Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-Traditional Etiology in Mesoamerica and Other Regions

March 20 – 22, 2019 - San José, Costa Rica

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Report From the Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-Traditional Etiology in Mesoamerica and Other Regions

March 20-22, 2019
San José, Costa Rica

Workshop organized by:

Consortium for the Epidemic of Nephropathy in Central America and Mexico (CENCAM), National Institutes of Health: National Institute of Environmental Health Sciences (NIEHS) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and World Health Organization Regional Offices: Pan American Health Organization (PAHO) and South-East Asia Regional Office (SEARO)

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About This Report

Chronic kidney diseases of uncertain etiology (CKDu)[1] describes a pattern of chronic kidney disease not explained by traditional risk factors, appearing endemic to some rural communities along the Pacific Coast of Mesoamerica and in specific regions of Sri Lanka and India. To address this issue and present recent research progress, the Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-Traditional Etiology in Mesoamerica and Other Regions was held in San José, Costa Rica, March 20-22, 2019, with the participation of 137 individuals from 15 countries. The meeting was hosted by the Central American Program for Health, Work, and Environment (SALTRA) and the Regional Institute for Studies on Toxic Substances (IRET) of the National University (UNA), Costa Rica.

The workshop represented a collaboration between the Consortium for the Epidemic of Nephropathy in Central America and Mexico (CENCAM), the National Institute of Environmental Health Sciences (NIEHS) and the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health in the United States, the Central American Institute for Studies on Toxic Substances (IRET), and World Health Organization (WHO) Regional Offices, Pan American Health Organization (PAHO), and South-East Asia Regional Office (SEARO).

Workshop participants included specialists from diverse backgrounds including nephrology, epidemiology, occupational health, environmental health, public health, toxicology, pathology, social sciences, and exposure assessment. Face-to-face interactions at workshops enabled additional exchange of information, such as new scientific findings, lessons learned, resources or training available, and opportunities for new or strengthened collaborations.

Discussions at workshops are not always summarized in publications, which can lead to the loss of valuable information. Therefore, this report, in addition to the brief summary report (Crowe et al., 2019) published shortly after the workshop, aims to detail scientific evidence and ongoing research discussed at the workshop. It will provide the reader with information about where consensus exists and where questions or different perspectives remain, as well as specific recommendations for future research to reduce the burden of CKDu. By making this report freely available online, it is our hope that it is a meaningful tool to advance future efforts addressing this critical issue.

Reference

[1] This illness is also called *chronic kidney disease of non-traditional origin (CKDnt)*. Regional names also exist including, *Mesoamerican Nephropathy*, a term that has been in use in Central America and Mexico since the first International Workshop in 2012. In Sri Lanka, it has been called *Chronic Interstitial Nephritis in Agricultural Communities (CINAC)*. Workshop organizers recognize all of the names used for the disease; however, the term *chronic kidney disease of unknown etiology (CKDu)* is used in this report with the intent to simplify the text.

Sobre este informe

Las enfermedades renales crónicas de etiología no conocida (CKDu por sus siglas en inglés)[2] describen el patrón de la enfermedad renal crónica no explicado por factores de riesgo tradicionales, que parece endémico en algunas comunidades rurales a lo largo de la costa del Pacífico de Mesoamérica y en regiones específicas de Sri Lanka e India. Para abordar este problema y mostrar avances en investigaciones recientes, se organizó el Tercer Taller Internacional sobre Enfermedades Renales Crónicas de Etiología No-conocida/No-Tradicional en Mesoamérica y otras regiones que se llevó a cabo en San José, Costa Rica, del 20 al 22 de marzo de 2019, con 137 participantes de 15 países. La reunión fue organizada por el Programa Salud, Trabajo y Ambiente en América Central (SALTRA) y el Instituto Regional de Estudios en Sustancias Tóxicas (IRET) de la Universidad Nacional (UNA), Costa Rica.

El taller fue el resultado de una colaboración entre el Consorcio para la Epidemia de Nefropatía en Centroamérica y México (CENCAM), el Instituto Nacional de Ciencias de Salud Ambiental (NIEHS) y el Instituto Nacional de Diabetes y Enfermedades Digestivas y Renales (NIDDK) de los Institutos Nacionales de Salud en los Estados Unidos, el Instituto Regional de Estudios en Sustancias Tóxicas (IRET) y las Oficinas Regionales de la Organización Mundial de la Salud (OMS), la Organización Panamericana de la Salud (OPS) y la Oficina Regional del Sudeste Asiático (SEARO).

En el taller participaron especialistas de diversas áreas como nefrología, epidemiología, salud ocupacional, salud ambiental, salud pública, toxicología, patología, ciencias sociales y evaluación de la exposición. La discusión entre los especialistas en el taller permitió un intercambio de información incluyendo nuevos hallazgos científicos, lecciones aprendidas, recursos o capacitaciones disponibles, y nuevas oportunidades de trabajos en conjunto.

Los resultados de talleres no siempre terminan en publicaciones, lo que puede conducir a la pérdida de información valiosa. Es por eso que, además de un breve informe (Crowe et al., 2019) publicado poco después del taller, el presente informe tiene como objetivo detallar evidencia científica e investigaciones analizadas en el taller. Este informe proporcionará al lector información sobre los consensos, dudas y perspectivas existentes, así como recomendaciones específicas para futuras investigaciones con el fin de reducir la carga de CKDu. Al estar este informe disponible gratuitamente en línea, esperamos que sea una herramienta importante para avanzar en los esfuerzos futuros que aborden este problema crítico.

Referencia

[2] Esta enfermedad también se llama enfermedad renal crónica de origen no tradicional (CKDnt). También existen nombres regionales que incluyen, Nefropatía Mesoamericana, un término que se ha utilizado en Centroamérica y México desde el primer Taller Internacional en 2012. En Sri Lanka, se ha denominado Nefritis Intersticial Crónica en Comunidades Agrícolas (CINAC). Los organizadores del taller reconocen todos los nombres utilizados para la enfermedad, sin embargo, el término enfermedad renal crónica de etiología desconocida (CKDu) en este informe con la intención de simplificar el texto.

Executive Summary

Background

Chronic kidney diseases of uncertain etiology (CKDu)[3] is a term that describes a pattern of endemic, non-diabetic, non-hypertensive kidney disease, characterized by reduced glomerular filtration with mild or no proteinuria or other features of glomerulonephritis. Although unclear whether it is the same disease in all regions of the world, clinically similar hotspots have been most extensively described in rural communities along the Pacific Coast of Mesoamerica and in specific regions of Sri Lanka and India. Although not entirely restricted to agricultural workers, the disease appears to be most prevalent in field workers in hot and humid climates. Clinically, CKDu cases typically present predominantly among male individuals in their third to fourth decade of life with a low-grade or non-proteinuric nephropathy characterized by a progressively declining glomerular filtration rate. Traditional risk factors for most common chronic renal diseases (e.g., hypertension, diabetes mellitus, obesity, or advanced age) are generally absent. The disease has devastated many of the communities where it exists and has overwhelmed health care systems in affected countries, causing unknown morbidity and tens of thousands of deaths over the last 20 years in Mesoamerica alone. Despite international research efforts, the specific causes of the disease remain unknown, creating an enormous need for research, patient care, and socio-economic interventions that can only be appropriately addressed through international and interdisciplinary collaboration.

The Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-Traditional Etiology in Mesoamerica and Other Regions in 2019 (referred to here as the Third International Workshop) built on previous international workshops organized by SALTRA in 2005, 2009, 2012, and 2015, as well as a 2016 workshop organized by SEARO and a 2018 workshop organized by NIDDK and NIEHS. The 2019 Third International Workshop reflected a collaboration across CENCAM, NIEHS, NIDDK, IRET, WHO, PAHO, and SEARO. This global interinstitutional collaboration is a testament to the increasing awareness about the seriousness of CKDu – an enormous change from the workshops held as recently as 2012 when the disease had yet to be formally recognized and cross-country and cross-disciplinary collaboration was in its infancy. Over the last few years, the literature describing CKDu research has grown substantially, with more than 250 publications in the last five years, increasing in number each year (20 in 2015; 33 in 2016; 40 in 2017; 71 in 2018; 95 in 2019; and five in January 2020).[4] The Third International Workshop demonstrated the continued advances made in confronting CKDu. Attendance was higher and more diverse than previous workshops, with 137 participants from 15 countries, as well as a pre-workshop training for 120 health care providers and personnel responsible for health surveillance in a CKDu-endemic region.

The pre-workshop training was jointly organized by the Costa Rican Health Care and Social Security System (CCSS), the Costa Rican Association of Nephrologists, the Latin American Society of Nephrology and Hypertension (SLANH), and CENCAM. This pre-workshop took place in Liberia, Costa Rica, and was directed to physicians and health workers in direct contact with patients with CKDu. During the event, multiple voices of those in the field taking care of patients were heard, training facilitated discussions of patient treatment options and clinical course, ways to create where absent and to improve registries, and approaches to collaboration across institutions.

Reference

[3] This illness is also called *chronic kidney disease of non-traditional origin (CKDnt)*. Regional names also exist including, *Mesoamerican Nephropathy*), a term that has been in use in Central America and Mexico since the first International Workshop in 2012. In Sri Lanka, it has been called *Chronic Interstitial Nephritis in Agricultural Communities (CINAC)*. Workshop organizers recognize all of the names used for the disease; however, the term *chronic kidney disease of unknown etiology (CKDu)* is used in this report with the intent to simplify the text.

[4] Publication counts reflect searches of PubMed, Web of Science, and Scopus databases for articles indexed describing original research or reviews related to chronic kidney disease of uncertain etiology and related terms. Full search query available by contacting corresponding authors.

Workshop content and structure

The workshop began with an inaugural session with authorities from participating countries and agencies, and a World Café event designed for face-to-face meetings of all participants to express perspectives and share experience related to CKDu research. The workshop included several plenary presentations, and optional early morning sessions covering specific scientific topics. Poster sessions that included 41 abstracts presenting insights into the science of CKDu, such as pathology, epidemiology, and treatment and prevention efforts, demonstrated the promise of the ongoing efforts on this issue. More detailed information on the abstracts is available in the workshop meeting book, "Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-Traditional Etiology in Mesoamerica and Other Regions: Meeting Book, 2019." Most time over the three days was dedicated to working groups comprised of participants with experience and expertise in specific scientific topics. This report includes the summaries from each working group: Analytical Epidemiology; Biomarkers of Abnormal Kidney Function; Clinical Presentation and Treatment; Heat Load/Dehydration and Workload; Molecular Approaches to CKDu: Genetics, Epigenetics, and Infectious Disease; Pathology; Pesticides and Metals; and Surveillance and Screening. Joint sessions with two or more working groups also took place and are included in the working group summaries.

Working groups

Analytical Epidemiology

The Analytical Epidemiology Working Group discussions focused on several priority areas relevant to advancing the epidemiological understanding of CKDu. Clinical definitions of chronic kidney disease (CKD) and acute kidney injury (AKI) are relevant for case control studies and clinical epidemiology. A separate priority was the consideration of studying early stages of kidney damage necessary to understand the natural history of CKDu. This requires development of variables accepted as biological measures for population studies of early kidney damage. Equally important is developing standardized measures of priority exposure variables that have been identified as the most likely to be related to CKDu or AKI. Study design discussions favored prospective and/or intervention studies, with both community- and occupation-based populations felt to play a complementary role. Finally, best approaches to methodological challenges discussed a focus on healthy worker selection effect, time between exposure and detectable event, and misclassification in epidemiological studies of CKDu. Sharing information about existing and planned work was a priority to promote collaboration or common core elements of studies to enhance comparisons between study results. Priorities for CKDu research noted by the analytical epidemiology group were 1) large longitudinal studies that measure short-term change (days) in serum creatinine, long-term change (months to years) by eGFR, and continually assess protocols in settings such as the Third International Workshop; 2) intervention studies and their assessment; 3) cross-talk between studies; 4) deep engagement with community and occupational study participants; and 5) establish material repositories for questionnaires, protocols, and community engagement tools.

Biomarkers of Abnormal Kidney Function

The Biomarkers Working Group agreed that biomarkers play a key role in diagnosis and research in all kidney diseases, including CKDu. The group aimed to describe the evidence for the use of currently established renal biomarkers such as serum creatinine, cystatin C, and urinary albumin, and discuss the relevant strengths and weaknesses of these markers with respect to the CKDu field. Although there is a clear research need, as well as a likely clinical utility, for a marker of early disease in CKDu, evidence in support of the use of any such novel biomarker was limited. The group agreed that diagnosis of CKDu along with studies of detection, burden, and associations are going to be based on estimates of glomerular function from serum markers, at least for the foreseeable future. A clear understanding of those factors, which might influence the interpretation of these measurements, is therefore needed by all investigators in the field.

Clinical Presentation and Treatment

The Clinical Presentation and Treatment Working Group discussed the clinical features of CKDu, similarities and differences in these clinical features among the regional entities termed CKDu, and the areas where there is insufficient evidence to create a comprehensive case definition or definitions. Given the lack of published clinical guidelines for the management of CKDu at the time of this meeting, the group presented a consensus on clinical features, which should prompt clinicians to consider CKDu in a patient, as well as management recommendations when CKDu is suspected.

The group agreed that the current understanding of CKDu is limited, and it remains to be seen whether CKDu is a single disease or a collection of diseases with similar clinical features, as this is often the case in a multiplicity of scenarios of advanced chronic kidney disease. Similarities in the characteristics of CKDu across India, Sri Lanka, and Mesoamerica include similar male-to-female ratio, familial concordance, living in poverty, rural residence, occupations involving physical labor, hypokalemia, hyponatremia, minimal proteinuria, and sterile pyuria. Differences across regions include variable age distribution and the presentation of hyperuricemia and urate crystalluria, the latter two reported only in Mesoamerica. Despite limited available renal biopsies, patients appear to consistently demonstrate non-specific tubulointerstitial nephritis with a lymphocytic infiltrate. Clinicians in both regions described concurrent acute kidney injury (AKI) episodes and it has been hypothesized that they may portend progressive CKDu.

The group agreed that clinical management in early stages of CKD focuses on appropriate hydration with clean water, minimization of exposure to heat and agrochemicals, correction of electrolyte disturbances, and avoidance of nephrotoxins, especially nonsteroidal anti-inflammatory drugs and herbal medications. The treating nephrologists in the group reported that timely diagnosis and appropriate CKD care is highly likely to delay progression of renal disease, although currently there is insufficient data to support this. The group noted the need for 1) more systematic and comprehensive studies on the clinical aspects of CKDu; 2) the inclusion of clinicians in affected regions in research studies; 3) centralized disease reporting; 4) enhanced local renal biopsy capacity; and 5) expanded collaboration across basic and environmental scientists, nephrologists, and nephropathologists experienced in this disease, including regional, national, and international partnerships.

The group discussed the need for policies focusing on occupational health and safety protections, particularly in professions with high levels of heat exposure; basic services provision with a focus on safe and adequate drinking water; and development of health care infrastructure to increase access to medical care in CKDu-affected regions. More research is needed to further understand the pathophysiology, natural history, and treatment of the disease.

Heat Load/Dehydration and Workload

Heat strain and dehydration have recently been in the spotlight globally due to emerging evidence of their association with adverse renal health issues including chronic kidney disease, especially in working populations. The probability of heat stress is greater when the temperature of the environment surpasses a person´s core temperature, a situation common for outdoor workers in tropical settings where temperatures can easily surpass 37°C.

The working group reviewed 36 epidemiological studies that included heat as a possible risk factor for adverse renal health including CKDu. Epidemiological studies in Central America have reported adverse heat stress impacts in agricultural workers, especially, but not only, in the sugarcane industry. In addition to exposure to high temperatures, evidence exists that high sweat rates and limited fluid intake can lead to functional and sub-clinical acute kidney injuries (AKI), and that repetitive AKIs on a daily basis could also lead to compromised kidney function and finally end up in the development of chronic kidney disease (CKD). It has also been reported that heat stress and high workload may cause AKI and accelerate disease progression in populations with a pre-existing illnesses caused by hypovolemia.

Although the specific role of heat, dehydration, and workload in the causal pathway for CKDu continue to be debated, their association with CKD and CKDu has been shown repeatedly. Studies published to date have used a wide array of methods to measure heat, dehydration, workload, and renal health, which makes it difficult to compare study results and assess HS/D as part of the causal pathway.

A wide range of equipment, questionnaires, quantitative measurements, and methodologies exist for measuring heat stress and dehydration. Their use varies depending on cost, feasibility in the field, and other logistical factors. Having a standardized method of measuring the qualitative and quantitative indicators of heat exposure, workload, and dehydration will help scientific community to understand the link these factors have with the disease. When possible, gold standard devices (e.g., core temperature pill, wet bulb globe temperature, heart rate, and accelerometers) should be used. Likewise, valid and reliable outcome measures (e.g., urine specific gravity instead of a urine color chart or self-report of water consumption) should be considered in order to increase accuracy. Workplace interventions specific to heat are needed to tackle disease prevention and progression especially at the local level where poverty and reduced access to resources and health care complicate the situation for affected communities.

Molecular Approaches to CKDu: Genetics, epigenetics, and infectious disease

The advanced molecular techniques that have transformed much of biomedicine in the 21st century have not been widely employed in the search to discover the causes of CKDu. Most studies to date have focused on single hypotheses using a combination of questionnaire assessment of exposure history and direct testing of a limited number of biological variables. In contrast, many of the molecular techniques are not limited by the need for specific hypotheses in advance because they can simultaneously test hundreds, thousands, or even millions of questions in parallel. This is a tremendous asset, but also raises several challenges for CKDu researchers, particularly in relation to the need for large sample sizes, collection of specific tissue types, high costs, and the requirement for interdisciplinary teams with both broad and deep expertise. This working group considered existing gaps in the field and critically evaluated how genetic, epigenetic, "-omic," pathogen detection, and other methodologies could help identify the causes of CKDu.

Genetics: Identification of genes that make individuals more susceptible to the environmental causes of the disease can help identify those environmental causes by focusing hypotheses on exposures that are related to the genes' function and/or focusing analyses on gene-environment interactions. Despite its challenges, the working group agreed that gene discovery should be approached through genome-wide studies using recently developed genome-wide arrays that are cost-effective. At the same time, it was noted that the coverage for population-specific variants may be suboptimal especially in poorly-studied geographic areas such as Central America. Whole genome sequencing data could be performed in a subset of individuals and used to improve imputation of variants in all study participants using study-specific inferred haplotypes. While genetics can play an important positive role in helping to identify the factors that cause CKDu, the potential also exists for negative consequences, such as employment discrimination, stigma, and psychological distress. Researchers must work with experts in these areas in order to minimize the likelihood of these harmful outcomes.

Epigenetics: By regulating when and in what quantities genes are expressed, the modifications that comprise the epigenome can have important effects on health and disease. Epigenetic tools have been developed only recently and show great promise to study health and disease. In the opinion of the working group, genome-wide DNA methylation arrays represent the technique most likely to be powerful and useful for the study of CKDu in the near term. These studies could potentially be used to identify specific environmental exposures and used in conjunction with other methodologies such as genetic studies to understand the environmental impact on gene expression. Because CKD can itself affect epigenetic patterns because of changes in the excretion of molecules (such as toxins) normally excreted by the kidney, longitudinal studies with measurement at several time points are likely to be the best study design for this purpose.

"-Omics": Technologies such as metabolomics, exposomics, and proteomics can potentially reveal unknown exposures by either identifying the exposure itself or by observing how exposure alters normal physiologic processes and subsequent metabolites. This may be key in the search for preventable causes of CKDu as affected workers and communities face multiple complex exposures. The major tools available for metabolomics are mass spectrometry and nuclear magnetic resonance (NMR). Mass spectrometry is a highly sensitive technique that may be well-suited for detecting environmental factors in the nanomolar range. NMR, though less sensitive, is highly quantitative and versatile with respect to the nature of the compounds it can identify; it may be especially useful for snapshots of an entire system of metabolites. These techniques can be applied to urine, serum, or even tissue. Furthermore, metabolomics could aid in the characterization of the exposome, defined as the totality of environmental exposures from conception onward. As noted above, longitudinal prospective studies with repeated measures can best help clarify the chronicity of changes.

Pathogens: The working group agreed that infectious disease is a plausible but under-studied potential contributor to CKDu. Changes in climate, high density of infectious disease vectors and reservoirs, limited access to clean water, limited resources for detecting and controlling infectious disease outbreaks, continued emergence of new pathogens and pathogen variants increasingly challenge kidney health in tropical and low-resource settings. While there have been a few studies focused on testing for known candidate pathogens (e.g., leptospira, hantavirus), a small but growing body of inconsistent findings suggest casting a wider net would be more productive. The working group was enthusiastic about moving toward unbiased screening, with case-control analyses and well-defined classification criteria. However, targeted, multiplexed testing (i.e., combining detection of multiple pathogens into a single PCR reaction) will also be informative if untargeted sequencing is not feasible. Partnerships to expand availability of technology to lower-resourced investigators and sharing/pooling of biospecimens for larger sample sizes will be important, as will be correctly classifying infection status (e.g., acute/current, past) and assigning temporality (i.e., infection occurred prior to CKDu).

Pathology

The Pathology Working Group engaged a small group of nephropathologists, clinical nephrologists, and toxicologic pathologists to compare observations and perspectives on key pathologic features of CKDu. Group members had experience reviewing biopsy material from CKDu patients in Nicaragua, El Salvador, and Sri Lanka. Toxicologic pathologists from the U.S. National Toxicology Program (NTP) had experience modeling experimental kidney disease induced by environmental agents in animal studies. The primary aims of the working group were to 1) use collective experiences to identify key and consistent features among the samples studied; 2) speculate on potential pathogeneses with the intent to hypothesize etiologic contributors; 3) develop a descriptive histopathologic schema for biopsy characterization; 4) discuss opportunities to build a global partnership; and 5) discuss approaches to mechanistic investigation.

The group considered details of light microscopic and ultrastructural features of the biopsy material they have previously reviewed from patients suspected to have CKDu, from percutaneous needle biopsies mostly representing renal cortex. Pathologic changes in the samples reviewed demonstrated many consistent features including chronic interstitial fibrosis variably admixed with mixed mononuclear inflammatory cells, tubular atrophy, glomerular enlargement, global glomerular sclerosis, and signs of glomerular ischemia. Histologic changes in arteries such as intimal fibrosis, smooth muscle hyperplasia, and arteriolar hyalinosis were occasionally seen but not a consistent finding. In kidney biopsies from patients considered with acute CKDu, the main findings were described as tubulointerstitial nephritis with varying degrees of acute inflammation and chronic tubulointerstitial changes. Other research was shared including National Toxicology Program's (NTP) preliminary search of renal changes related to pesticide and heavy metals exposures in rodents using archived data.

The group also discussed critical gaps and how to address them, suggesting a standard template for CKDu renal biopsy characterization, an accompanying atlas of light microscopic and ultrastructural images to ensure consistent characterization of biopsies around the world, and the development of a user-friendly electronic template that could be used by pathologists who have or will review biopsy materials from CKDu patients. The value of a central repository for biopsy images, patient data, and biopsy characterizations was also discussed, but with notable challenges in adoption and implementation. The group recognized the importance of engaging and educating clinicians in the localities where the disease is believed to occur and suggested the development of a training module. Lastly, participants discussed the need for mechanistic investigation and how that might improve current understanding of the etiology and pathogenesis of CKDu. Though animal studies were postulated, welfare issues would need to be considered. The group hoped to continue a global and collaborative venue for consultation with pathologists who did not attend the workshop and felt that integrating or aligning continued effort by the Pathology Working Group participants with other working group participants will likely yield the most impact.

Pesticides and Metals

The Pesticides and Metals Working Group aimed to review and discuss the state of the science addressing the potential for pesticides or metals to adversely affect kidney function and potential CKDu susceptibility, compare research findings across regions (Mesoamerica, Sri Lanka, and India), discuss integrated methodologies to evaluate exposure to contaminants in CKDu research settings, and explore possible associations and interactions with other CKDu risk factors, particularly between contaminants and heat stress.

The working group generally discussed the potential for pesticides to act as environmental risk factors associated with CKDu, and in certain cases, discussed specific pesticides. Agrochemicals, such as fertilizers, growth hormones, and components in the commercial formulation other than active ingredients, were not specifically addressed and could be considered in future discussions. The review of studies in Mesoamerica, Sri Lanka, and India found no conclusive evidence that pesticides cause CKDu. Glyphosate, the most widely used herbicide around the globe, remains a concern and there is limited epidemiologic evidence evaluating an association with CKDu.

The group also discussed pesticide formulations and noted that studies of commercial pesticide formulations are complicated because they are heterogenous across brands and consist of a combination of active pesticidal ingredient(s), a variety of additives, and possibly metal contaminants such as arsenic, cadmium, and lead. Working group participants concluded there is a need for more robust study designs to evaluate the potential role of pesticides and other agrochemicals in CKDu etiology in affected regions, in combination with other risk factors. In the discussion of the importance of metals and CKDu, there were two viewpoints proposed by working group participants, both stressing the need for high quality exposure assessment. One group thought metals were unlikely to be a major driver of the CKDu epidemics, and emphasized the importance of sound exposure assessment and epidemiological designs in regions with high risk of CKDu to enable a better understanding before looking into any interactions. Others thought metals may be involved in CKDu etiology and emphasized that, even if environmental exposure levels are low, daily exposure might be moderate-high with higher water consumption or more intense inhalation. Additionally, multiple metals are present at low-moderate levels in most environments and it is unknown whether metal mixtures act synergistically or antagonistically with other risk factors in relation to kidney injury.

There was agreement about the importance of designing interdisciplinary studies, such as geological studies collecting samples that are useful in health studies or leveraging studies on other diseases that have biobanked specimens available for ancillary investigations. Importantly, studies should be done to contrast exposures in high and low prevalence areas. The group recommended measuring exposure to metals and pesticides in an integrated way and to consider interactions between pesticides and metals, as well as mixtures of environmental chemicals in statistical modeling of CKDu studies. The timing of exposures was considered important as early life exposures may

impact CKDu susceptibility. There was broad agreement that the epidemic state of CKDu warrants intervention and that where there is suggestive evidence of risk factors associated with CKDu, there must be work done to reduce these exposures, and hopefully the burden of CKDu, while advancing our understanding of etiology.

The overall recommendations from the working group were to 1) propose a set of core information that should be collected about occupational and environmental exposures to pesticides and metals across studies; 2) pursue interdisciplinary research to leverage current expertise and resources; 3) enable data and sample sharing for environmental and occupational exposures (for past and future studies); 4) identify sites with and without CKDu to compare risk factors across study populations and over time; and 5) prioritize intervention studies on modifiable factors related to CKDu.

Surveillance and Screening

The Surveillance and Screening Working Group had participants from eight countries (Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, and Sri Lanka), and: 1) reviewed prevalence studies and screening data; 2) proposed recommendations for future studies or surveillance; and 3) addressed aspects of ethics related to surveillance and screening CKDu in vulnerable populations.

Progress in CKDu surveillance and screening has occurred in different ways in each of the affected countries. Two initiatives were highlighted: PAHO has published an initial case definition and methodology for public health surveillance; and the DEGREE protocol for CKDu prevalence studies has been developed and is currently being tested in several countries.

The group recommends that any institution establishing a CKDu surveillance system (SS) should consider the following questions: 1) What type of surveillance is needed? 2) What will be the target populations? 3) Which definition of CKDu will be used? 4) How will the issue of CKD vs. CKDu be addressed? and 5) What are the objectives of the SS?

It is important to use standard protocols for case definition and case evaluation in the different contexts of clinical research, surveillance, screening, and epidemiological research. It is also important to identify key participants from different sectors in the SS to ensure the sustainability of the system and to link results to critical action. The need to link SS and research was also regarded as essential given the unclear causal mechanisms of CKDu. Furthermore, the group addressed aspects of ethics related to surveillance and screening CKDu in populations in conditions of vulnerability, highlighting the need for developing clear national guidelines to link the results of CKDu surveillance and screening activities to the appropriate policies, programs, and activities.

Representatives from Guatemala, Nicaragua, Honduras, Costa Rica, Panama, El Salvador, Mexico, and Sri Lanka reported new data on CKD and CKDu at the national and, in some cases, sub-national level. Some of the countries have implemented incipient surveillance systems for CKDu, and El Salvador included questions on CKDu and non-traditional risks in a STEP-WISE national survey on NCDs.

The group also reviewed prevalence studies and screening data published or made available since the Second International Workshop. To do that, ecological, cross-sectional, and screening studies selected by published systematic reviews were used as the basis for discussion. A major issue identified was lack of comparability between the studies for most parameters.

Great progress has been made in collecting and analyzing health service records in each country and these developments should be supported and strengthened. However, much work is still needed to obtain comparable population prevalence data. It is recommended that the DEGREE protocol be the core protocol (with additional questionnaires for the specific needs of the study) for prevalence surveys. As well as being a key part of surveillance, such surveys are a key part of the search for the causes of CKDu, as they can identify high-risk

populations that could be targeted for future research. Such surveys can also identify potential 'hotspots' of the disease and can be used to generate hypotheses. There are specific ethical considerations for different types of surveillance and screening. The need to link surveillance to medical attention is important to decrease disease morbidity and mortality.

Conclusions

Key accomplishments from the Third International Workshop on CKDu included face-to-face interactions in small and large group settings and the productive working group efforts on focused science areas, updating of the state of the art by experts from various countries and disciplines, and an increase in the number and scope of institutions involved in the workshop. CKDu remains a public health crisis while a clear understanding of its etiology is elusive. The lives that have been lost and the toll on families and communities have not subsided, despite gains in research, public health, and clinical efforts over the last decade. The Second International Workshop (Program on Work Environment and Health in Central America (SALTRA), Central American Institute for Studies on Toxic Substances (IRET), and Universidad Nacional (UNA), 2015) reported a high priority for coordinated regional approaches to study prevalence, etiology, and evaluate interventions. These priorities remain moving forward from this workshop. Despite the breadth of knowledge and experience shared on CKDu to date, the scientific evidence is insufficient to stop the disease. We continue to believe that interdisciplinary research that involves international scientists and clinicians is critical to enable progress as quickly as possible. This document aims to summarize the most current information in order to assure that new research builds on previous work.

Resumen Ejecutivo

Antecedentes

Enfermedad renal crónica de etiología no-conocida (ERCu) es un término que describe un patrón de enfermedad renal endémica, no diabética y no hipertensiva caracterizada por filtración glomerular reducida con proteinuria leve o nula y ausencia de características de la glomerulonefritis. Aunque no está claro si es la misma enfermedad en todas las regiones del mundo, los puntos críticos clínicamente similares se han descrito más ampliamente en las comunidades rurales a lo largo de la costa del Pacífico de Mesoamérica y en regiones específicas de Sri Lanka e India. Aunque no está completamente restringida a los trabajadores agrícolas, la enfermedad parece ser más frecuente en los trabajadores de campo en climas cálidos y húmedos. Clínicamente, los casos de ERCu generalmente se presentan predominantemente entre individuos masculinos en su tercera a cuarta década de la vida con una nefropatía de bajo grado o no proteinúrica caracterizada por una tasa de filtración glomerular en declive progresivo. Los factores de riesgo tradicionales para las enfermedades renales crónicas más comunes (por ejemplo, hipertensión, diabetes mellitus, obesidad o edad avanzada) generalmente están ausentes. La enfermedad ha devastado muchas de las comunidades donde existe y ha abrumado a los sistemas de salud en los países afectados, causando morbilidad desconocida y decenas de miles de muertes en los últimos 20 años solo en Mesoamérica. A pesar de los esfuerzos de investigación internacionales, las causas específicas de la enfermedad siguen siendo desconocidas, lo que crea una enorme necesidad de investigación, atención al paciente e intervenciones socioeconómicas que solo pueden abordarse adecuadamente a través de la colaboración internacional e interdisciplinaria.

El Tercer Taller Internacional sobre Enfermedades Renales Crónicas de Etiología Desconocida/ No Tradicional en Mesoamérica y Otras Regiones en 2019 (denominado aquí como el Tercer Taller Internacional) tuvo como base los talleres internacionales organizados previamente por SALTRA en 2005, 2009, 2012 y 2015, así como un taller de 2016 organizado por SEARO y un taller de 2018 organizado por NIDDK y NIEHS. El Tercer Taller Internacional de 2019 reflejó una colaboración entre CENCAM, NIEHS, NIDDK, IRET, OMS, OPS y SEARO. Esta colaboración interinstitucional e global es un testimonio de la creciente conciencia sobre la gravedad de la ERCu, la cual representa un cambio enorme de los talleres celebrados tan recientemente como 2012 cuando a enfermedad aún no se había reconocido formalmente y la colaboración entre países y entre disciplinas estaba en su infancia. En los últimos años, la literatura que describe la investigación de ERCu ha crecido sustancialmente, con más de 250 publicaciones en los últimos cinco años, aumentando en número cada año (20 en 2015; 33 en 2016; 40 en 2017; 71 en 2018; 95 en 2019; y cinco en enero de 2020). El Tercer Taller Internacional demostró los continuos avances logrados en la confrontación de ERCu. La asistencia fue mayor y más diversa que los talleres anteriores con 137 participantes de 15 países. Adicionalmente, se realizó una capacitación previa al Taller, la cual se realizó para 120 proveedores de atención médica y personal responsable de la vigilancia de la salud en una región endémica de ERCu. Dicha capacitación fue organizada de forma colaborativa por la Caja Costarricense de Salud y Seguridad Social (CCSS), la Asociación Costarricense de Nefrología (ASCONE), la Sociedad Latinoamericana

Referencia

[5] Esta enfermedad también se llama enfermedad renal crónica de origen no tradicional (ERCnt). De igual manera, existen nombres regionales entre ellos, Nefropatía Mesoamericana, un término que se ha utilizado en Centroamérica y México desde el primer Taller internacional en 2012. En Sri Lanka, se ha denominado la enfermedad, Nefritis Intersticial Crónica en Comunidades Agrícolas (CINAC). Los organizadores del taller reconocen a todos los nombres utilizados para la enfermedad, sin embargo, se utiliza el término enfermedad renal crónica de etiología desconocida (ERCu) en el presente informe con la intención de simplificar el texto.

[6] Los números de publicaciones reflejan búsquedas en las bases de datos PubMed, Web of Science y Scopus de artículos indexados que describen investigaciones originales o revisiones relacionadas con la enfermedad renal crónica de etiología incierta y términos relacionados. Consulta de búsqueda completa disponible contactando a los editores del presente informe.

de Nefrología e Hipertensión (SLANH) y CENCAM. La capacitación se realizó en Liberia, Costa Rica y fue dirigida a médicos y trabajadores de salud quienes tengan contacto directo con pacientes de CKDu. Durante la actividad, se escuchó sobre las experiencias de las personas quienes cuiden pacientes con ERCu, se facilitó las discusiones sobre opciones del tratamiento del paciente, las formas de crear y mejorar los registros y los enfoques de colaboración entre las instituciones.

Contenido y estructura del taller

El taller comenzó con una sesión inaugural con autoridades de los países y agencias participantes y un evento denominado "World Café" (Café Mundo) diseñado para brindar la posibilidad a todos los participantes expresar perspectivas y compartir experiencias relacionadas con la investigación de ERCu. El taller incluyó varias presentaciones plenarias y sesiones matutinas opcionales que cubrían temas científicos específicos. Las sesiones de póster incluyeron a 41 resúmenes que presentaban información sobre la ciencia de la ERC; la patología; la epidemiología; tratamiento y prevención, demostrando la potencial de los esfuerzos actuales en estas áreas. Información más detallada sobre los resúmenes está disponible en el programa del taller. La mayor parte del tiempo durante los tres días se dedicó a grupos de trabajo compuestos por participantes con experiencia y conocimientos en temas científicos específicos. Este informe incluye los resúmenes de cada grupo de trabajo: Epidemiología Analítica; Biomarcadores de la Función Renal Anormal; Presentación Clínica y Tratamiento; Carga de Calor / Deshidratación y Carga de Trabajo; Enfoques Moleculares de ERCu: Genética, Epigenética y Enfermedades Infecciosas; Patología; Plaguicidas y Metales; y Vigilancia y Tamizaje. También se realizaron sesiones conjuntas las cuales combinaron dos o más grupos de trabajo, los informes de los cuales están incluidos en los resúmenes de los grupos de trabajo.

Grupos de trabajo

Epidemiologia Analítica

Las discusiones del grupo de trabajo de Epidemiología Analítica se centraron en mejorar la comprensión de varias áreas prioritarias relevantes para avanzar en la comprensión epidemiológica de ERCu. Las definiciones clínicas de enfermedad renal crónica (ERC) y lesión renal aguda (AKI por sus siglas en inglés) son relevantes para los estudios de casos y controles y la epidemiología clínica. Una prioridad adicional fue el estudio de las primeras etapas del daño renal necesario para comprender la historia natural de la ERC. Esto requiere el desarrollo de variables aceptadas como medidas biológicas para estudios poblacionales de daño renal temprano. Igualmente importante es el desarrollo de medidas estandarizadas de variables de exposición prioritarias que se hayan identificado como las más probables de estar relacionadas con ERCu o AKI. Las discusiones sobre los diferentes diseños de estudio favorecieron los estudios prospectivos y / o de intervención, ambos con poblaciones comunitarias y ocupacionales ya que pueden desempeñar un papel complementario. Finalmente, los mejores enfoques para los desafíos metodológicos discutidos se centran en el efecto de selección de trabajadores sanos, el tiempo entre la exposición y el evento detectable y la clasificación errónea en los estudios epidemiológicos de ERCu. Compartir información sobre el trabajo existente y planeado era una prioridad para promover la colaboración o elementos centrales comunes de los estudios para mejorar las comparaciones entre los resultados de las investigaciones.
Las prioridades para la investigación de ERCu señaladas por el grupo de epidemiología analítica fueron 1) Grandes estudios longitudinales que miden el cambio a corto plazo (días) en la creatinina sérica, el cambio a largo plazo (meses a años) mediante eGFR, realizando una evaluación de los protocolos de forma continua mediante entornos como el Tercero Taller internacional; 2) Estudios de intervención y su evaluación; 3) Dialogo abierto entre las diferentes investigaciones; 4) Compromiso profundo con los participantes del estudio comunitario y ocupacional; y 5) Establecer un repositorio de cuestionarios, protocolos y herramientas de participación comunitaria.

Biomarcadores de la Función Renal Anormal

El grupo de trabajo de Biomarcadores acordó que los biomarcadores desempeñan un papel clave en el diagnóstico y la investigación en todas las enfermedades renales, incluida la ERC. El grupo tuvo como objetivo describir la evidencia del uso de biomarcadores renales establecidos actualmente incluyendo a la creatinina sérica, la cistatina C y la albúmina urinaria, y analizar las fortalezas y debilidades relevantes de estos marcadores con respecto al ERCu. Aunque existe una clara necesidad de investigación, así como una probable utilidad clínica, para un marcador de temprana de la ERCu, la evidencia en apoyo del uso de cualquiera de estos nuevos biomarcadores es limitada. El grupo acordó que el diagnóstico de ERCu junto con los estudios de detección, carga y asociaciones se basarán en estimaciones de la función glomerular estimado mediante los marcadores séricos, al menos en el futuro previsible. Por lo tanto, todos los investigadores en el campo necesitan una comprensión clara de los factores que podrían influir en la interpretación de estas mediciones.

Presentación Clínica y Tratamiento

El grupo de trabajo de Presentación Clínica y Tratamiento discutió las características clínicas de ERCu, las similitudes y diferencias en las características clínicas reportadas en las diferentes regiones geográficas, y las áreas donde no hay evidencia suficiente para crear una definición o definiciones de caso exhaustivas. Dada la falta de guías clínicas publicadas para el manejo de ERCu en el momento de esta reunión, el grupo presentó un consenso sobre las características clínicas que deberían incitar a los médicos a considerar la posibilidad de una diagnosis de ERCu en un paciente, así como recomendaciones de manejo cuando se sospecha de ERCu.

El grupo acordó que la comprensión actual de la ERCu es limitada, y queda por ver si a ERCu es una enfermedad única o una colección de enfermedades con características clínicas similares, ya que este es a menudo el caso en una multiplicidad de escenarios de enfermedad renal crónica avanzada. Las similitudes en las características de la ERCu en India, Sri Lanka y Mesoamérica incluyen una relación hombre-mujer similar; concordancia familiar; la vivencia en la pobreza; residencia rural, ocupaciones que involucran trabajo físico; hipocalemia; hiponatremia; proteinuria mínima y piuria estéril. Las diferencias entre las regiones incluyen la distribución variable por edad y la presentación de hiperuricemia y cristaluria de urato, las dos últimas solo se ha informado en Mesoamérica. A pesar de las limitadas biopsias renales disponibles, los pacientes parecen demostrar consistentemente nefritis tubulointersticial no específica con un infiltrado linfocítico. Los médicos en ambas regiones describieron episodios concurrentes de lesión renal aguda (AKI) y se ha planteado la hipótesis de que pueden presagiar una ERCu progresiva.

El grupo acordó que el manejo clínico en las primeras etapas de la ERC se centra en la hidratación adecuada con agua limpia, la minimización de la exposición al calor y los agroquímicos, la corrección de las alteraciones electrolíticas y la evitación de nefrotoxinas, especialmente medicamentos antiinflamatorios no esteroideos y medicamentos a base de hierbas. Los nefrólogos tratantes del grupo informaron que el diagnóstico oportuno y la atención adecuada de la ERC es muy probable que retrasen la progresión de la enfermedad renal, aunque actualmente no hay datos suficientes para respaldar esto. El grupo señaló la necesidad de 1) estudios más sistemáticos e integrales sobre los aspectos clínicos de ERCu; 2) la inclusión de médicos en las regiones afectadas en estudios de investigación; 3) informe centralizado de la enfermedad; 4) mayor capacidad de biopsia renal local; y 5) una mayor colaboración entre científicos básicos y ambientales, nefrólogos y nefropatólogos con experiencia en esta enfermedad, incluidas las asociaciones regionales, nacionales e internacionales.

El grupo discutió la necesidad de políticas centradas en las protecciones de salud y seguridad ocupacional, particularmente en profesiones con altos niveles de exposición al calor; provisión de servicios básicos con un enfoque en agua potable segura y adecuada; y el desarrollo de infraestructura de salud para aumentar el acceso a la atención médica en las regiones afectadas por ERCu. Se necesita más investigación para comprender mejor la fisiopatología, la historia natural y el tratamiento de la enfermedad.

Carga de Calor/Deshidratación y Carga de Trabajo

El estrés térmico por calor y la deshidratación han estado en el centro de atención recientemente a nivel mundial debido a la evidencia emergente sobre su asociación con problemas adversos de salud renal, incluida la enfermedad renal crónica, especialmente en poblaciones laborales. La probabilidad de estrés térmico por calor es mayor cuando la temperatura del ambiente supera la temperatura central de una persona, una situación común para los trabajadores al aire libre en entornos tropicales donde las temperaturas pueden superar fácilmente los 37 ° C.

El grupo de trabajo revisó 36 estudios epidemiológicos que incluyeron el calor como un posible factor de riesgo para efectos negativos en la salud renal, incluida la enfermedad renal crónica. Estudios epidemiológicos en América Central han reportado impactos adversos de estrés térmico por calor en los trabajadores agrícolas, especialmente pero no solamente en la industria de la caña de azúcar. Además de la exposición a altas temperaturas, existe evidencia de que las altas tasas de sudoración y la ingesta limitada de líquidos pueden conducir a lesiones renales agudas (por sus siglas en inglés) funcionales y subclínicas, y que las AKI repetitivas a diario también pueden conducir a una función renal comprometida y finalmente terminar en el desarrollo de enfermedad renal crónica (ERC). También se ha informado que el estrés por calor y la alta carga de trabajo pueden causar AKI y acelerar la progresión de la enfermedad en poblaciones con enfermedades preexistentes causadas por hipovolemia.

Aunque el papel específico del calor, la deshidratación y la carga de trabajo en la vía causal de la ERCu continúan siendo debatidos, su asociación con la ERC y la ERCu se ha demostrado repetidamente. Los estudios publicados hasta la fecha han utilizado una amplia gama de métodos para medir el calor, la deshidratación, la carga de trabajo y la salud renal, lo que dificulta la comparación de los resultados del estudio y la evaluación del estrés térmico por calor/deshidratación como parte de la vía causal.

Existe una amplia gama de equipos, cuestionarios, mediciones cuantitativas y metodologías para medir el estrés por calor y la deshidratación. Su uso varía según el costo, la factibilidad en el campo y otros factores logísticos. Tener un método estandarizado para medir los indicadores cualitativos y cuantitativos de la exposición al calor, la carga de trabajo y la deshidratación ayudará a la comunidad científica a comprender el vínculo que estos factores tienen con la enfermedad. Cuando sea posible, se deben utilizar dispositivos del estándar de oro (por ejemplo, píldora de temperatura basal, la temperatura globo de bulbo húmedo, la frecuencia cardíaca y acelerómetros). Del mismo modo y con el fin de aumentar la precisión, se deben utilizar como variables dependientes (outcome measures) medidas válidas y confiables (p. Ej., Gravedad específica de la orina en lugar de una tabla de colores o en lugar del auto-informe del consumo de agua). Se necesitan intervenciones específicas para el calor en el lugar de trabajo para abordar la prevención y la progresión de la enfermedad, especialmente a nivel local, donde la pobreza y el acceso reducido a los recursos y la atención médica complican la situación de las comunidades afectadas.

Enfoques moleculares de ERCnt: genética, epigenética y enfermedades infecciosas

Las técnicas moleculares avanzadas que han transformado gran parte de la biomedicina en el siglo XXI no se han empleado ampliamente en la búsqueda de las causas de la ERCnt (enfermedad renal crónica de origen no tradicional). La mayoría de los estudios hasta la fecha se han centrado en hipótesis únicas utilizando una combinación de evaluación mediante cuestionario de los antecedentes de exposición y pruebas directas de una cantidad limitada de variables biológicas. Por el contrario, muchas de las técnicas moleculares no están limitadas por la necesidad de hipótesis específicas de antemano porque pueden evaluar simultáneamente cientos, miles o incluso millones de preguntas en paralelo. Este es beneficio tremendo, pero también plantea varios desafíos para los investigadores de la ERCnt, particularmente en relación con la necesidad de grandes tamaños de muestras, la recolección de tipos de tejidos específicos, los costos altos y la necesidad de equipos interdisciplinarios con experiencia amplia y profunda. Este grupo de trabajo consideró las lagunas existentes en el campo y evaluó críticamente cómo las metodologías genéticas, epigenéticas, "-ómicas", de detección de patógenos y de otra naturaleza podrían ayudar a identificar las causas de la ERCnt.

Genética: la identificación de genes que hacen que los individuos sean más susceptibles a las causas ambientales de la enfermedad puede ayudar a identificar esas causas ambientales al enfocar las hipótesis en las exposiciones que están relacionadas con la función de los genes o al enfocar los análisis en las interacciones gen-ambiente. A pesar de los desafíos, el grupo de trabajo acordó que el descubrimiento de genes debe abordarse a través de estudios de todo el genoma utilizando matrices de todo el genoma desarrolladas recientemente que sean rentables. Al mismo tiempo, se observó que la cobertura para variantes específicas de la población puede ser subóptima, especialmente en áreas geográficas poco estudiadas como Centroamérica. Los datos de secuenciación del genoma completo podrían realizarse en un subconjunto de individuos y usarse para mejorar la imputación de variantes en todos los participantes del estudio utilizando haplotipos inferidos específicos del estudio. Si bien la genética puede desempeñar un papel positivo e importante en ayudar a identificar los factores que causan la ERCnt, también existe la posibilidad de consecuencias negativas, como la discriminación laboral, el estigma y la angustia psicológica. Los investigadores deben trabajar con expertos en estas áreas para minimizar la probabilidad de estos resultados perjudiciales.

Epigenética: al regular cuándo se expresan los genes y en qué cantidades, las modificaciones que componen el epigenoma pueden tener efectos importantes sobre la salud y la enfermedad. Las herramientas epigenéticas se han desarrollado recientemente y son muy prometedoras para estudiar la salud y la enfermedad. En opinión del grupo de trabajo, las matrices de metilación de ADN de todo el genoma representan la técnica con más probabilidades de ser potente y útil para el estudio de la ERCnt en el corto plazo. Estos estudios podrían usarse para identificar exposiciones ambientales específicas y junto con otras metodologías, como estudios genéticos, para comprender el impacto ambiental en la expresión genética. Debido a que la ERC por sí misma puede afectar los patrones epigenéticos debido a cambios en la excreción de moléculas (como toxinas) que normalmente excreta el riñón, es probable que los estudios longitudinales con medición en varios momentos específicos sean el mejor diseño de estudio para este propósito.

"-ómicas": las tecnologías como la metabolómica, la exposómica y la proteómica pueden revelar potencialmente exposiciones desconocidas al identificar la exposición en sí misma o al observar cómo la exposición altera los procesos fisiológicos normales y los metabolitos posteriores. Esto puede ser clave en la búsqueda de causas prevenibles de la ERCnt, ya que las comunidades y los trabajadores afectados enfrentan múltiples exposiciones complejas. Las principales herramientas disponibles para la metabolómica son la espectrometría de masas y la resonancia magnética nuclear (RMN). La espectrometría de masas es una técnica muy sensible que puede resultar muy adecuada para detectar factores ambientales en el rango nanomolar. La RMN, aunque menos sensible, es muy cuantitativa y versátil con respecto a la naturaleza de los compuestos que puede identificar y puede ser especialmente útil para obtener instantáneas de un sistema completo de metabolitos. Estas técnicas pueden aplicarse a orina, suero o incluso tejido. Además, la metabolómica podría ayudar en la caracterización del exposoma, definido como la totalidad de las exposiciones ambientales desde la concepción en adelante. Como se señaló anteriormente, los estudios prospectivos longitudinales con medidas repetidas pueden ayudar mejor a aclarar la cronicidad de los cambios.

Patógenos: el grupo de trabajo estuvo de acuerdo en que las enfermedades infecciosas son un contribuyente potencial plausible pero poco estudiado de la ERCnt. Los cambios en el clima, la alta densidad de vectores y reservorios de enfermedades infecciosas, el acceso limitado a agua potable, los recursos limitados para detectar y controlar los brotes de enfermedades infecciosas, la aparición continua de nuevos patógenos y variantes de patógenos desafían cada vez más la salud renal en entornos tropicales y de bajos recursos. Si bien se han realizado algunos estudios centrados en las pruebas de detección de patógenos candidatos conocidos (p. ej., leptospira, hantavirus), un conjunto pequeño pero creciente de hallazgos inconsistentes sugiere que abordar un rango más amplio sería más productivo. El grupo de trabajo se mostró entusiasmado por avanzar hacia una evaluación imparcial, con análisis de control de casos y criterios de clasificación bien definidos. Sin embargo, las pruebas multiplexadas focalizadas (es decir, la combinación de la detección de múltiples patógenos en una sola reacción de PCR) también serán informativas si la secuenciación no focalizada no es factible. Serán importantes las asociaciones para ampliar la disponibilidad de tecnología a investigadores con menos recursos y para compartir/combinar bioespecímenes para lograr tamaños de muestra más grandes, al igual que clasificar correctamente el estado de la infección (p. ej., aguda/actual, pasada) y asignar la temporalidad (es decir, la infección ocurrió antes de la ERCnt).

Patología

El grupo de grabajo de Patología involucró a un pequeño grupo de nefropatólogos, nefrólogos clínicos y patólogos toxicológicos para comparar observaciones y perspectivas sobre las características patológicas clave de ERCu. Los miembros del grupo tenían experiencia revisando material de biopsia de pacientes con ERCu en Nicaragua, El Salvador y Sri Lanka. Los patólogos toxicológicos del Programa Nacional de Toxicología (NTP) de EEUU tenían experiencia en el modelado de la enfermedad renal experimental inducida por agentes ambientales en estudios con animales. Los objetivos principales del grupo de trabajo fueron 1) utilizar experiencias colectivas para identificar características clave y consistentes entre las muestras estudiadas; 2) especular sobre posibles patogénesis con la intención de hipotetizar los contribuyentes etiológicos; 3) desarrollar un esquema histopatológico descriptivo para la caracterización de la biopsia; 4) discutir oportunidades para construir una asociación global; y 5) discutir enfoques para la investigación mecanicista.

El grupo consideró los detalles de las características microscópicas y ultraestructurales del material de biopsia que revisaron previamente de pacientes con sospecha de ERCu, de biopsias con aguja percutánea que en su mayoría representan la corteza renal. Los cambios patológicos en las muestras revisadas demostraron muchas características consistentes, la hiperplasia del músculo liso y la hialinosis arteriolar, pero no es un hallazgo consistente. En las biopsias renales de pacientes considerados con ERCu aguda, los hallazgos principales se describieron como nefritis tubulointersticial con diversos grados de inflamación aguda y cambios tubulointersticiales crónicos. Se compartió otras investigaciones, incluida la búsqueda preliminar del Programa Nacional de Toxicología (NTP) de cambios renales relacionados con la exposición a plaguicidas y metales pesados en roedores utilizando datos archivados.

Adicionalmente, el grupo discutió las brechas críticas y cómo abordarlas, sugiriendo una plantilla estándar para la caracterización de la biopsia renal ERCu, un atlas de imágenes microscópicas y ultraestructurales para garantizar una caracterización consistente de las biopsias en todo el mundo, y el desarrollo de un sistema electrónico fácil de usar con una plantilla que podrían usar los patólogos quienes revisarán biopsias de pacientes con ERCu. También se discutió el valor de un repositorio central para imágenes de biopsias, datos de pacientes y caracterizaciones de biopsias, pero con desafíos notables para la adopción e implementación. El grupo reconoció la importancia de involucrar y educar a los médicos en las localidades donde se cree que ocurre la enfermedad y sugirió la idea de desarrollar un módulo de capacitación. Por último, los participantes discutieron la necesidad de una investigación mecanicista y cómo eso podría mejorar la comprensión actual de la etiología y la patogénesis de ERCu. Aunque se postularon estudios en animales, habría que considerar los retos de bienestar animal. El grupo esperaba continuar como un espacio para colaboración y consultas internacionales con patólogos quienes no asistieron al taller. De igual manera, el grupo consideró que el mayor impacto se podría lograr mediante un esfuerzo continuo de los participantes del grupo de trabajo de Patología con participantes del grupo de trabajo.

Plaguicidas y Metales

El grupo de trabajo sobre Plaguicidas y Metales tuvo como objetivo revisar y discutir el estado de la ciencia que aborda el potencial de los plaguicidas o metales para afectar negativamente la función renal y la susceptibilidad potencial a la ERCu, comparar los resultados de la investigación en todas las regiones (Mesoamérica, Sri Lanka e India), discutir metodologías integradas, evaluar la exposición a contaminantes en entornos de investigación de ERCu y explorar posibles asociaciones e interacciones con otros factores de riesgo de ERCu, particularmente contaminantes y estrés por calor.

En términos generales, el grupo discutió el potencial de los plaguicidas para actuar como factores de riesgo ambientales asociados con ERCu, y en ciertos casos, discutió plaguicidas específicos. Los agroquímicos como los fertilizantes, las hormonas de crecimiento y los componentes de la formulación comercial que no sean ingredientes activos no se abordaron específicamente y podrían considerarse en futuros debates. La revisión de

estudios en Mesoamérica, Sri Lanka e India no encontró evidencia concluyente de que los plaguicidas causen ERCu. El glifosato, el herbicida más utilizado en todo el mundo, sigue siendo motivo de preocupación y existe evidencia epidemiológica limitada que evalúa una posible asociación con ERCu.

El grupo también discutió las formulaciones de plaguicidas y señaló que los estudios de formulaciones de plaguicidas comerciales son complicados porque son heterogéneos en todas las marcas y consisten en una combinación de ingrediente (s) pesticida (s) activo (s), una variedad de ingredientes 'inertes' y posiblemente contaminantes metálicos como el arsénico. , cadmio y plomo. Los participantes del grupo de trabajo concluyeron que existe la necesidad de diseños de estudio más robustos para evaluar el potencial papel de los plaguicidas y otros agroquímicos en la etiología de ERCu en las regiones afectadas, en combinación con otros factores de riesgo. En la discusión sobre la importancia de los metales y ERCu, los participantes del grupo de trabajo propusieron dos puntos de vista, ambos enfatizando la necesidad de una evaluación de exposición de alta calidad. Un grupo pensó que era improbable que los metales fueran un importante impulsor de las epidemias de ERCu y enfatizó la importancia de una evaluación de exposición sólida y diseños epidemiológicos en regiones con alto riesgo de ERCu para permitir una mejor comprensión previa la investigación de posibles interacciones. Otros pensaron que los metales pueden estar involucrados en la etiología de ERCu y enfatizaron que, incluso si los niveles de exposición ambiental son bajos, la exposición diaria podría ser moderada-alta con un mayor consumo de agua o una inhalación más intensa. Además, múltiples metales están presentes en niveles bajos-moderados en la mayoría de los entornos y se desconoce si las mezclas de metales actúan de forma sinérgica o antagónica con otros factores de riesgo en relación con la lesión renal.

Hubo acuerdo sobre la importancia de diseñar estudios interdisciplinarios, como los estudios geológicos que recolectan muestras que son útiles en estudios de salud o el aprovechamiento de estudios sobre otras enfermedades que tienen especímenes biobancos disponibles para investigaciones auxiliares. Es importante destacar que se deben realizar estudios para contrastar exposiciones en áreas de alta y baja prevalencia. El grupo recomendó medir la exposición a metales y plaguicidas de manera integrada y considerar las interacciones entre plaguicidas y metales, así como las mezclas de químicos ambientales en el modelado estadístico de los estudios de ERCu. El momento de las exposiciones se consideró importante ya que las exposiciones tempranas pueden afectar la susceptibilidad a la ERCu. Hubo un amplio acuerdo en que el estado epidémico de ERCu justifica la intervención y que, cuando existe evidencia sugestiva de factores de riesgo asociados con ERCu, se debe trabajar para reducir estas exposiciones y, con suerte, la carga de ERCu, mientras se avanza en nuestra comprensión de la etiología.

Las recomendaciones generales del grupo de trabajo fueron: 1) proponer un conjunto de información central que debería recopilarse sobre exposiciones ocupacionales y ambientales a plaguicidas y metales a través de los estudios; 2) realizar una investigación interdisciplinaria para aprovechar la experiencia y los recursos actuales; 3) permitir el intercambio de datos y muestras para exposiciones ambientales y ocupacionales (para estudios pasados y futuros); 4) identificar sitios con y sin ERCu para comparar los factores de riesgo entre las poblaciones de estudio y con el tiempo; y 5) priorizar los estudios de intervención sobre factores modificables relacionados con ERCu.

Vigilancia y Tamizaje

El grupo de trabajo de vigilancia y tamizaje contó con participantes de ocho países (Costa Rica, El Salvador, Guatemala, Honduras, México, Nicaragua, Panamá y Sri Lanka) y: 1) revisó los estudios de prevalencia y los datos de tamizaje; 2) propuso recomendaciones para futuros estudios o vigilancia; y 3) abordaron aspectos de ética relacionados con la vigilancia y detección de ERCu en poblaciones vulnerables.

El progreso en la vigilancia y detección de ERCu se ha producido de diferentes maneras en cada uno de los países afectados. Se destacaron dos iniciativas: la OPS ha publicado una definición inicial de caso y una metodología para la vigilancia de la salud pública; y el protocolo DEGREE para estudios de prevalencia de ERCu se ha desarrollado y actualmente se está probando en varios países.

El grupo recomienda que cualquier institución que establezca un sistema de vigilancia ERCu (SS por sus siglas en español) considere las siguientes preguntas: 1) ¿Qué tipo de vigilancia se necesita? 2) ¿Cuáles serán las poblaciones meta? 3) ¿Qué definición de ERCu se utilizará? 4) ¿Cómo se abordará el problema deERC vs. ERCu? y 5) ¿Cuáles son los objetivos de las SS?

Es importante utilizar protocolos estándar para la definición y evaluación de casos en los diferentes contextos de investigación clínica, vigilancia, detección e investigación epidemiológica. También es importante identificar participantes clave de diferentes sectores en la SS para garantizar la sostenibilidad del sistema y vincular los resultados con la acción crítica. La necesidad de vincular SS y la investigación también se consideró esencial dado los mecanismos causales poco claros de la ERCu. Además, el grupo abordó aspectos de la ética relacionados con la vigilancia y detección de ERCu en poblaciones en condiciones de vulnerabilidad, destacando la necesidad de desarrollar directrices nacionales claras para vincular los resultados de las actividades de vigilancia y detección de ERCu con las políticas, programas y actividades apropiadas.

Representantes de Guatemala, Nicaragua, Honduras, Costa Rica, Panamá, El Salvador, México y Sri Lanka informaron nuevos datos sobre ERC y ERCu a nivel nacional y, en algunos casos, subnacional. Algunos de los países han implementado sistemas de vigilancia incipientes para ERCu, y El Salvador incluyó preguntas sobre ERCu y riesgos no tradicionales en una encuesta nacional de STEP-WISE sobre enfermedades no transmisibles.

El grupo también revisó los estudios de prevalencia y los datos de detección publicados o disponibles desde el Segundo Taller Internacional. Para hacer eso, los estudios ecológicos, transversales y de cribado seleccionados por revisiones sistemáticas publicadas se utilizaron como base para la discusión. Un problema importante identificado fue la falta de comparabilidad entre los estudios para la mayoría de los parámetros.

Se han logrado grandes avances en la recopilación y el análisis de los registros de los servicios de salud en cada país y estos desarrollos deben ser apoyados y fortalecidos. Sin embargo, aún se necesita mucho trabajo para obtener datos comparables de prevalencia de la población. Se recomienda que el protocolo DEGREE sea el protocolo central (con cuestionarios adicionales para las necesidades específicas del estudio) para las encuestas de prevalencia. Además de ser una parte clave de la vigilancia, tales encuestas son una parte clave de la búsqueda de las causas de la ERCu, ya que pueden identificar poblaciones de alto riesgo que podrían ser objeto de futuras investigaciones. Dichas encuestas también pueden identificar posibles "puntos críticos" de la enfermedad y pueden usarse para generar hipótesis. Existen consideraciones éticas específicas para los diferentes tipos de vigilancia y detección. La necesidad de vincular la vigilancia con la atención médica es de particular importancia para disminuir la morbilidad y mortalidad de la enfermedad.

Conclusiones

Los logros más importantes del Tercer Taller Internacional sobre ERCu incluyeron interacciones cara a cara en grupos pequeños y grandes y los esfuerzos productivos de los grupos de trabajo en áreas científicas enfocadas, actualización del estado del arte por expertos de varios países y disciplinas, y un aumento en el número y alcance de las instituciones involucradas en el taller. La ERCu sigue siendo una crisis de salud pública y una comprensión clara de su etiología sigue siendo difícil de alcanzar. Las vidas que se han perdido y el costo para las familias y las comunidades no han disminuido, a pesar de los avances en investigación, salud pública y esfuerzos clínicos en la última década. El Segundo Taller Internacional (Program on Work Environment and Health in Central America (SALTRA) et al., 2015) reportó una alta prioridad para los enfoques regionales coordinados para estudiar la prevalencia , etiología y evaluación de intervenciones. Estas prioridades siguen avanzando desde este taller.
A pesar de la incremento en conocimiento y la experiencia compartida sobre la ERCu hasta la fecha, la evidencia científica es insuficiente para detener la enfermedad. Seguimos creyendo que la investigación interdisciplinaria que involucra a científicos y clínicos internacionales es fundamental para permitir el progreso lo más rápido posible. El objetivo de este documento es resumir la información y los puntos de vista más actualizados provenientes del taller con el fin de asegurar que futuras investigaciones sean realizadas en base de los esfuerzos anteriores.

Workshop Purpose, Content, and Structure

Background

Chronic kidney diseases of uncertain etiology (CKDu)[7] reflects a pattern of endemic kidney diseases described as progressively declining glomerular filtration rate (GFR) with no or mild proteinuria, and the absence of typical risk factors for CKD (e.g., hypertension, diabetes, immune-mediate glomerular disease, and heritable renal disease). Although unclear whether it is the same disease in all regions of the world, clinically similar hotspots have been most extensively described in rural communities along the Pacific Coast of Mesoamerica and in specific regions of Sri Lanka and India (N. T. Athuraliya et al., 2011; Badurdeen et al., 2016; Fischer et al., 2017; Johnson, Wesseling, and Newman, 2019; Kupferman et al., 2016; Pearce et al., 2019; Trabanino, Aguilar, Silva, Mercado, and Merino, 2002). Though not entirely restricted to agricultural workers, the disease appears to be most prevalent in field workers in hot and humid climates. Clinically, these workers present as relatively young men (20-60 years old, varying by region) with a non- or minimally proteinuric nephropathy characterized by a progressively declining glomerular filtration rate. Usual risk factors for chronic renal disease (e.g., hypertension, diabetes mellitus, obesity, and advanced age) are generally absent.

Pre-workshop meeting for clinical training

A pre-workshop training meeting in Liberia, Costa Rica, was held on March 19, 2019, for 120 health care providers and personnel responsible for health surveillance in a CKDu-endemic region. This event was jointly organized by the Costa Rican Health Care and Social Security System (CCSS), the Costa Rican Association of Nephrologists, the Latin American Society of Nephrology and Hypertension (SLANH), and CENCAM. Dr. Ricardo Correa-Rotter (CENCAM /SLANH, México), Dr. Ramón García-Trabanino (CENCAM/SLANH, El Salvador), and Dr. Jennifer Crowe (CENCAM, Costa Rica) gave presentations. The training allowed for a discussion of patient treatment, ways to improve registries, and approaches to collaboration across institutions.

Reference

[7] This illness is also called *chronic kidney disease of non-traditional origin (CKDnt)*. Regional names also exist including, *Mesoamerican Nephropathy (MeN)*, a term that has been in use in Central America and México since the first International Workshop in 2012. In Sri Lanka, it has been called *Chronic Interstitial Nephritis in Agricultural Communities (CINAC)*. Workshop organizers recognize all of the names used for the disease; however, the term *chronic kidney disease of unknown etiology (CKDu)* in this report with the intent to simplify the text. Some exceptions are made in working group reports.

History of workshops

This workshop built on previous international workshops organized by SALTRA. The first took place in Nicaragua in 2005 (Program on Work Environment and Health in Central America (SALTRA), Central American Institute for Studies on Toxic Substances (IRET), and Universidad Nacional (UNA), 2005), and the second took place in Costa Rica in 2009 (Program on Work Environment and Health in Central America (SALTRA), Central American Institute for Studies on Toxic Substances (IRET), and Universidad Nacional (UNA), 2009), including nephrologists, occupational health, and epidemiologists from the Central American region. These were followed by larger workshops in Costa Rica in 2012 (Program on Work Environment and Health in Central America (SALTRA), Central American Institute for Studies on Toxic Substances (IRET), and Universidad Nacional (UNA), 2012) and 2015 (Program on Work Environment and Health in Central America (SALTRA) et al., 2015), a workshop on CKDu organized by SEARO in 2016 (World Health Organization (WHO), 2016), and a 2018 workshop organized by NIDDK and NIEHS (National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and National Institute of Environmental Health Sciences (NIEHS), 2018). Previous workshops have aimed to summarize scientific knowledge or hypotheses about the possible etiologies of the disease and increase cross-disciplinary and international collaborations. This 2019 meeting had the highest attendance of any meeting on CKDu to date, and included the greatest diversity across CKDu impacted regions, with participants from Sri Lanka, India, Mexico, and Central American countries, as well as researchers from the United States and Europe.

Meeting objectives

The specific aims of the 2019 Third International Meeting were to:

- Update the current knowledge and knowledge gaps related to Mesoamerican Nephropathy and other CKDu epidemics identified around the globe.
- Discuss research agendas to compare disease occurrence between different countries and fill knowledge gaps (including evidence for potential risk factors of CKDu, methodological considerations, and current understanding of CKDu etiology).
- Increase and facilitate collaborations and relationships between researchers and clinicians studying and involved in the care of CKDu.
- Compile and communicate evidence that can be used by policymakers to improve prevention and treatment of CKDu epidemics.
- Take advantage of the presence of experts at the workshop to organize a pre-workshop course to share existing knowledge about CKDu and related topics.

Agenda and meeting materials

The workshop was intended to be a space for productive collaboration, and as such, much of the three-day event was dedicated to working group discussions. The complete program can be found on the workshop website: www.niehs.nih.gov/news/events/pastmtg/2019/ckd_2019/index.cfm.

The Meeting Book includes the agenda, poster abstracts, working group descriptions, and list of participants: www.niehs.nih.gov/news/events/pastmtg/assets/docs_c_e/ckd_meetingbook_508.pdf.

Inaugural session

The meeting opened with remarks from:

1. Dr. Denis Angulo Alguera, Vice Minister of Health, Costa Rican Ministry of Health
2. Dr. Linda Birnbaum, Director, National Institute of Environmental Health Sciences (NIEHS) and National Toxicology Program (NTP), National Institutes of Health (NIH), U.S.
3. Dr. Enrique Pérez-Flores, Pan American Health Organization (PAHO)/World Health Organization (WHO), Costa Rica
4. Dr. Nalika Gunawardena, in representation of Dr. Poonam Khetrapal Singh, Regional Director, WHO South-East Asia Region (SEARO)
5. Dr. Norman Solorzano, Representative, Vice Rectory for Research, Universidad Nacional Costa Rica (UNA)
6. Dr. Ricardo Correa-Rotter, Consortium for the Epidemic of Nephropathy in Central America and Mexico (CENCAM)
7. Dr. Jennifer Crowe, Central American Program for Health Work and Environment Program (SALTRA), IRET-Universidad Nacional, Costa Rica

Inaugural session with comments from the institutions represented on the workshop organizing committee. Left: Opening comments from NIEHS director, Dr. Linda Birnbaum. Middle: Dr. Nalika Gunawardena delivers a message from Dr. Poonam Khetrapal Singh, Regional Director, WHO South-East Asia Region at the opening ceremony. Right: The inaugural session was followed by a keynote presentation from Dr. Catharina Wesseling, La Isla Network/ Karolinska Institutet, Sweden.

World café

Since workshop participants came from a wide variety of disciplines, countries and languages, the first interactive activity involved round table discussions following a "World Café" format (The World Café, 2019), designed to allow participants to meet as many new people as possible and to express their opinions and thoughts about three main questions: What do we know about the disease? What do I/we need to know about the disease? How can I/we move forward? This session enabled communication across the 137 workshop attendees. Highlighted points expressed in this session were listed in the published brief summary report (Crowe et al., 2019) for the workshop and also incorporated in the content of this report.

Morning sessions

Optional morning sessions were available for all workshop participants and most included bilingual presentations or simultaneous translation. The first three sessions listed were designed as introductory sessions for trainees and researchers who do not have prior experience on the topic. The last two sessions in the list were designed for all workshop participants.

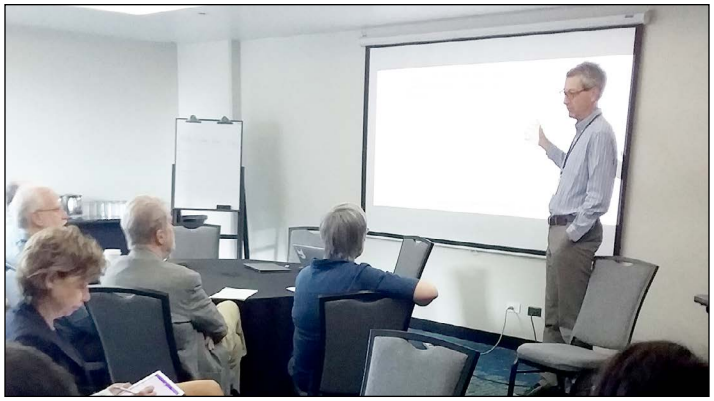
1. Heat exposure assessment for epidemiology studies, led by Dr. Vidhya Venugopal, Sri Ramachandra University, India; Dr. Daniel Rojas, Universidad Nacional, Costa Rica; Dr. Andrés Robles, SALTRA, TEC, Costa Rica

2. Epidemiology study design, led by Dr. Ben Caplin, University College London; Dr. Marvin González-Quiroz, Research Center on Health, Work, and Environment (CISTA)/UNAN-León, Nicaragua

3. Pesticides and agrochemicals exposure assessment, led by Dr. Aurora Aragon, Research Center on Health, Work, and Environment (CISTA)/UNAN-León, Nicaragua; Dr. Katherine James, University of Colorado-Anschutz Medical Campus; Dr. Alison Sanders, Icahn School of Medicine at Mount Sinai; Dr. Andres Cardenas, University of California, Berkeley; Dr. Lesliam Quiros-Alcala, University of Maryland

4. Update on biomarkers of renal function, led by Dr. Carl-Gustaf Elinder, Karolinska Institutet, Sweden

5. Ethical considerations in CKDu research, organized by Dr. Agnes Soares and Dr. José Escamilla of PAHO, with a presentation from Costa Rican bioethicist Mr. Jorge Villalobos Alpízar



Morning training sessions were held for new researchers as well as experts. Dr. Carl-Gustaf Elinder (Sweden) led a session on biomarkers.

Policy session

The policy session was intended as a space for discussion about the potential implications of research findings and workshop conclusions for intersectoral public policy decisions in CKDu-impacted countries. It provided a platform to learn from regional and national mechanisms of policy-making processes, and to explore how researchers and policy makers from different sectors could play a role in those processes. While recognizing that each country has its own dynamics and contexts that affect policymaking related to CKDu, this session was designed to discuss the opportunities presented by existing national and international technical and political integration mechanisms where the workshop conclusions could be presented, translated, and incorporated into public policymaking.

During the policy session, Dr. Olivia Brathwaite, PAHO Subregional Adviser for Non-Communicable Diseases for Central America and Dominican Republic presented an overview of the intersectoral response to CKDu in Central America. Intersectoral represented actions of government, private, nonprofit organizations, and other entities outside of the health sector that may impact CKDu-related policy and public health action.

The session included presentations from the following:

1. Dr. Denis Angulo Alguera, Vice-Minister of Health in Costa Rica

2. Dr. Asela Iddawela, Project Director of the Presidential Task Force on Prevention of Chronic Kidney Disease, Sri Lanka

3. Dr. Olivia Brathwaite, PAHO Subregional Adviser for Non-Communicable Diseases for Central America and Dominican Republic

Costa Rica´s Vice-Minister of Health opened the session with a welcome and announced that legislation had just been signed regarding a case definition for CKDu that will allow for official CKDu surveillance soon. Opening comments were followed by presentations and a panel discussion moderated by Olivia Brathwaite. Jennifer Crowe and Agnes Soares served as coordinators of the session.

Panel discussion

A panel discussion on collaboration and cooperation took place on the final day of the workshop to discuss the successes and barriers in collaborative studies on CKDu and potential next steps. The panel included one representative from each working group, WHO, and NIH. Panelists and audience members were encouraged to think creatively, beyond financial restrictions, regarding what could be considered in collaborative research agendas. An example question was posed, "If money is not an issue, what would you prioritize for collaborative research, with the ultimate goal of reducing CKDu?" Big picture considerations as well as smaller scale research priorities were discussed. Recommendations from this panel included:

1. Improve and standardize exposure assessment across studies.

2. Promote the use of harmonized core questionnaires in epidemiological studies.

3. Identify early characteristics of disease and conduct extensive follow up of patients to better understand clinical trajectories.

4. Strengthen government CKDu public health surveillance tied to health care access for patients to known occupational and environmental risks.

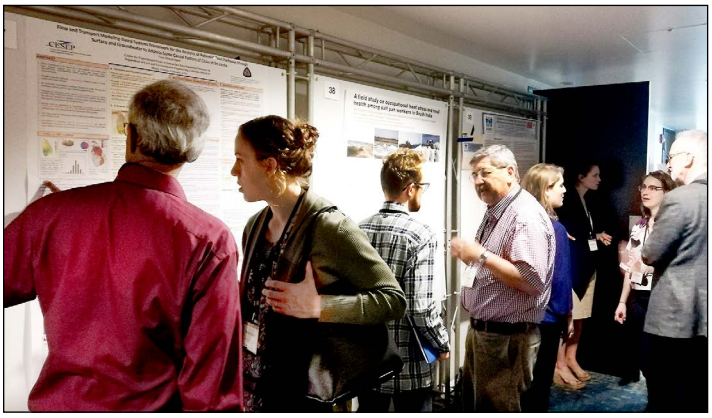
5. Continue the network of CENCAM and partners.

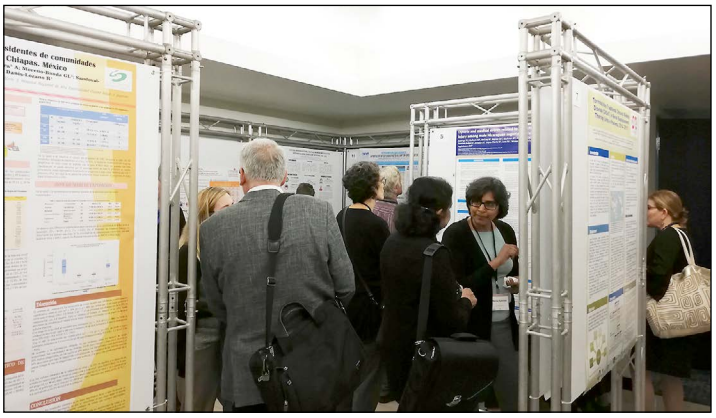
The last day of the workshop included a scientific summary by CENCAM president, Dr. Ricardo Correa-Rotter (left) and a panel with presentations from all working groups, as well as WHO and NIH, led by Dr. Bonnie Joubert of NIEHS (right).

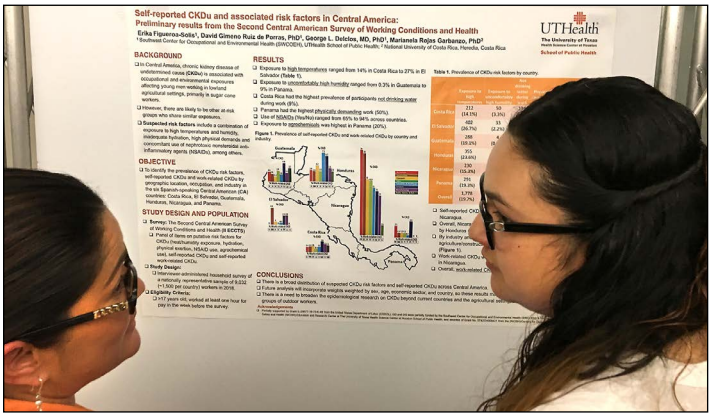
More detailed points also covered expanded surveillance, interventions, pathology studies, and additional omics data in epidemiology studies. Some of the barriers to collaboration raised in the discussion included competition across scientists for publications; the diversity of CKDu across affected countries; coordination across institutions and agencies; language barriers; and limited resources in affected communities. Studies of the disease are likely impacted by confounding and bias which need to be planned for, and causality is challenging to establish. Despite these challenges, more research is clearly needed, as well as intervention and policy efforts.

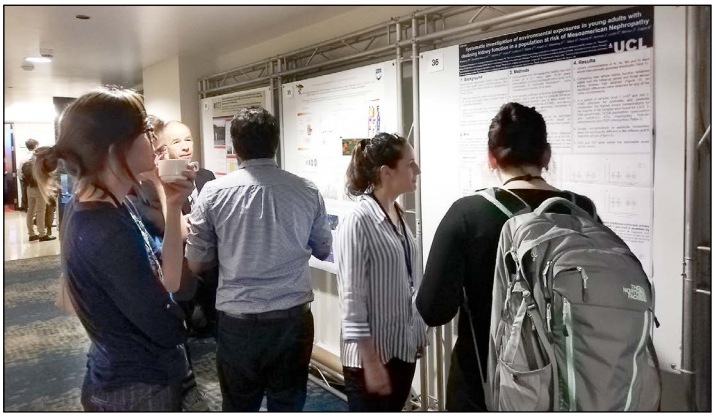
Posters

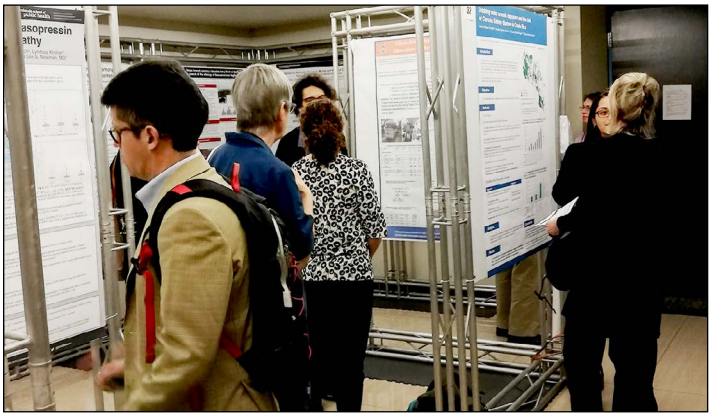
Forty-two poster presentations displayed recent progress on current studies and plans for future work. Full abstracts are included in the meeting book available in English and Spanish on the workshop website. Poster presentations facilitated leadership opportunities for many trainees and stimulated ongoing discussions across all workshop participants.











Posters demonstrated the most recent work available including ongoing (unpublished) studies from across the globe.

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Working Group Reports

All workshop attendees participated in at least one working group. The working groups were led by researchers from a wide range of organizations and countries. Joint sessions between working groups allowed for multidisciplinary discussion and harmonization of recommendations. Working groups had a total of five sessions to complete their work. A summary of the current evidence, discussion during the workshop, conclusions, and recommendations are included below for each group. Groups are listed in alphabetical order. Please see the Executive Summary section for brief summaries in English and Spanish.

Analytical epidemiology

David Wegman, USA
Shuchi Anand, USA
Christer Hogstedt, Sweden
Marvin Gonzalez-Quiroz, Nicaragua
Joaquin Barnoya, Guatemala

Background

Well-designed epidemiological studies can test multiple hypotheses regarding the potential causes of Mesoamerican nephropathy/CKDu. Door-to-door community surveys, designed to sample by altitude, and community workforce (Peraza et al., 2012; Torres et al., 2010), provided the initial convincing evidence regarding the burden of abnormal kidney function in sea-level communities and a strong association with agricultural work. Since then, in the Mesoamerican region several occupation-based reports, including cross-shift (Garcia-Trabanino et al., 2015) and cross-harvest (J. Butler-Dawson et al., 2018; R. L. Laws et al., 2015), studies have demonstrated small but consistent declines in kidney function in workers undertaking strenuous work at high temperatures. In a community-based study (Gonzalez-Quiroz, Smpokou, et al., 2018), men and participants undertaking outdoor tasks had substantially higher risk for rapid decline in kidney function. In Sri Lanka, the association of CKD with farm work and the higher prevalence among men has been confirmed (Anand, Montez-Rath, et al., 2019; N. T. Athuraliya et al., 2011; Nanayakkara et al., 2014). In addition, heavy metal exposure through ground water has been explored in detail using a case-control design (Diyabalanage, Fonseka, Dasanayake, and Chandrajith, 2017) without any evidence for higher bioburden in matrices such as hair and nails. While a very similar phenomenon (i.e., high prevalence of non-proteinuric CKD in agricultural workers or rural areas) is described in some specific states in India (O'Callaghan-Gordo et al., 2019), detailed examination of risk factors in these populations are not yet available. In summary, epidemiological research is slowly adding knowledge but no major breakthroughs have occurred to date.

Goals

Most of the epidemiological work performed to date has been cross-sectional, case-control, or cross-harvest, with only one community-based study reporting data from two years of follow up (Gonzalez-Quiroz, Smpokou, et al., 2018). Several studies have tested a single hypothesis only, and many have used different case definitions or exposure assessment and outcome measurements. The analytical epidemiology work group thus outlined three major goals and structured its activities accordingly. The group planned to: 1) evaluate the literature studies according to their design, and use these to highlight the epidemiology concepts and challenges relevant to CKDu, 2) discuss ongoing field studies, providing real-time feedback to researchers, and 3) identify potential areas of overlap or potential collaboration as a next step to facilitate rigorous testing of multiple candidate hypotheses.

Proceedings of the analytical epidemiology work group

Epidemiological analysis of CKDu in community and occupational settings was examined and discussed at the previous two International Workshops (2012, 2015). The outcome of those efforts was reviewed for the participants. The 2012 discussions provided a review of different epidemiological approaches emphasizing the special place for cohort epidemiology accompanied by suggestions for common definitions/measurements of outcome and exposure. The 2015 discussions added depth to understanding exposure measurement, possible early markers of adverse kidney effects, and the value that could be added from a common core questionnaire. New discussions of intervention and surveillance broadened the considerations for applied epidemiology.

Prior to the start of the 2019 session, the analytical epidemiology work group facilitators prepared spreadsheets to aid participants in a) updating their knowledge about CKDu epidemiology focused on Central America and Sri Lanka, and b) familiarizing themselves with ongoing studies that might engage their interest or collaboration. Active research is ongoing in several countries in Central America, in Sri Lanka and in India. The group was provided additional background on the epidemiological evidence for occupational factors through Dr. C. Wesseling's keynote and with valuable information added about the core biomarkers in a presentation from C-G Elinder and B Caplin. These emphasized the priority importance of established analytes, specifically creatinine and cystatin C.

Key epidemiology concepts relevant to CKDu

Four studies were presented: one case-control (see poster abstract by Yih et al. in the workshop meeting book, (Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-Traditional Etiology in Mesoamerica and Other Regions: Meeting Book, 2019) and three with long-term follow up. Dr. Gonzalez-Quiroz presented results from a community-based longitudinal follow-up study, emphasizing the importance of selecting healthy participants and evaluating longitudinal trajectory of eGFR as a potential outcome rather than incident CKD, which is typically defined as eGFR < 60 ml/min/1.73m2 and may require longer follow-up to achieve adequate power for testing correlations(Gonzalez-Quiroz, Smpokou, et al., 2018).

Dr. Vlahos presented an analysis from 10-year follow-up from an elderly population of 1000 participants from Uppsala, Sweden. In this study, eGFR decline was associated with baseline serum p,p'-DDE (1-dichloro-2,2-bis (p-chlorophenyl) ethylene)(Jayasinghe, Lind, Salihovic, Larsson, and Lind, 2018). The strengths of the analysis were length of follow-up, and appropriate exposure assessment, since p,p'-DDE can persist in bloodstream. While its total body concentration may vary over time, its measurement in serum may serve as a broader marker for 'agrochemical exposure.'

In discussing the analysis by Fischer et al. (Fischer, Vangala, Mandayam, et al., 2018), the group discussed outcome assessment, particularly as related to changes in kidney function over a period of three to six months, rather than days as is typically used to describe AKI or years as done for CKD. Further, the nature of serum creatinine-based eGFR assessments requires relatively steady body composition and diet, and these may particularly change cross-shift and possibly cross-harvest in agricultural workers.

Ongoing studies

Five ongoing studies were presented: a community-based follow up from Gonzalez-Quiroz et al. (Gonzalez-Quiroz, Smpokou, et al., 2018), a cohort study of established CKDu cases in Sri Lanka (Vlahos et al., 2018), an occupational prospective study [Scammell, et al] and two intervention studies (Wegman, et al; Butler-Dawson et al). Two additional studies [Crowe, et al; Ruwanpathirana T, et al] were identified but not presented (Table 1).

Community-based: Dr. Gonzalez-Quiroz has described a marked decline in kidney function in one out of 10 males (18 mL/min/1.73m2/year) and one out of 30 females (14 mL/min/1.73m2/year) with normal kidney function at baseline. Rapid decline in kidney function was associated with agricultural work, outdoor work, and lack of shade during a work break(Gonzalez-Quiroz, Smpokou, et al., 2018). In addition, for the detection of those a risk of drop in eGFR over time at least two or more measurements at six and 12 months are needed (Gonzalez-Quiroz, Smpokou, Pearce, Caplin, and Nitsch, 2019). Based on these findings, the cohort study was extended and expanded through 2020 and the current sample size is more than seven hundred participants. The outcomes for the second phase are decline in kidney function over time and rate of progression of CKD.

Dr. Vlahos described an ongoing case-cohort in Sri Lanka where nearly 300 participants with clinically diagnosed CKDu are being followed for two years (Vlahos et al., 2018). The goals are to 1) describe the natural history of CKDu, 2) determine if a subset of participants experience more rapid progression, and 3) elucidate risk factors for rapid progression. The latter could identify factors for secondary prevention, and potentially primary prevention if ongoing exposure to causative agent(s) is leading to rapid progression. This study uniquely pairs measurements of specific environmental exposures (in particular, drinking water source organic and inorganic compounds) with disease ascertainment.

Occupation-based: An occupational prospective cohort study was presented by Dr. Scammell. The aim of this study is to identify occupational exposures associated with kidney injury and drop in kidney function over time. They have recruited more than 500 workers between 18-45 years old from different industries in El Salvador and Nicaragua. She provided an overview how the participants were recruited and what lab analysis they will perform and the environmental measurement that they are taking as a part of data collection.

Intervention studies

Two active studies with intervention components were discussed: 1) the LIN WE-Adelante study in Nicaragua examining an intervention program effectiveness in sugarcane field workers and 2) the University of Colorado Pantaleon Collaboration in Guatemala.

LIN WE-Adelante study in Nicaragua

This project aims to assess effectiveness of an intervention program designed to reduce heat stress and dehydration and thus prevent or minimize kidney injury among sugarcane field workers. Results suggest possible positive impact, but not successful for jobs with most intensive physical demands. Improvements have been recommended and will be assessed in subsequent two years including feasibility of sufficient protection. Study design is pre-post and not experimental. The challenges to a true experimental intervention design were discussed.

UColorado Pantaleon Collaboration in Guatemala

Two intervention trials were carried out among sugarcane workers and effects on kidney function within a larger cohort study of total worker health. One examined different levels of electrolytes with evidence that workers felt better when they consumed 5-10 liters of electrolyte solution per day. The other examined shorter work days and found reduction in creatine phosphokinase (CPK) levels but workers were concerned that shorter days resulted in too much pressure to cut the same quota within a shorter period.

Table 1. Describes the main ongoing longitudinal and interventions that are in process in Central America and Sri Lanka

Contact investigator	Country	Study design	Sample size	Age range	Start – end dates	Outcome
Cohort studies						
Gonzalez-Quiroz, M.	Nicaragua	Community-based prospective cohort	767	18 – 30 years	2014 – 2020	Decline in kidney function over time
Scammell, M.	Nicaragua and El Salvador	Occupational prospective cohort	570	18 – 45 years	2017 – 2022	Decrease in eGFR over period of follow-up
Crowe, J.	Costa Rica	Occupational cohort	82	18 – 62 years	Feb – May 2018	Changes in kidney function pre/post-shift and over three months of follow-up
Vlahos, P.	Sri Lanka	Prospective cohort	296	21– 65 years	2017 – 2019	Rate of progression
Ruwanpathirana, T.	Sri Lanka	Community-based prospective cohort	729	18 – 60 years	2018 – 2022	Decline in kidney function over time
Intervention studies						
Wegman, D.	Nicaragua	Occupational intervention study	427		2017 – 2020	1. Changes in kidney function across harvest 2. Incident kidney injury
Butler-Dawson, J.	Guatemala	Occupational intervention study	50		2017 – 2018	Changes in kidney function across the harvest

Analytical Epidemiology joint sessions

Joint session: Analytical epidemiology and biomarker working groups

This short joint session discussed the recommendations from the biomarker working group on best available methods for detecting kidney injuries in epidemiology studies of CKDu (see also the report from the biomarker working group).

The biomarker group had agreed that diagnosis of CKDu along with studies of detection, burden, and associations are going to be based on estimates of glomerular function from serum markers, at least for the foreseeable future. Acute kidney injury (AKI) and chronic kidney disease (CKD) of all causes have been based on the estimated glomerular filtration rate (eGFR) calculated from serum creatinine (and/or cystatin C). Validated equations have been developed that provide a quantitative estimate of glomerular filtration for any serum creatinine (or cystatin C) level adjusting for age and sex along with ethnicity to a limited extent.

There are several circumstances, however, that create difficulties in comparisons and conclusions when serum creatinine and associated estimates of GFR are used in epidemiologic studies. Serum creatinine-based estimates of eGFR have been shown to be subject to a high degree of inter- and intra-laboratory variation in the absence of adequate external quality assurance procedures. Hence care should be taken to use a single laboratory with a validated method or to be certain that different laboratories are using the same analytic methods for serum creatinine or cystatin C.

Reliance on single measures of serum creatinine may lead to substantial misclassification of CKD. According to the biomarker working group the substantial between-person variability in eGFR makes detecting the initial stages of disease from one-time measurements of serum markers impossible (i.e., the only way to detect early evolution of renal dysfunction using serum biomarkers is to obtain serial measures in the same individual over time). Examining the slope of changes in serum biomarkers can be used in epidemiology studies.

Population distributions are not easily comparable between regions. Acute changes in renal clearance are not easily quantified from short-term changes in serum markers. The biomarker group reported that eGFR equations are only valid if kidney function is stable. Hence, acute changes in serum creatinine are used in nephrology guideline-based definitions for acute kidney injury. However, in the cross-shift and cross-harvest settings, changes in hydration and/or body composition could complicate interpretation of small changes in serum creatinine. Urinary biomarkers may be useful for identifying episodes (and/or types) of AKI, but none have yet been identified as reliable markers for AKI.

The biomarker group also called attention to evidence that suggests the most precise estimates of renal clearance can be obtained by using a combination of serum creatinine and cystatin C. However, cystatin C measures currently have several disadvantages including cost and laboratory standardization but can have a role in certain circumstances.

Joint session: Analytical epidemiology and heat stress working groups

This joint session on interventions focused attention primarily on effective measurement of heat stress in working populations. The recommendations from CENCAM 2015 (Program on Work Environment and Health in Central America (SALTRA) et al., 2015) were described and agreed to be still appropriate for epidemiology field study use. Attention was paid to the importance of formal intervention evaluation for effectiveness of any effort to reduce cumulative heat stress. Importance to study customized interventions with sensitivity towards local customs in other high-heat occupational sectors other than agriculture (such as steel, foundries, and glass) was discussed. Low-cost (locally available and traditionally used) and feasible interventions are the priority.

Joint session: Analytical epidemiology and pesticides/metals working groups

The analytical epidemiology and pesticides groups organized a joint session where participants discussed the existing evidence related to pesticide exposure and kidney damage. The groups agreed that there is poor evidence that agrochemicals alone explain the great increase in CKDu in recent decades. This lack of evidence is attributed by some to epidemiological studies having used poor exposure assessments (e.g., questionnaire data). Some studies have not sufficiently controlled for potential confounders or evaluated interactions across pesticides, and most studies have been cross-sectional. There is an urgent need to improve the epidemiological methods used to estimate exposure to pesticides and metals and to conduct prospective/retrospective cohort studies or case-control studies that can evaluate the association between properly measured exposure to environmental toxins (agrochemicals and heavy metals) and CKDu.

In addition, both groups commented that there is an urgent need to develop a core questionnaire that can capture the agrochemical exposures by designing a pesticide-exposure matrix (list of active ingredients used) in conjunction with self-reported information about past and current exposures (types of pests treated, crops, regions, apply and/or mix, how frequently, etc.). With such data an exposure index can be constructed to determine exposure gradients among workers or individuals. This would allow us to make regional and within municipalities comparisons in each country.

In summary, participants suggested combining different approaches such as self-reported exposure, exposure indices from combining external and individual data (see above), biological monitoring, and environmental monitoring in order to advance efforts to evaluate the degree to which agrochemical exposures play a role in the etiology of CKDu. Also, it is vital that each country provides information about the quantity and types of pesticides imported and use data for crops and regions over time.

Joint session: Analytical epidemiology and clinical working groups

In this session, clinicians provided input regarding potential markers for diagnosing CKDu at earlier stages. Clinicians shared that even among persons with near-normal kidney function, they sometimes find helpful signs such as marked hypokalemia, hyperuricemia, and defects in urine concentrating ability, and clinical history of dysuria (otherwise rare among men) and cramps/weakness. Epidemiology work group members agreed that validating such earlier signs would result in substantial progress, since in persons with earlier stage disease, the time-lag between exposure and disease could be shorter.

The role for biopsies as a gold standard for diagnosis, but also as an important tool to assess exposures and disease pathophysiology was discussed. There seems to high heterogeneity in capacity for biopsy across regions, but regions with lower capacity were willing to bolster these, recognizing the value of tissue-based investigations into cause(s).

Finally, clinicians argued for study designs that provide more information about risk factors for CKDu and/or CKDu natural history, yet also include interventions that could help reduce its inexorable progression. Clinicians felt that a set of standard clinical care measures (e.g., avoidance of NSAIDs, prescription of allopurinol for those with high uric acid, and prescription of sodium bicarbonate for metabolic acidosis) were likely to reduce progression yet evidence of this is lacking in this specific population. A stepped wedge clinical trial (where treatments are randomly provided to different groups (or steps) over time (Hussey and Hughes, 2007) was felt particularly relevant.

Analytical epidemiology summary conclusions

1. Large longitudinal studies are now ongoing and such studies are a priority.

    a. Short-term change (days) should be measured in serum creatinine, long-term change (months to years) by eGFR.

    b. Continual assessments of protocols in settings like this workshop have been fruitful.

2. Intervention studies and their assessment are also a priority.

3. Cross-talk between studies should be encouraged.

4. Deep engagement with community and occupational study subjects is essential.

5. Material repositories for questionnaires, protocols and community engagement tools are encouraged.

Surveillance and Screening Working Group meeting (left). Joint session with the Analytical Epidemiology and Pesticides/ Agrochemicals working groups (right).

Dr. Vidhya Venugopal (India) presents during the Heat Working Group session. All working groups were responsible for presenting advances to the other participants for open discussion. Dr. Lee Newman (USA) asks a question after a presentation from Dr. Ben Caplin (UK) for the Biomarkers Working Group (right).

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Biomarkers of Abnormal Kidney Function

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Background

Across nephrology, biomarkers are fundamental to surveillance efforts, diagnosis, determination of prognosis, etiological investigation, identification of exacerbating factors, and quantification of the effect's interventions. The global renal community has developed widely accepted guidelines on the use of biomarkers in the diagnosis and stratification (staging) of both acute kidney injury (AKI) and chronic kidney disease (CKD) of all causes (Kidney Disease: Improving Global Outcomes (KDIGO), 2012; Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, 2013). This is based on both the estimated glomerular filtration rate (eGFR) calculated from creatinine (and/or cystatin C) and urinary albumin excretion in the case of CKD. Although these guidelines are widely accepted, and extremely valuable for the clinical diagnosis, there remain several challenges around use of these biomarkers in low- and middle-income countries and/or when applied specifically to CKD of unknown cause (CKDu). These include:

- Serum creatinine-based estimates of eGFR (and presumably those based on cystatin C, as well, though this has been less well studied) have been shown to be subject to a high degree of inter- and intra-laboratory variation in the absence of adequate external quality assurance (eQA) procedures (Coresh et al., 2002).
- Demonstration of chronicity of renal abnormalities (e.g., low eGFR or albuminuria present for more than three months) is challenging in many populations at risk of CKDu and reliance on single measures may lead to substantial misclassification of CKD (Benghanem Gharbi et al., 2016).
- Equations used to calculate eGFR have not been validated in the populations affected by CKDu and hence population distributions are not comparable between regions (Stevens et al., 2011).
- For cross-shift workplace studies, acute changes in renal clearance are not easily quantified from short-term changes in serum markers (Kidney Disease: Improving Global Outcomes (KDIGO), 2012).
- Serum markers in the 'normal' range can accompany substantial underlying renal damage, particularly in younger age groups (Bohle, Mackensen-Haen, von Gise, 1987).
- Albuminuria, an established urinary marker of early disease and prognosis in many forms of CKD, is not typically elevated in many of those with CKDu (Torres et al., 2010).

Goals of the working group

The goals of the working group were to: describe the advantages and disadvantages associated with the use of the established biomarkers for different purposes as applied to the CKDu field; examine the evidence for the utility of novel biomarkers in the CKDu field; and, if possible, *suggest* potential biomarker strategies that might be applied to a range of different clinical and research questions.

Report on the working group discussions

At the outset, the group agreed there is no 'one size fits all' solution and different biomarker choices should be made depending on several factors: clinical versus research use; detection versus prognostication; stage of disease in which the biomarker is to be used; pre-analytical and analytical factors; as well as resource implications.

Serum biomarkers

Serum biomarkers and relationship to glomerular filtration rate

Impaired kidney function is the hallmark of CKDu and it was agreed that studies in the CKDu field are likely to be based on estimates of glomerular function from serum markers at least for the foreseeable future. Endogenous markers are routinely used as a surrogate for renal clearance (hence, estimated glomerular filtration rate, or eGFR) as using exogenous markers is impractical and resource intensive. The two widely used serum/plasma endogenous markers in this context are creatinine, a breakdown product of phosphocreatine originating from skeletal muscle, and cystatin C, a ubiquitous cysteine protease produced in all cells that has more recently been adopted to estimate kidney function. The serum concentration of these molecules will be inversely proportional to the renal clearance assuming stable kidney function and constant production/handling of the marker (see Table1 for both renal and non-renal factors affecting creatinine concentration).

Validated equations have been developed that provide a quantitative estimate of glomerular filtration rate for any serum creatinine (or cystatin C) level adjusting for age, sex, and ethnicity (which are surrogates of serum creatinine or cystatin C production) (Inker et al., 2012; Levey et al., 1999).

Table 1. Factors influencing eGFR when calculated from serum creatinine

Between-person factors unrelated to kidney clearance	Within-person factors unrelated to kidney clearance (also relevant between-person)	Within-person factors due to changes in kidney clearance (also relevant between-person)
Muscle mass (although this can also change within a person over extended periods)	Animal protein meal	Functional decrease in GFR (e.g., reduced glomerular blood flow) due to volume depletion or vasoactive drugs
Ethnicity (may at least partially be a reflection of muscle mass)	Strenuous exercise leading to muscle breakdown	Irreversible loss of nephrons (functional units of the kidney) due to tubular or glomerular cell damage (i.e., progressive CKD potentially leading to end-state renal disease (ESRD))
Sex (may at least partially be a reflection of muscle mass)	Drugs that alter creatinine handling (e.g., trimethoprim)	
Age (may at least partially be a reflection of muscle mass)	Changes in plasma volume (impacts all solutes in the plasma, e.g., short-term dehydration of overhydration)	
Genetic background		

An understanding of some of the limitations of serum biomarkers is required for researchers in the CKDu field:

AKI: eGFR equations are only valid if kidney function is stable, as it takes time for a serum marker to accumulate following a drop in glomerular filtration. For example, it will typically be at least eight hours for an acute 50% drop in glomerular filtration to lead to an increase in serum creatinine ~20umol/l (0.23mg/dL; ~25% of baseline) [C-G Elinder, personal communication; Figure 1]. This, in combination with the potential contribution of serum creatinine production (e.g., originating from dietary meat intake or muscle breakdown) and changes in intravascular volume, make the quantification of any change in filtration rate difficult to determine from the increases in serum creatinine that have been described in cross-shift studies in CKDu research. Indeed, the inability to detect kidney injury in the first few hours after an insult is a key motivation underlying the search for urinary biomarkers of AKI even in the non-CKDu arena (see section on urinary low-molecular weight proteins below).

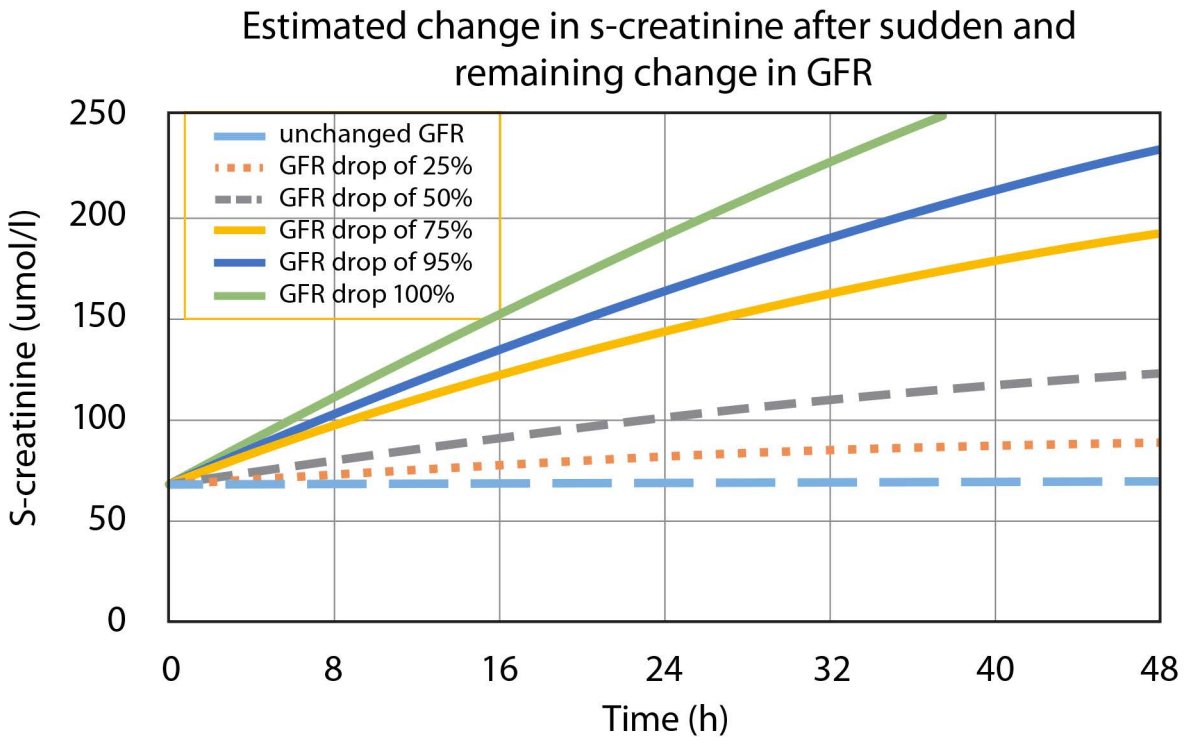

Figure 1. Changes in serum (s-) creatinine following an acute drop in GFR.

Comparisons between individuals and populations: Renal clearance is a function of age and sex so reports of summary data need to be stratified on these variables to allow robust comparisons between populations. Furthermore, there remain two additional key unknowns when comparing the range of eGFR in the population between regions:

- How well do eGFR equations developed in the USA and Europe (e.g., MDRD or CKD-EPI) reflect actual GFRs in populations affected by CKDu? (See Stevens et al., 2011 for examples.)
- What is the 'normal' population distribution of the actual GFR (renal clearance) in populations affected by CKDu? For example, is a GFR of 80mL/min/1.7m2, 'normal' in working age South Asians even though it is 'abnormal' in European young adult populations (Barai et al., 2005)?

Neither of these issues can be easily addressed in the short term, but ideally population studies investigating the burden of CKDu (e.g., prevalence of eGFR <60mL/min/1.7m2) should be interpreted in the context of the normal/healthy population eGFR distribution from the same region. Furthermore, and more challenging, eGFR estimating equations need to be validated in all populations at risk of CKDu.

Coexistence of substantial kidney injury with serum markers in the normal range

Studies outside the CKDu arena have demonstrated that substantial underlying histological kidney scarring can coexist with a serum creatinine concentration in the normal range. Similarly, an eGFR above 60mL/min/1.7m2 despite not meeting internationally accepted criteria for CKD, may nonetheless also reflect substantial underlying kidney damage, particularly in a young adult. This issue, combined with the substantial between-person variation of eGFR in renal health, makes detecting the initial stages of disease from one-time measurements of serum markers close to impossible. In turn this implies that serial measures in the same individual, to quantify change in eGFR over time, are required to detect the evolution of renal dysfunction, and hence early-stage CKDu when using serum biomarkers. Although there is an increasing research interest in within-individual changes in eGFR over time outside the CKDu field, many questions remain. These include, but are not limited to, the optimal time intervals and frequency of sampling to estimate eGFR slope, rates of decline that reflect underlying disease versus physiological variability/ageing and the utility of quantifying absolute versus percentage change in eGFR.

Renal functional reserve

The group then discussed the concept of renal functional reserve (RFR), defined as the capacity for an individual to increase their eGFR in response to a physiological challenge. It has been proposed that the RFR may underlie the above observation that resting eGFR is maintained even while there is substantial underlying kidney damage (i.e., an individual with underlying kidney damage will utilize their RFR to maintain the serum creatinine in the normal range). Hence, (although challenging to reproducibly demonstrate) loss of RFR may be the earliest detectable sign of underlying kidney damage using serum biomarkers.

Equally importantly however, loss of RFR may translate into amplified short-term changes in creatinine in the face of any renal insult, as these individuals are unable to recruit reserve capacity in response to a (patho)physiological stressor. In turn, this loss of RFR may be manifest in an increase in eGFR variability when study participants with subclinical kidney disease have serial eGFR measures performed over time in observational studies. This phenomenon has been studied in other forms of CKD, and it is clear that individuals with greater eGFR variability (i.e., greater variance from a theoretical line of best fit through a plot of eGFR over time) are at higher risk of adverse renal (and cardiovascular) outcomes independent of baseline eGFR, specifically progression to end-stage renal disease (Al-Aly, Balasubramanian, McDonald, Scherrer, and O'Hare, 2012; Tseng et al., 2015).

These issues have two implications:

- Tests of (absence of) RFR might be a useful marker of disease whilst the eGFR remains in the normal range.
- Observed short-term increases in serum creatinine may be as much a reflection of an amplified response to a ubiquitous renal insult (e.g., dehydration or nephrotoxic drugs) in those with existing subclinical renal dysfunction (undetectable using a one-off eGFR measure) as a signpost to a primary cause of disease.

Pre-analytical and analytical factors

Evidence suggests preanalytical handling of serum creatinine/cystatin C is reasonably straightforward. Separated serum (or serum gel tubes) can be stored for several days at room temperature and prolonged periods if frozen (personal communication, Clinical Trials Service Unit, Oxford, UK).

Laboratory standardization of serum creatinine, in practice assays using isotope dilution mass spectrometry (IDMS) referenced standards for external quality assurance, is critical for the comparison of both eGFR measures between laboratories and eGFR measures performed at different time points in the same laboratory (Coresh et al., 2002). This is particularly true in population or workplace-based studies of CKDu where the eGFR of the participants is likely to be in the normal or near-normal range, values where small changes in creatinine concentration can lead to large changes in the absolute eGFR.

Advantages of cystatin C over serum creatinine

Evidence suggests the most precise estimates of renal clearance can be obtained by using a combination of creatinine and cystatin C (e.g., CKD-Epicys-creat equation) (Inker et al., 2012). However, cystatin C measures currently have several disadvantages, including cost (most laboratories charging three to 10 times the per-sample cost of creatinine). Furthermore, laboratory standardization is even less well established than for serum creatinine. Nonetheless, the group agreed that there might be a role for using this newer marker in some specific circumstances, e.g., international comparisons where ethnicity/muscle mass-dependent bias may be less of an issue with cystatin C (although there is not yet any empirical evidence this is the case) or in studies where changes in biomarkers are expected to be small (e.g., cross-shift studies) or might be confounded by meat consumption/muscle breakdown.

Point of care (POC) devices

Several portable devices are available for the measurement of serum creatinine. Although there may be practical advantages to performing serum creatinine assays in the field, most published data suggest that any POC device requires calibration against standard laboratory serum creatinine measures for each population/study site in which it is used (and potentially each device where more than one is used, as even those devices of the same type may perform differently) (Shephard et al., 2010). This calibration will require standard venous blood sampling in at least a subset of participants, which may in turn undermine the benefits of the POC device. Per-sample testing is also likely to be more expensive than routine laboratory testing although there may also be associated savings in terms of repeat site visits to provide participants results if POC results are reported back immediately.

Urinary biomarkers

Potential uses of urine biomarkers

Given some of the issues described with serum biomarkers of renal clearance, a number of potential roles for urinary biomarkers were identified specific to the CKDu arena:

- Detecting early chronic disease using a one-time test (while serum creatinine remains in the normal range).
- Determining prognosis in established disease.
- Identifying acute tubular injury (and potentially differentiating tubular injury from hemodynamic changes in renal clearance; see Table).

Urinary albumin

Increases in urinary albumin are a well-established biomarker for both detection and prognostication in CKD in general. Albuminuria indicates glomerular damage and can be present in the context of a preserved eGFR. However, unlike in many forms of CKD, most studies of CKDu have demonstrated low-level or absent albuminuria, at least in the early stages of disease (e.g., eGFR>45mL/min/1.7m2).

Urinary low-molecular weight proteins

Low-molecular weight proteins (LMWP, proteins smaller than albumin that typically pass freely across the glomerular filtration barrier) have been extensively investigated in the wider kidney research landscape as markers of kidney injury, as the urinary concentration of a number of these molecules are elevated in the presence of tubular dysfunction. These increases in urinary LMWP can be the result of either reduced reabsorption of the filtered protein through dysfunctional tubular-cell transporters (e.g., β2-microglobulin, α1-microglobulin or retinol-binding protein), enzymes released

from tubular cells due to cellular stress or death (e.g., kidney injury molecule-1), or molecules released by infiltrating inflammatory cells (e.g., neutrofil gelatinase-associated lipocalin, NGAL; or interleukin-18, IL-18).

A number of these molecules, often described as novel biomarkers, have been shown to be useful in predicting in-hospital AKI (e.g., predicting renal complications of cardiac surgery), but none have been robustly shown to provide predictive power over and above albuminuria in studies of CKD of all causes (B. Caplin and Nitsch, 2017). Some of these LMWPs have been examined in the CKDu arena, specifically in workplace studies of sugarcane workers. NGAL has been reported to fall across the sugarcane cutting shift (Garcia-Trabanino et al., 2015) but rise across the cutting season (along with IL-18) (Laws et al., 2016). NGAL levels were also reported to be quantitatively higher across the young adult population in those who sustain decline in eGFR over two years; however, the differences were small and predictive power limited (Gonzalez-Quiroz, Smpokou, et al., 2018).

The group also expressed the hope one of these molecules (or similar LMWPs, or indeed a combination) might have potential as an early marker of disease (particularly given the problems with serum markers in this context, as described above). The lack of existing population-based longitudinal studies was seen as a major obstacle in achieving this aim. There were no known studies describing the use of urinary biomarkers in predicting the prognosis of CKDu in those with established disease.

Pre-analytical and analytical factors for urinary LMWP tests

Studies have demonstrated that measurements of LMWPs are subject to substantial storage-dependent variation with freeze-thaw cycles particularly affecting results (Parikh et al., 2014; van de Vrie, Deegens, van der Vlag, and Hilbrands, 2014). Costs of these assays is often high and laboratory standardization almost entirely absent. Although a number of POC tests are also available, again cost and standardization remain significant challenges.

Finally, correction of urinary biomarker values for urinary concentration is usually required. Creatinine is typically used but urinary creatinine excretion varies by age and diet which may be particularly relevant in communities affected by CKDu. Alternatives might include correction with urinary specific gravity (USG).

Non-protein urinary biomarker and dynamic tests of tubular function

The group discussed the possibility of testing more easily measurable biomarkers of early tubular dysfunction; suggestions included urinary concentration (with good experience in the field of using a refractometer) or electrolyte concentrations. Given the wide-variation in the concentration of these test values in health, it was agreed that physiological challenge tests might be required (e.g., urinary concentration in response to water deprivation over several hours). None of these ideas have been tested in the CKDu research field to anyone in the group's knowledge.

Summary, Conclusions, and Next Steps

The group presented the summary of their discussion at the workshop plenary session. The group agreed that studies of detection, burden, natural history and etiology in CKDu are going to be based on estimates of glomerular filtration rate from established serum markers such as serum creatinine, at least for the foreseeable future. Additional conclusions and suggestions are presented in the box.

Box 1. Conclusions and suggestions from the biomarker working group.

Estimates of low eGFR prevalence and eGFR distributions should be reported with age and sex stratification or standardization.
Standardization of biomarker assays between laboratories, and between batches over time, is critical for accurate comparisons (using IDMS methods for serum creatinine).
Normal population eGFR distributions need to be described in populations at risk of CKDu.
Adequately sized population-based longitudinal studies are needed to identify biomarkers of early disease.
Practical estimates of (loss of) renal reserve may be worth exploring as a tool to characterize the earliest stages of kidney injury (i.e., while eGFR/creatinine is in the 'normal' range).
Low molecular weight protein urinary biomarkers may currently be useful for identifying episodes (and/or types) of AKI in areas impacted by CKDu.
Multiplex urinary low molecular weight protein biomarker panels may have more utility than single markers for early CKDu disease detection but pre-analytical factors and cost remain major obstacles.
(Dynamic) tests of tubular physiology could be explored as an early marker of disease.
Care should be taken in invoking disease causality when observing exposures associated with short-term changes in serum creatinine in CKDu research. Such changes may reflect suboptimal renal responses, due to loss of renal reserve, to ubiquitous kidney stressors in a population with high rates of underlying subclinical renal dysfunction.

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Clinical Presentation and Treatment

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Working group description

The Clinical Presentation and Treatment Working Group was composed of clinicians in attendance at the Third International Workshop who provide care for CKDu patients in India, Sri Lanka, Mexico, Guatemala, El Salvador, Nicaragua, and Costa Rica, as well as researchers from around the world who study the disease. Over the course of three days, using an open discussion format structured around a series of pre-prepared questions, we discussed aspects of the clinical management, policy priorities, and needed research to provide better care for CKDu patients. The group did not perform a systematic review of medical records and did not survey the wider nephrology community in different regions, which could be a next logical step.

Clinical Features

While it is unknown if CKDu in Mesoamerica has the same etiology to that seen in India and Sri Lanka, there are important similarities in the clinical between regions among individuals in early and moderately advanced stages of disease (eGFR >45 ml/min), which have led us to consider them under the same CKDu syndrome. We acknowledge potential biases in observation, since the data collection was not systematic and may be skewed based upon who presents for medical care and how or where they receive care. Clinicians who attend to CKDu patients report that individuals affected tend to be young, with disease often manifesting in the third or fourth decade of life. There is a high male-to-female ratio, and significant familial concordance. Affected individuals almost universally live in poverty and have limited health literacy and access to health care. Individuals in both regions frequently live in rural areas, although there is increasing evidence that this is not a disease limited to agricultural workers. Clinical features are also quite similar (Table 1 on next page), and are similar to clinical features described in the literature (N. T. Athuraliya et al., 2011; Badurdeen et al., 2016; Kupferman et al., 2016; Wijkstrom et al., 2017).

Table 1: Clinicians' impressions of features of CKDu in India and Sri Lanka and Mesoamerica (Guatemala, El Salvador, Nicaragua, and Costa Rica).

	India and Sri Lanka	Mesoamerica
Demographics		
Age at presentation	20s – 60s	20s – 40s
Male:Female ratio	5:1 or greater	10:1 or greater
Family concordance	High	High
Laboratory Findings		
Hypokalemia	Frequent (>50%) but not universal	Nearly universal >90%
Hyponatremia	Frequent (>50%) but not universal	Frequent (>50%) but not universal
Hyperuricemia	Uncommon (<25%)	Common (>50%)
Creatine Kinase	Normal or mildly elevated	Normal or mildly elevated
Proteinuria	Minimal/low grade (<500 mg/day)	Minimal/low grade (<500 mg/day)
Urinalysis and urine sediment	Sterile pyuria No RBCs WBC casts No crystalluria	Sterile pyuria No RBCs WBC casts Urate crystalluria
Radiology Findings		
Ultrasound	In early stages, there is lack of ultrasound studies; In more advanced disease, there is non-specific increased echogenicity and reduced renal volume	In early stages, there is lack of ultrasound studies; In more advanced disease, there is non-specific increased echogenicity and reduced renal volume

As individuals in both Central America and South Asia progress to end-stage renal disease (ESRD) their clinical manifestations cannot be reliably distinguished. In advanced CKD, clinical manifestations of CKDu in both regions of the world are very similar to those seen in tubulointerstitial diseases. Once an affected individual reaches ESRD, clinical presentation may be indistinguishable from that seen in almost any progressive renal disease.

AKI Episodes

In both South Asia and Central America, clinicians describe a pattern of AKI episodes occurring among individuals who may either have or be at risk for CKDu. These episodes are recognized when individuals present to their physician with symptoms of weakness, cramping/myalgia/ arthralgia, and fever (either subjective or measured), with occasional headache, dysuria, and nausea. In some instances, episodes of heat stroke are present in affected individuals. There is concomitant use of NSAIDs in a fraction of the cases; we cannot determine how much of a contribution that represents. Clinical features of these AKI episodes are like those described in Table 1, with the exception that reduced GFR seems frequently to recover. The relationship between these AKI episodes and CKDu development and progression remains unknown.

Natural History of CKDu

The natural history of CKDu development and progression overall remains largely unknown. The disease is considered to result in progressive decline in renal function, but the pattern and speed of decline is not known, and it is likely that early disease exists for extended periods and most often is unrecognized. Both slow and fast progressors have been described, with certain patients progressing to ESRD in as little as two to four years and others taking a decade or more, if they ever fully progress to ESRD. In anecdotal observations, progression appears to slow in individuals who receive routine care under the guidance of a nephrologist, suggesting the disease course may be modifiable through appropriate clinical management.

Clinical Management of CKDu

To date, there are no standard guidelines for the management of CKDu patients, and treatment strategies employed by health care providers vary widely. Clinicians in Mesoamerica have prescribed allopurinol to CKDu patients in response to their elevated uric acid levels; there are anecdotal reports of stabilization of renal function with this approach. Some have also prescribed angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), and do not consider adverse effects to be problematic. However, among the assembled nephrologists there was concern regarding the use of ACEIs and ARBs therapy in a disease characterized by dehydration and episodic AKI, and consensus was not reached by the group regarding the advisability of this form of therapy. Health care providers in India and El Salvador have infrequently used corticosteroids when biopsies demonstrated a lymphocytic infiltrate suggestive of a possible tubulointerstitial nephritis and report some anecdotal improvement in kidney function with sustained responses in the few patients who have had sufficient follow up.

Clinicians in Mesoamerica, Sri Lanka, and India report some success with reducing exposure to suspected inciting factors for CKDu, although a causal relationship between these factors and disease progression remains unproven. In India and Sri Lanka, this frequently means provision of clean water; in Mesoamerica this more often entails interventions reducing exposure to heat in conjunction with provision of appropriate hydration. In all affected regions, provision of routine nephrology care, including the correction of electrolyte abnormalities and counseling on avoidance of nephrotoxins, is anecdotally associated with improved outcomes.

Based on our clinical experience and the current understanding of the etiology and pathophysiology of disease, the following treatments have been utilized by experts in Mesoamerica, India, and Sri Lanka. We propose the following management guidelines when caring for CKDu patients:

Table 2. Recommendations for clinical management of patients with CKDu by health care providers.

1. Ensure adequate access to safe drinking water and promotion of appropriate hydration.
2. Encourage pursuing frequent rest breaks and reducing heat exposure in the occupational setting (rest and shade).
3. Emphasize avoidance of nephrotoxic medications, especially NSAIDs and over the counter medications not requiring oversight by a health care provider.
4. Emphasize avoidance of all herbal medications.
5. Encourage minimization of alcohol consumption, with a focus on avoidance of home-brewed alcohol.
6. Counsel on using appropriate personal protective equipment and avoiding agrichemical exposures.
7. Establish regular follow-up and care with physicians, ideally nephrologists, who can diagnose early the presence of renal dysfunction and manage sequelae of chronic kidney disease, including electrolyte and acid-base disturbances.
8. There is insufficient evidence at present to support the use of pharmacotherapy to prevent progressive renal function decline in CKDu, including ACEIs and ARBs, allopurinol, and corticosteroids. However, use of these and other medications in appropriate clinical situations arising concurrently with CKDu may be appropriate for some patients.

Research priorities for CKDu moving forward

One of the most striking features reported by clinicians who perform early biopsies in CKDu, largely in India and Sri Lanka, is the degree of tubulointerstitial nephritis. This corroborates the findings described in the literature in both Sri Lanka and Nicaragua (Fischer, Vangala, Truong, et al., 2018; Nanayakkara et al., 2012). Less than 40 total biopsies of early-stage disease have been published to date. Further characterizing the histopathology of early disease is crucial in advancing our understanding of its development and progression. Early biopsies also represent an opportunity for further molecular and genetic studies using kidney tissue. We propose this is best achieved by expanding the capacity to perform biopsies by nephrologists working directly in affected regions, in partnership with international nephrologists and nephropathologists.

Understanding the natural history of CKDu is an important component of further elucidating its etiology and developing effective prevention strategies. At present, there are a few active cohort studies which may ultimately provide important insights, and more work is needed.

Finally, despite the significant gaps that exist in our understanding of CKDu, we are at a point where intervention studies could be considered. There is evidence that reducing exposure to heat stress may help retard the development or progression of CKDu, although to what extent remains unclear (J. Butler-Dawson et al., 2018; Correa-Rotter and Garcia-Trabanino, 2019; Correa-Rotter, Wesseling, and Johnson, 2014). While there is a lack of evidence for agrochemicals as direct cause of CKDu, reducing exposure to any pesticide or herbicide is of major importance for health in general and could also be important for this specific health problem. No studies of outcomes from any of the various pharmacotherapies already being employed by nephrologists as described above for management of CKDu exist and establishing evidence even as basic as case series from already established patients would form an important starting point for broader intervention studies.

With all clinical research into CKDu moving forward, we suggest the following be prioritized:

- Consistent clinical data collection.
- Data sharing to create meaningful registries.
- Formation of alliances between practitioners and researchers.
- Collaboration with at risk communities to shape research priorities and expand participation of affected individuals and their families.

Government policy priorities for CKDu moving forward

The group discussed where the tools needed to appropriately care for patients affected by CKDu fall beyond the scope of individual clinical practice and into the realm of government policy. Those working in Sri Lanka and Costa Rica applauded strong commitments by government to support people affected by the disease, as well as programs and research to prevent its spread. Other countries have begun to establish similar commitments, and the group encouraged the ongoing process of translating those commitments into widespread action. Our group identified several priorities for governments and health ministries in their efforts to address CKDu:

- Establish disease registries and surveillance programs specifically for CKDu or incorporated into broader chronic disease programs.
- Create and enforce laws protecting against unsafe working conditions, particularly conditions which expose individuals to undue heat stress, harsh labor, inappropriate hydration practices, and inadequate protection against agrochemical products.
- Develop infrastructure focused on the provision of safe, clean, and widely available drinking water.
- Train more nephrologists and provide additional training to other health care and social service providers in the specifics of caring for CKDu.

- Expand the medical infrastructure needed to care for CKDu patients, particularly facilities which can conduct kidney biopsies; expand and modernize the capacity for renal replacement therapy provision.
- Develop educational campaigns to expand awareness and recognition of CKDu.
- Engage with affected individuals and communities to understand their priorities regarding the management of CKDu.

Conclusions

CKDu is a major health issue, which has proven catastrophic in affected populations in Mesoamerica, India, and Sri Lanka. Our understanding of the disease is still limited, and it remains to be seen whether CKDu is a single disease or a collection of diseases with similar clinical features. Nevertheless, the disease course may be modifiable through simple, cost-effective clinical management. More research is needed to further understand the pathophysiology, natural history, and treatment of the disease. We present here our consensus on clinical features, which should prompt clinicians to consider CKDu in a patient, as well as management recommendations when CKDu is suspected. Unfortunately, many people affected by CKDu do not yet have access to even basic health care, let alone the specialist care required to adequately manage their disease. This gap in coverage is one which we must focus on addressing, through strong and consistent messaging to governments in affected regions.

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Heat Load/Dehydration and Workload

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Background and scope

The terms heat stress and heat strain represent the relationship and difference between external factors and the body's core temperature control mechanisms. Heat stress is defined as the net heat load to which a person is exposed. Physical exertion, environmental factors, and clothing all contribute to heat stress, and heat strain is the physiological response to heat stress (e.g., sweating). Heat strain and dehydration have recently been in the spotlight globally due to emerging evidence of their association with adverse renal health issues, including chronic kidney disease, especially in working populations (Hansson et al., 2019; Tord Kjellstrom, Holmer, and Lemke, 2009; Catharina Wesseling et al., 2016). Effects of global warming have been highlighted, and in the last century, the trend of rising temperature has been steep with predicted rise in mean temperature expected to increase up to 7°C without appropriate interventions (Tord Kjellstrom et al., 2009).

The working group discussed epidemiological studies in Central America that have reported adverse workplace heat stress impacts in agricultural communities, especially in sugarcane workers (Hansson et al., 2019; Catharina Wesseling et al., 2016). The probability of heat stress appears to be greater when environmental temperature surpasses a person´s core temperature (37°C), and outdoor workers in tropical settings are regularly exposed to temperatures well above 37°C. Exposure to high temperatures, particularly when co-occurring with heavy work (metabolic) load, can adversely affect the kidney, possibly through functional and sub-clinical acute kidney injuries (AKI). It has also been reported that high sweat rates and limited fluid intake can lead to AKI, independent of heat exposure. For example, a recent study demonstrated that well-hydrated workers exposed to heat and heavy workload also have biomarkers of kidney injury [3]. Repetitive AKIs on a nearly daily basis may lead to compromised kidney function and, ultimately, the development of chronic kidney disease (Hansson et al., 2019).

Much of the working group discussion precipitated into one single question: "Why does CKDu only occur in certain regions?" Geographical regions reporting high rates of CKD and CKDu have been documented in several countries and it seems likely that more will be identified (Abraham et al., 2016; Fiorentino, Grandaliano, Gesualdo, and Castellano, 2018; Obrador and Levin, 2019; Tippet, Stofan, Lacambra, and Horswill, 2011). The populations affected are generally marginalized and unmeasured, so for these populations, the adage "absence of evidence is not evidence of absence" is relevant. There is a need for a structured review of occupation, socioeconomic standards, environmental conditions (heat and humidity), land use, water sources, and other risk factors in CKDu endemic regions to inform future studies. Such a review could involve different study designs, such as cross-sectional, cohort, and case-control studies that measure heat as a risk factor for CKDu/CKDnt and evaluate the potential association between heat strain/dehydration (HS/D) and CKDu.

Studies published to date have used many methods to measure heat, dehydration, workload, and indicators of adverse renal health, which makes it difficult to compare study results and assess HS/D as part of the causal pathway. Different kinds of questionnaires, equipment, quantitative measurements, and self-reported health symptoms have been utilized, depending on the resources, feasibility of implementation in the field, and other logistical factors. A standardized

method of measuring the qualitative and quantitative indicators of heat exposure, workload, and dehydration is needed. Future studies of CKDu should ideally use gold standard devices (e.g., ingestible core temperature sensors, wet bulb globe temperature (WBGT) measurements, accelerometers) and other standardized and reliable methods (e.g., blood and urine biomarkers) to increase accuracy and enable comparison of studies across regions.

The working group members felt that getting an overall/global picture of the disease is essential for making strategic plans and policies at the local level to prevent and control the disease. At the same time, workplace interventions such as the Adelante Initiative (Wegman et al., 2018) specific to heat are needed to tackle disease prevention and progression especially at the local level where poverty and reduced access to resources and health care complicate the situation for affected communities. With this background, three primary topics were discussed. First, the group conducted a literature review to identify and describe studies examining HS/D as a possible risk factor for adverse renal effects. Second, the group discussed the possibility of developing a standardized assessment method/best and feasible practices for heat and CKDu research, to facilitate data comparisons. Third, the group discussed the need to intervene to reduce HS/D exposure while simultaneously working to improve epidemiologic evidence regarding the association between heat and CKDu. A collaborative session for intervention was discussed with the analytical epidemiology working group and the summary is presented by that group.

Literature review of heat exposure and adverse renal outcomes

We identified 36 epidemiological studies that evaluated heat as a risk factor for adverse renal health, including CKDu. Of these, 12 cross-sectional studies were from Australia, Central America, and India; one case-control study from Sri Lanka; one cohort study in Thailand; one exploratory study from Central America; one longitudinal study from Central America; and one feasibility study each in Sri Lanka and Central America. Three prevalence studies were conducted in Italy and Japan, and three retrospective studies were from Brazil, Australia, and Italy.

The cross-sectional studies reveal heat as a risk factor for decreased kidney function in countries like Central America (Garcia-Trabanino et al., 2015), Sri Lanka (Jayasekara et al., 2019), Brazil (da Cunha França et al., 2010), and California, USA (Moyce et al., 2017). In tropical settings like India, high-heat conditions and minimum cooling interventions could contribute to kidney-related illnesses without appropriate work practices (Venugopal et al., 2017). Occupational groups with high exposures to heat and workload were found to be most vulnerable to decreased kidney function, such as agricultural workers, among other occupations (Rebecca L Laws et al., 2015; Moyce et al., 2017; Peraza et al., 2012; Torres et al., 2010; C. Wesseling et al., 2016). Research conducted in non-agricultural high-heat occupations also reveals similar adverse kidney consequences. Singh et al. in 2016 reported altered kidney function in commercial kitchen workers due to continuous heat exposure (Singh et al., 2016), and Krishnamoorthy et al. have demonstrated that steel workers had higher sweat rates and were dehydrated during the shift, as shown by high urine-specific gravities (Krishnamurthy et al., 2017).

A prevalence study in Singapore reported that urinary stone disease was five times higher in outdoor workers (5.2%) compared to indoor workers (0.85%), providing evidence that high ambient environmental temperature is a risk factor for adverse kidney outcomes (Pin, Ling, and Siang, 1992). Kidney stones have been shown to be more common among physically active outdoor workers in the tropics in jobs such as quarry work (Pin et al., 1992), postal delivery workers (Pin et al., 1992), and glass industry workers (Borghi et al., 1993). Harvesters of burnt sugarcane in Brazil had decreased estimated glomerular filtration rate (eGFR) and increased serum creatinine consistent with AKI that was associated with a combination of dehydration and other factors (Santos, Zanetta, Terra-Filho, and Burdmann, 2015). A longitudinal of study of 29 Nicaraguan sugarcane workers and 25 office workers reported a remarkable decrease in eGFR and increased serum creatinine in sugarcane workers that may have been caused by dehydration and heavy labor during nine weeks of harvesting (Catharina Wesseling et al., 2016). In a Thai cohort study with 37,816 workers, significant associations between heat stress and incident kidney disease were observed in men (adjusted odds ratio [OR] = 1.48, 95% CI: 1.01–2.16). Among men exposed to prolonged heat stress, the odds of developing kidney disease were 2.22 times that of men

without such exposure (95% CI 1.48–3.35, p-trend <0.001). The incidence of kidney disease was even higher among men aged 35 years or older in a physical job: 2.2% exposed to prolonged heat stress developed kidney disease compared to 0.4% of those with no heat exposure (adj. OR = 5.30, 95% CI 1.17–24.13) (Tawatsupa et al., 2012).

The above-mentioned evidence confirms that there are some environmental, physiological, and other socioeconomic and working conditions in common between populations and geographical places with reports of adverse renal outcomes including CKDu.

Quantifying heat, workload, and physiological strain

Worker heat exposure can be assessed by using a Wet Bulb Globe Temperature (WBGT) monitor, from meteorological data (Met data recorded in meteorological centers, using standard data loggers, or from online heat index calculators such as Climate CHIP software (ClimateCHIP, 2016) or National Centers for Environmental Information (NOAA). WBGT remains one of the most widely used occupational heat stress indices (Casa et al., 2010; Kakaei, Omidi, Ghasemi, Sabet, and Golbabaei, 2019; Methner and Eisenberg, 2018; Miller and Bates, 2007; Venugopal, Chinnadurai, Lucas, and Kjellstrom, 2016; Venugopal et al., 2017; Venugopal, Krishnamoorthy, Venkatesan, Jaganathan, and Paul, 2018; Venugopal, Rekha, et al., 2016; Catharina Wesseling et al., 2016) since the 1950s (Parsons, 2014; Yaglou and Minaed, 1957) and provides a more conservative assessment philosophy (proposing limits that allowing for much shorter working times) than those predicted using the Predicted Heat Strain (PHS) model. Heat exposure estimated using Met data has been used by researches for various reasons including strong scientific backing, lower cost, and the ability to cover large areas (Blazejczyk, Epstein, Jendritzky, Staiger, and Tinz, 2012; Lemke and Kjellstrom, 2012). WBGT can be estimated from the Met data using Lemke models or using online tools such as Climate CHIP (ClimateCHIP, 2016; Lundgren-Kownacki, Kjellberg, et al., 2018). However, the use of meteorological data for occupational heat stress assessment is limited because weather stations do not directly measure important climate factors, such as solar radiation or local workplace environmental conditions, such as local heat source. In addition, meteorological data does not account for personal factors, such as metabolic heat production, based on the workload or clothing properties of the worker, both of which impact the heat exchange between the body and the environment. In locations where meteorological data is inaccessible, data loggers are used to collect continuous environmental data (Lemke and Kjellstrom, 2012; Patel, Mullen, and Santee, 2013). Worker perception of heat exposure is assessed in many studies across the world using a validated HOTHAPS (The High Occupational Temperature, Health, and Productivity Suppression) questionnaire (T. Kjellstrom, Gabrysch, Lemke, and Dear, 2009; Pin et al., 1992; Pogacar et al., 2018; Venugopal, Chinnadurai, et al., 2016; Xiang, Hansen, Pisaniello, and Bi, 2015) that can be modified and validated for local conditions. Heat strain responses are assessed by various parameters including Core Body Temperature (CBT) (Kalkowsky and Kampmann, 2006; Özgünen et al., 2010), Sweat Rate (SwR) (Krishnamurthy et al., 2017; Nielsen and Krog, 1994; Tippet et al., 2011) and Urine Specific Gravity (USG) (Borghi et al., 1993; Singh et al., 2016; Venugopal et al., 2017). In the case of CBT, accuracy varies depending on the method of measurement (gastrointestinal, tympanic, oral, or rectal). Although tympanic CBT is attractive due to being quick and non-invasive (Krishnamurthy et al., 2017; Nagano et al., 2010; Venugopal et al., 2017; Yeoh et al., 2017), it is widely discouraged for heat studies as it can be inaccurate for assessing core temperature. Rectal temperature is highly accurate, but impractical for field studies (Kalkowsky and Kampmann, 2006). Ingested temperature pills allow researchers to assess gastrointestinal temperature in a validated and relatively simple, but costly method (Tippet et al., 2011). Commonly used heat strain indicators, such as SwR (Tippet et al., 2011; Venugopal, Chinnadurai, et al., 2016; Venugopal et al., 2017), urine specific gravity (USG) (Borghi et al., 1993; Pin et al., 1992; Carlos Roncal-Jimenez et al., 2016; C Roncal-Jimenez, Lanaspa, Jensen, Sanchez-Lozada, and Johnson, 2015), and urine osmolality (Carlos Roncal-Jimenez et al., 2016) also reflect the hydration status of the workers (Montazer et al., 2013). Studies have also demonstrated the potential of specific gravity and urine color as an indicator of dehydration (Armstrong et al., 1998; Khorami et al., 2012), often using urine color chart (Nevola, 1998; Ramphal-Naley, 2012; C. Wesseling et al., 2016). Workload assessment can be measured physiologically, subjectively, and/or performance-based. Heart Rate (HR) as an indicator of heat strain and measure of workload has been reported (Bates and Schneider, 2008) and is usually measured using heart rate monitors (Bates and Schneider, 2008; Kalkowsky and Kampmann, 2006), accelerometers (Florea, Dobrescu, Popescu, and Dobrescu, 2013; Hoff et al., 2004; Quiller, 2016) and by observations (Adomat and Hicks, 2003; Lundgren-Kownacki, Kjellberg, et al., 2018; Rabiul Ahasan, Väyrynen, and Kirvesoja, 1996; Venugopal, Chinnadurai, et al., 2016; Venugopal et al., 2017; Venugopal et al., 2018).

Heat stress interventions

Feasible and sustainable interventions, such as the Worker Health and Efficiency (WE) program (Bodin et al., 2016) for outdoor workers, have benefited multiple stakeholders. Intervention studies conducted to control heat at workplaces especially with focus of averting adverse renal health are not available in abundance, but the few successful available studies have used different methods to control factors that influence heat stress, such as workload, in the presence of heat (Bodin et al., 2016; Jaime Butler-Dawson et al., 2019; Lundgren-Kownacki, Dahl, et al., 2018; Wegman et al., 2018). The second iteration of WE, the Adelante Initiative in Nicaragua, is addressing these shortcomings and provides an opportunity to better characterize heat stress and strain, understand its impact on kidney function, and the ability of interventions to ameliorate damage (Abraham et al., 2016). Evaluations of interventions used for community and occupational settings and the feasibility of the suggested interventions in field conditions are required for rolling out successful intervention programs. Customization of the interventions considering the local climate, culture, and socioeconomic conditions are essential to ensure its acceptability by the workers. Mechanisms by which the resources for the interventions could be sustained must be established to ensure the success of the interventions. Examples of possible funding include Corporate Social Responsibility (CSR) funds, government public health programs, or labor programs and development banks.

Summary and future directions

Most studies that assess heat in association with CKDu have not adjusted for other factors that may be associated with CKDu. Although the specific roles of heat, dehydration, and workload in the causal pathway for CKDu continue to be debated, they are indeed associated with both CKD and CKDu. Existing studies have used varying methods to measure heat, dehydration, workload, and indicators of adverse renal health, which makes it difficult to compare study results and assess HS/D as part of the causal pathway. Having a standardized method of measuring the qualitative and quantitative indicators of heat exposure, workload, and dehydration will help the scientific community understand the impact of these factors on disease. Additionally, future efforts to assess CKDu should use gold standard devices for measuring HS/D (e.g., core temperature pill, WGBT, accelerometers) and other valid and reliable methods (e.g., blood and urine tests) in order to increase accuracy and comparability across CKDu research studies. Workplace interventions specific to heat are needed to tackle disease prevention and progression, especially at the local level where poverty and reduced access to resources and health care complicate the burden for affected communities.

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Molecular Approaches to CKDu: Genetics, Epigenetics, and Infectious Disease

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Background and rationale

The CKDu hotspots in Mesoamerica, Sri Lanka, and India have all emerged as public health crises within the last 25 years, and it is likely that there are other foci of CKDu that have not yet been discovered. Since these initial observations, dozens of studies have attempted to characterize potentially causal factors. Most studies have focused on either occupational exposures such as heat stress and agrichemicals, toxins known to cause kidney injury under certain conditions such as heavy metals, and pathogens with known associations with kidney disease such as leptospirosis and hantavirus. Many investigators suspect that no one exposure may be solely responsible for CKDu, instead favoring a multifactorial model more consistent with most examples of chronic disease.

While an array of powerful molecular techniques has transformed fields ranging from cancer to HIV, the power of these approaches has not been brought to bear on CKDu, a disease typically occurring in countries with limited resources to devote to public health work and medical care. These tools are especially important in a disease like CKDu because they can assess pre-specified risk factors, but also identify new potential causal factors due to their non hypothesis-based nature—i.e., many of these techniques are not limited by the need for specific hypotheses in advance because they can simultaneously test hundreds, thousands, or even millions of questions in parallel. This is a tremendous asset, but also raises several challenges for CKDu researchers, for example related to the need for large sample sizes, specific tissue types, high costs, and the requirement for interdisciplinary teams with both broad and deep expertise.

Topics of discussion (gaps in knowledge)

We organized our discussions around a set of questions relating to ways that molecular techniques could help identify the cause of CKDu:

1. What kinds of genetic studies are likely to be most powerful for CKDu? What characteristics are genetic variants that drive CKDu likely to have? How large do genetic studies need to be?

2. Is there a role for current epigenetics tools to study CKDu pathogenesis? Are there new technologies that could expand the role of epigenetics?

3. What are the best tools for identifying pathogens with possible roles in CKDu? Should we be focusing on CKDu or acute presentations that appear related to CKDu?

4. How can we use technologies like metabolomics and proteomics to find novel biomarkers for CKDu?

5. What molecular techniques can be applied to kidney biopsy tissue that may teach us about possible etiologies? How can we increase the number of biopsies being performed to facilitate these studies?

6. How should resources be divided between molecular testing of candidate hypotheses and unbiased screens?

7. If/when we find important multi-omic factors that predispose or predict CKDu, what could be the real-world impact (both good and bad) of these discoveries?

Summary of discussion

Based on the questions we hoped to answer and the techniques currently available, our discussion was organized into five general categories:

Genetic factors

CKDu is likely a complex disease under the influence of genetic factors and environmental triggers (Friedman, 2019). Populations with Amerindian ancestry have shown increased genetic susceptibility to CKD, although mostly in the context of metabolic disease and Type 2 diabetes (Brown et al., 2017). Current unpublished evidence suggests it is most likely that multiple variants with small-to-moderate effects contribute to CKDu susceptibility rather than a single variant explaining the phenotype as in Mendelian disorders. The early onset of disease in young adult males in manual labor occupations suggests strong environmental factors acting as initiator or promoting progression of kidney injury in individuals with a potential underlying genetic susceptibility. To date, studies have included CKD cases with reduced kidney function in the absence of other CKD risk factors, and controls without kidney disease obtained from the same population. Family studies can help to uncover risk variants that segregate in pedigrees. Challenges to studying the genetic component of CKDu include the lack of a clearly defined phenotype and lack of early markers of disease. Cohort studies are appropriate to assess early changes over time in estimated glomerular filtration rate (eGFR) and to study changes in biomarkers including multi-omics. It would be important to obtain detailed measures of environmental factors for the study of gene-environmental interactions (Anand, Caplin, et al., 2019).

The working group consensus for gene discovery included unbiased genome-wide studies (association and admixture mapping) using recently developed genome-wide arrays that are cost-effective, although the coverage for population-specific variants may be suboptimal especially in poorly-studied geographic areas such as Central America. Whole genome sequencing data could be performed in a subset of individuals and imputed to remaining study participants using study-specific inferred haplotypes. Challenges of genome-wide association approaches are the low statistical power with small sample sizes if the variants have small effects, and the lack of suitable replication cohorts for any identified risk variant. However, multiple variants with small effect sizes could be leveraged to construct polygenic risk scores of CKDu (Lambert, Abraham, and Inouye, 2019). If successful, identifying genetic variants may point directly toward specific environmental factors in the context of gene X environment interactions and/or by being able to stratify susceptible vs non-susceptible individuals for more powerful studies of environmental risk factors.

While genetics can play an important positive role in helping to identify the factors that cause CKDu, participants also stressed the potential for negative consequences, such as employment discrimination, stigma, and psychological distress. Researchers must work with experts in these areas in order to minimize the likelihood of these harmful outcomes.

Epigenetics

The environment alters the genome through biochemical or chemical modifications that alter gene behavior without altering genetic sequence. These epigenetic changes may include modification to DNA itself (e.g., methylation), to the histones that help package and regulate DNA (e.g., acetylation), and to other elements that affect gene regulation and subsequent expression. By regulating when and in what quantities genes are expressed, the modifications that comprise the epigenome can have important effects on health and disease (Feinberg, 2018). The epigenome may also be thought of as the physical site of gene X environment interactions. Theoretically, the epigenome serves in this capacity as a biomarker of environmental exposures and subsequent phenotype.

Epigenetic tools have been developed only recently and show great promise in the study of health and disease. Epigenetic studies include both candidate approaches (testing alterations to specific pre-identified genes or pathways) and genome-wide, hypothesis-free approaches. In the opinion of the working group, genome-wide DNA methylation arrays represent the technique most likely to be powerful and useful for the study of CKDu in the near term. These studies could potentially be used to identify specific environmental exposures ("fingerprinting") and used in conjunction with other methodologies, such as genetic studies, to understand the environmental impact on gene expression (Ladd-Acosta and Fallin, 2016). Linking epigenetic studies with exposome studies is another potentially very powerful approach to uncover new environmental risk factors for CKDu by providing an unbiased approach in the absence of clear drivers.

Epigenetic studies may be most effective when they are tied to specific outcome measurements or responses to environmental stimuli. One challenge is that CKD itself may have a significant influence on DNA methylation patterns independent of etiology due to accumulation of molecules (such as toxins) normally excreted by the kidney. For that reason, temporal changes in DNA methylation pattern and longitudinal study designs are likely to be a necessary adjunct to single measurements (e.g., case-control studies). Additionally, the tissue and cell-type specificity of epigenetic marks raises the question as to whether commonly collected blood samples are appropriate to study CKDu. However, it is possible to obtain nucleated cells for DNA methylation analyses from urine samples, which provides target cells to study CKD (Lecamwasam et al., 2018). If successful, epigenetic studies could serve as quantifiable sequential biomarkers of environmental exposures and potentially early biomarkers of the disease process.

"Omics," particularly metabolomics

"Omic" technologies can survey tissues and body fluids for clues to physiologic dysregulation. A major strength of these approaches is that they could potentially reveal unknown exposures by either identifying the exposure itself or by observing how exposure alters normal physiologic processes and subsequent metabolites. This could be a key step in the search for preventable causes of CKDu as affected workers and communities face multiple complex exposures.

Small biological molecules or metabolites provide information not only on the underlying DNA sequence of an organism but also on environmental influences and biological processes of disease. The major tools available for metabolomics are mass-spectrometry and nuclear magnetic resonance (NMR) (Emwas, 2015; Hocher and Adamski, 2017). Mass spectrometry is a highly sensitive technique that may be well-suited for detecting environmental factors in the nanomolar range. NMR, though less sensitive, is highly quantitative and versatile with respect to the nature of the compounds it can identify; it may be especially useful for snapshots of an entire system of metabolites. These techniques can be applied to urine, serum, or even kidney tissue. Two major approaches commonly implemented for metabolomic research include targeted and untargeted metabolomics. In targeted analyses, researchers look for known metabolites or groups of metabolites with the aid of available validated libraries for matching signals by disease status or exposures, while untargeted metabolomics can provide novel information about the disease process with the major challenge of identifying unknowns that don't match to available libraries (Bingol, 2018). Furthermore, metabolomics could aid in the characterization of the exposome, defined as the totality of environmental exposures from conception onwards (Rappaport and Smith, 2010). Capturing endogenous and exogenous influences on the metabolome could also be extremely valuable for understanding similarities and differences of the metabolomic signature of CKDu relative to CKD and other diseases (Athersuch and Keun, 2015).

Since many compounds are cleared by the kidney, measurements will be confounded by kidney function. However, the collection of urine samples might also aid in understanding disease progression. Longitudinal prospective studies with repeated measures may help address this inherent limitation by factoring in the chronicity of changes. If successful, metabolomics could help identify exposures or relevant biological pathways that were not previously considered as hypotheses and address differences between CKDu and CKD.

Infectious disease

Infectious diseases are an important contributor to renal morbidity in areas affected by the CKDu epidemic and could be one of the factors that underlie its emergence. Changes in climate, high density of infectious disease vectors and reservoirs, limited access to clean water, limited resources for detecting and controlling infectious disease outbreaks, and continued emergence of new pathogens and pathogen variants increasingly challenge kidney health in tropical and low-resource settings (Prasad and Patel, 2018). The presence of these factors in CKDu-burdened regions, along with endemicity and hyperendemicity of several candidate pathogens; a preceding acute, febrile syndrome in some who develop CKDu; and epidemic curves in disease hotspots suggestive of pathogen emergence lend credence to the hypothesis of an infectious etiology.

While targeted testing for the most likely candidate pathogen is a judicious and focused approach, a small but growing body of inconsistent findings suggest casting a wider net would be more productive. Leptospira and hantavirus infection, widely assumed the most likely culprits, and a few others have been explored through targeted testing; these studies

so far fail to provide strong evidence of their involvement in the epidemic, but do confirm infections are commonly occurring in the populations and may suggest important risk factors (Gamage et al., 2017; Wijkstrom et al., 2018; Yih et al., 2019). Untargeted techniques, (e.g., 16S, shotgun RNA/DNA sequencing) are generally resource-intensive and expensive but have the sensitivity to detect an unexpected or even novel pathogen. However, recent unbiased (untargeted) exploratory analysis in a small group of patients with AKI in Nicaragua did not implicate any single known bacteria or virus (Fischer R.S. abstract in workshop meeting book (2019). The consensus among working group members was that additional testing using newer technologies is warranted to understand or rule out the role of an infectious disease process in CKDu.

The working group was enthusiastic about moving toward unbiased screening, with case-control analyses and well-defined classification criteria. However, targeted, multiplexed testing (i.e., combining detection of multiple pathogens into a single PCR reaction) will also be informative if untargeted sequencing is not feasible. Partnerships to expand availability of technology to lower resourced investigators and sharing/pooling of biospecimens for larger sample sizes will be important, as will be correctly classifying infection status (e.g., acute/current, past) and assigning temporality (i.e., infection occurred prior to CKDu). For example, analysis of acute infection should utilize biospecimens from the earliest symptoms of CKDu. A thorough approach would test multiple specimen types (ideally, whole blood, serum, urine, and renal tissue) and prospectively monitor renal and infection status over time.

It is important to note that if identified, an infectious pathogen (or pathogens) may only play only a role in CKDu, such as providing one 'hit' in a multifactorial disease, initiating an immune-disruptive systemic process, or aggravating an existing, subclinical renal injury. Even so, identification of an infectious etiology of CKDu could offer hope of treatment and strategies that prevent additional morbidity and mortality through modifiable risk factors and interrupting transmission cycles.

Future directions

Many additional emerging technologies were cited as potentially useful in the search for the cause(s) of CKDu. Transcriptomics of kidney biopsy tissue could serve as both an important way to study etiology and also serve as a biomarker to define CKDu; overcoming the obstacles to kidney biopsy will be an important priority for other advanced approaches as well. Some panel members were enthusiastic about the prospect of using patient-derived cells to evaluate responses to risk factors (e.g., heat). Studies of the microbiome as a possible factor in CKDu pathogenesis remain totally unexplored. However, in the absence of clear drivers, panel members emphasized the used of untargeted measurements to accelerate discovery to address the current crisis rapidly.

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Pathology

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Background

Existing evaluations of renal biopsy samples from human patients have followed usual conventions of clinical nephropathology variably including traditional special histochemical stains for light microscopy, immunofluorescence, and transmission electron microscopy. Descriptions of the morphologic changes in those samples have also followed usual diagnostic conventions. Alternatively, the field has lacked a consistent approach to evaluating these materials such as using the Banff Classification (Roufosse et al., 2018) for semiquantification of e.g. chronic tubulointerstitial changes (a classification applied to renal allograft pathology) which significantly complicates our ability to consistently prognosticate disease progression and compare pathologic features of this disease across affected patient cohorts.

Research gaps

Gaps in this field of study include:

- An agreed technical approach to the assessment of renal biopsy material from patients with suspected CKDu, including a descriptive and severity grading schema.
- A consistent set of complementary clinical data.
- A central repository/registry of clinicopathologic data.
- An articulation of clinical gaps in our current data sets (e.g., which patients haven't been sampled and should be).
- A strategic, structured and hypothesis-driven approach to experimentally modeling this enigmatic disease that might enable insights into its pathogenesis.

Content and scope

The Pathology Working Group engaged a small group of nephropathologists, clinical nephrologists, and toxicologic pathologists to compare observations and perspectives on key pathologic features of this unique disease. Collectively, the group had experience reviewing biopsy material from CKDu patients in Nicaragua, El Salvador, and Sri Lanka. Toxicologic pathologists from the U.S. National Toxicology Program (NTP) also had experience modeling experimental kidney disease induced by environmental agents in animal studies. The primary aims of the working group were:

- To use our collective experiences to identify key and consistent features among the samples studied.
- To speculate on potential pathogeneses with the intent to hypothesize etiologic contributors.
- To develop a descriptive histopathologic schema for biopsy characterization.
- To discuss opportunities to build a global partnership.
- To discuss approaches to mechanistic investigation.

Additionally, the group planned to have discussions with complementary working groups, including Biomarkers, Clinical Diagnosis and Treatment and Molecular Mechanisms to understand how to align and integrate our efforts and action plans.

Individual members of the working group shared presentations highlighting key light microscopic and ultrastructural features of the biopsy material they have previously reviewed from patients suspected to have CKDu (Anand, Montez-Rath, et al., 2019; Fischer, Vangala, Truong, et al., 2018; Wijkstrom et al., 2017; Wijkstrom et al., 2018; Wijkstrom et al., 2013). All the experiences shared were derived from percutaneous needle biopsies thus mostly representing renal cortex. Also, biopsies were generally collected from patients with chronic kidney disease stage 2-4, i.e. eGFR between 90-16 ml/min/1.73m^2. Pathologic changes in the samples reviewed by the contributors demonstrated several consistent features, including chronic interstitial fibrosis variably admixed with mixed mononuclear inflammatory cells, tubular atrophy, glomerular enlargement, global glomerular sclerosis, and signs of glomerular ischemia. Histologic changes in arteries such as intimal fibrosis, smooth muscle hyperplasia, and arteriolar hyalinosis were occasionally seen but not a consistent finding.

In kidney biopsies from "acute CKDu" patients, the main findings were described as tubulointerstitial nephritis with varying degrees of acute inflammation and chronic tubulointerstitial changes (Fischer, Vangala, Truong, et al., 2018). In some cases, in both chronic and acute CKDu patients' neutrophils have been found in the tubular lumen, but urine cultures from these patients have been negative. Ultrastructural changes evaluated with electron microscopy were mostly consistent with the light microscopic observations. Immune complex deposits have not been found. Glomerular ischemia was characterized by collapsed glomerular tufts, periglomerular fibrosis or wrinkled capillary basement membranes. Podocytic changes and foot process effacement have been reported in some cases. Tubular cells show varying degrees of degenerative changes and atrophy. Large dysmorphic lysosomes within tubular epithelial cells previously described as a distinctive feature were occasionally seen but not considered pathognomic.

In addition to the clinical experiences, the group also reviewed a high-level summary of the U.S. National Toxicology Program's (NTP) experience testing environmental agents in rodent models. A preliminary search of previous studies of pesticides and heavy metals (included in lists of putative etiologies for CKDu) has revealed studies in which renal changes were identified as test article-related changes. While these investigations will continue, the group was also introduced to the breadth of technical capabilities of the NTP and how they might be leveraged to advance CKDu interests.

Participants reflected on the consistency of the morphologic features across the experiences that were shared and discussed potential insights into etiologies and pathogenesis. Participants recognized the breadth of hypotheses emerging from various groups studying this disease including heat stress, dehydration, agricultural chemicals, heavy metals, and even infectious agents. The chronicity of the changes and absence of typical features of more common causes of chronic progressive renal disease (e.g., diabetic glomerulopathy, immune complex deposits, hypertensive vascular changes) complicates identification of a particular etiology.

The group also discussed what more we would like to know and how to support efforts to improve our current understanding. Gaps in our current knowledge include a consistent approach to characterizing biopsy material, a central registry or repository for the observations collected, collection of biopsies from patients earlier in the course of their disease, a more complete clinical characterization of the progression of disease (particularly, the association of intermittent acute injury), and a more complete global community of renal pathologists and clinicians who could work together to harmonize approaches and integrate experiences. The group considered a number of solutions for these gaps. A standard template for CKDu renal biopsy characterization akin to the Banff Classification of Renal Allograft Rejection Pathology (Roufosse et al., 2018) was considered a useful addition to the field. It was also agreed that an accompanying atlas of light microscopic and ultrastructural images would ensure consistency in characterization of biopsies in disparate parts of the world. A draft template was developed during the meeting with a commitment by attendees to complete the development after the workshop. Representatives of the U.S. NTP offered to support the development of a user-friendly electronic template that could be used by pathologists who have or will review biopsy materials from CKDu patients.

The value of a central repository for biopsy images, patient data, and biopsy characterizations was discussed by participants. It was viewed as a challenge given that most, if not all, of the biopsies that have been collected to date have not been collected under a specific research protocol and would not have included necessary patient permissions. It was thought that the anonymized observational data might be collated for review and analysis. If a research protocol were to be developed, there is precedent for a centralized pathology registry as supported by NIH and NCI for the NEPTUNE and CureGN studies (Gadegbeku et al., 2013; Mariani et al., 2019).

The group also recognized the importance of engaging and educating medical clinicians in the localities where the disease is believed to occur. Our best opportunity for getting sufficient insights from patient samples and associated clinical data lies with local medical staff. Accordingly, the group discussed and agreed on the usefulness of developing a training module of renal biopsy collection, handling, and evaluation that might be used to engage clinicians in affected regions.

Several nephropathologists with experience reviewing biopsy material from patients suspected to have CKDu were not present at the meeting. The Pathology Working Group discussed approaches for engaging this broader community of experts and considered the U.S. NTP Pathology Working Group model. The NTP routinely brings together pathologists from different organizational and geographical venues to collaborate and develop consensus on pathology observations generated in NTP studies. The infrastructure to facilitate these collaborations includes digital and telepathology capabilities. This infrastructure could be leveraged to broaden the international collaboration of CKDu pathologists.

The Pathology Working Group also met with other working groups with complementary interests, including Biomarkers and Clinical Diagnosis and Treatment. The three groups aligned on an interest and need to get pathologic insights into earlier stages of the disease and recognized the need for sensitive and specific biomarkers of early disease to enable early diagnosis and opportunity for biopsy sampling. There was general agreement that usual biomarkers of progressive renal disease of glomerular origin (i.e., proteinuria) or acute tubular injury in isolation will not likely be sufficient.

Lastly, participants discussed the need for mechanistic investigation and how that might add to our current understanding of the etiology and pathogenesis of CKDu. Though the NTP will continue to review its experiences with environmental agents that induce chronic, non-glomerular renal injury, working group members were aligned on the challenges of initiating targeted experimental work in the absence of sufficient epidemiologic or clinical data to support specific etiologic hypotheses. Alternative to that is the common environmental context (i.e., heat, dehydration, heavy workload, etc.) that could be a focus of investigation as a contributing or susceptibility factor (Johnson et al., 2019). Though animal studies were postulated, welfare issues would need to be considered. This is an area requiring additional consideration.

Conclusions

The pathology working group concluded with a discussion of a strategy for moving these interests forward. Participants committed to completing the biopsy characterization template and supporting it with an electronic framework. It was agreed that an accompanying atlas of typical light microscopic and ultrastructural changes would be useful to ensure consistency in recording the key characteristics. The group also expressed an interest in collaborating on a training module that might be shared with local and regional health care workers as well as organizing a more global and collaborative venue for consultation with pathologists who did not attend the workshop. Integrating or aligning continued effort by the participants of the pathology working group with other groups will likely yield the most impact.

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Pesticides and Metals

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Preparation of pre-workshop materials

All pre-workshop materials noted below and in this working group report are available on the workshop website: www.niehs.nih.gov/news/events/pastmtg/2019/ckd_2019.

Note: Pre-workshop reading materials reflect the views and work of the authors listed in each posted file. The material has not been peer reviewed and may not represent opinions or input from all working group participants.

1. Pesticides and CKDu, Mesoamerica (ppt). Catharina Wesseling, Karolinska Institutet, Sweden; La Isla Network, USA
2. Chronic kidney disease of unknown etiology in Sri Lanka: The potential role of pesticides, heavy metals and drinking water as environmental risk factors. Kamani Wanigasuriya, Pathmalal M. Manage, Keith E. Levine, Jill Lebov, Jennifer Hoponick Redmon.
3. Glyphosate-based herbicides (ppt). Channa Jayasumana, Rajarata University, Sri Lanka
4. Measurement strategies to evaluate occupational exposure to pesticides (pdf). Berna van Wendel de Joode, Universidad Nacional, Costa Rica
5. Summary of current understanding of the role of metals in CKDu epidemic (pdf). Kathy James, University of Colorado at Denver, USA
6. Heavy metal exposure and CKDu, Sri Lankan perspective (ppt). Kamani Wanigasuriya and Pathmalal Manage, University of Sri Jayewardenepura, Sri Lanka, Jennifer Hoponick Redmon and Jill Lebov, RTI International, USA
7. Exposure to metals and decreased renal function? (ppt). Kristina Jakobsson, University of Gothenburg, Sweden

Objectives

The overall objectives of the working group were to:

a. Review the state of the science regarding the potential for pesticides or metals to adversely affect kidney function as risk factors of the CKDu epidemics, and compare research findings across regions (Mesoamerica, Sri Lanka and India).

b. Discuss the potential for pesticides or metals to act as environmental risk factors for CKDu based on the current research to date.

c. Discuss integrated methodologies needed to properly evaluate the exposure to potential contamination risk factors and explore possible associations and interactions with other CKDu risk factors, especially between contaminants and heat stress.

To build on prior workshops, we focused on evidence from research published after the 2015 report from the Second International Research Workshop on MeN (Program on Work Environment and Health in Central America (SALTRA) et al., 2015).

Pesticides

The working group generally discussed the potential for pesticides to act as environmental risk factors associated with CKDu, and in certain cases, discussed specific pesticides. Agrochemicals, such as fertilizers, growth hormones, and components in the commercial formulation other than active ingredients as well the role of chemical mixtures, impacts of long-term exposure to low-levels of chemical contaminants, and altered susceptibility to chemicals due to other risk factors (e.g., nutrition) were not specifically addressed and should be considered in future discussions.

State of the science – recent research

A systematic review by Valcke et al. in 2017 examined 21 epidemiologic studies of the associations between pesticide exposures and any outcome measure of CKD (not CKDu specifically) globally (Valcke, Levasseur, Soares da Silva, and Wesseling, 2017). The findings from this paper were discussed in the working group (pre-workshop material 1). Eleven studies represented research from Mesoamerica, five from Sri Lanka, two from the United States, and three from other countries. Three studies used a cohort design, five were case-control, and 13 were cross-sectional (five with nested case-control analyses). The reviewed studies, reporting both positive and negative associations between pesticides and CKD, were limited by cross-sectional design, confounding, and selection bias. Different pesticides were measured across studies, and studies considered to use robust study designs were mostly conducted in regions not experiencing CKDu epidemics. The results of the global review by Valcke et al (2017) on associations between pesticide exposures and CKD concluded that there is limited evidence that certain specific pesticides, as well as acute poisonings in general, can produce acute and chronic kidney damage in humans, but do not provide much evidence in the context of the CKDu epidemics; however, the review noted that exposure assessment had been deficient (Valcke et al., 2017). The evidence that exists comes from studies with stronger design and better exposure assessment and included an association between CKDu and glyphosate in an endemic area in Sri Lanka (Jayasumana, Paranagama, et al., 2015), CKDu and organochlorine pesticides in urban Delhi in India (Siddarth et al., 2012; Siddarth et al., 2014), and end-stage kidney disease and past exposure to alachlor, atrazine, metalochlor, paraquat, pendimethalin, and permethrin among U.S. farmers (Lebov et al., 2016).

Additional research published after the 2017 Valcke review was also discussed (pre-workshop material 1). In Mesoamerica, one recent study measured residues of two nephrotoxic herbicides (glyphosate and 2,4-D) in urine of study participants and found low to negligible exposure in Salvadorian sugarcane cutters at high risk for CKDu (Curwin, 2016). A community cohort in Nicaragua collected information on agrochemical use including glyphosate, paraquat, cypermethrin, and methomyl use within the last six months of the second follow-up visit. Adjusting for age and education level, the authors did not observe an association between the combined effect of agrochemical use and rapid decline in eGFR among male participants (Gonzalez-Quiroz, Smpokou, et al., 2018). A case control study nested in this community cohort documented

substantial exposures to pyrethroid pesticides and the organophosphate chlorpyrifos, but not to glyphosate. Baseline levels of 11 mostly commonly used pesticides or their metabolites (2,4-D, glyphosate, MCPA, 4F-3PBA, 3-PBA, DCCA, CFCA, OH-PYR, TEB-OH, 5-OH-TBZ, ETU) were similar for cohort members with stable kidney function over two years and for those who rapidly declined kidney function in this CKDu hotspot in Nicaragua (Smpokou et al., 2019).

Many working group participants concluded that the Valcke et al. 2017 review and recent studies in Mesoamerica do not support an association between pesticide exposure and CKDu, beyond the possibility of chemicals acting as a disease progressing factor or interacting with heat stress. However, there was not a consensus across working group participants on the potential etiologic role of toxic substances in CKDu in Mesoamerica. It was agreed that most studies to date are still limited by crude exposure assessment, sample size, and/or inadequate study design, and there was agreement on the limitations in pesticide studies so far for CKDu.

A review to compile the most recent research on pesticides and CKDu in Sri Lanka and India was prepared prior to the workshop (pre-workshop material 2). Some recent work in Sri Lanka includes evidence that metals and glyphosate were significantly different between CKDu cases and controls, but the sample size was very small (n=10 cases from CKDu endemic area, 10 controls from endemic area, 10 controls from non-endemic area) (Jayasumana, Gunatilake, and Siribaddana, 2015). A study in Delhi, India, of 100 patients with CKDu and CKD of known etiology (CKDk) and 100 healthy controls detected nine organochlorine pesticides or their isomers (OCs) and evaluated their impact on eGFR in the three groups (Ghosh et al., 2017). The results indicated associations between increased blood levels of four OCs and CKDu and two with CKDk. However, this cross-sectional study represented an urban population and only addresses nephrotoxicity of OCs, not an association of OCs with epidemic CKDu. The review of studies in Sri Lanka and India reiterated the findings from Mesoamerica in that there is not conclusive evidence that pesticides cause CKDu.

Glyphosate (e.g., Roundup), the most widely used herbicide around the globe, remains a concern (preworkshop material 3). Glyphosate-metal complexes that form in the presence of hard water have been hypothesized as contributing to CKDu etiology in Sri Lanka (Jayasumana, Gunatilake, and Senanayake, 2014), and there is limited epidemiologic evidence for an association between glyphosate and CKDu in Sri Lanka (Jayasumana, Gunatilake, et al., 2015; Jayasumana, Paranagama, et al., 2015). Studies in El Salvador and Nicaragua that addressed glyphosate exposure and its relationship with CKDu did not detect substantially elevated exposure levels or associations with renal function (preworkshop material 1; (Curwin, 2016; Gonzalez-Quiroz, Smpokou, et al., 2018; Smpokou et al., 2019; Valcke et al., 2017). No studies have been conducted in India, and other potential hotspot regions. A study of the presence of glyphosate residues and its metabolite aminomethylphosphonic acid (AMPA) in topsoil, water, and sediment samples in an agricultural region impacted by CKDu in Sri Lanka, detected very low levels of glyphosate in all sediment samples (85-1000 ug/kg) and in all groundwater samples (1-4 mg/L), and of AMPA (2-11 mg/L) less frequently. Glyphosate levels were far below the maximum contaminant level (MCL) of the EPA (700 ug/L) for drinking water, but remain high in the sediment samples.

It was pointed out in the working group that commercial pesticide formulations not only consist of the active ingredient(s), but also contain a variety of additives and may contain metal contaminants such as arsenic, cadmium, and lead (Cox and Surgan, 2006) and Jayasumana et al. addendum in the report from the first international research workshop on MeN (Program on Work Environment and Health in Central America (SALTRA) et al., 2012). Studying formulations is challenging due to the heterogeneity across brands and between lots. Despite the heterogeneity, identifying potentially common elements and formulations of additives across different agrochemicals used may help examine or eliminate a potential role for these additives. The working group participants agreed that there is a need for more robust study designs to evaluate the potential role of pesticides and other agrochemicals in CKDu etiology in affected regions, in combination with other risk factors.

The research question is: "Do (certain) pesticides have a role in the etiology of CKDu?" Of note, this question is different from, "Are (certain) pesticides nephrotoxic?" While these remain two different research questions, they are intricately linked. If future studies observe nephrotoxic compounds present in biological or environmental samples in CKDu endemic regions, closer examination of the route of exposure to humans, biotransformation, mixtures, and acute and long-term renal effects will be needed. Until recently, studies Until recently, studies have been limited by inadequate

exposure measurement methods (such as use of crude ever or current use of pesticides or agrochemicals, inappropriate methods for selecting pesticides (low-use or non-nephrotoxic pesticides), insufficient quantification of selected pesticides, limited time-frames for exposure assessment), poor characterization of CKDu, or study designs that are inadequate to address the pertinent research question (such as cross-sectional designs and studies conducted solely in non-CKDu endemic/epidemic areas). Future epidemiologic research on pesticides and CKDu should meticulously address all these issues, to provide insight on a potential etiologic role of pesticides in disease generation and progression. Encouraging is that several recent studies have used more appropriate approaches to measure potentially putative exposures (Curwin, 2016; Gunarathna et al., 2018) and to assess associations of common-use pesticides and longitudinal kidney function decline (Smpokou et al., 2019) in regions and populations at high risk for CKDu.

Topics addressed in the working group regarding pesticides as a potential environmental risk factor for CKDu

Working group participants reasoned that for a specific pesticide to be a key cause of an epidemic of the magnitude seen in Mesoamerica, Sri Lanka, and India, it must be present in a diversity of agricultural settings in multiple countries, with elevated and widespread chronic occupational or environmental exposures. Some of the participants thought that the existence of a common "bad actor" chemical is unlikely to explain the cause of CKDu. Alternatively, different pesticides may act through common mechanisms that increase the risk of CKDu or further disease progression. However, it was reiterated that there is no research in CKDu endemic areas with a strong longitudinal design examining lifetime exposures to specific pesticides or chemical groups with similar toxicological actions. Such research is necessary to elucidate the role, if any, of pesticides or other agrochemicals in this epidemic. In addition, epidemiologic or experimental research on interactions between pesticide exposures and heat exposure (or other risk factors) is absent and should be addressed.

Other participants postulated that chemical exposures are a likely cause and that multiple compounds could be potentially interacting. However, trying to find the chemical agent may be taking us down the wrong path. Consistent with this line of thinking, broader exposure assessment technologies (exposomics), including non-targeted chemical analyses, were proposed. Silicone wristbands to measure exposure to pesticides as well as other environmental chemicals, such as polycyclic aromatic hydrocarbons (PAHs), over the course of a week was thought to be a way forward to the use of new and affordable non-targeted analysis methods and for contemporary exposure assessment. It was noted that this approach does not solve the cross-sectional limitations of exposure assessment, however, and more research is needed to understand which internal doses are reflected in the concentrations measured by passive exposure sensors, such as silicone wristbands.

It was not specifically discussed in the working group, but emerged later, whether to focus on pesticides or on agrochemicals, including fertilizers and any other type of chemical compounds used in agriculture, like growth hormones, adjuvants of pesticide formulations and microcontaminants. Such considerations are important and other agrochemicals should be kept in mind.

Ecological comparisons were proposed to examine pesticide imports, history of land use, technologies, and CKDu incidence. Historic pesticide data (i.e., common use pesticides over time by crop and region) can inform chemicals to study. The methods that have already been established for Central America must be restarted by SALTRA and extended to Sri Lanka and beyond. A key Global Historical Database for imported pesticides and use on crops should be a goal. Although this is a valid approach, especially as a starting point to direct future research, this study design alone is only hypothesis generating and individual data are needed in order to be able to establish causal associations.

Regarding specific pesticides, working group participants discussed common use pesticides and within that group prioritize suspected nephrotoxic pesticides (and in Mesoamerica especially those used on sugarcane), such as glyphosate, 2,4-D, atrazine, and paraquat. Pesticides causing acute organ damage (including kidney), such as paraquat, needs to be examined in impacted regions. It was proposed that glyphosate, propachlor, propanil, and diazinon, which are variably but commonly used pesticides in Sri Lanka and Central America, should be addressed together as a nephrotoxic group due to their neurocalcerin inhibiting properties with similar effects as those seen in patients

taking cyclosporin. Paraquot was measured in migrant workers with CKD in Houston, but this information was considered anecdotal and not widely replicated. However, exposure to paraquat in this population was anecdotal and not present in all patients. In Sri Lanka, it was mentioned that paraquat has not been detected in wells recently, possibly due to its prohibition in 2010.

Long-term, low-dose pesticide exposure through drinking water remained a concern, especially in Sri Lanka. The issue is complex as persistence of chemicals in the water systems is unknown; pesticides have different half-lives; seasonal variations call for studies in dry and rainy seasons; concentrations vary depending on closeness to paddy fields; geochemical characteristics influencing runoff from the fields are unknown; there are multiple chemicals in these wells including pharmaceuticals; the presence of arsenic, cadmium, and other toxic compounds in commercial pesticide and fertilizer formulations may also be a factor to consider. In the endemic area of Sri Lanka, clean water is now provided with reverse osmosis plants and the water quality is evaluated, but it will take time to see any potential impact on health. In addition, no monitoring studies were conducted prior to the implementation of the reverse osmosis plants, so evaluation of their impact on water quality will be challenging. Contamination of fertilizers and pesticides with metals, such as arsenic and cadmium, needs to be addressed not only in water but also in food, especially rice.

A project of interest that was discussed is the "Global Network for Pesticide Exposure Research," coordinated from Lund University, Sweden. It is an example of exposure assessment of a wide range of chemicals in multiple locations. Biosamples from existing cohorts around the globe are collected and analyzed for pesticide residues, providing a cross-sectional snapshot. Although studies used different protocols and the results do not address lifetime exposure, these data could serve as a baseline. Prospective studies were considered important in the discussions, as well as standardizing the questions about pesticide exposure across cohorts. Furthermore, it was considered that intervention studies on pesticide exposure should be done once there is more clarity whether specific pesticides impact CKDu etiology.

Two key points raised were that i) all future studies on CKDu should use a core protocol of pesticide exposure assessment for valid comparisons, and, whenever possible, ii) all studies on CKDu should include both a pesticide exposure, as well as a workload and heat exposure component.

Integrating methodologies to identify pesticides as a potential CKDu risk factor

Considering the inadequate pesticide exposure assessment in most previous studies (Valcke et al., 2017), an overview was prepared about how to properly go about this task, also considering previously heavily used but currently discontinued pesticides (preworkshop material 4). It is essential to establish a strategy based on contextual information and have an agent-specific approach focusing on commonly used pesticides in crops and regions of interest. It is a general principle of exposure assessment to contrast exposure levels with characterization of duration and intensity of exposures, on the individual level when possible, or on the group level with workers performing similar tasks. Measurement strategies of current exposure should consider variability between and within workers or members of study populations through repeated measurements over time. To investigate the role of pesticides in the CKDu epidemics, a life-time exposure approach is needed, including current as well as past exposure assessment.

Quantitative Biological Assessment. To investigate the role of pesticides in the CKDu epidemics, a life-time exposure approach is needed, including current as well as past exposure assessment that includes questions about specific exposures that go beyond binary responses (e.g., have you ever been exposed to pesticides?). Quantitative measures of pesticide residues in biological samples such as urine are often viewed as the preferred media for exposure assessment, whereas questionnaire data may be considered 'subjective' and prone to recall bias. However, biomarker data should be combined with information on pesticide use over time, especially since residues of current-use pesticides in urine, in general, only reflect recent exposures to pesticides and most have short half-lives, from hours to days. Depending on the chemical, other biological matrices could be considered including blood, hair, and nails. The group agreed that it is essential to understand how pesticide residue concentrations vary between persons and in time and evaluate grouping strategies that maximize contrasts in exposure levels of specific pesticides between study participants. Unless a putative agent is a persistent chemical or a ubiquitous compound causing sustained daily exposures over prolonged time periods

in the population at risk, causal agents may be missed in biological samples, especially without multiple measurements over time and space. Also, pertinent pesticides may not be addressed, for example when the chemical analytical methods and detection limits of a laboratory define which pesticides are analyzed instead of criteria of frequent use and nephrotoxicity.

Overall, working group participants agreed that the following integrated analytical approaches are essential:

- Repeated measurements in study populations and monitoring over time to improve assessment of recent exposures by accounting for variability.
- Biobanking to allow retrospective identification of etiologically important pesticides combined with sufficient data on pesticide use.
- Crosslinking and georeferencing biological samples with environmental and food samples for comparative analysis.
- Non-targeted analysis methods made possible by novel mass spectrometry techniques to analyze environmental contaminants across a broad range as a starting point for determination of specific environmental contaminants to evaluate further.

Retrospective pesticide exposure assessment. Although retrospective pesticide exposure assessment is a challenging task, it can provide critical data through implementation of well-designed questionnaire methods. Questionnaires remain an essential tool to address life-long or long-term pesticide use. Experiences exist in Central America about how to prepare efficient and reliable questionnaires based on combining data extracted from external sources on pesticide use organized by regions and crops with individual use data from questionnaires, the latter to reduce recall bias and increase validity of exposure assessment (Monge et al., 2005; Monge et al., 2004; Valcke et al., 2005).

In Central America, hundreds of active ingredients are registered and sold, but according to the pesticide import database at IRET-Universidad Nacional in Costa Rica, in Central America, only 20 active ingredients composed approximately 80% of pesticide imports between 2010-2014 (Box 1). Import data can be a proxy for use (Valcke et al., 2005) and the pattern of a limited number of pesticides accounting for a high proportion of total pesticide use has been observed for decades (Bravo et al., 2011).

Box 1: Most imported pesticides in Central America

- Imports can be a proxy for use.
- Analysis of pesticide imports in Central America for 2010-2014:
  - 20 active ingredients represent 80% of pesticide imports regionally.
- These active ingredients are in descending order of amount imported:
  - Mancozeb, glyphosate, 2,4-D, 1,3-dichloropropene, paraquat, chlorothalonil, chloropicrin, diuron, propineb, atrazine, metam sodium, ametryn, methyl bromide, ethephon, terbutryn, diazinon, pendimethalin, chlorpyrifos, terbufos, sulfur.

Source: Central American Institute for Studies on Toxic Substances (IRET)
Universidad Nacional, Costa Rica

The group discussed drafting a list of pesticides most commonly used over the last decades. From this list, suspected nephrotoxic pesticides and pesticides of concern in general can be selected, such as glyphosate, 2,4-D, paraquat, or some organophosphate and pyrethroid insecticides, for further prioritization in a pesticide exposure assessment strategy. Such data can be organized in the form of a crop-exposure matrix, job-exposure matrix and even task-exposure matrix (Baldi et al., 2017; Carles et al., 2018). Consultations with crop experts are especially useful in the preparation of questionnaires

restricted to a limited amount of pesticides, organized by time period, region, and specific crop for prompting recall (Monge et al., 2005; Monge et al., 2004). In addition, questionnaires can collect individual information about total days of application during life of specific pesticides or groups of pesticides with similar toxicological action, re-entry work, use of specific pesticides at home, and use of personal protective equipment. Because questionnaire data, even with special methods such as icon-based interviews (Monge et al., 2004), remain subject to recall bias, data may be improved by combining questionnaire data with biomonitoring data. The combination of external and individual exposure data could possibly be refined using ranking strategies or other quantitative approaches (Mong et al., 2005).

Overall recommendations to identify pesticides as a potential CKDu risk factor

The main recommendations presented to the group by the working group leads for future research on pesticides and CKDu include the following:

Note, these recommendations were not considered comprehensive or reflective of views and input of all working group participants.

- Mixed/multi-faceted data collection methods are necessary to assess life-time exposure to specific pesticides.
- Priority pesticides (and other agrochemicals if pertinent) should be specified, based on use data and nephrotoxic potential.
- An international historical database should be created cataloging importation and crop use of pesticides.
- For sound retrospective pesticide exposure assessment, crop-exposure matrices over time and regions can be combined with pesticide use data at a personal level to assess life-time exposures.
- To address current exposure, there is a need for repeated measurements of urinary pesticide concentrations with short half-lives to understand how exposures vary between and within jobs and subpopulations.
- Contamination of drinking water by pesticides should be addressed while considering physical-chemical parameters of water.
- To be able to compare results from different studies, a core module/questionnaire for assessment of pesticide exposure should be developed.

Metals and metal mixtures

The working group generally discussed the potential for metals to act as environmental risk factors associated with CKDu (preworkshop material 5) and in certain cases, specific metals or metal mixtures (preworkshop materials 2, 6, and 7).

State of the science – recent research

The group discussed the following published research. In Sri Lanka, for over a decade studies have been conducted on associations between various metals, metalloids, and minerals and CKDu, especially arsenic (As), cadmium (Cd), lead (Pb), and fluoride (F), and to a lesser extent also mercury (Hg), cobalt (Co), iron (Fe), manganese (Mn), molybdenum (Mo), strontium (Sr), thallium (Tl), uranium (U). A preworkshop review of eight biomarker studies in Sri Lanka, presented in the working group (preworkshop material 7), did not observe higher urine levels of Pb and Cd in controls from endemic areas as compared to controls from non-endemic areas, and there was no consistent finding of higher metal levels for cases compared to controls (Bandara et al., 2008; Chandrajith et al., 2011; HMA et al., 2018; Jayasumana, Gunatilake, et al., 2015; Jayatilake, Mendis, Maheepala, and Mehta, 2013; Nanayakkara et al., 2014; Rango, Jeuland, Manthrithilake, and McCornick, 2015; Wanigasuriya, Jayawardene, Amarasiriwardena, and Wickremasinghe, 2017). One study in Sri Lanka, using a metallomics /mineralomics approach, analyzed a broad panel of elements in local rice and freshwater fish from two towns within the endemic North Central Province region of Sri Lanka areas, as well as residential drinking water and soil samples, and blood and hair of 84 male "patient participants" (no further description) (Levine et al., 2016). Notably, non-endemic areas were not investigated for comparison. The mean and max blood levels of Cd and Pb

exceeded US general population reference levels from NHANES data 2009-2010, but were well within OSHA occupational standards, and arsenic was mostly non-detectable. Regarding water samples, in some samples F, Fe, Pb and sodium exceeded WHO international drinking water standards or US-EPA MCL (Levine et al., 2016).

In 2017, Herath et al. noted based on the results from chemical analysis of a large number of water samples throughout the country, that Sri Lankan well water needed treatment for fluoride, nitrate, aluminum, manganese, and hardness for drinking purposes especially in CKDu endemic regions, but this study did not perform biological measurements in individuals (Herath et al., 2017). In 2018, this same research group extended their analyses to cadmium, chromium and lead along with a review of results from previous Sri Lanka studies. The authors concluded that arsenic, cadmium, lead, and chromium concentrations in human urine samples from CKDu-endemic areas were not significantly different from those from non-endemic areas and are thus not likely causes of CKDu (HMA et al., 2018). The most recent, comprehensive, evaluation published in 2019 (Nanayakkara et al., 2019) state: "…the levels of all minerals and trace elements analyzed including Cd and As from affected and unaffected areas were within the levels recommended by World Health Organization and Sri Lanka drinking water guidelines and did not suggest any form of contamination. Analysis of biological samples, including urine, hair and renal tissue from biopsies, did not provide evidence to support Cd or As toxicity in CKDu patients." However, this does not rule out the effect of total metal burden may have on kidneys, given that As, Cd, Pb, and V (all with nephrotoxic properties) were found in the drinking water in the impacted regions in Sri Lanka.

One issue raised in the working group discussions with the research in Sri Lanka was that, in most studies, cases included were based on proteinuria, whereas CKDu is considered mostly a non-proteinuric disease. Also, etiologic research for cadmium was often based on measurement of Cd in urine, which is unsuitable because of the risk of reverse causation.

In Mesoamerica, there are few data on metal levels in the general population. In Mexico, a small study with 50 cases of CKDu and 50 healthy controls in Toluca, a highly polluted area in the western part of the State of Mexico, observed an association between CKDu in people age 15-40 and hair levels of arsenic and also non-significantly with cadmium, in addition to a number of associations with occupational and behavioral risk factors (Bustamante-Montes et al., 2018). In Nicaragua, no increased metal levels in urine were found in sugarcane workers (McClean et al., 2012). A case control study nested in a community cohort in León and Chinandega in Nicaragua, the area with the highest prevalence and incidence of CKDu known in Mesoamerica, found elevated concentrations of aluminium and total arsenic but no associations between twelve metals and metalloids and rapid decline of kidney function over the course of two years (Smpokou et al., 2019).

At this workshop, although abstracts of several ongoing studies on metals were presented for both regions, the working group generally agreed that current data on metals and CKDu are limited, and while presently not suggestive for metals as being sole risk factors for CKDu, there is a need for better exposure monitoring in populations at risk.

Topics addressed in the working group regarding metals, metal mixtures, and other environmental exposures

Methods considerations for exposure assessment of metals and metal mixtures in analytical epidemiologic studies in high-risk populations

The group discussed the following considerations for research assessing exposure to metals:

- Ensure that the best matrix for the elements of interest is used; consider representative time window of biomarker (recent exposure, long-term body burden), risk of contamination (hair, nails), and specificity of marker (total As versus speciation or metabolites). The possibility of reverse causality when using biomarkers of exposure in cross-sectional and case-control studies should also be addressed by designing longitudinal studies where possible.
- Studies should concurrently collect environmental samples (e.g., water, sediment, air, soil, food) and biological samples (blood, urine), along with data on smoking and diet so that, if internal exposure is identified, it may be possible to identify the source of the exposure.
- Metal degradation is not a concern in biobanked specimens.

- Repeated samples of environmental and/or biological samples will provide a temporal picture of the chronic/acute status of the exposure.
- Low levels of contamination in various elements may add up to high-level internal exposures if large quantities of the vehicle being consumed/exposed (e.g., dust all day vs. for an hour or liters of water a day, etc.).
- Inductively coupled plasma-mass spectrometry (ICPMS) methods identify multiple elements. Patterns of metals occurring together can be identified by principal component analyses.
- Need to account for hard water and bioavailability, both for individual elements and mixtures.
- Pesticides and fertilizers may be contaminated with metals, and pesticide-metal interactions need to be considered.

Except for hard water mentioned above, other environmental contaminants as potential causes of CKDu were minimally discussed. Some working group participants felt that other potential causal toxic agents need to be explored, such as phthalates, per- and polyfluoroalkyl substances (PFAS), cyanotoxins, and polycyclic aromatic hydrocarbons (PAHs). Exposure to silica was also raised as possibly of concern, and the need for characterization of exposure was stressed (soluble, amorphous, crystalline; inhalation or ingestion).

Points of agreement and disagreement on metals and metal mixtures

As in the discussion of pesticides, part of the working group participants felt strongly that a substantial role of metals in CKDu in Mesoamerica and Sri Lanka is highly unlikely. It was considered important to find a driver that can be manipulated to prevent the disease, even if we do not fully understand it, and to prioritize prevention studies.

Other participants advocated that metals may not be the primary cause or even a major contributor to CKDu, but workers who are exposed to multiple sources of metals in addition to other environmental contaminants should be offered protection from these exposures. And these exposures should be more adequately evaluated in the context of possible interactions with other low-dose exposures (Backhaus, Blanck, and Faust, 2010).

Table 1, on the next page, presents a perspective of current general knowledge (A) and then integrates exposure data from Mesoamerica (B), to compare with accepted understanding of metals as a cause of CKDu, as presented at the Second International Workshop on Mesoamerican Nephropathy (Elinder, 2015). This table is now adapted and includes an additional evaluation for Sri Lanka (C).

Summarizing current knowledge (as in Barregard and Elinder, 2015 (Barregard and Elinder, 2015) discussed by the working group participants (Table 1A), it was accepted that very high exposure to Pb, Cd, inorganic Hg, and inorganic As can cause acute kidney injury, and high occupational exposure to Pb, Cd, and inorganic Hg is associated with increased risk for end stage renal disease (ESRD). It was also accepted by most working group participants that occupational exposure to Pb, Cd, and Hg is associated with markers of adverse renal function. While there is evidence supporting a role of heavy metals in CKD, a clear link to CKDu is yet to be uncovered. Data on the general population and markers of renal dysfunction indicate Pb exposure being associated with declining eGFR, Cd with tubular protein excretion, and mercury and arsenic possibly with proteinuria.

Data in Mesoamerica (Table 1B) do not suggest a causal relationship between metals and CKDu, because i) no particularly high exposures have been reported, and ii) the clinical presentation in affected workers is not in accordance with metal toxicity (Wijkstrom et al., 2017; Wijkstrom et al., 2018). Per group discussions and review above, the data from studies in Sri Lanka (Table 1C) was considered limited.

Table 1: Metal exposure and renal effects †

Metal	Arsenic*	Cadmium	Lead	Lithium	Mercury
A. Agreement between morphology and renal toxicity from exposure to metals					
Evidence of nephrotoxicity in humans	+	+ + +	+ +	+ + +	+ + +
Specific clinical and morphological findings in human nephrotoxicity	–	+ + +	+	+ +	+ +
Specific clinical and/or morphological findings in animal nephrotoxicity	–	+ + +	+ +	+ +	+ +
Established dose-effect and dose-response relations between exposure and renal effects in humans	–	+ +	–	+	+ +
The possibility to use measurements in blood and or urine (biological monitoring) to assess exposure	+	+ + +	+ + +	+ + +	+ + +
Established dose-effect and dose-response relations between results from biological monitoring and renal effects in humans	–	+ +	–	+ +	+ +
B. Likelihood of association with CKDu in Mesoamerica and exposure to metals					
Evidence of **significant exposure** in CKDu endemic areas from environmental measurements or biological measurements	+*	–	–	–	–
Clinical concordance between cases with CKDu in endemic areas with what has been reported from humans exposed to metals	–	–	+	+	–
Renal pathology in concordance between cases with CKDu in endemic areas with of what has been reported from humans exposed to metals	–	+	+	–	–

Metal	Arsenic*	Cadmium	Lead	Lithium	Mercury
C. Likelihood of association with CKDu in Sri Lanka and exposure to metals					
Evidence of **significant exposure** in CKDu endemic areas from environmental measurements or biological measurements	++*	+	–	–	–
Clinical concordance between cases with CKDu in endemic areas with what has been reported from humans exposed to metals	–	–	+	+	–
Renal pathology in concordance between cases with CKDu in endemic areas with what has been reported from humans exposed to metals	–	+	+	–	–

* *Studies have measured only total arsenic, without speciation of organic As (mostly from ingestion of seafood and non-toxic) and inorganic As (mostly from soil and highly toxic), and environmental data from the endemic zones in Sri Lanka have primarily been derived from soil, water, and sediment.*

† *This table (parts A and B) was adapted from a table in the Report from the Second International Research Workshop on MeN created by Dr. Carl Gustaf Elinder (Elinder, 2015).*

Recommendations for future research on metals and CKDu

There were two viewpoints proposed by working group participants, both stressing the need for high quality exposure assessment:

1. Those who thought metals were unlikely to be a major driver of the CKDu epidemics, emphasized the importance of sound exposure assessment and epidemiological designs in regions with high risk of CKDu to have a basic understanding before looking into any interactions, especially if hitherto unknown from other settings.

2. Those who thought that metals may be an important etiology emphasized that, even if environmental exposure levels are low, daily exposure might be moderate-high with higher water consumption or more intense inhalation. Additionally, multiple metals are present at low-moderate levels in most environments and it is unknown whether metal mixtures act synergistically or antagonistically in relation to kidney injury. Environmental mixtures need to be considered in epidemiological studies, including exposures to chemicals in water and personal exposures to metals and other environmental pollutants.

Future Considerations

There was agreement within the working group regarding the importance of designing interdisciplinary studies, such as geological studies collecting samples that are useful in health studies, or health studies on other diseases biobanking specimens for future evaluation of contaminants or evidence of CKDu. Importantly, studies should be done to contrast exposures in high and low prevalence areas. Exposure to metals and pesticides from environmental media needs to be assessed in an integrated way. Interactions between pesticides and metals were considered. Statistical methods for modeling environmental exposures including mixtures were also noted as an important consideration in future research. Early life exposures that may predispose individuals to kidney damage at a more advanced age, especially during work,

should be considered. There was also some discussion on the need to consider non-human models, such as rodent models and higher throughput cell culture and non-human models to evaluate the impact of pesticides, metals, or other toxic exposures on kidney outcomes that can inform future epidemiological studies of CKD and CKDu. There was agreement that the epidemic state of CKDu warrants intervention. Where there is suggestive evidence of risk factors associated with CKDu, there must be work done to reduce these exposures, and hopefully the risk of CKDu, while advancing in understanding etiology.

Overall recommendations of the working group regarding pesticides and other agrochemicals, metals and metal mixtures, and other environmental exposures

1. Propose a set of core information that should be collected about occupational and environmental exposures to pesticides and metals across studies.
   - Occupational studies should include information about drinking water sources, at home, and on the job.
   - Environmental studies should include information on occupational aspects, type of job, heat stress, pesticides, and other relevant exposures, as well as information about intensity and duration of exposure/occupational practices.
   - Develop standardized questionnaire on occupational and environmental exposures.
   - Add collection of biospecimens and exposure data for understanding of etiology.
2. Interdisciplinary research is key for leveraging expertise and funding to better understand multi-factorial etiology of CKDu.
3. Enable data and sample sharing for environmental and occupational exposures, not only from past studies, but as a mandate for future studies.
4. Identify sites with and without CKDu, for example using the DEGREE protocol, to compare chemical exposures and heat stress indicators across study populations.
5. Over time, prioritize intervention studies on modifiable factors related to CKDu.

Combined/joint working group sessions

Joint session of surveillance, pesticides, and heat stress working groups

In this session the use of standardized descriptive protocols and preparation of modules of standard simple exposure assessment of heat and pesticides within those protocols were emphasized.

- Descriptive epidemiology should be used more often to identify high risk populations and potential causes.
- Standardized DEGREE prevalence studies are needed for international comparisons.
- Select farming endemic areas versus non-farming areas. Spatial variability and temporal variability need to be considered also within endemic areas.
- Modules with simple assessment of the exposures of main interest should be added to these standardized descriptive protocols, including occupation, heat, and pesticides.
- Both individual data and contextual data is needed.
- How can we better understand what the pesticide exposures are? Many times, individuals do not know what they use. A better way to capture this information may be to gather information from government records and then conduct qualitative interviews of knowledgeable informers in the regions of interest. For more details see section 3.3.
- How to measure heat and pesticide in the general population?

- For external heat, use historical meteorological (national and regional) data (also in ecological studies), but ensure a proper spatial and temporal resolution.
- For individual heat exposure (internal + external heat), a job - heat matrix should be developed to have a crude estimate (low – medium – high) linked to a simple questionnaire.
- For pesticides, a crop-matrix, adapted to the study area, can be developed using data for most common AND nephrotoxic pesticides – this method has already been developed and can be shared.
- The DEGREE organizers asked for five questions that can capture heat and pesticide exposure to be included in a survey.
- There are many existing studies that have measured cardiovascular biomarkers, including serum creatinine. These data can be used for retrospective assessments of kidney function(O'Callaghan-Gordo et al., 2019).

Joint session with Molecular and Genetic Working Group

The joint session consisted mostly of an interesting explanation of the Molecular and Genetic Working Group on how genetic, epigenetic, and molecular methods can contribute to clarify the potential role of toxins in CKDu.

- Genetics is worth pursuing for gene environment interactions. It is more effective to evaluate environmental toxins in subgroups of susceptible individuals rather than whole populations.
- Using epigenetic technologies may help identify specific environmental exposures and serve as biomarkers that predict kidney outcomes; miRNA and exosomes in urine were discussed as novel biomarkers of disease.
- Genome-wide DNA methylation arrays are powerful tools for identifying the impact of environment on the genome, potentially leading to the identification of new exposures.
- Molecular studies may allow us to move from associations to causal relationships as happened in the Balkan nephropathy. Measuring DNA methylation across the genome may help identify down-stream effects from pesticides, metals and other environmental exposures on pathways and genes.
- The molecular-genetics group advocated for unbiased (non-hypothesis based) screens such as metabolomics, to identify new causal candidates, both at work and at home. This approach could illuminate the complex interplay between exogenous environmental exposures and endogenous metabolites.
- Also, metabolomics could help us find accumulation of downstream metabolites of known candidate pesticides that are no longer detectable in their original form, because of external (e.g., cane burning) and endogenous influences (metabolism).
- Reverse causation is a problem in epigenetics. Prospective designs are needed in molecular epidemiology.

There was not enough discussion time for comparing the advantages of continuing with traditional exposure assessment versus starting from zero ("unbiased screens") in this field. Mutual strengthening of these two fields should be brought into the discussion.

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Surveillance and Screening

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Objectives of the working group

The objectives of the working group were to:

1. Review and critically assess prevalence studies and screening data (strengths, weaknesses, and needs).

2. Propose recommendations for future studies and surveillance.

3. Address aspects of ethics related to surveillance and screening for CKDu in populations in conditions of vulnerability.

Introduction

Prevalence studies, screening, and surveillance for CKDu have been discussed previously in several settings. Recommendations from a 2015 workshop in Costa Rica included: 1) developing a core set of questions to be used in a baseline one-time questionnaire of varying lengths for adults at work or in the communities; 2) revising collected questions already tested in previous studies to develop the core elements for a questionnaire; and 3) circulating and field testing them (Program on Work Environment and Health in Central America (SALTRA), Central American Institute for Studies on Toxic Substances (IRET), and Universidad Nacional (UNA), 2019). Recommendations from a 2016 workshop in Sri Lanka included: 1) developing an international consensus on a case definition for CKDu (suspected, probable, and confirmed); 2) enhancing community-based surveillance, including agrochemical use and water quality; and 3) strengthening the national renal registry (World Health Organization WHO) and (NSF), 2016). The International Consortium of Collaborators on Chronic Kidney Disease of Unknown Etiology (i3C) published a report on approaches

to population-level detection strategies and recommendations for a minimum dataset that was also considered by the group in preparation for this workshop (Ben Caplin et al., 2019). Currently, there remains an urgent need to discover which parts of the world are experiencing endemic/epidemic CKDu, as it is not clear whether CKDu is confined to particular areas, with different local risk factors playing a key role, or whether there is a global epidemic with common risk factors across regions (Pearce and Caplin, 2019).

During the Surveillance and Screening Working Group session, data were presented on CKD or CKDu surveillance from eight countries (Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, and Sri Lanka). The participants assessed developments in each of the affected countries in Mesoamerica and Sri Lanka. In general, considerable progress has occurred in recent years, though it is not homogeneous across all geographical settings or all activities. Two initiatives in particular were highlighted. The first was the PAHO publication of a provisional case definition and methodological basis and approaches for public health surveillance, a result of a consultation process in the region of the Americas (Pan American Health Organization). The second was the development, publication and implementation of a protocol for prevalence studies—the Disadvantaged Populations eGFR Epidemiology Study (DEGREE) (B. Caplin et al., 2017). The initial results of studies using DEGREE were presented and discussed. A very practical multilevel case definition for surveillance and epidemiological studies was also presented by Sri Lanka, and briefly discussed in the group. After the workshop, but prior to the publication of this report, a paper was published proposing three tiers of diagnosis: (i) "suspected CKDu," relevant for the primary care level; (ii) "probable CKDu," for epidemiologic surveillance; and (iii) "confirmed CKDu," for clinical diagnosis. (Wijewickrama, Gunawardena, Jayasinghe, and Herath, 2019)

Surveillance systems for CKD/CKDu

Two main documents were used for this discussion: One from PAHO (Pan American Health Organization), and one from the Sri Lanka workshop on CKDu (World Health Organization WHO) and (NSF), 2016). Box 1 summarizes the recommendations for each main component of a surveillance system for CKDu (or, in its absence, CKD) as discussed in the group. Heath service records, such as disease registries, screening data, and population surveys, are usually structured to capture renal failure or decreased renal function using biomarkers, but do nothing to report causes. These components are all part of surveillance "system" that can be used for different purposes in broad or limited populations. For example, registries could be universal or sentinel. Likewise, screening could be done in the whole population or in a high-risk group (e.g., agricultural or construction workers, rural communities, migrants, others). The working group recommended that even when initiated in a limited setting, the different systems of surveillance of CKD/CKDu should be integrated as much as possible within the existing public health surveillance system and the existing health care system to avoid unsustainable parallel processes. For example, cases (suspected/probable) identified in surveys or screenings should be referred for further clinical investigation and/or treatment.

Box 1 - Components, targets, and scope of CKDu surveillance

Types/ components	Focus/ target	Scope	Case definition*
Prevalence studies	population	• Prevalence/severity of a disease in a given population. • Assessment of distribution of environmental/ occupational and lifestyle risks/ exposures potentially associated with the disease. • Does not require accurate individual diagnosis (e.g., a single eGFR is sufficient). • Comparisons over time within and between countries/regions. • Provides clues as to causes of population patterns – basis for further research. • Estimates the population burden of disease.	suspected

Types/ components	Focus/ target	Scope	Case definition*
Screening	individual	• Prevalence/severity of a disease in a given population. • Assessment of distribution of environmental/ occupational and lifestyle risks/ exposures potentially associated with the disease. • Does not require accurate individual diagnosis (e.g., a single eGFR is sufficient). • Comparisons over time within and between countries/regions. • Provides clues as to causes of population patterns – basis for further research. • Estimates the population burden of disease.	probable
Registries	individual	• Accurate clinical identification of disease in individuals. • Clinical management and follow-up. • Basis for analytical studies (e.g., case-control studies).	confirmed

** based on recommendations and experience from Sri Lanka*

There was some discussion in the group regarding screening the general population for CKD. Based on the reviewed literature, there is no evidence to support screening for stages 1-3 because of insufficient evidence for benefit (Bochud, 2015). A review paper suggests that CKD screening could be an opportunity to scale up CKD care and strengthen health care systems in low-income to middle-income countries, but applied different screening criteria to define the need for screening (George, Mogueo, Okpechi, Echouffo-Tcheugui, and Kengne, 2017). The working group did not come to a conclusion on the matter, but indicated that screening should be prioritized in places where this would be required for specific interventions, such as agricultural workers and other populations with increased vulnerability (e.g., occupational settings or CKDu hotspots), and should always be linked to follow-up in clinical settings. Screening should not be done in places where the prevalence of CKD/CKDu is expected to be low and where early diagnosis (Stages 1-3) would be of limited benefit (e.g., increasing costs and the likelihood of introducing unnecessary burden to the individuals and to the health services).

The group also noted that prevalence studies are an important component of surveillance of CKD/CKDu and indicated the need to use common protocols to allow for comparability between different countries and different areas within a country. The DEGREE protocol is designed to estimate prevalence of reduced renal function (reduced eGFR), not CKDu. Therefore, it can be incorporated into (but not replace) existing non-communicable diseases surveillance systems, adding value to the data collected periodically in many countries. Special attention should be given to exclusion criteria when analyzing data from surveys, as the prevalence of CKDu may change drastically depending on those criteria (e.g., to estimate the prevalence of CKDu, persons with diabetes, hypertension, and high proteinuria should be excluded).

Prevalence of CKDu in cross-sectional and screening studies (2015-2019)

The working group reviewed 24 CKDu cross-sectional and ecological studies found in peer-reviewed literature for the period 2015-2019 and in the references of CKDu review articles (Chapman et al., 2019; Gonzalez-Quiroz, Pearce, Caplin, and Nitsch, 2018; Lunyera et al., 2016; Pearce and Caplin, 2019; Valcke et al., 2017). A major issue was lack of comparability across most study parameters. In fact, age and sex were the only variables included in all the 24 studies. A summary of the main variables included in each of the selected studies is presented in Table 1 on the next page.

Table 1 - Variables identified in selected studies

Types of questions	Study variables	Number of papers that included the variable (n=24)
Sociodemographic questions	Age	24
	Sex	24
	Education	8
	Monthly household income per person in family	4
	Water source	4
	Low altitude	1
Anthropometric measurements	Blood pressure	10
	Weight	11
	Height	11
Occupation questions	Past and current occupation	18
	Year worked in the current occupation	11
	Current hours worked per week	2
	Number of hours worked per day	2
	Current social security	1
	Rapid work pace	1
	Shade available during work break	1
	Water intake at work or electrolyte solution	3
Environmental questions	Temperature/heat stress	7
	Land use	4
	Heavy metal	3
	Pesticide exposure	8
	Infectious disease	2
	Snake bites	1
	Dehydration symptoms	4

Types of questions	Study variables	Number of papers that included the variable (n=24)
Lifestyle	Smoking	13
	Alcohol	13
Chronic disease and medications consumption questions	HT	12
	Diabetes	12
	NSAIDs	8
	Family history of CKDu	9

The working group also discussed two systematic review papers (Chapman et al., 2019; Gonzalez-Quiroz, Pearce, et al., 2018). Although their objectives, methods, and inclusion criteria differ, both papers showed a clear positive association between CKDu and males (vs. females), agricultural work, low altitude, and family history of CKD. In general, the quality of cross-sectional studies was medium, with incomplete adjustments for confounding factors, and potential of reverse causality. A problem identified in one of the reviews was lack of a validated eGFR formula for the Latin-American population (Gonzalez-Quiroz, Pearce, et al., 2018). The working group urged the development, validation and use of a minimum set of questions in all descriptive and analytical studies for more reliable analysis and comparison across countries and regions within each country.

An overview of current knowledge about CKDu prevalence in different countries is presented in Table 2.

Table 2. State of the knowledge on the prevalence of CKDu

Country	Main findings
Costa Rica	CKD is one of the top causes of premature mortality in Costa Rica, comprising 10% of premature deaths from non-transmissible diseases in 2016. CKD prevalence is drastically higher in the Guanacaste province where peritoneal dialysis patients are predominantly young agricultural workers ages 35-59 years; in these age groups the male:female ratio is ≥4:1. (Costa Rican Ministry of Health and Dirección de Vigilancia de la Salud, 2019)
	– A mortality study between 1970–2012 reported age-adjusted mortality rates in the northwestern coastal province of Guanacaste increased among men from 4.4 to 38.5 per 100,000 vs 3.6–8.4 in the rest of the country. In women, the rate increased from 2.3 to 10.7 in Guanacaste per 100,000 vs 2.6–5.0 in the rest of the country. Excess mortality among men in Guanacaste can be seen in the mid-1970s, a trend that has continued to steeply increase. Female excess mortality appeared a decade later, remaining more stable. Male age-specific rates were high in Guanacaste for age categories ≥30, and since the late 1990s also for age range 20–29. (Wesseling et al., 2015)
El Salvador	The National Survey of Chronic Non-communicable Diseases in adult population (ENECA-ELS) used the KDIGO definition to characterize CKD. Findings confirm that CKDu is not a national phenomenon, but rather is concentrated in the Paracentral and Oriental departments where the prevalence of CKDu is twice that of other departments. Prevalence is higher in male farmer workers in rural areas. (Ministerio de Salud/Insituto de Salud)

Country	Main findings
El Salvador	64% of CKD cases are under the age of 40 and 25% of the CKD cases are 41 years or older. Being a farmer is associated with both CKD and CKDu, but the association with CKDu is 1.5 times higher. (Herrera-Valdés et al., 2019; Orantes-Navarro et al., 2019)
Guatemala	Rates of dialysis enrollment per 100,000 residents are highest in the Southwest region of the country where sugarcane plantations are located and daytime temperatures during the harvest season (November to April) are higher than the rest of the country. (Laux, Barnoya, Guerrero, and Rothstein, 2015)
Honduras	In 2018, there were a total of 3,160 dialysis patients. 60% of the patients are from Benque SPS, Comayaguela and Choluteca, all areas where average temperatures are higher. (Unpublished data presented in the workshop Surveillance and Screening working group)
Nicaragua	Many occupational and prevalence studies have been conducted in Nicaragua since 2010. (Gonzalez-Quiroz, Pearce, et al., 2018) Prevalence of high serum creatinine or low eGFR (single measure) reported in the following occupational populations: – 26% in males and 7% in females in the mining/subsistence farming village – 22% in males and 6% in females in banana/sugarcane – 13% in male and 4 % in fishing – 7% in male and 0% in female in coffee – 0% in male and 1% in females in services – 0% in male coffee workers (Laux et al., 2012; Torres et al., 2010) – 14% in male and 0% in female brickmaking workers (confirmed with two eGFR measurements 3 months apart) (Gallo-Ruiz et al., 2019)
	Prevalence reported in population-based studies: – Quezalguaque shows a male prevalence of 20% and general prevalence of 13%; in Leon, a male prevalence of 13.8% with a general prevalence of 9%.(O'Donnell et al., 2011), (Lebov et al., 2015) – A study in Leon found an 11% prevalence of decreased kidney function among males compared to 4% in females. (Mejia-Reyes) – A study in the La Isla community found a prevalence of 42% in males and 10% in females. Risk factors included: increased lifetime days cutting sugarcane during the dry season, nondeliberate pesticide inhalation, and sugarcane chewing. (Raines et al., 2014) – One study in a high-altitude village did not show the presence of CKD in males and found low prevalence of CKD in females. (Laux et al., 2012) – Preliminary results of a population-based national survey for the most affected departments showed the following prevalence of CKD: Rivas (22.3%); Leon (20.9%); Chinandega (20.5%); and Granada (18.8%). – Prior to this study, the Rivas Department was not considered a high prevalence area; therefore, household visits for verbal autopsies followed the study and confirmed the findings. (Unpublished data from the Ministry of Health Nicaragua, presented in the Surveillance and Screening working group.) – A population based cross-sectional study in Tierra Blanca reported a prevalence of reduced eGFR (≤60) of 12.4%. (Mendoza-Gonzalez et al., 2012)

Country	Main findings
Mexico	– In the period 2008-2018, 30 of the 57 patients with ESRD referred for renal transplant to the Instituto Nacional de Cardiología, Mexico met the criteria for CKDu; both agricultural work and family history of CKD were independent risk factors. Biopsies in 5 out of 6 patients who underwent kidney biopsy showed atrophy, mild sclerosis, tubulointerstitial nephritis, and inflammatory infiltrate, all compatible with previously reported Mesoamerica Nephropathy biopsies. (De Arrigunaga et al.)
	– Tierra Blanca has been identified as a hotspot. A cross-sectional study with 6717 participants age 20-60 years from some communities of Tierra Blanca reported a prevalence of 25% of probable CKD based on KDIGO definition, and 44% of them could be considered CKDu cases (Aguilar and Madero, 2019). ESRD cases of Tierra Blanca represent 25% of the State's waiting list for kidney recipients, although the municipality represents only 1.3% of the State's population. – Poncitlán, Jalisco has also been identified as a CKDu hotspot. The main findings to support this claim come from population screenings for CKD that are performed in the State of Jalisco since 2007 (Garcia-Garcia et al., 2019). The prevalence of CKD in adults from Poncitlán is twofold higher than average state prevalence (CKD: 20.1 vs. 10.4%, $p = 0.002$); RRT prevalence is also twice as high as the rest of the state. Prevalence of traditional risk factors for CKD in Poncitlán did not differ with the rest of the State, and the prevalence of diabetes mellitus was significantly lower. (De Arrigunaga et al.)
Panama	– Data suggestive of CKDu clusters were identified in the areas of Coclé, Chiriqui, and Bocas del Toro. – The Social Security Fund reported more than 2,200 patients receiving dialysis treatment in 2016, and CKDu represents 43% of the total cases, with a rate of 15.14 per million inhabitants in 2018. (Unpublished data from the Ministry of Health Nicaragua, presented in the Surveillance and Screening working group.)
Sri Lanka	CKDu hotspots have been reported in several locations. The main findings supporting evidence of endemic areas are: – Based on a survey of 4,777 individuals in endemic areas and 250 individuals in non-endemic areas, the prevalence of CKDu was higher in Anuradhapura, Polonnaruwa and Badulla, with 25%, 20.6% and 22.9% prevalence, respectively. (Jayatilake et al., 2013) – GPS mapping shows that areas reporting higher incidence of CKD/CKDu are clustered together, mainly around paddy fields and irrigation tanks, and even within the high incidence areas there is geographic clustering. (Ranasinghe et al., 2019) – Prevalence of CKDu was 4.2 in Medawachchiya, and 0.2% in other control areas. – Based on review of clinical records (n=392), CKDu cases represented 54% of CKD cases in the North Central Province and 82% in Kandy and Anuradhapura. (N. T. Athuraliya et al., 2011) (T. N. Athuraliya, Abeysekera, Amerasinghe, Kumarasiri, and Dissanayake, 2009)

Country	Main findings
India	– CKDu hotspots have been suggested in several locations, and CKDu has been reported as the second most common form of CKD after diabetic nephropathy. (Rajapurkar et al., 2012) – Analysis from cross-sectional data from three population-based studies that provide information from three different geographical areas of India (CARRS; UDAY; and the ICMR-CHD studies) included 12,500 participants. The main findings of these studies suggest that clusters of CKDu may be occurring in India. – In Southern India, older age, being male, and rural residence were the main risk factors for decreased eGFR. – In Northern India, older age was the main risk factor for decreased eGFR. The findings are consistent with the definition of CKDu in rural settings of Southern India (Vishakhapatnam district). (O'Callaghan-Gordo et al., 2019)
Tanzania	Two prevalence studies were identified: – A randomly selected, community-based prevalence study in Northern Tanzania (Moshi, Kilimanjaro Region) in 2015 reported that half of the CKD cases were not associated with diabetes, hypertension, or HIV, with an overall prevalence of 7%. (Stanifer et al., 2015) – A prevalence study in two communities in Kisarawe, a rural district located at the Pwani region (coastal area) approximately 40 km from Dar es Salaam, reported a 12.5% prevalence of CKD stages 3-5, with a higher prevalence of stage V among the youngest age group (18-36 years). Again, CKD was not significantly associated with diabetes and hypertension. (Ploth et al., 2018)

Visualizing data across countries

The working group considered ways that routine surveillance or registry data can be used to visualize patterns in the occurrence of CKDu, which might help in the design of future studies. Erik Hanssen presented preliminary data as an example of a possible approach. A summary of these techniques and data can be found in poster abstract #15 in the meeting book available online (Third International Workshop on Chronic Kidney Diseases of Uncertain/Non-Traditional Etiology in Mesoamerica and Other Regions: Meeting Book, 2019).

International collaboration

The CENCAM workshop in 2012 and 2015 had recommended to strengthen the relationships and increase collaboration between researchers and clinicians across the region and country by conducting epidemiological and clinical research. Since the last meeting, the working group identified noteworthy collaborations between researchers from Mesoamerica/USA, Mesoamerica/Europe, USA/Asia, and Europe/Asia. These collaborations have generated new knowledge and contributed to an increase in the number of scientific publications on CKDu.

Footnote

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Mohan S, Jarhyan P, Ghosh S, et al. UDAY: A comprehensive diabetes and hypertension prevention and management program in India. *BMJ Open 2018;8:e015919*.

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The way forward

Mapping of national capacities for surveillance of CKDu in the context of CKD surveillance in Central America

In 2017, the Pan American Health Organization (PAHO/WHO) conducted a survey to assess the strengths and weaknesses of national public health surveillance systems for CKDu in the context of surveillance of CKD in Central America. The purpose was to identify information gaps and suggest priority areas of intervention to improve the CKDu surveillance, and to facilitate the planning of their systems. PAHO country professionals responsible for non-communicable disease (NCD) surveillance were responsible for the collection of information. The preliminary results of this exercise were presented and discussed in the working group and are available from PAHO upon request.

Out of six invited countries, responses were received from four: Costa Rica, Guatemala, Honduras, and Panama. Although most of the respondents were professionals at the Ministry of Health responsible for different aspects of public health surveillance systems, other Ministries such as Labor and Agriculture also participated. As in many surveys, limitations on the results arise because the analysis was based on the information provided by the respondents rather than through objective assessments. The information provided by this exercise during the workshop helped the discussion on facilitators and barriers to implement a CKDu surveillance system.

Participants presented an overview about the current status of their CKD/CKDu surveillance systems in place in their countries of origin. A summary of this information is presented in Table 3.

Table 3 - Overview of CKD/CKDu Surveillance Systems in affected countries

Country	Main features
Costa Rica	Notification of CKD is compulsory; it is in the list of notifiable diseases and conditions (though this is recent and data from obligatory report was not available during the workshop). The system recognizes three levels of attention, and the notification is done electronically. Data is consolidated and analyzed by the department of epidemiological surveillance of the Caja Costarricense del Seguro Social (CCSS) and the MOH. The decree N 39147 on occupational health requires hydration, rest, shade, and regular tests of serum creatinine in occupational populations exposed to heat, but monitoring compliance remains a challenge, as it encompasses three different institutions.
	The country is implementing the World Health Organization – STEPwise approach to NCD surveillance (WHO STEPS) that now includes measuring creatinine levels. As diabetes and hypertension are measured in the same population, this will allow more precise population-based estimates of prevalence for CKD and CKDu (by exclusion).
El Salvador	Notification of CKD/CKDu includes outpatient in primary care facilities, hospitalizations, hospital deaths, and a national registry for replacement therapies. The most important challenges are: lack of a unique number for the patient (multiple entries are possible); not all providers are included in the notification system; data collected from routine patient care are frequently incomplete; it is not possible to disaggregate information by stage of the disease. The country has invested in improving the quality of death certificates but recognizes that incorrect reporting of CKD/CKDu as cause of death remains a problem. CKD/CKDu replacement therapy registry in 2019 shows that almost 53% of the cases have an unknown etiology, and a little over 40% of the patients are workers in the field of agriculture, forestry, and fishery.
Guatemala	No formal surveillance system in place. 58% of the CKD patients are treated by the public health care system provided by the Ministry of Health, and the remaining 42% are treated by the Guatemalan Institute of Social Security. Institutional data is available per unit where the service is delivered, and the University hospital has an automatized registry, which facilitates the recovery of the information. However, data is limited, partly because of the small number of nephrologists in the country.

Country	Main features
Honduras	There is a system that collects information on dialysis developed by the Universidad Autonoma Nacional de Honduras that covers eight CKD health care centers. A national surveillance system on CKD/CKDu is being implemented in a network of primary health care centers, and on an active surveillance system in sentinel sites (The University hospital and the Mario Catarino Rivas Hospital).
Nicaragua	A national population-based prevalence study included screening people 15 years and older as the basis for a national surveillance system of CKD/CKDu. Preliminary results show a national prevalence of 13.3% with large differences among different parts of the country.
Mexico	The country is in the process to implement a national registry for ESRD and dialysis, and is negotiating including questions on CKD in the National Health and Nutrition Surveys.
Panama	CKD/CKDu surveillance has been regulated through a 2018 national policy, but it has not been fully implemented. Challenges include consolidating information provided by different public institutions and by private health care providers.
Sri Lanka	The existing data sources are community-based screening programs, the national registry maintained by the Ministry of Health and a renal registry maintained by the Sri Lanka Nephrology Association. Linkage with monitoring data on toxins in food, water, and the environment has been recommended. The system is not entirely integrated yet.

Standardized protocols to estimate prevalence around the world

The working group discussed the importance of population comparisons and surveillance, as many of the major discoveries and important epidemiological hypothesis derive from population comparisons and/or analyses of trends over time. There are many unanswered questions regarding CKD/CKDu. See Box 2 for surveillance questions.

Box 2 - Surveillance questions

- Who gets this disease? Does it occur in all tropical/subtropical regions of the world, or only in some?
- Does this look like the same disease in different parts of the world (i.e., are we experiencing a single epidemic in different parts of the world, or are different epidemics occurring in parallel)?
- What is the prevalence in various parts of the world which have exposures to some of the risk factors, but not others (e.g., in areas which are hot and involve strenuous work, but not pesticide exposure and vice versa)?
- Within at-risk regions, which population subgroups are most at risk? Is it always rural male agricultural workers?

The working group reviewed the protocol developed for the DEGREE Study, an international collaboration for the epidemiology of impaired renal function, and agreed to recommend its use as a minimum set of comparable questions, methodologies and parameters for future prevalence studies (B. Caplin et al., 2017). Although countries can add to the minimum set of questions recommended in DEGREE, having a standard minimum will increase the possibility of answering the questions summarized in Box 2.

Ethical issues in public health surveillance of CKD/CKDu

According to the WHO Guidelines on ethical issues in public health surveillance (World Health Organization, 2017):

"Countries have an obligation to develop appropriate, feasible, sustainable public health surveillance systems. Surveillance systems should have a clear purpose and a plan for data collection, analysis, use and dissemination based on relevant public health priorities." (Guideline 1)."

It is also essential that all information collected must be used, and to ensure that health care coverage is guaranteed to all suspected, probable and confirmed cases of CKD.

The working group reviewed the guidelines and the current literature on the issue and concluded that a proposal to develop or strengthen surveillance systems to include CKDu (in the context of CKD surveillance) is necessary in countries where hotspots have been identified. In addition, the working group also concluded that it is necessary to assess CKD and CKDu separately, as current criteria for surveillance and screening of CKD is not necessarily sensitive for CKDu.

Another topic that needs attention are the unwanted consequences of population screening. In Sri Lanka, for example, it was reported that unmarried young people identified as having CKD, even in stage 1, can be considered unfit to get married, introducing a heavy burden for both the individuals and the community. In Central America, participants stressed that when screening agricultural workers, it is necessary to think about safeguards, as they fear that this could eventually leave affected workers without means to provide for their families and could lead to higher exposure of children to harsh work, as they would need to take their father's role earlier in life.

In summary, the working group recommends that all aspects of surveillance of CKD and CKDu be planned to include measures to avoid unintended harmful consequences to individuals and communities. The measures must be sensitive to cultural and socioeconomic background and should be communicated in plain language to health care workers, as well as to the target populations.

Conclusions and Recommendations

Surveillance and screening

There is a common understanding that current surveillance systems do not capture the true burden of CKD/CKDu. However, public health surveillance of CKD/CKDu cannot be dissociated from the need to guarantee universal access to good quality health care services.

The working group indicated the need to strengthen primary health care units to guarantee access to care in the initial phase of the disease, improve collection of basic data, and strengthen registries. This is particularly important because current CKDu cases are consuming public health budgets while still failing to meet the needs of all patients.

Great progress has been made in collecting and analyzing health service records in each country. The working group praises the progress and indicates that these developments should be supported and strengthened, with training throughout the health system.

The group recommends implementing CKD/CKDu surveillance systems that are adequate for the situation of each country and indicates the need to strengthen collaboration between academic research and public health surveillance systems to provide reliable and up-to-date information to policy decision-makers.

The group also discussed the opportunity to strengthen the surveillance of CKD as part of broader NCD surveillance, including questions that would make it possible to exclude traditional risks and identify CKDu.

The group acknowledged there is compelling evidence in Central America and Sri Lanka supporting claims that the disease is mainly work-related, in particular to agricultural communities. Therefore, particular attention should be given to develop reliable surveillance systems at the workplace and strengthen capacity of the health sector to incorporate screening of workers' health in primary care settings because, at least in Central America, a large part of the workforce works with no or temporary contracts, which is a barrier to screening and follow-up of newly identified cases.

Major knowledge gaps remain about the prevalence of CKDu in various parts of the world. The question whether the epidemics of CKDu represent a global phenomenon or whether there are different regional or national causes for the same clinical and epidemiological outcomes is still unanswered. Therefore, the working group recommends the use of the DEGREE protocol as the minimum set of questions and parameters in future surveys (in isolation or as part of surveillance systems and screening activities), recognizing that each country may add its own components or questions.

It was also recommended that the PAHO protocol for research on CKDu that is in development be consistent with DEGREE to facilitate comparisons of the results between regions of the world.

Recommendations for future research

Screening and prevalence studies need to be done both in CKDu-endemic areas, as well as other regions that are similar in working conditions and environmental exposures but have no reports of CKDu to help refine our knowledge of the distribution of the disease. The same could be done where there is reported CKDu but the conditions and exposures differ from the known hotspots.

In the "CKDu hotspots," it is also necessary to include questions and study designs that help identify the population groups at highest risk within a region, but also across regions.

Individual-level associations cannot be inferred from area-level associations, but ecological studies may be suitable to study some risk factors (e.g., climate-related; crop or pesticide type related etc.) as variations between individuals within a smaller study area may be too small, hiding important correlations and the possible identification of potential CKDu hotspots. Available area-based data in different parts of the world could be used for this purpose, prior to developing community-based prevalence studies and population-based screening.

The working group concluded that the question about "who gets the disease" has not been fully answered with current data, and calls for the use of harmonized questionnaires and study design to allow comparisons between different countries and study areas.

The working group recognized that many questions remain unanswered regarding whether there are specific environmental risks that increases the odds of developing CKDu in different settings. These questions should be adequately answered in future research to inform surveillance systems and support sound policy interventions to minimize or eliminate those risks. Table 4 summarizes the recommendations for surveillance and screening for different settings and objectives as discussed in the working group.

Table 4 - Summary of recommendations for surveillance and screening

Focus	Interventions	Prevalence Studies	Surveillance and Screening	Other Recommendations
Health care facilities	Create awareness of CKDu and training for Primary Health Care facilities staff for early diagnosis and screening. Assure appropriate referral mechanisms for specialized centers for all cases identified in clinical settings and cases of CKD/CKDu identified a part of the surveillance and research done in the area, as appropriate.	Follow protocols for community surveys and screening (questions and tests compatible with DEGREE protocols) in catchment area.	Create/improve renal replacement registries. Create/improve a disease registry (suspected, probable or confirmed). Create/improve information flow and analysis of disease registries on mortality, incidence, and prev-alence of CKD and CKDu. Create/improve screening of CKD/CKDu in the workplace (in suspected, probable, or confirmed work-related CKDu hotspots)	Ensure comparable, reliable and robust data. Build upon what has been achieved until now. Strengthen collaboration between researchers and public health officials for prevalence studies, screening, and public health surveillance. Surveillance of CKD/ CKDu should be linked to actions or policies and interventions at the workplace and affected workers, families, and communities.

Focus	Interventions	Prevalence Studies	Surveillance and Screening	Other Recommendations
Policy and decision makers	Create or strengthen multidisciplinary and inter-sectoral mechanisms for decision and policy-making on CKD/CKDu to deliver more comprehensive interventions (e.g., improving access to health care for early diagnosis of CKD/CKDu and RRT for ESRD; developing sound and specific prevention and health promotion strategies in different settings and for different work categories). Provide the means for the sustainability of CKD/CKDu surveillance systems (e.g., institutional governance, human resources, laboratory capacity, and budgetary funds).	Provide the means for the incorporation of evidence-based decision-making in the policy-making process (e.g., screening the scientific literature and the reports of the surveillance system, and sharing the information with all interested parties regularly).	Provide enough resources and monitor progress of implementation of surveillance and screening activities.	Use the Health in All Policies framework (HiAP) to build the political will and support for the policies and interventions.* Involve the political mechanisms of integration with neighboring countries to strengthen support for concerted interventions (e.g., the Central American Integration System [SICA]).

** HiAP is an approach to public policies across sectors that systematically considers the health implications of decisions, seeks synergies, and avoids harmful health impacts in order to improve population health and health equity. For additional information see WHO. Health in All Policies Global Framework for Country Action.* Available at: www.paho.org/hq/index.php?option=com_docmanandtask=doc_downloadandgid=24802andlang=en

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Conclusions from the Workshop

This workshop represented the largest international effort on CKDu research to date. Since the first workshop in 2012, impressive efforts have been made in the realm of research, and the amount of published data has grown exponentially. This, along with the collaboration across disciplines and borders, shows promising opportunity to eliminate this devastating disease. Nonetheless, CKDu remains a major public health crisis. The lives that have been lost and the toll on families and communities have not subsided, despite gains in research, public health, and clinical efforts over the last decade.

The Second International Workshop on MeN recommended 1) coordinated regional approaches to document prevalence, study etiology, and evaluate interventions and 2) a need for standardized protocols to assess exposures and risk factors of interest. Although progress has been made in both areas, they remain priorities moving forward from the 2019 workshop. While advances have been made in research on CKDu, more research is essential to understand the underlying cause(s) of CKDu in different regions, as well as the underlying mechanisms that may explain associations reported so far. Most researchers agree that CKDu is caused by a combination of more than one casual factor and that more research is urgently needed to identify its causes. At the same time, it is important to act to reduce exposures to known risk factors in populations vulnerable to CKDu, particularly reducing poverty and assuring fair and decent work, including adequate water rest and shade for heat-exposed workers.

Ethical concerns remain, including access to health care, lost work as a consequence of CKDu diagnosis, and cultural stigmas related to the disease that have socioeconomic impacts. These issues require interdisciplinary and intersectoral efforts to make sure that the scientific response helps improve these issues rather than contribute to them.

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