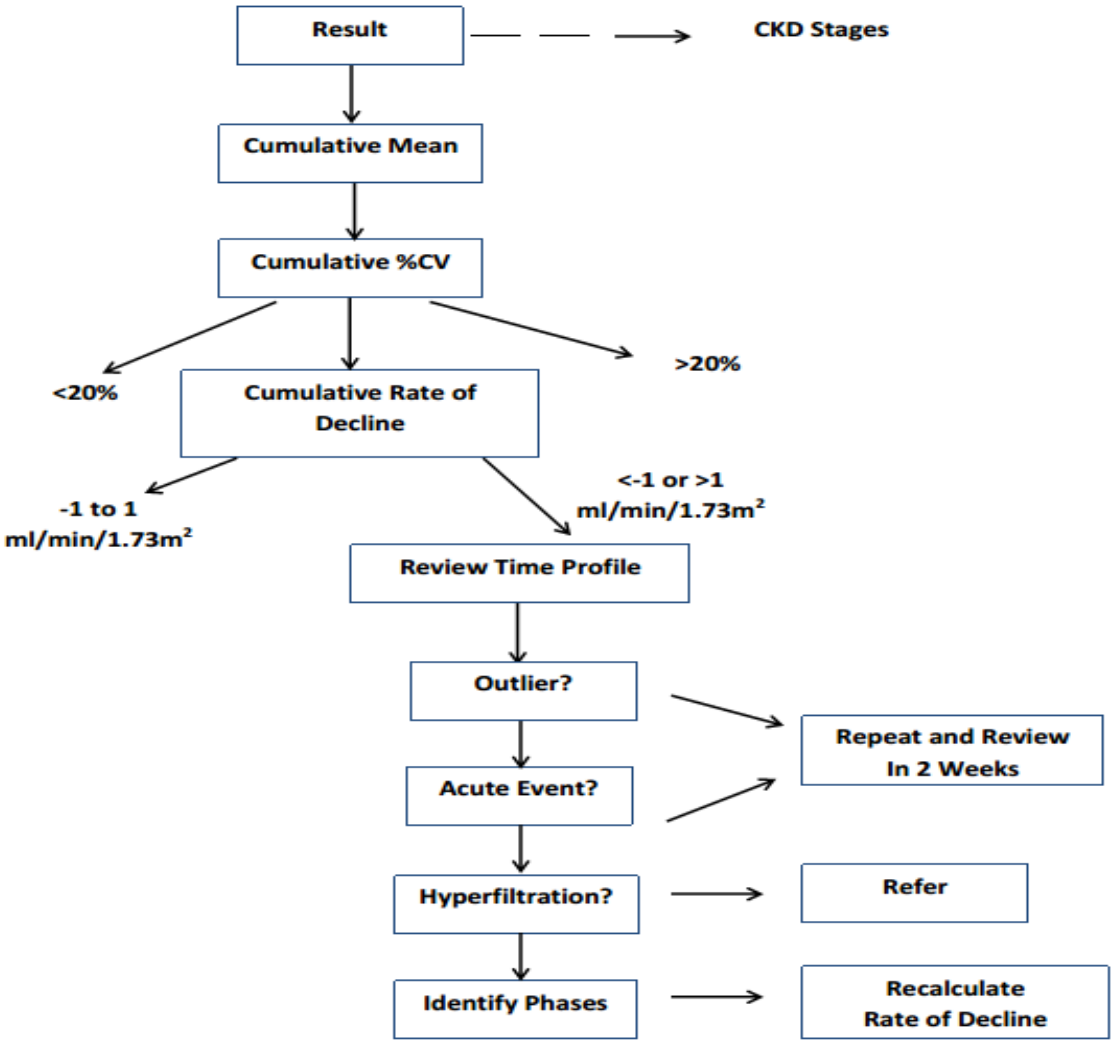
slope, patients are in essence being used as their own baseline for interpretation of new data[17]. An efficient approach would be to review, as needed, statistical regression of the data with consideration of the p value (Figure 13). Judicious visual selection of the time points to be included would be a more powerful and appropriate analysis for serial eGFR interpretation. CV_i's could then be used as an indicator to flag subjects with increased variation (*i.e.* > 20%), whose time profiles need to be visually reviewed as a final interpretation and, RCVs may be more appropriately used in the detection of acute disease such as AKI, as recently suggested by Gardner *et al.*[27]

Several studies have suggested that a patient's initial rate of decline could be used as their own control[17] or as a stratification variable[18] in therapeutic trials. In their 1977 report on 63 adults on hemodialysis, Rutherford *et al.* concluded that "functional nephron loss is either exponential (log CREA) or constant" (1/CREA), with 84% of their subjects demonstrating a linear decline by either one or the other model[17]. They pointed out that "the varied rates of progression among patients with the same disease suggest that individual host factors are important", and thus that there is an "inherent difficulty in using any group of patients as controls since patients with the same disease have diverse courses". Their data focused on retrospective CREA results that were significantly elevated as a "small" change in CREA was considered less than 530 µmol/L (6 mg/dL).

Two questions which are not easy to address are: "How to identify outliers?" and "What results to include in the estimation of slope?" Given the potentially modifiable nature of the disease process and the availability of an inexpensive and universally available test such as eGFR, it would be prudent to routinely monitor patients, in a risk related fashion. With statistical regression analysis, the need to identify and possibly omit potential outliers may be mitigated by the advantage of multiple samples over time from a subject. The model in this study employed weighted analysis and, the study exclusion criteria addressed incongruous results at either end of the time profiles. Due to the retrospective nature of the study, it was not possible to identify and investigate potential outliers in a

real time fashion to identify mislabeled specimens (*i.e.* from a different subject) or suboptimal samples (*i.e.* contamination from a central line, interference from a drug or vitamin). Given the infrequent rate of verifying a result as incorrect (*i.e.* < 1 - 5%), and the importance of identifying the actual biological variation routinely encountered, potential outliers were not identified or deleted prior to data analysis in this study.

FIGURE 13. Approach to identifying pCKD and monitoring CKD.



CHAPTER 6: STRENGTHS, LIMITATIONS AND FUTURE INVESTIGATIONS OF THIS STUDY

This study has several important strengths, the first of which is the review of individual time profiles to ascertain "linearity" and the second of which is the use of JOINPOINT to objectively determine the best estimates of rate of early CKD. Use of MLM optimized the data analysis with respect to statistical options (*e.g.* RANDOM intercepts and slopes) and to the information generated (*e.g.* ICCs, group specific probabilities). Controls were age- and sex-matched, and were not healthy or "normal", but had the disease most important to distinguish from the cases (*i.e.* controls had CKD). The inclusion criteria of "declining eGFR" selected the populations that are of specific clinical interest; thus, the results may be more accurate than studies on a more heterogeneous population. Finally, inter-assay variability was minimized by measuring CREA in the same laboratory using the same IDMS standardized method.

The inclusion/exclusion criteria had several potential limitations, including the requirement for 12 or more observations in the 5 year study period which may have selected subjects with more co-morbidities, or who were seeing more specialists, or who were less healthy than those with fewer observations. Other studies have reported on community-dwelling subjects with 2 or more but less than 12 observations[20]. In addition, the requirement for at least 3.8 years of observation data may be a relatively short time period to study a chronically progressive disease.

Selection bias may have been introduced during the process of selecting the subjects by visual inspection of their eGFR time profiles, especially as this step was performed by one investigator only. However, subject enrollment with JP time profile review was completed both as a convenience sample and following a random selection protocol (PROC SURVEYSELECT), and both analyses yielded essentially the same eGFR-EPI J_SLOPE means. However, if visual inspection of time profiles is implemented clinically, it will most likely be performed by a single person. It should be noted that although JOINPOINT analysis is relatively automated, there are some judgment steps with respect to the selection of which data to include and which slopes to choose that may introduce some bias into the process.

Use of a hospital database may limit the generalizability of the results as hospital data is generally associated with specific patient health issues (severely ill patients with acute events, chronic patients, and follow-up patients), and with the referring practices by regional physicians, as well as the ordering practices of clinical specialists. There was a wide range in the number of eGFR results per patient in this study. Proposed selection strategies to minimize the number of data points (*e.g.* the annual median or the first result annually) or to obtain a consistent number of data points across subjects were not employed as they may have resulted in conservative estimates and obscured the true data heterogeneity. It is possible that data associated with *acute events* may need to be identified and eliminated, or at least accounted for, in slope estimations. Omission of AKI observations may be justified in future studies as non-specific in-vitro and in-vivo effects, such as hemodynamic alterations and cross-reacting substances, may be increased during acute illnesses and treatment, and thus may contribute to result variability in these samples.

Finally, some subjects experienced a period of hyperfiltration (eGFR > 120 mL/min/1.73m^2, included in the classification of Stage 1 CKD) prior to renal function deterioration. This was not an exclusion criteria for this study, and subjects with hyperfiltration were not identified in this study, so final results may have been biased by this factor (*e.g.* the baseline eGFR-EPI, or correlations between slope and baseline eGFR-EPI).

POTENTIAL FUTURE INVESTIGATIONS BASED ON THIS STUDY:

The findings of this study will be further tested in the retrospective study which is currently recruiting cases with known ESRD and age- and sex-matched CKD controls. This study with patient consent will allow collection of all patient CREA data as well as information on other risk factors, such as diabetes, hypertension, and obesity (*e.g.* HbA1c, blood pressures, urinary albumin to creatinine ratio, urea, uric acid, weight and height).

Implementation of enhanced interpretation using linear regression for the calculation of rate of change will require a change in monitoring practice. The optimal testing frequency may be dependent on a variety of factors including risk level, co-morbidities, patient treatment preferences and options, age, CREA method performance and local economic justification. In addition, enhancement of routine monitoring may be warranted following AKI. Implementation will require: processes for real time identification and handling of potential outliers, with automatic requests for repeat sampling; a mechanism to review and flag results not to be included in rate of change calculations; a process to deal with results associated with AKI and its recovery phase; and the ability to define different sequential phases, which ultimately could be linked to the results in the LIS and or medical record.

Investigation of the utility of personalized medicine for CKD with the determination and monitoring of individual homeostatic set points and intra-individual variation appears warranted as information based on significant changes will likely be more reliable than comparison with generic population reference ranges.

CHAPTER 7: CONCLUSIONS

This study demonstrated a significant difference in the eGFR rate of decline during CKD Stages 1 to 3 for cases with presumptive ESRD and controls with CKD; suggesting that early rate of decline may be a useful predictor for pCKD. The difference in the slopes was substantial at approximately -10 mL/min/1.73m^2, with cases having a mean slope of -13 mL/min/1.73m^2 (95%CI: -17 to -9), while controls had a mean slope of -2.9 mL/min/1.73m^2 (95%CI: -3.3 to -2.4). Intra-individual biological variation (CV_{a+i}) was also significantly different for cases (24% median) and controls (9.5% median), and may prove to be a useful screening parameter. The RCV in a control subject needed between two serial values to detect a significant decrease was -25%.

CREA, and thus eGFR, is considered to be a test with "marked individuality", and as such would be more reliably interpreted by comparison to a subject's own homeostatic set point. It is time to consider a shift in the interpretation of monitoring (serial) results from comparison with a population reference interval or a medical decision limit, or from basic consideration of RCVs, to the potential of statistical regression analysis in the setting of personalized medicine. Although use of a clear decision limit such as an eGFR < 60 mL/min/1.73m^2 for the consistent diagnosis of CKD was an important step forward, in order to reliably detect pCKD earlier, statistical regression with visual review of the time profile will probably be necessary. pCKD is a treatable disease which has eGFR as an inexpensive and widely available screening test. Individuals at high risk need to be monitored at appropriate time points with a useful frequency in order to take full advantage of the testing that is performed and the potential to significantly modify patient outcomes.

The main strengths of this study were review of subjects' individual time profiles to identify and define the decline in renal function during the early stages of CKD, and use of CKD subjects with declining eGFR as controls. Calculation of slopes using all available data was a reasonable approach for

controls whose intra-individual variation was less than 20%; however, 67% of cases had an eGFR-EPI J_SLOPE which was different than their overall SLOPE.

Considerable heterogeneity in homeostatic set points, intra-individual biological variation, and individual time profiles was observed. pCKD might be better modeled as a step-wise or event-to-event process on top of a potentially cumulative decline. Given this observation, analysis of pCKD presuming linearly declining slopes within individuals or summarized across a study should be approached cautiously. The clinical challenge of interpreting sometimes limited and highly variable data was clearly observed in this study.

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APPENDIX i : Research Ethics Board (REB) Approval

QUEEN'S UNIVERSITY HEALTH SCIENCES AND AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD ANNUAL RENEWAL

Queen's University, in accordance with the "Tri-Council Policy Statement 2, 2010" prepared by the Interagency Advisory Panel on Research Ethics for the Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada and Social Sciences and Humanities Research Council of Canada requires that research projects involving human participants be reviewed annually to determine their acceptability on ethical grounds.

A Research Ethics Board composed of:

Dr. A.F. Clark, Emeritus Professor, Department of Biomedical and Molecular Sciences, Queen's University (Chair)
Dr. H. Abdollah, Professor, Department of Medicine, Queen's University
Dr. C. Cline, Assistant Professor, Department of Medicine, Director, Office of Bioethics, Queen's University, Clinical Ethicist, Kingston General Hospital
Dr. R. Brison, Professor, Department of Emergency Medicine, Queen's University
Dr. M. Evans, Community Member
Ms. J. Hudacin, Community Member
Dr. B. Kisilevsky, Professor, School of Nursing, Departments of Psychology and Obstetrics and Gynaecology, Queens's University
Mr. D. McNaughton, Community Member
Ms. P. Newman, Pharmacist, Clinical Care Specialist and Clinical Lead, Quality and Safety, Pharmacy Services, Kingston General Hospital
Ms. S. Rohland, Privacy Officer, ICES-Queen's Health Services Research Facility, Research Associate, Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute
Dr. A. Singh, Professor, Department of Psychiatry, Queen's University
Dr. J. Walia, Assistant Professor and Clinical Geneticist, Department of Paediatrics, Queen's University and Kingston General Hospital
Ms. K. Weisbaum, LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)

has reviewed the request for renewal of Research Ethics Board approval for the project **Early detection of progressive chronic kidney disease by monitoring change in eGFR in CKD stages 1, 2 & 3** as proposed by **Dr. Christine P. Collier** of the **Department of Pathology and Molecular Medicine**, at **Queen's University** . The approval is renewed for one year, effective **December 11, 2013.** If there are any further amendments or changes to the protocol affecting the participants in this study, it is the responsibility of the principal investigator to notify the Research Ethics Board. Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other adverse events must be reported within 15 days after becoming aware of the information.

Albert F. Clark

_____Date: November 11, 2013
Chair, Health Sciences Research Ethics Board
Renewal 1[X] Renewal 2 [ ] Extension [ ] **Code# PATH-130-12 Romeo file# 6007598**

APPENDIX ii: JOINPOINT (JP) Statistical Software

JOINPOINT Regression Program, Version 4.0.4 - May 2013;
Statistical Methodology and Applications Branch, Surveillance Research Program,
National Cancer Institute. 9609 Medical Center Drive, Bethesda, MD 20892
http://surveillance.cancer.gov/joinpoint/

A "joinpoint" (JP) is the inflection point between two linear regression lines. "The [JP] software takes trend data (*e.g.* cancer rates) and fits the simplest joinpoint model that the data allow. The user supplies the minimum and maximum number of joinpoints. The program starts with the minimum number of joinpoint (*e.g.* 0 joinpoints, which is a straight line) and tests whether more joinpoints are statistically significant and must be added to the model (up to that maximum number). This enables the user to test that an apparent change in trend is statistically significant. The tests of significance use a Monte Carlo Permutation method. The models may incorporate estimated variation for each point (*e.g.* when the responses are age adjusted rates) or use a Poisson model of variation. In addition, the models may also be linear on the log of the response (*e.g.* for calculating annual percentage rate change). The software also allows viewing one graph for each joinpoint model, from the model with the minimum number of joinpoints to the model with maximum number of joinpoints." (http://surveillance.cancer.gov/joinpoint/)

The main assumptions of linear regression and thus JP are: linearity and additivity, homoscedasticity (constant variance versus time, the predictions and any independent variable), statistical independence of the errors (uncorrelated errors), and normality of the error distribution.

As this program can currently only handle databases with the same number of observations for each subject, the data was individually submitted (as txt files) to the program for each subject. Examples of the output tables from JP follow below.

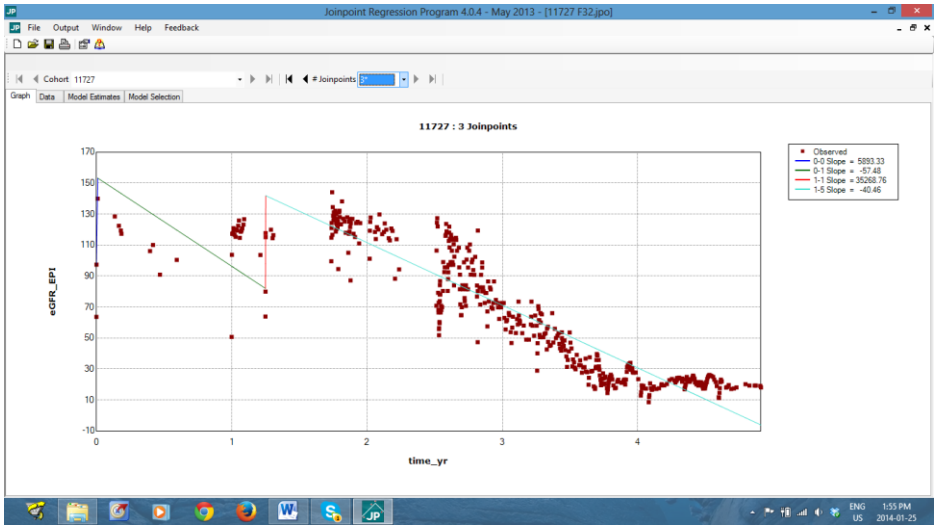
Although this program uses a default of a maximum of 5 joinpoints per subject, the process becomes limiting above 100 observation points. The default was set at 3 joinpoints for the purposes of this analysis (resulting in a maximum of 4 individual slopes). The program automatically provided the calculations (slopes) and graphs for 0, 1, 2, and 3 joinpoints (0 = straight line through all data).

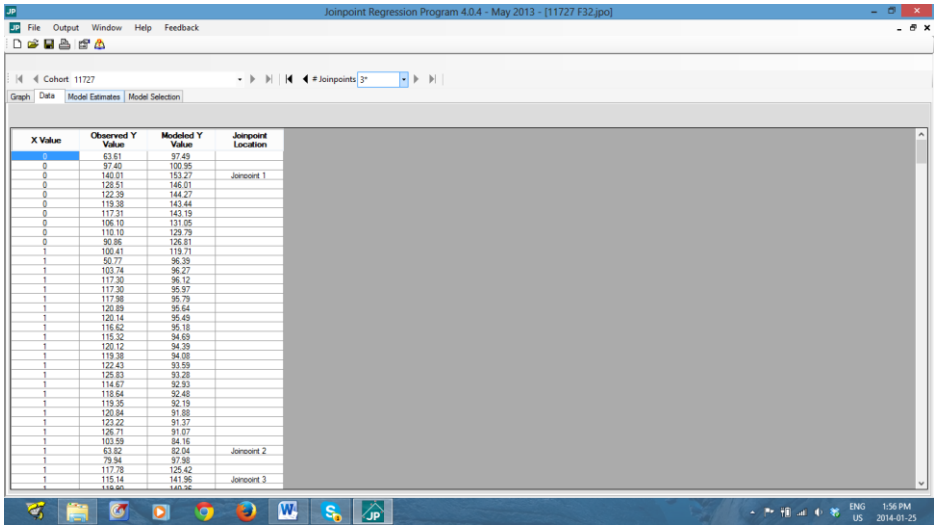
Screen 1 – Data Import Wizard: Dependent Variable Information: x Provided; x Other
Screen 2 – Specifications:
By Variables: ID;
Dependent Variable: eGFR_EPI; x Constant Variance (Homoscedasticity); No Log Transformation
Independent Variable: time_yr; Maximum number of Joinpoints – 3 (default was 5)
Screen 3 – Advanced:
Method – Grid Search (default)
Autocorrelated Errors Options - x Fit an autocorrelated errors model based on the data
Number of Observations
Minimum number of observations from a joinpoint to either end of the data - 3 (default)
(including the first or last joinpoint if it falls on an observation)
Minimum number of observations between two joinpoints – 4 (default)
(including any joinpoint that falls on an observation)
Number of points to place between adjacent observed x values in the grid search – 0 (default)
Model Selection Method – x BIC (Bayesian Information Criterion)
Permutation Test Options:
Overall significance level for the permutation tests - 0.05 (default)
Number of randomly permuted data sets for permutation test – 4499 (default)
Early Stopping Options – x Fixed
Screen 4 – Comparison:
Comparison Type: x None (At least 1 By Variable Required)

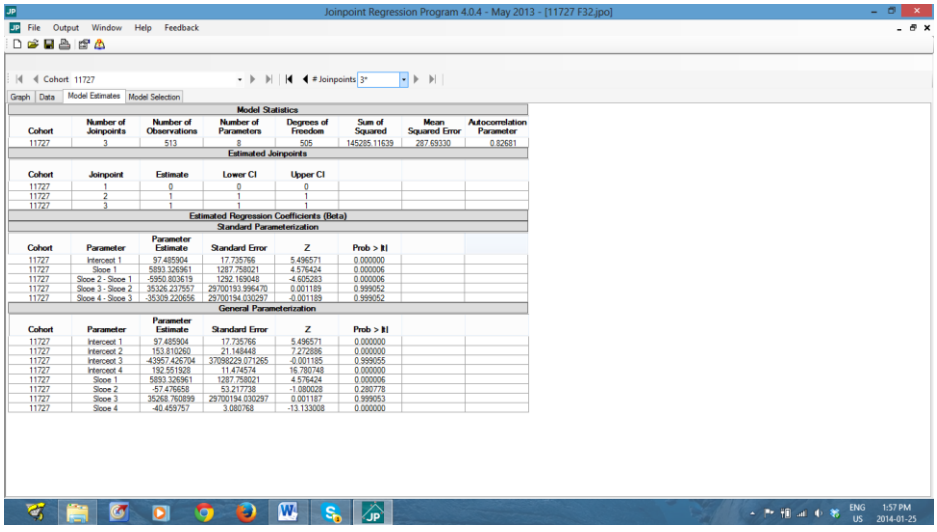
NOTES: JP program does not like extra lines at the bottom of the text file
JP program does not handle duplicate data points

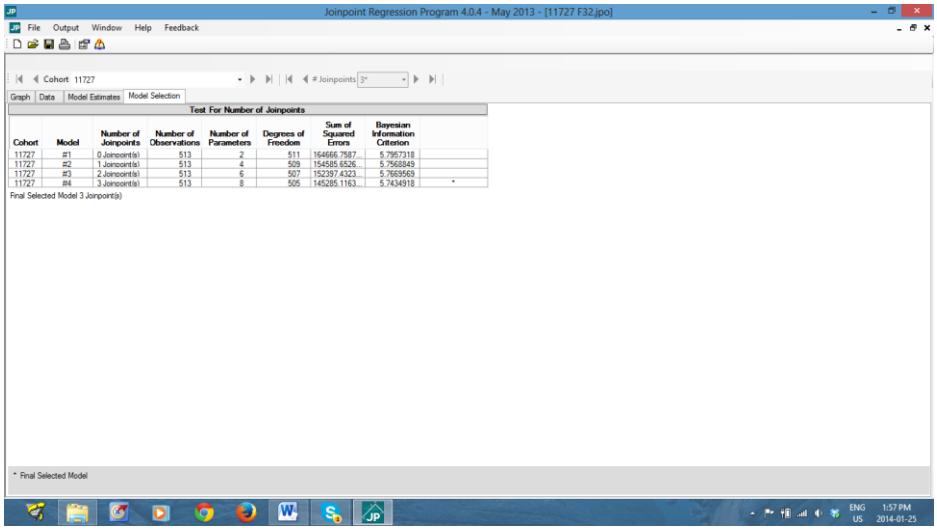
Kim HJ, Fay MP, Feuer EJ, Midthune DN. "Permutation tests for joinpoint regression with applications to cancer rates" *Statistics in Medicine* 2000; 19:335-351: (correction: 2001;20:655).

Example of JOINPOINT output tables:

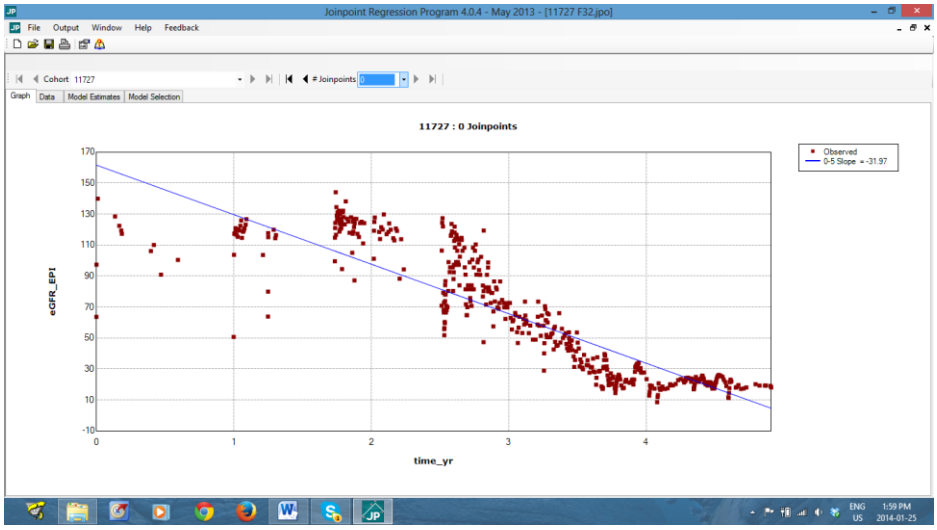








Regression with no "joinpoints" (= 0)….*example of linear regression assumption*



APPENDIX iii: SAS EXAMPLE PROGRAMS

To handle the 'datetime19.' format provided in the LIS database, the following SAS protocol was used to import the original files:

```
PROC IMPORT OUT= work.eGFR
DATAFILE= "C:\Users\Christine\Desktop\Christine Files\SASdata2\CREA_JUNE6.xlsx"
 DBMS=EXCEL REPLACE;
 SHEET="Sheet2";
  GETNAMES=YES;
  USEDATE=NO; /*use DATETIME. format for data/time column */
  SCANTIME=YES; /* use TIME. if only time values found */
RUN;
```

Removed data with unknown sex or if age is less than 18 years.:

```
if sex ne 'U';
if age >=18;
```

Removed data reported as comments (eg NAV – not available, CHK – Checked, NSQ – insufficient sample quantity, HIDE) in SAS for eGFR data. For HbA1c and ACR data, the 'CHK'ed comments were manually removed in EXCEL after sorting for these.

```
IF test result = 'NAV' ('CHK','NSQ' or'HIDE')then test result = '.' (missing);
```

Numeric data were provided as text/characters in original database, so new numerical variables were created.

```
CREAn=input(CREA,7.);format CREAn 7.; CRn=input(CR,7.);format CRn z7.;
```

Results above or below the reportable range were transformed to the number just above or below the range. (NOTE, that sometimes the results were not consistently reported as the defined URL or LRL (upper reporting limit, lower reporting limit), so there may have been several limits that needed to be identified and changed.)

```
if eGFR = ">120" then eGFRn = 121;
if eGFR = "<15" then eGFRn = 14;
if CREA = "<30" then CREAn = 29;
if CREA = "<1" then CREAn = 29;
if URIC = "<30" then URICn = 29;
if UREA = "<2.0" then UREAn = 1.9;  if UREA = "<0.5" then UREAn = 0.4;
```

eGFR-EPI is a new calculation with better accuracy at higher levels. As it is not currently reported at KGH, it was calculated as follows:

```
if sex = 'F' and CREAn le 61.9 then eGFR_EPI = 144*(CREAn/61.9)**(-0.329)*(0.993)**age;
if sex = 'F' and CREAn gt 61.9 then eGFR_EPI = 144*(CREAn/61.9)**(-1.209)*(0.993)**age;
if sex = 'M' and CREAn le 79.6 then eGFR_EPI = 141*(CREAn/79.6)**(-0.411)*(0.993)**age;
if sex = 'M' and CREAn gt 79.6 then eGFR_EPI = 141*(CREAn/79.6)**(-1.209)*(0.993)**age;
```

CKD Stages (1-5) were calculated as follows:

```
if eGFR_EPI GE 90 then Stage=1;
if 60 <= eGFR_EPI < 90 then Stage=2;
if 45 <= eGFR_EPI < 60 then Stage=3;
if 30 <= eGFR_EPI < 45 then Stage=3.5;
if 15 <= eGFR_EPI < 30 then Stage=4;
if eGFR_EPI < 15 then Stage=5;
```

SEX and GROUP were coded as follows:

```
IF SEX = 'F' THEN SEXg=1; IF SEX = 'M' THEN SEXg=0; FORMAT SEXg 1.;

IF GROUP =1 THEN GRP=1; IF GROUP =2 THEN GRP=0; IF GROUP =3 THEN GRP=0;
IF GROUP =1 THEN GRPtime=1; IF GROUP =2 THEN GRPtime=2; IF GROUP =3 THEN GRPtime=2;
label GRP = 'GRP case=1 ctrl=0'; label GRPtime = 'GRPtime case=1 ctrl=2';
```

Calculations from baseline data ('_0 '):

```
time_yr = (collected - collected_0)/31536000; /*in years*/
chg = eGFR_EPI - eGFR_EPI_0; /*absolute change*/
p_chg = chg*100/ eGFR_EPI_0; /*percent change*/
yr_chg =chg/time_yr; /*rate of change per year*/
```

Calculations for point-to-point changes (using lag1(variable) and first.ID):

```
pt_time = time_hr - time_lag; /* time between two consecutive visits */
pt_chg = eGFR_EPI - eGFR_EPI_lag; /* crea change between two consecutive visits */
pt_rchg = pt_chg/pt_time; /* rate of change between two consecutive visits */
```

Calculating summary data for merging to main databases:

```
1a)     proc summary data=database_eGFR n mean std cv max min median noprint;
        class CRn;  var eGFR_EPI; output out= eGFR_stat n=nobs mean=mean_eEPI    …etc

1b)     data eGFR_n; set eGFR_stat;
        if _type_ ne 0; keep CRn nobs mean_eEPI …etc

2)      proc sort data=eGFR_n; by CRn;

3)      data eGFR_721_2; merge database_eGFR eGFR_n; by CRn; ….
```

Calculating baseline (eg database_0) data for merging to main databases, by using "nodupkey" with **proc sort**:

```
1a)     proc sort DATA=ACR1; by CRn collected;
1a)     proc sort DATA=ACR1 OUT=ACR1_0 nodupkey; by CRn;

2)      data ACR1_0; SET ACR1_0;
        date_0 = Collected; format R_date_0 datetime19.;
        ACRn_0 = ACRn; format ACRn_0 7.1;
        age_0 = age; format R_age_0 6.2;
        KEEP CRn age_0 date_0 ACRn_0;

3)      data ACR2; merge ACR1 ACR1_0 ACR_STATs; by CRn;
        time_yr = (collected - date_0)/31536000; format R_time_yr 6.2; /*TIME IN YEARS*/
```

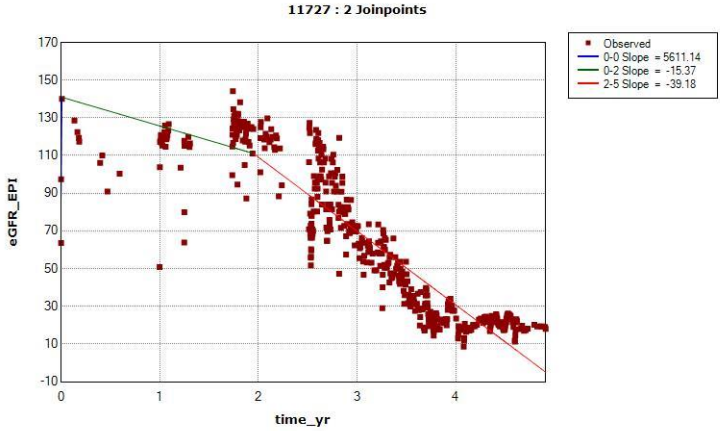
Simple MLM – Solving for J_SLOPE:

```
        /*  J_SLOPE subset data - eGFR_1201 (n=3612); baseline data - eGFR_1201_0 (n=90; 88 used)*/

        proc sort data=eGFR_1201_0; by TRIAD descending casectrl descending sexg; run;

        ods graphics on;
        proc mixed data=eGFR_1201_0 order=data covtest noclprint method=ml;
        CLASS       TRIAD casectrl sexg;
        WEIGHT    wgt_J_SE2;     /* = 1 /(J_SE2*J_SE2)*/
        MODEL      j_slope2 = casectrl age_02 sexg / solution;
        RANDOM   TRIAD(sexg) / GROUP=casectrl;
        LSMEANS    casectrl / at means cl diff;
        RUN;        ods graphics off;
```

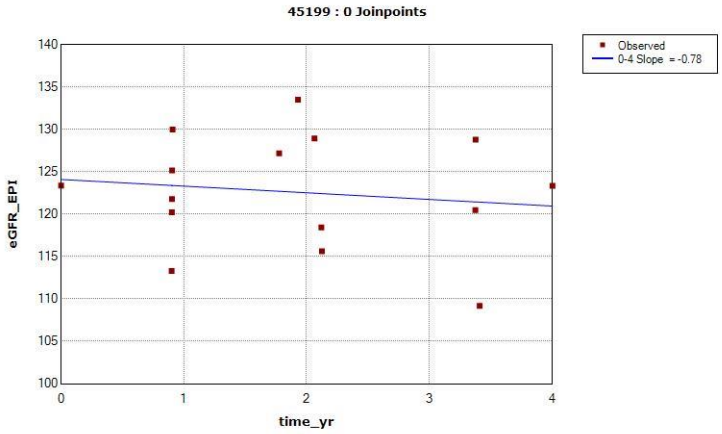
APPENDIX iv: Examples of JP time profile TRIADS: a) Cases, b) sex-age-matched Controls

TRIAD 1

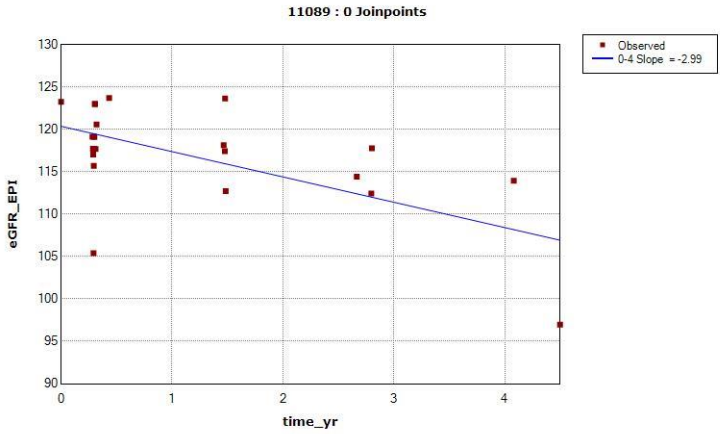
F32



F33

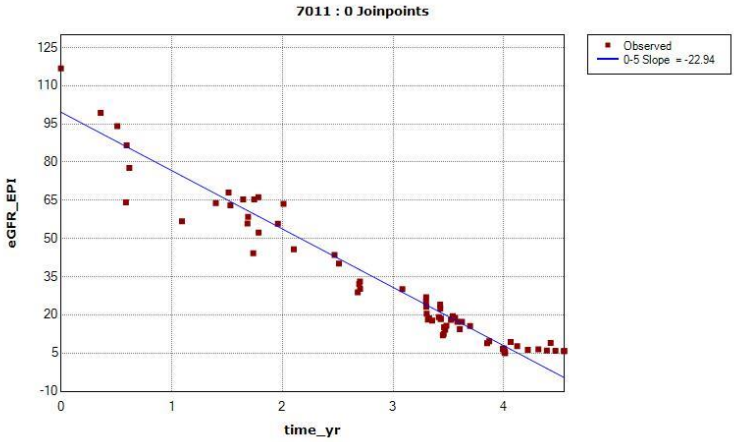


F33

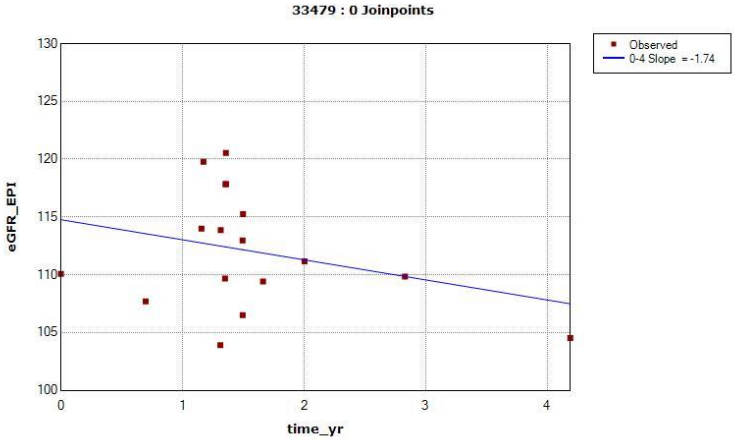


TRIAD 3

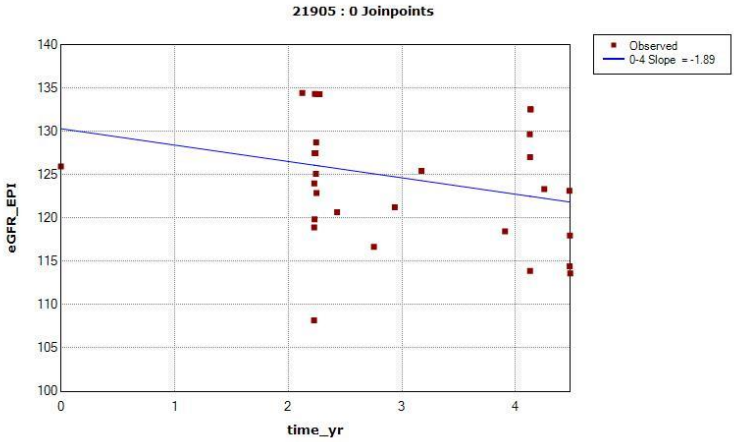
F39



F42

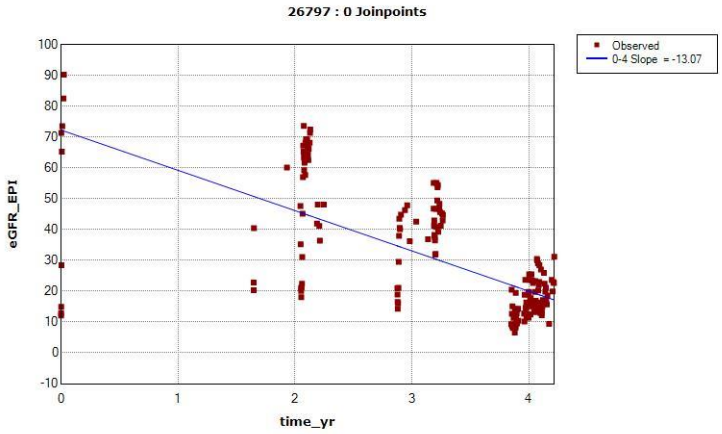


F43

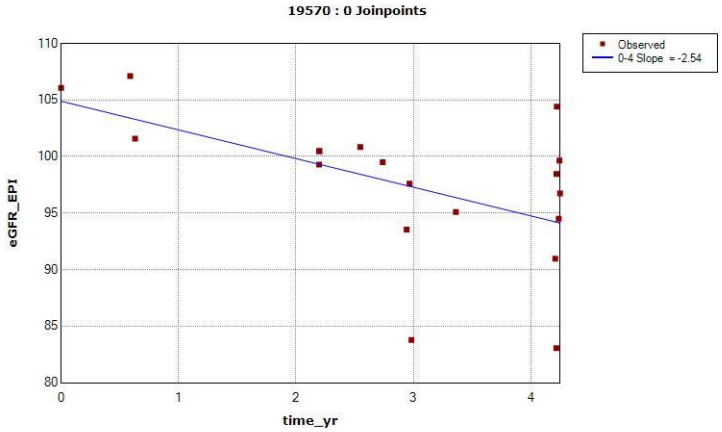


TRIAD 8

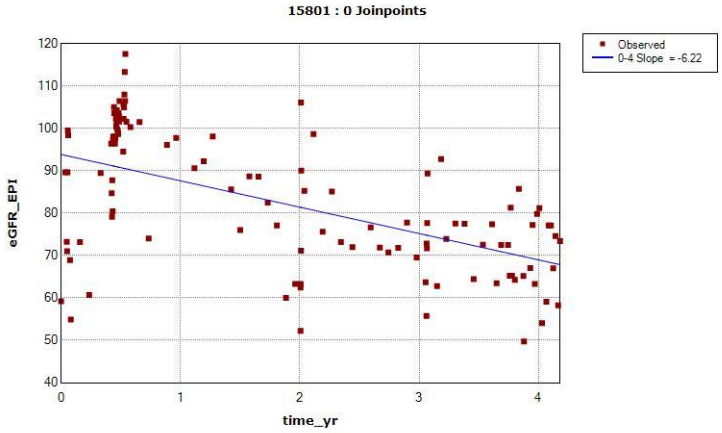
F58



F55

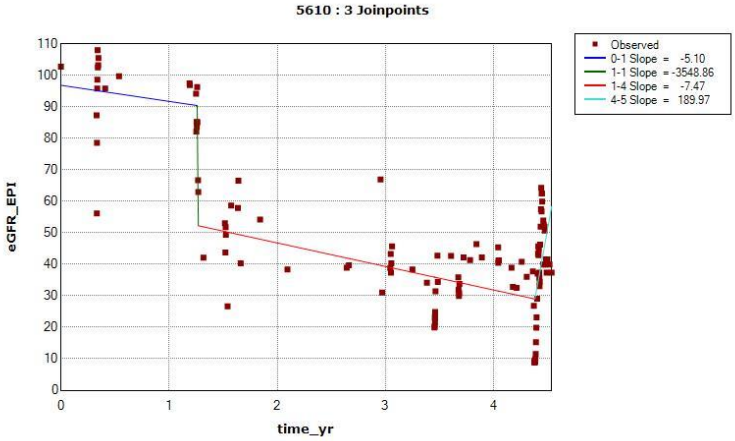


F56

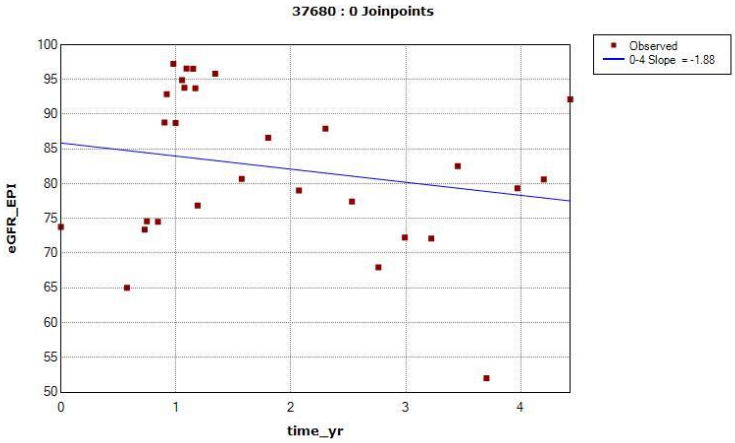


TRIAD 9

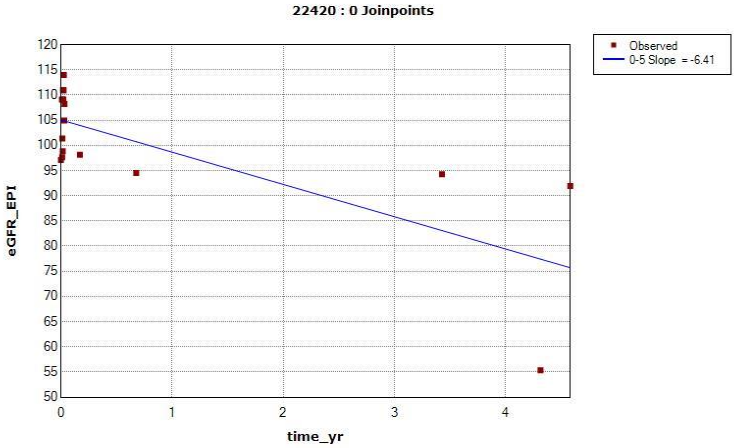
F60



F62

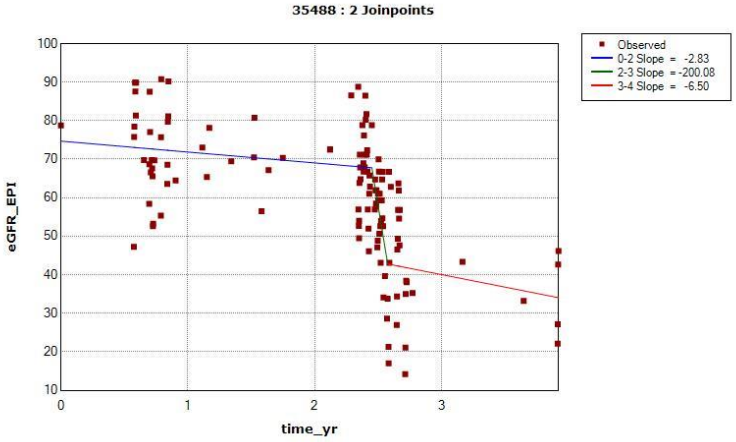


F60

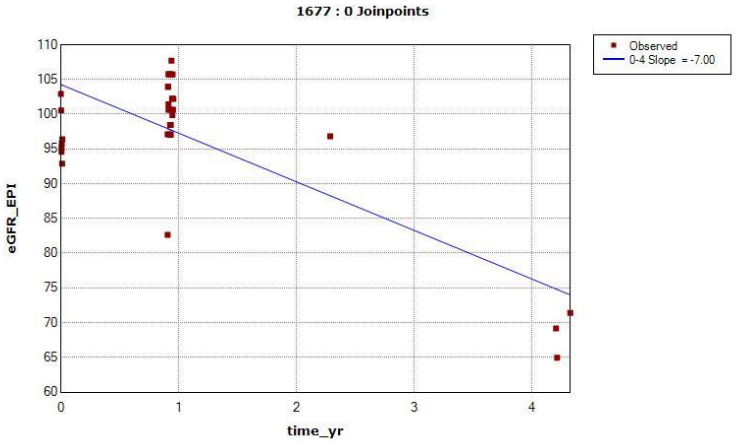


TRIAD 12

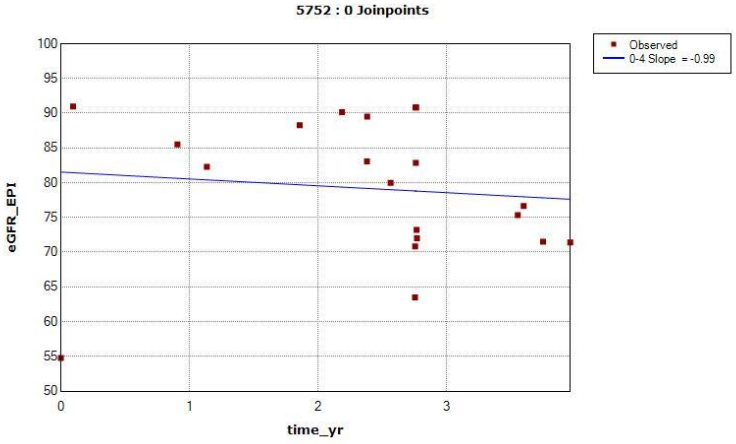
F67



F67



F68



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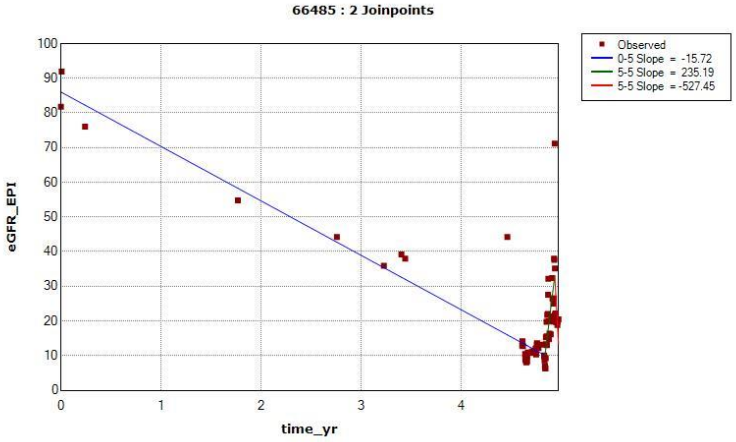

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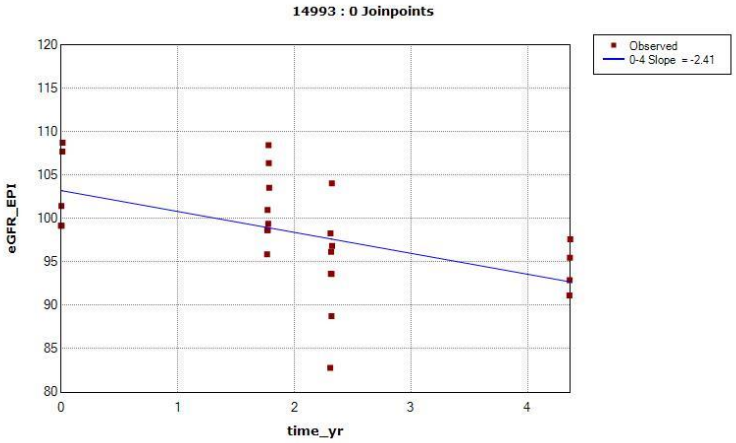

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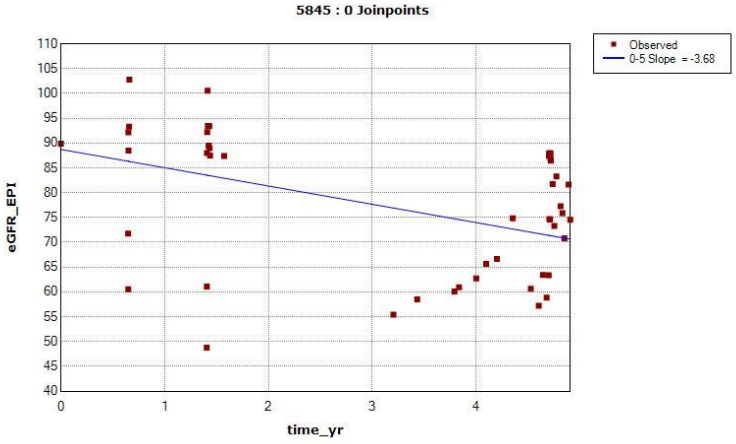

TRIAD 16

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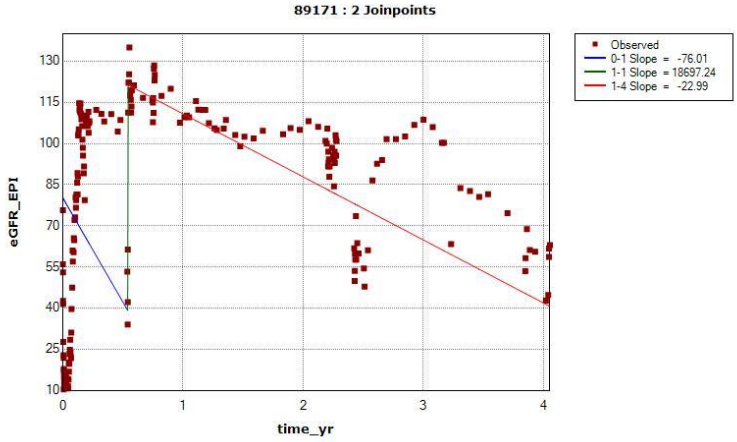

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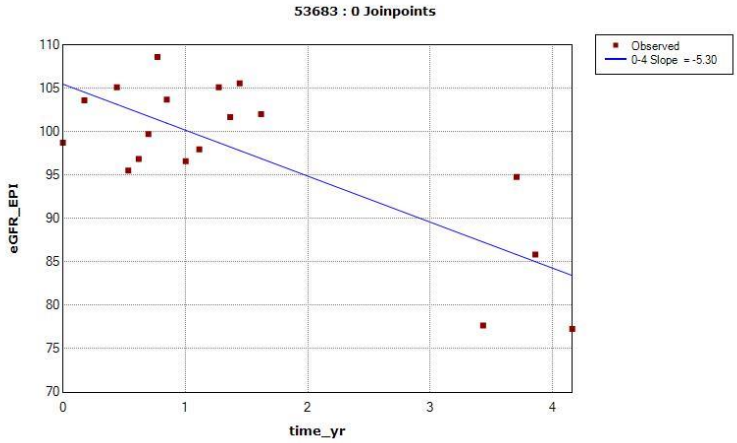
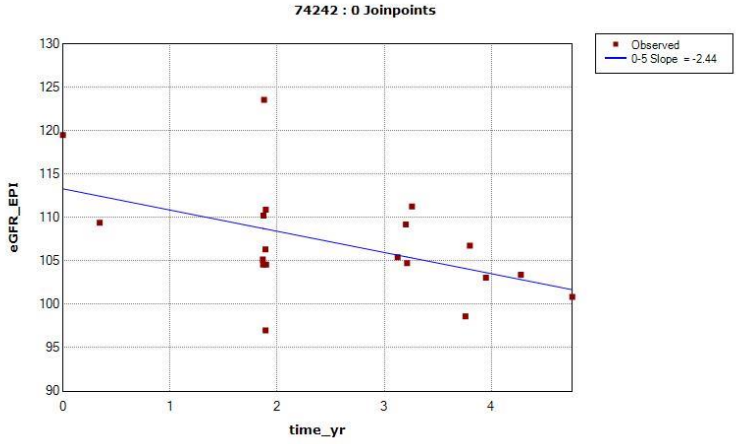


M44



TRIAD 18
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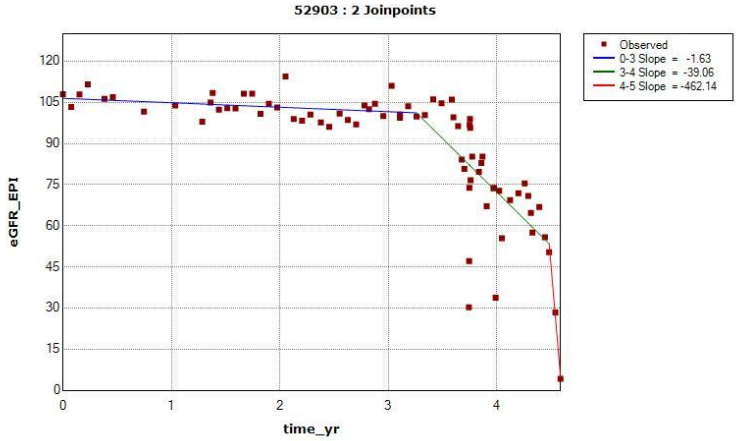

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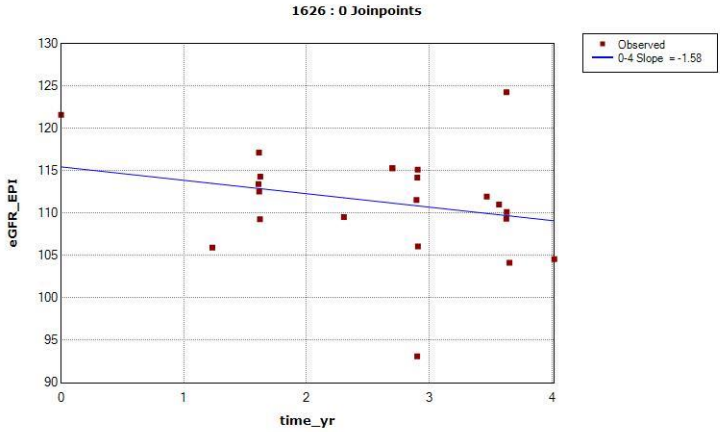

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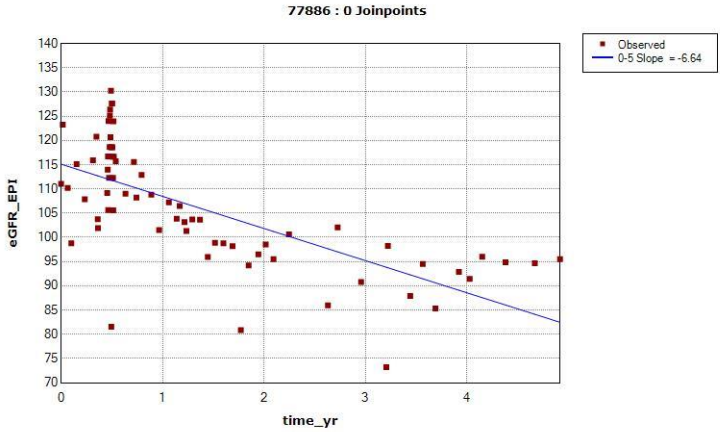

TRIAD 20

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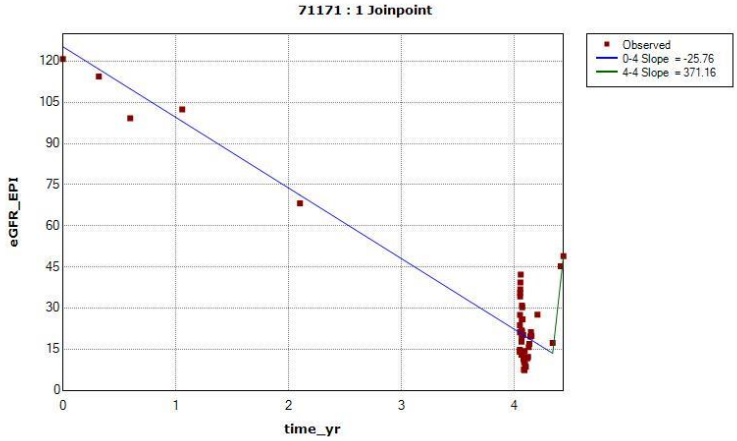

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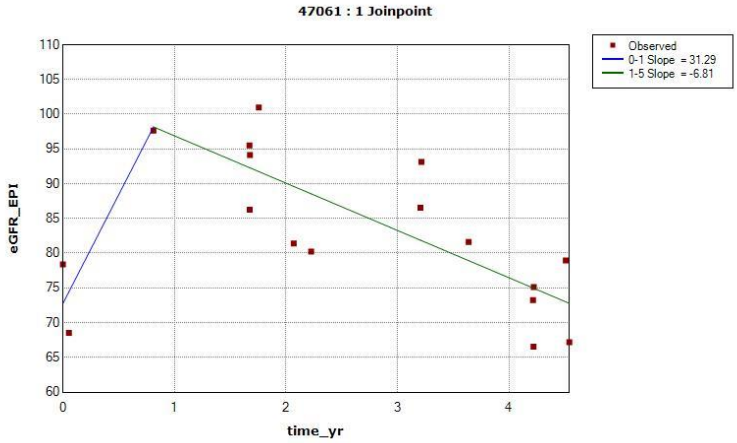

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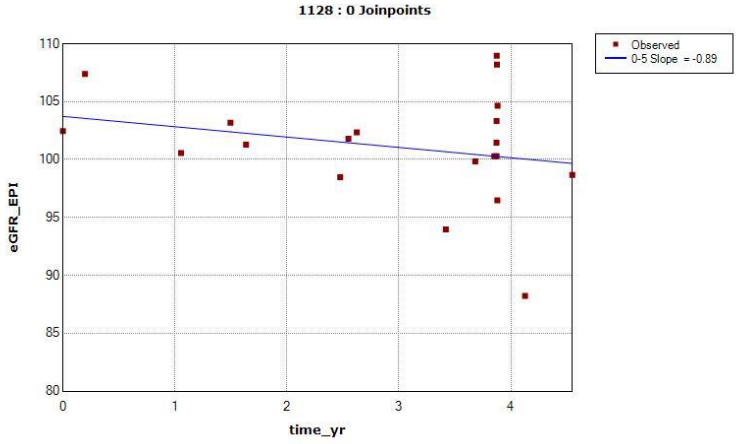
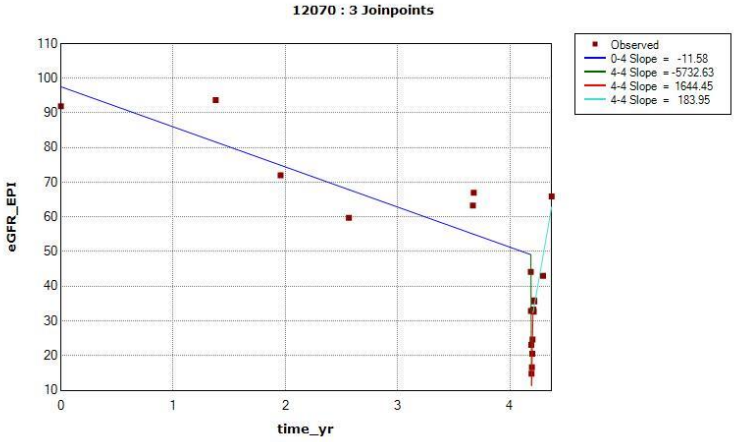
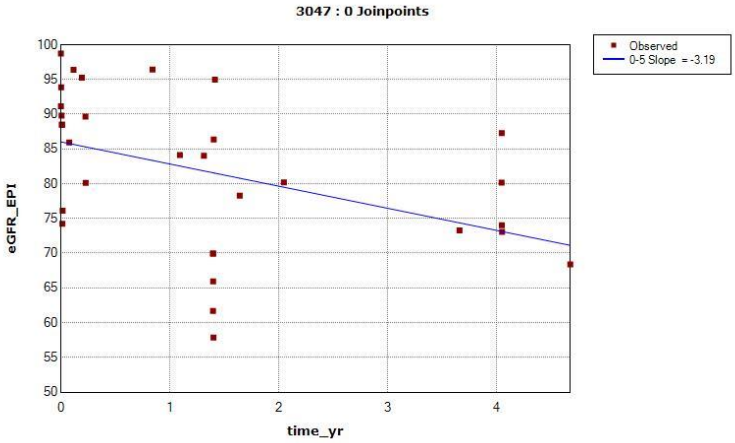


TRIAD 24

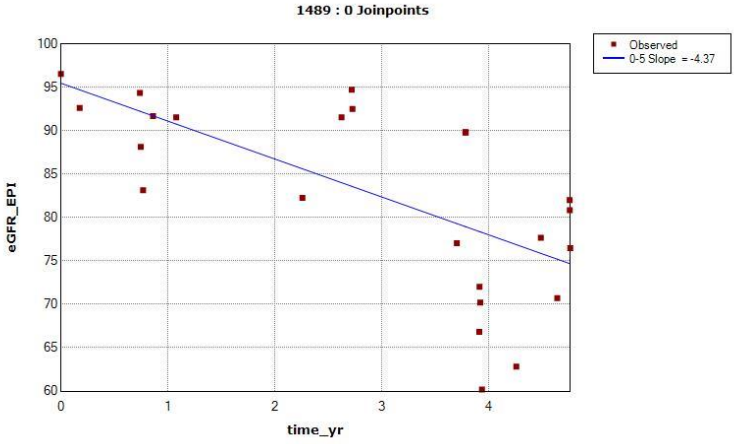
M58



M59



M63



TRIAD 26

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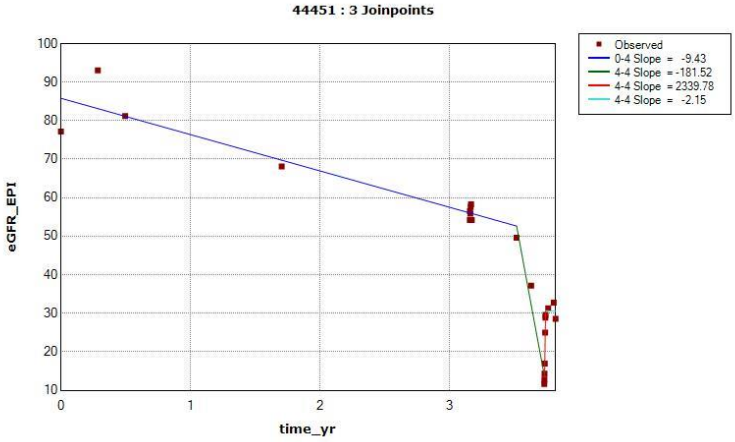
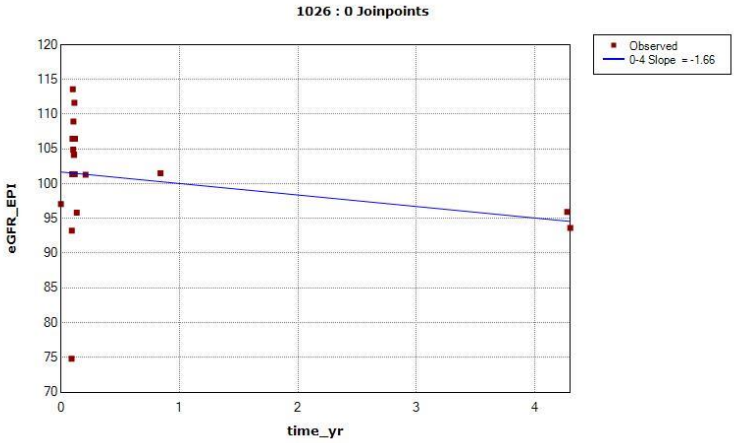


M62



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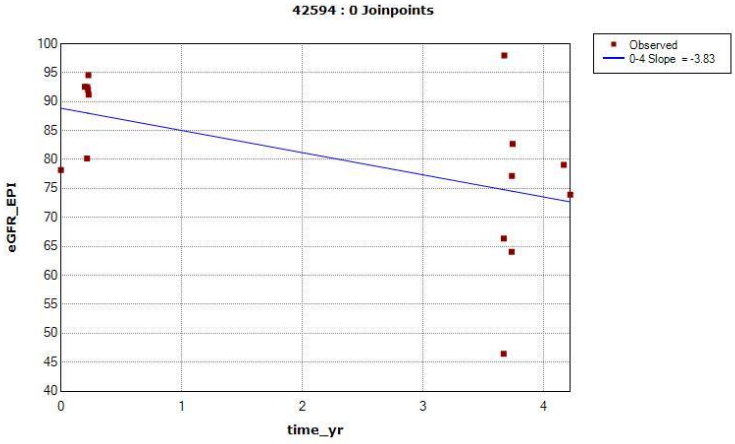

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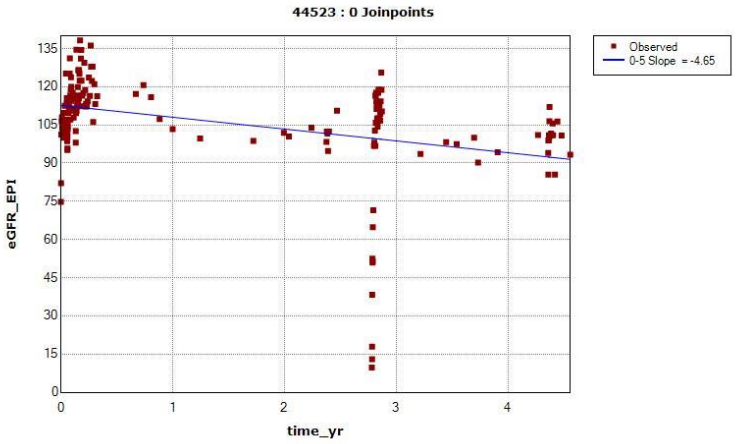

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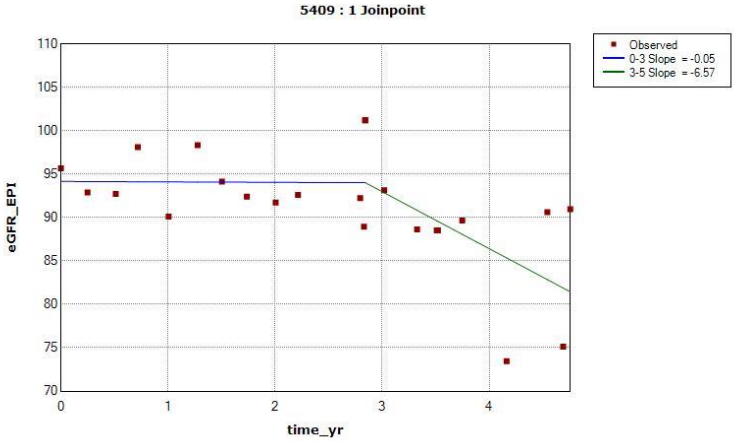

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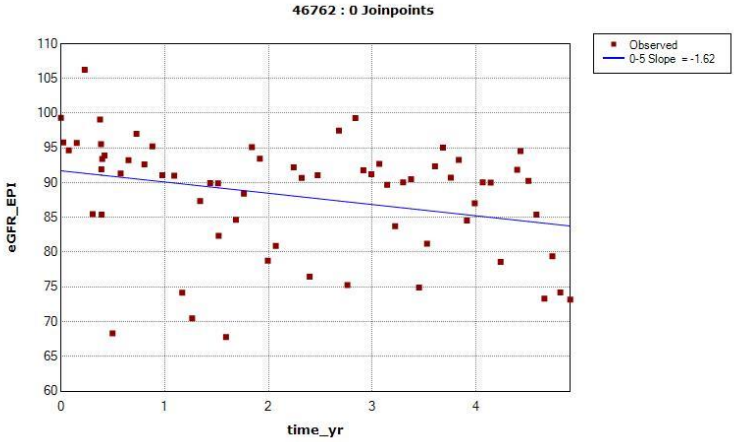

TRIAD 28

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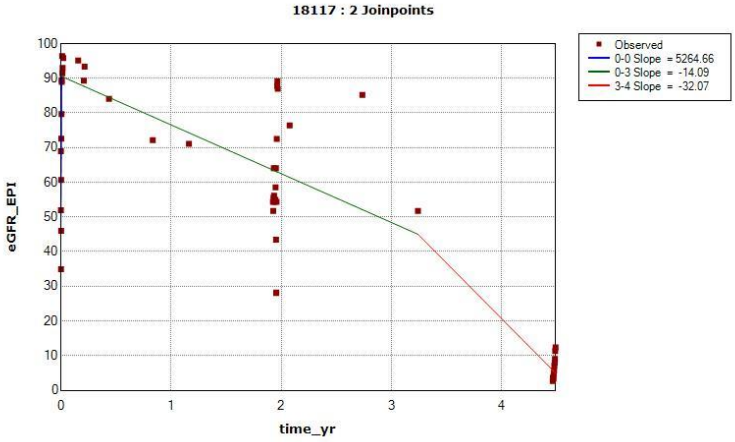

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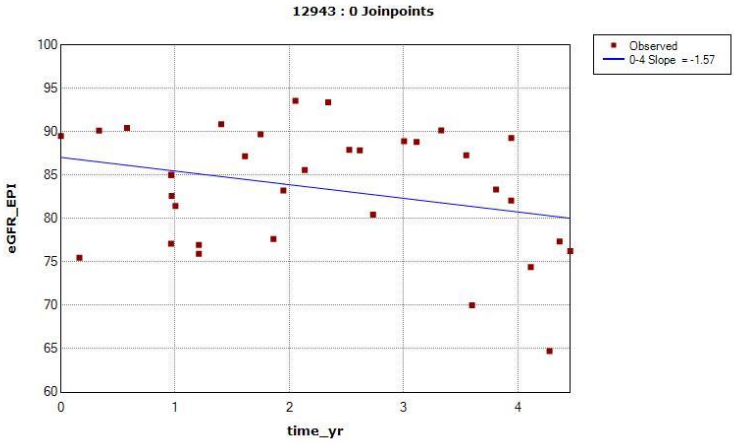

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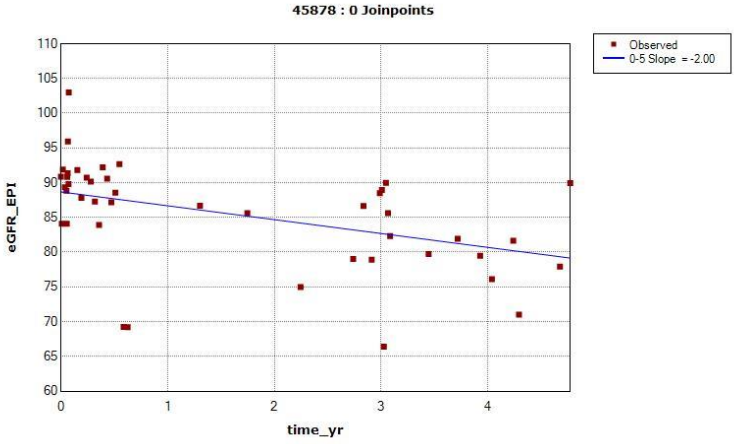

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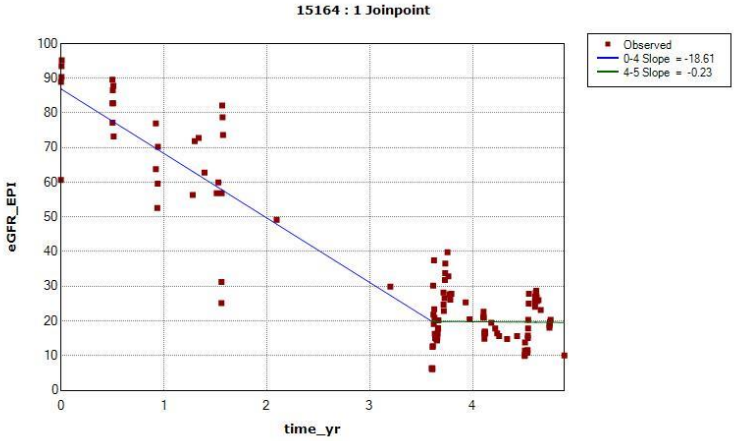

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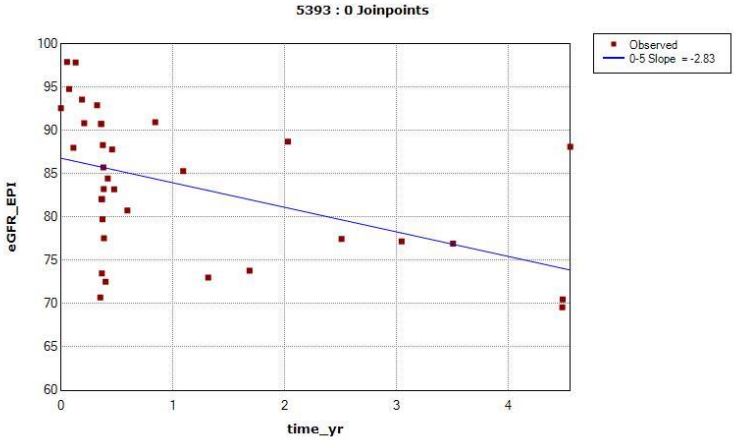

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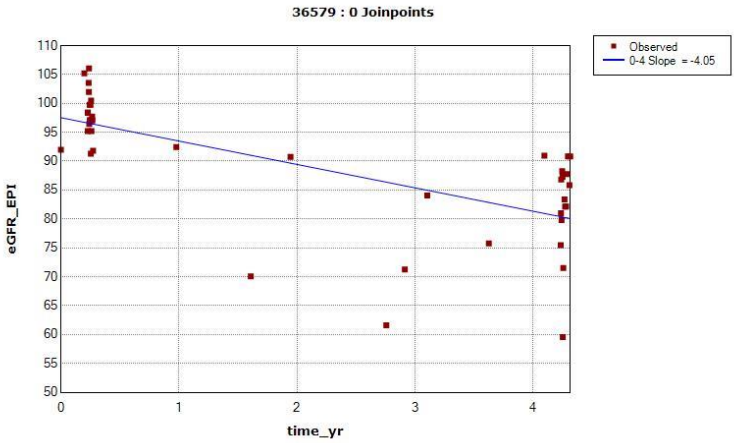
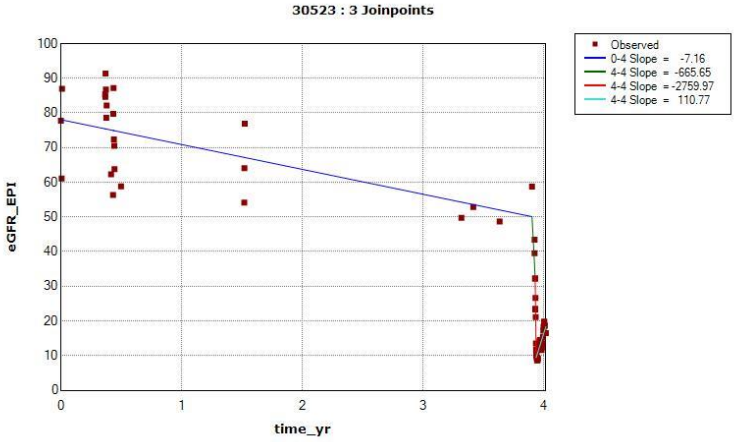
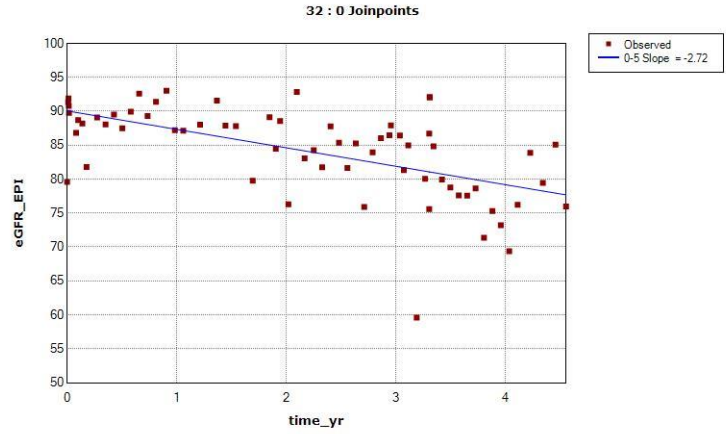


TRIAD 30

M71



M70



M72