What We Know (and don't know) about CKDu in Agricultural Communities in the Americas and Worldwide

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Since its first descriptions in 2002, a progressive tubulo-interstitial kidney disease (termed variably, CKDu, MEN, KDUCAL, CINAC) is resulting in a heavy death toll in working-age people. Despite significant and interdisciplinary effort to uncover its extent and cause, we have yet to wholly understand either.

Several studies from Meso-America report a prevalence beyond 20% in high-risk populations, and one reports rapid glomerular filtration rate decline in 10% of healthy adults. Population-based evidence using uniform, standardized approaches to track prevalence, incidence, and trends is lacking, as is an understanding of regions affected. Even in areas that have been studied, comparisons and conclusions are hampered partly by the lack of a clinical (non-invasive) case definition.

Pathology reviews in two regions recognized as hot spots, i.e., lowland regions in Sri Lanka, Nicaragua, and El Salvador, note a tubulo-interstitial nephritis with little vascular or primary glomerular pathology. Both regions also report 'symptomatic' acute interstitial nephritis. Yet we don't have definitive evidence that the regions face the same disease phenomenon: demographics are certainly similar, as is timing of emergence, but the environments, while they share many features, do not overlap exactly.

Investigations into etiology from Meso-America have primarily consisted of occupation-based studies, which demonstrate a potential association between 'end-harvest' or 'end-of-workday' kidney dysfunction with high-energy work in hot environments. Water source, heavy metals, and pesticides have been investigated in Sri Lanka. An increasing number of case-control or case-cohort studies are underway. While work done so far has been limited due to small sample size or inexact criteria for study entry, it seems clear that compared with persons with 'traditionally' progressive kidney disease, CKDu-afflicted patients are behaviorally, environmentally, and clinically unique. A collaborative, inter-disciplinary approach will add rigor and clarity as investigations into extent and cause move forward toward the ultimate goal of prevention.

How to create an exposure paradigm for a novel disease

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The Exposome is defined as all health relevant human environmental exposures from conception to death. While an enormous undertaking conceptually, there have been a number of major developments in the last 5 years that demonstrate the power and promise of exposomic research. Like genetics, all diseases, even monogenetic diseases, have an environmental component. Measures of the exposome are relevant to all complex diseases. CKDu in particular may benefit from exposomic research, as diseases with a rise in incidence over the last 20 years typically have concurrent environmental risk factors that are also rising in prevalence. Exposomics encompasses chemicals, nutrition, the physical environment and social factors and its data analytical procedures must also factor in the time varying nature of environment. A useful starting point is to divide the exposome into the internal and external environment. Internal exposomic measures include untargeted chemical assays that utilize high resolution mass spectrometry. Other subtypes of exposomic assays include PHiP-Seq which can be used to measure the human virome in plasma or serum. Unlike many 'omic' sciences, not all exposomic measures are estimated from biological assays (noise, air pollution, temperature) but are instead modeled from sensors and from public databases. Recent advances in satellite remote sensing, wearable devices as well as the internet of things (IoT) have led to novel breakthroughs in measuring such environmental risk factors often in real time. Finally, recent advances have combined mass spectrometry with novel biological matrices such as deciduous teeth that maintain a record of past environmental exposures in their histological matrices. These assays have the added advantages of "going back in time" and assigning exposure levels and dates of exposure, reconstructing events that occurred years prior to sample collection. In sum, these advances should be leveraged in future epidemiologic research into the origins of CKDu and if integrated with genomic, epigenomic and proteomic measures, could provide new insights to the underlying etiology of CKDu as well as potential prevention and treatment measures. This talk will outline these exposomic technologies and propose a framework for incorporating them into future CKDu research studies.

Solving the Mystery of Balkan Nephropathy

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Balkan endemic nephropathy (BEN) is a chronic, progressive renal disease affecting residents of rural farming villages located near the tributaries of the Danube River in Croatia, Serbia, Romania, Bulgaria, and Bosnia and Herzegovenia. Significant epidemiologic features include a familial but not inherited pattern of disease, occurrence only in individuals over 18 years of age and a strong association with urothelial carcinoma of the upper urinary tract (UTUC). Our insight into the etiology of BEN was informed by reports of chronic renal failure and UTUC among a group of Belgian women who had ingested the Chinese herb, *Aristolochia fangchi* as part of a weight loss regimen. The nephrotoxin/carcinogen responsible for both nephrotoxicity and cancer proved to be aristolochic acid (AA), a component of all *Aristolochia* plants, including those used in traditional herbal medicine. A pilot epidemiologic study in Croatia confirmed reports that *Aristolochia clematitis* (birthwort) grows in cultivated fields. Traditional methods used for harvesting and milling of wheat allowed seeds of *A.clematitis* to comingle with grain used to prepare bread, a dietary staple in the endemic region. Subsequently, aristolactam (AL)-DNA adducts were detected in the renal cortex and tumor tissue of patients with BEN/UTUC. Moreover, a unique pattern of A:T → T:A mutations was found in the TP53 gene, establishing a causal relationship between exposure to AA and BEN. BEN is now known as aristolochic acid nephropathy (AAN), which proved to be a harbinger of a global iatrogenic disease (*Environ Mol Mutagen*, 2013;**54**:1–7).

What Can the USRDS Tell Us?

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This presentation will provide an overview of the capabilities of the United States Renal Data System (USRDS), in investigating the potential existence of and supporting investigations designed to understand the growing epidemic of kidney disease among agricultural communities (i.e., CKD of unknown origin or CKDu). Briefly, the USRDS is a comprehensive kidney disease surveillance system that processes and analyzes data from a variety of existing data sources for both end stage renal disease (ESRD and chronic kidney disease as well as acute kidney injury. In addition it assists in the tracking Healthy People 2020 objectives for kidney disease. A team at the USRDS Coordinating Center based at the University of Michigan, in Ann Arbor, MI, generates the well-known web-based Annual Data Report (ADR; www.usrds.org), with data updates annually in the form of 22 chapters (9 for CKD, 12 for ESRD and 1 on HP 2020), an executive summary, as well as numerous reference tables, reference tables and methodology sections. In addition quarterly updates provide incidence and prevalence counts of ESRD in the country. Major functions of the USRDS include providing standard analysis files (SAFs) to researchers upon request, as well as respond to data merge request from large health systems and research studies. This talk will cover a key example of findings from the USRDS that have stimulated both practice and policy change with resulting benefit, and also highlight a preliminary examination of the USRDS database looking for evidence for the existence of CKDu in this country.

Insights into Pathogenesis of CKDu from Renal Biopsies

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Thirty years after the detection of a chronic interstitial nephritis in agricultural communities (CINAC), there is no consensus on its etiology[1,2]. Heat stress/dehydration, and toxic agrochemical exposure are the two most likely etiologies[3–8]. Thirty-seven renal biopsies (24 Sri Lanka, 11 El Salvador, 1 India, 1 France) with a diagnosis of CINAC (CKD 1-3A, 3B) were examined by light microscopy (LM) and electron microscopy (EM). In addition to previously described histopathological changes[1,9,10], there was a unique constellation of proximal tubular cell findings including increased size and number of dysmorphic lysosomes with light-medium uniform electron-dense matrix containing dispersed dark electron-dense non-membrane bound "aggregates." Identical renal lesions were observed in patients on calcineurin inhibitor (CNI) therapy, and in a subset of patients with light chain disease. Control biopsies (n = 58) of normal kidney, toxic nephropathies, and overt proteinuric patients with several renal diseases did not show the tubular cell changes observed by LM and EM in CINAC and CNI-treated patients. Rats treated with cyclosporine for 4 weeks developed similar tubular cell lysosomal alterations, absent in a dehydration group. These lesions are best detected on LM using methenamine silver (Jones) stain and autofluorescence, with confirmatory EM.

A specific renal tubular cell lesion was detected associated with CINAC and CNI nephrotoxicity, suggesting CINAC patients are experiencing a tubulotoxic mechanism similar to CNI nephrotoxicity. Pesticides having a CNI activity strongly supports this hypothesis[11–16].

1. Jayasumana, C. et al. Chronic interstitial nephritis in agricultural communities: A worldwide epidemic with social, occupational and environmental determinants. *Nephrol. Dial. Transplant.* **32,** 234–241 (2017).
2. Weaver, V. M., Fadrowski, J. J., and Jaar, B. G. Global dimensions of chronic kidney disease of unknown etiology (CKDu): a modern era environmental and/or occupational nephropathy? *BMC Nep* **16,** 2–8 (2015).
3. Glaser, J. et al. Climate change and the emergent epidemic of CKD from heat stress in rural communities: the case for heat stress nephropathy. *Clin. J. Am. Soc. Nephrol.* **11,** 1472–1483 (2016).
4. Roncal Jimenez, C. A. et al. Fructokinase activity mediates dehydration-induced renal injury. *Kidney Int.* **86,** 294–302 (2014).
5. Wanigasuriya, K. P., Peiris-John, R. J., Wickremasinghe, R., and Hittarage, A. Chronic renal failure in North Central Province of Sri Lanka: an environmentally induced disease. *Trans. R. Soc. Trop. Med. Hyg.* **101,** 1013–1017 (2007).
6. Shah, M. D., and Iqbal, M. Diazinon-induced oxidative stress and renal dysfunction in rats. *Food Chem. Toxicol.* **48,** 3345–3353 (2010).
7. Tripathi, S., and Srivastav, A. K. Nephrotoxicity induced by long-term oral administration of different doses of chlorpyrifos. *Toxicol Ind Heal.* **26,** 439–447 (2010).
8. Rankin, G. O., Racine, C., Sweeney, A., Kraynie, A., and Anestis, D. K. *In vitro* nephrotoxicity induced by Propanil. *Env. Toxicol.* **2,** 435–442 (2008).
9. Wijkström, J. et al. Morphological and clinical findings in Sri Lankan patients with chronic kidney disease of

unknown cause (CKDu): Similarities and differences with Mesoamerican Nephropathy. *PLoS One* **13,** (2018).
10. Wijkström, J. et al. Clinical and pathological characterization of Mesoamerican Nephropathy: a new kidney disease in Central America. *Am. J. Kidney Dis.* **62,** 908–918 (2013).
11. Enan, E., and Matsumura, F. Specific inhibition of calcineurin by type II synthetic pyrethroid insecticides. *Biochem. Pharmacol.* **43,** 1777–1784 (1992).
12. Jabłońska-Trypuć, A., Wołejko, E., Wydro, U., and Butarewicz, A. The impact of pesticides on oxidative stress level in human organism and their activity as an endocrine disruptor. *J. Environ. Sci. Heal. Part B* **52,** 483–494 (2017).
13. Ghosh, M. C., Wang, X., Li, S., and Klee, C. Regulation of calcineurin by oxidative stress. *Methods Enzymol.* **366,** 289–304 (2003).
14. Lúcia de Liz Oliveira Cavalli, V. et al. Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. *Free Radic. Biol. Med.* **65,** 335–346 (2013).
15. Uren Webster, T. M., and Santos, E. M. Global transcriptomic profiling demonstrates induction of oxidative stress and of compensatory cellular stress responses in brown trout exposed to glyphosate and Roundup. **16,** (2011).
16. Wang, X., Luo, F., Zhao, H., and Choi, J. Paraquat-induced reactive oxygen species inhibit neutrophil apoptosis via a p38 MAPK/NF-kB–IL-6/TNF-a positive-feedback circuit. (2014). doi:10.1371/journal.pone.0093837

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Assessing Environmental Exposures

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An epidemic of chronic kidney disease of unknown or nontraditional causes (CKDu) has been occurring in agricultural workers in warm regions of the word, in particular in sugarcane workers in Central America. The disease is thought to be driven by occupational factors, with the leading hypothesis that it is caused by heat stress and dehydration due to excessive workload and exacerbated by exposures to environmental toxicants. Specific environmental exposures of concern include pesticides (glyphosate), arsenic, and leptospirosis. Sugarcane workers may also be exposed to polycyclic aromatic hydrocarbons (PAHs) when harvesting burnt cane and silica from soil and cane bagasse, though these are not considered factors in CKDu. Most speculated causal scenarios involve the same mechanism: repeated subclinical insults to the kidney or overt acute kidney injury (AKI) episodes leading to CKD. However, CKD may also occur directly and environmental exposures can be involved in both mechanisms. Individuals receive exposures from both work and general environments in a unique manner. It is important to recognize that either mechanism of CKD is affected by individual susceptibility factors and that social determinants play a role in mediating the impact of any exposure or dose. Agricultural exposure sampling can be done in a variety of ways, including personal breathing zone sampling, area air sampling, wipe sampling and biomonitoring. However, several design and logistical issues need to be considered, including timing of sampling, security, health advisories, and locations that are hot, humid and rural.

Metal Exposures and Chronic Kidney Disease

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High-chronic metal exposure in occupational settings and in environmentally polluted areas can induce chronic kidney disease. Well-established nephrotoxicants at high exposure levels include lead, mercury and cadmium. These metals affect primarily the proximal tubule and under chronic conditions of exposure can result in tubulointerstitial fibrosis and chronic renal failure. Mitochondrial impairment is proposed as a major mechanism for metal-induced nephrotoxicity and could explain the impact of metals on the proximal tubule. Lead nephrotoxicity is generally accompanied by hypertension and impaired excretion of uric acid. Cadmium nephrotoxicity is characterized by increased levels of tubular proteins including kidney injury molecule-1 (Kim-1), β2-microglobulin and N-acetyl-β-D-glucosamidase (NAG) while chronic renal failure is less common. Mercury toxicity has been related to secondary focal segmental glomerulosclerosis and nephrotic syndrome. Less is known about potential metal-related neprotoxicity at low-chronic levels of exposure. High quality prospective studies of metal-related nephrotoxicity, including the potential for joint effects across metal mixtures and susceptibility factors (co-exposure to pesticides, solvents, presence of chronic kidney disease risk factors) are missing. In NHANES, increased blood lead levels were associated with reduced estimated glomerular filtration rate (eGFR) in both children and adults. While cadmium was also associated with reduced eGFR in NHANES, the association was stronger with albuminuria, a finding that is consistent with cadmium-induced tubular dysfunction in general populations. High quality prospective research is needed to understand the role of chronic metal exposure in the development of chronic kidney disease in general populations as well as in populations that are particularly susceptible to chronic kidney disease such as farming communities in Central America.

Studying Pesticide Exposure in Egyptian Agricultural Workers

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There is a critical need to investigate the susceptibility of children and adolescents to repeated occupational and environmental exposures to pesticides because the developing brain may be uniquely sensitive to the neurotoxic effects of these agents. Partnering with our colleagues at Menoufia University in Egypt, we have conducted a longitudinal study to investigate cumulative and potentially reversible effects of pesticide exposure among adolescent pesticide applicators. We recruited a cohort of 297 adolescents and conducted 44 test sessions across a 4-year period. Test sessions included the collection of biological samples (i.e., urine, blood, saliva), questionnaires and neurobehavioral testing. We characterized changes in biomarkers and neurobehavioral outcomes across the application season, identified increased symptoms, reduced lung function, neurobehavioral deficits, and a dose response association between ADHD symptoms and TCPy levels. Recognizing the need to reduce exposures, we partnered with the Ministry of Agriculture to develop an educational intervention, utilizing a behavioral change theory to reduce exposures targeting workplace behaviors and hygiene practices. In response to a needs assessment conducted as part of an R21, which identified lack of institutional support and limited training as barriers for seeking funding, a pilot grant program was established for researchers at Menoufia.

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The Role of Genetic Susceptibility in CKDu

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Both genes and environment impact most diseases. Genetic risk factors tend to be very rare and very powerful (Mendelian diseases) or quite common but also quite weak (for example, variants that contribute to the risk of hypertension or diabetes). Some populations, especially those with unusually high rates of kidney disease, may harbor genetic risk factors that have a more prominent role, such as the APOL1 kidney risk alleles that are both common and relatively powerful. Clustering of kidney disease in Central America, Sri Lanka, India, and perhaps other hotspots may be influenced by underlying genetic susceptibility combined with some as yet undefined environmental trigger. Finding the genetic variants that cause susceptibility and drive high rates of kidney disease in these populations could be helpful in identifying the potentially modifiable environmental trigger in at least three ways: 1) Defining susceptible individuals may make exposure studies more illuminating; 2) the biology of the genes and gene variants that increase or decrease disease risk may provide critical clues to the nature of the relevant environmental factor(s); and 3) overlap or non-overlap of genetic factors between CKDu hotspots might help us understand if the environmental factors affecting various CKDu clusters are similar. Work to date in Sri Lanka and Central America suggest that CKDu in these populations likely has a significant hereditable component.

Disclosures: DJF is a co-inventor on patents related to APOL1, is a founder and equity holder in APOLO1bio, and receives research funding from Vertex.

Epidemiological Challenges to Studying Agricultural Communities

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When investigating chronic kidney disease of unknown origin (CKDu) in agricultural communities, we should consider the potential for bias in epidemiologic studies. In particular, occupational cohort studies are being used to examine CKDu. A common source of bias in occupational studies is the healthy worker effect. This bias arises in two ways. First, when active workers are compared to the general population, the workers may appear to have a reduced risk of disease simply because they were already a healthier population and this would underestimate the association of interest. Second, the healthy worker survivor effect occurs in a study of workers only, yet the healthier workers remain at work the longest while less healthy workers move to jobs with lower exposure or leave work altogether, again underestimating the association. With the healthy worker survivor effect, the bias increases with increasing time since hire. A separate source of bias arises due to agricultural work being predominantly seasonal, raising the possibility of non-ignorable loss to follow-up if exposed workers become sick and don't return the following season. Lastly, there is the issue of left truncation, which describes that at the start of follow-up, long-term workers may not be representative of all previous workers and when analyzed concurrently with recent hires, a downward bias may be induced. In this lecture, we will review how these biases operate, their impact to study findings, and how they can be addressed.

Defining a disease cluster: Hantavirus Pulmonary syndrome in the Southwest U.S.

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In May 1993, an outbreak of severe respiratory illness occurred in the Southwestern United States. Illnesses considered in the initial differential diagnosis included pneumonic plaque, leptospirosis, inhalation anthrax, rickettsial infections, pulmonary tularemia, atypical bacterial and viral community acquired pneumonias, legionellosis, meningococcemia and other sepsis syndromes, and illness caused by viruses not commonly seen in the United states (Flaviviruses, Arenavirus, and Bunyavirus). There was no evidence of exposure to known toxic agents. Laboratory tests for bacterial and viral pathogens and a variety of toxic agents were negative and the initial autopsy findings suggested that bacterial or parasitic causes were unlikely. The results of laboratory studies of serum and tissues from several patients suggested an acute infection with a new species of hantavirus and since known as Hantavirus (Cardio)-Pulmonary Syndrome. Unexplained diseases could beneficiate of a systematic collection of clinical, epidemiological, and laboratory specimens. In case of fatal diseases, systematic pathology specimens collected during autopsies can also orient the diagnosis. Advances in molecular techniques, such as next generation sequencing, allow now to randomly search for an infectious etiology.

Nothing to disclose.

*Dr. Rollin was unfortunately unable to join us for this meeting because CDC required his expertise outside of the country.