

EDITORIAL COMMENT

Clinical proteomics in kidney disease as an exponential technology: heading towards the disruptive phase

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Abstract

Exponential technologies double in power or processing speed every year, whereas their cost halves. Deception and disruption are two key stages in the development of exponential technologies. Deception occurs when, after initial introduction, technologies are dismissed as irrelevant, while they continue to progress, perhaps not as fast or with so many immediate practical applications as initially thought. Twenty years after the first publications, clinical proteomics is still not available in most hospitals and some clinicians have felt deception at unfulfilled promises. However, there are indications that clinical proteomics may be entering the disruptive phase, where, once refined, technologies disrupt established industries or procedures. In this regard, recent manuscripts in *CKJ* illustrate how proteomics is entering the clinical realm, with applications ranging from the identification of amyloid proteins in the pathology lab, to a new generation of urinary biomarkers for chronic kidney disease (CKD) assessment and outcome prediction. Indeed, one such panel of urinary peptidomics biomarkers, CKD273, recently received a Food and Drug Administration letter of support, the first ever in the CKD field. In addition, a must-read resource providing information on kidney disease-related proteomics and systems biology databases and how to access and use them in clinical decision-making was also recently published in *CKJ*.

Exponential technologies double in power or processing speed every year, whereas their cost halves [1]. The development of exponential technologies follows well-characterized sequential stages, including deception and disruption stages [2]. Deception occurs when recently introduced technologies are dismissed as irrelevant, while they continue to progress, perhaps not as fast or with so many immediate practical applications as initially thought. According to PubMed, initial references to proteomics were published in 1997 and were hailed as presenting a rapidly rising technology in the Post-Genomic Era [3]. By 1998,

proteomics had been used to study cyclosporine A nephrotoxicity [4], and clinical proteomics was already a manuscript title in 2001 [5]. Fifteen years later, clinical proteomics is still not available in most hospitals. However, there are indications that clinical proteomics may be entering the disruptive phase, where, once refined, it may disrupt established industries or procedures. In this regard, recent manuscripts in *CKJ* illustrate how proteomics is entering the clinical realm, with applications ranging from the identification of amyloid proteins in the pathology lab, to a new generation of urinary biomarkers for chronic

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kidney disease (CKD) assessment, and discuss available general and kidney-specific systems biology databases [6–8].

Clinical proteomics represents one aspect of systems biology. Papadopoulos *et al.* [6] provide guidance on systems biology databases for use in kidney research. Systems biology refers to diverse analysis techniques that are frequently referred to as ‘omics’ and have in common the simultaneous assessment of the expression or levels of multiple molecules, even thousands of molecules, thus providing a systemic overview of biological processes and requiring solid bioinformatics tools for data interpretation. Systems biology approaches include genomics, epigenomics, transcriptomics, proteomics and its varieties (e.g. phosphoproteomics) and metabolomics [9]. While for many years, transcriptomics allowed the sensitive quantitative assessment of gene expression, proteomics appeared to lag behind. Against the backdrop of transcriptomics studies that identified novel mediators of kidney injury among thousands of differentially expressed genes within activated molecular pathways or novel biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL) [10], proteomics identified just a few high-expression proteins. In any case, systems biology research has generated huge amounts of data that are available to researchers as accessible databases. The review on existing databases in the kidney disease context and how to access them by Papadopoulos *et al.* [6] is a key resource and must-read manuscript for any researcher interested in kidney physiology and disease. Access to these databases allows later reanalysis from a different perspective or after collating with a different set of experiments or results. Moreover, multi-omics approaches combining epigenetics, transcriptomics, proteomics and metabolomics databases will greatly improve our capacity to apprehend the complexity of physiology and disease. These databases will be a key resource for clinicians in a new world of personalized medicine, which needs to embrace complexity and distill it into clinical decision-making. Most clinicians are still unaware of the potential impact of these technologies in the clinical setting within the near future. However, geneticists and specialists in rare diseases are already using on a daily basis population genetics databases in order to try to discern between single-nucleotide polymorphisms, hypomorphic genetic variants and disease-causing mutations [11–13]. Of the different systems biology approaches, it is likely, as illustrated below, that clinicians will become familiar with clinical proteomics in the next few years.

Picken [7] discusses the clinical use of proteomics in the pathology lab to identify the protein composition of amyloid deposits. This takes advantage of mass spectrometry (MS)-based proteomics and of the abundance of amyloid proteins in tissue where, frequently, they are the dominant proteins. Currently, at least 31 proteins may be deposited as amyloid, and therapy is aetiology specific [7]. Thus, it is necessary to identify the nature of amyloid deposits [14]. High-profile manuscripts have illustrated the misdiagnosis of more rare variants as one of the common [Amyloid A (AA) or Amyloid L (AL)] causes of amyloidosis [15]. Furthermore, exome sequencing has also drawn attention to the potential coexistence of two or more genetic defects, which result in atypical clinical presentations and non-Mendelian inheritance [16]. The current issue of *CKJ* presents a renal biopsy where both leukocyte cell-derived chemotaxin 2 (ALECT2) and AA amyloidosis were present in the same patient, and resulted from a genetic predisposition [17]. In this case, genetic analysis provided the clue, whereas a traditional approach, based on tissue immunofluorescence or immunohistochemistry initially for the most common variants, may

have resulted in an incomplete diagnosis. The need to test multiple potential aetiologies requires time-consuming step-by-step testing or expensive simultaneous testing of all the possibilities. It is precisely the multidimensional nature of the diagnostic tests used that suits proteomics best [7]. In this regard, ALECT2 amyloidosis, which is now recognized as one of the most common causes of systemic amyloidosis in North America, was identified through proteomics analysis [18]. In 2017, a proteomics analysis of a kidney biopsy identified for the first time amyloid ApoCII (AApoCII) amyloidosis [19].

Finally, Pontillo and Mischak [8] discuss the urinary peptidomics-based classifier CKD273. This classifier results from capillary electrophoresis–mass spectrometry-based analysis of 273 protein fragments in urine. Ever since its description in 2010 [20], as a biomarker that allowed specific detection of CKD among >600 patients and controls, evidence has accumulated of its potential usefulness in patient care. In a large cohort of 2672 CKD patients, CKD273 was a better predictor of CKD progression than urinary albumin in early CKD glomerular filtration rate (GFR) G categories [21]. Furthermore, CKD273 is responsive to therapy with renin–angiotensin system blockers, and a high CKD273 score was associated with a response to the anti-albuminuric effect of spironolactone [22]. A recent systematic review concluded that by 2014, initial promising evidence supported the utility of CKD273 in predicting CKD progression [23]. CKD273 obtained high-evidence levels (score range 1b) according to the Oxford Evidence-Based Medicine guideline, supporting its utility for predicting CKD progression, whereas lower scores (score ranges 1–4) were attained according to the Strength of Recommendation Taxonomy guidelines [23]. The evidence was derived from prospective cohort studies and disclosed that CKD273 performed independently of age and gender, predicting the development of pathological albuminuria categories A2 or A3 and fast CKD progression, defined as >5% annual decrease in estimated GFR (eGFR). In June 2016, the US Food and Drug Administration issued a biomarker letter of support for the CKD273 classifier [24] meant to improve its visibility and to encourage ‘the further development of CKD273 to be used in combination with current measures (i.e., albuminuria, serum creatinine) in early phase clinical trials in diabetic kidney disease to identify patients with early stage disease who may be more likely to progress’. CKD273 is now available as an *in vitro* diagnostic device in Germany for the early detection of CKD in diabetic patients, although only for privately insured patients [8].

Further testing is ongoing. The precise role of CKD273 as a tool in the patient risk stratification workup and therapeutic decision-making is being explored in an ongoing European Union-funded multicentric randomized clinical trial (PRIORITY) [25]. PRIORITY addresses whether risk stratification of diabetic patients with albuminuria category A1 based on the CKD273 classifier with subsequent therapeutic decision-making based on CKD273-based risk stratification offers outcome advantages in early diabetic kidney disease. Patients at high risk of progression, based on CKD273 results, were randomized to spironolactone or placebo.

Thus, CKD273 may provide solutions for two unmet needs. First, the development of novel surrogate endpoints that avoid the current need to follow patients over many years and which allow enriching population for accelerated progression of CKD beyond currently available markers, urinary albumin:creatinine ratio (UACR) and eGFR. Secondly, the need for new tools for the earlier diagnosis of CKD. Current tools to diagnose CKD only allow a late diagnosis of the condition (Figure 1), resulting in the

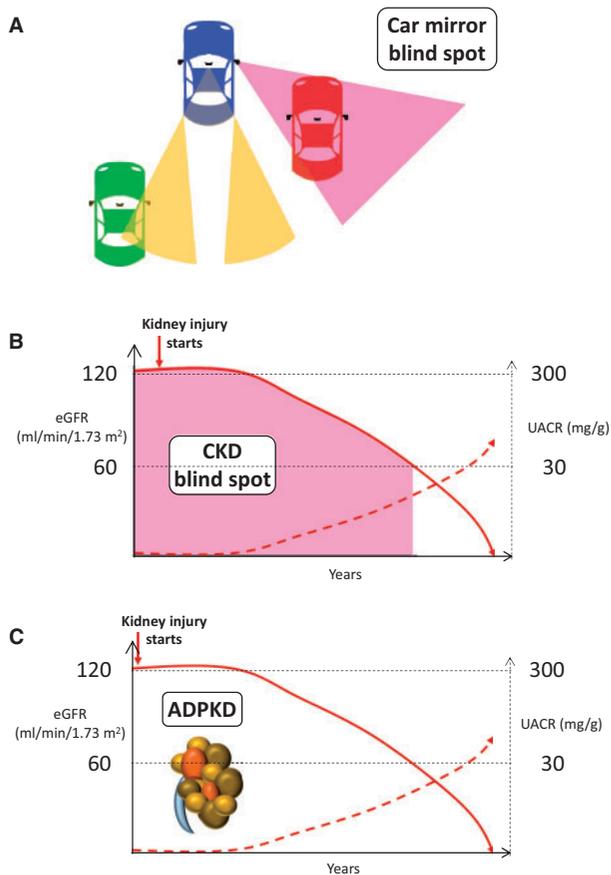


Fig. 1. The CKD blind spot. (A) Car mirror blind spot. (B) CKD blind spot. The most frequently used diagnostic and staging criteria for CKD, based on GFR and UACR, are late events, allowing a blind spot that may last years, characterized by progression of undiagnosed kidney injury. As is the case with driving, a blind spot may be fatal, since specific therapy may be initiated too late to prevent the need for renal replacement therapy or premature death. (C) Imaging addresses the blind spot in ADPKD, allowing the diagnosis of CKD decades before GFR or UACR values allow diagnosing CKD. A key research priority is the search for biomarkers that allow closing the blind spot in other causes of CKD.

existence of a blind spot for early-stage CKD: kidney injury is present but we lack tools to identify and diagnose it. Thus, physiological albuminuria in young healthy individuals is usually under 5 mg/g. However, UACR is allowed to increase 6-fold to 30 mg/g before a diagnosis of CKD is entertained [26]. The diagnosis may be even later when proteinuria is assessed, since it may remain within normal limits when albuminuria is already pathological; GFR criteria for the diagnosis of CKD also allow for roughly 50% of renal function to be lost before a CKD diagnosis is made. A clear illustration of the potential magnitude of the problem is provided by autosomal dominant polycystic kidney disease (ADPKD). In this regard, the availability of an early diagnosis technique, renal ultrasound, allows the diagnosis of CKD a full decade or more before UACR or eGFR become pathological. However, this is only the tip of the iceberg, since ADPKD kidneys are already abnormal at birth. Thus, the diagnosis of CKD may still be delayed, despite the use of imaging techniques.

Clinical proteomics may contribute to close the blind spot in the general CKD population, in the same manner as imaging does in polycystic kidney disease. In this regard, the current 3D initial approach to the patient with CKD (eGFR, albuminuria/proteinuria and glomerular haematuria) may in the future be

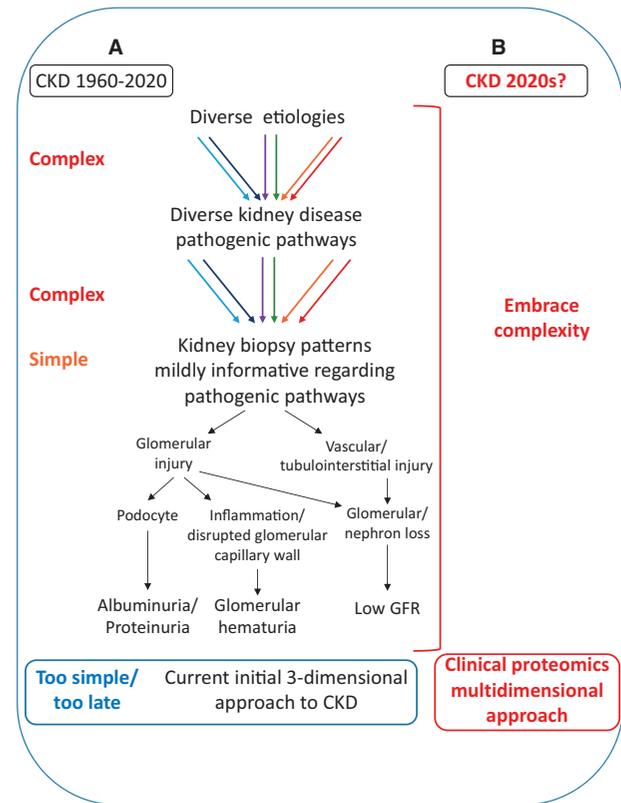


Fig. 2. Clinical proteomics for assessment of CKD aetiology and major molecular pathways involved. (A) Since the 1960s, the initial diagnostic approach to CKD has been 3D, based on the assessment of a limited number of parameters (mainly albuminuria/proteinuria, glomerular haematuria, GFR) that summarize, in perhaps too simple a way, the interplay of diverse aetiologies and diverse molecular pathways of kidney injury. The result has been a considerable delay in the diagnosis of CKD and also in the development of novel therapeutic approaches to kidney disease. (B) Systems biology and, specifically, clinical proteomics allow the nephrologist to embrace complexity in order to develop personalized medicine approaches that provide information within a single diagnostic test that leads to the diagnosis of CKD, to identification of aetiology and molecular pathways (and, thus, of the most appropriate therapy) and outcome prediction.

replaced by a multidimensional systems biology approach (Figure 2). Further refinement may one day lead to the potential for non-invasive diagnosis of aetiology [27] or, perhaps, more interestingly, of a molecular pathophysiological pathways-based classification that may help to choose a specific therapeutic approach, effectively yielding a nephrological 'liquid biopsy' based on assessment of urine, perhaps within a multi-omics systems biology approach. In this regard, applying peptidomics technology similar to CKD273, novel urinary peptides classifiers showed good-to-excellent accuracy for discrimination of seven different causes of CKD from one another (area under the receiver operating characteristic curve ranged from 0.77 to 0.95) and the component peptides provided insights into pathophysiological mechanisms [27]. The aetiologies tested encompassed major causes of end-stage kidney disease, such as diabetic kidney disease, hypertensive nephrosclerosis, diverse primary glomerulonephritides (primary focal segmental glomerulosclerosis, IgA nephropathy, membranous nephropathy) and lupus nephritis.

In conclusion, a series of signals suggests that clinical proteomics has entered the disruptive phase of exponential technologies and it is likely that it will be incorporated in routine clinical care in the short term, together with other systems

biology-based approaches, within an integrated personalized medicine framework. The availability and physician familiarity with systems biology databases is a key step towards achieving this goal.

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Conflict of interest statement

None declared.

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