Hypoxia-Inducible Factor 1 α (HIF-1 α), Angiopoietin-2 (ANG-2) and Endocan: Novel Biomarkers of Disease Progression Involving Polycystic Kidney Disease

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Discovery and validation of biomarkers comprise a critical step in making progress in the fight against autosomal dominant polycystic kidney disease (ADPKD). Biomarkers can be on 3 different levels: diagnostic, prognostic, and therapeutic. The implementation of these biomarkers in the design and feasibility of clinical trials depends on their accuracy, reproducibility and dynamic response to an intervention.

Two novel imaging markers, renal artery blood flow (RABF) and height-adjusted total kidney volume (HtTKV), are considered prognostic biomarkers in ADPKD [1–3]. HtTKV was approved by the United States Food and Drug Administration in 2014 for use as an enrichment biomarker in clinical trials. Change in HtTKV, used as the primary endpoint in clinical trials, has been associated with an unprecedented enthusiasm for interventional trials in ADPKD over the past 15 years, leading to the marketing of the first disease-modifying agent (tolvaptan) outside of the United States [4]. A predictive model utilizing HtTKV, age, sex, race, and serum creatinine has been developed as a risk stratification tool for disease progression in ADPKD [5]. Clinical trial de-

sign utilizing the Irazabal classification system allows for the enrollment of “fast progressing” patients, making clinical trial design shorter with fewer subjects ultimately being less expensive and less burdensome on the patient population.

Both HtTKV and RABF require an MRI or MRA, which remain cumbersome, expensive (if it cannot be performed in resource-limited countries), and technically difficult to obtain (especially for RABF). Therefore, a continued search for more accurate biomarkers of disease progression has gained a significant momentum over the past decade. These biomarkers include urinary concentrations of copeptin, mitochondrial pyruvate carrier (MPC-1), neutrophil gelatinase-associated lipocalin, interleukin 8, heart-type-fatty acid binding protein, kidney injury molecule 1, and serum levels of high density lipid cholesterol, vascular endothelial growth factor (VEGF), angiopoietin-1, uric acid levels and MPC-1, and tumor necrosis factor alpha [6].

In this issue of the journal, Raptis and colleagues report the first human comparison of the serum levels of 3 novel biomarkers of endothelial function (endocan also

called Endothelial cell-specific molecule 1 or ESM-1), angiogenesis (angiopoietin-2), and renal hypoxia (HIF-1 α) [7]. They evaluated 26 normal controls, 26 patients with early (estimated glomerular filtration rate [eGFR] >70 mL/min) and 26 with more advanced ADPKD (GFR 45–70 mL/min) [7]. The authors show significantly higher serum values of all 3 biomarkers in ADPKD patients with more advanced compared to both less advanced and normal controls, suggesting that these molecules may represent early markers of disease progression in ADPKD. Furthermore, they show modest to moderate correlations between eGFR and endocan ($r = -0.258$), angiopoietin-2, and HIF-1 α ($r = -0.468$ and -0.465 respectively) levels. Even more intriguing is the fact that they demonstrate strong correlations between the ADMA levels (an established marker of endothelial dysfunction in ADPKD) and endocan ($r = 0.908$), angiopoietin-2 ($r = 0.983$), and HIF-1 α ($r = 0.998$) levels. The authors speculate on the role of potential intermediary molecules that may link these distinct pathways together (VEGF and nitrogen oxide).

While this report is limited by its sample size, the authors pose legitimate questions, generate new hypotheses, and offer practical implications on the direction of future clinical and translational research in ADPKD.

The addition of HIF-1 α signaling pathway to the previously reported correlation between RABF and renal function raises an interesting question: Is the decline in GFR that follows the fall in RABF mediated through pure hemodynamic changes without hypoxia, through hypoxia, or both? Investigating the relationship between these 2 factors, may lead to a better understanding of the disease progression in the early stages of ADPKD.

Similarities that exist between ADPKD tubular epithelial cell behavior and neoplastic cell behavior (de-differentiation, abnormal trafficking, and targeting of proteins such as the epidermal growth factor receptor, polarization defects, increased cell-matrix and reduced cell-cell adhesion, high rates of division, and apoptosis), explain the recent rise of interest in investigating clinical pathways or molecules that have shown some relevance in oncology (bosutinib, tesevatinib, and others) [8]. This report adds specific molecules (HIF-1 α , endocan, and angiopoietin 2) that are involved in 2 critical steps of neoplastic process: hypoxia and angiogenesis. Furthermore, it links these molecules to a previously known marker of endothelial dysfunction, asymmetric dimethylarginine (ADMA). The strong correlation between ADMA and the 3 novel biomarkers, belonging to 3 distinct signaling pathways, may be suggestive of either a central place (through unknown intermediary molecules,

may be nitric oxide, or VEGF) or a very distal (downstream) place for ADMA in those pathways. Only future studies may elucidate that point.

However, our experience with previously identified candidate biomarkers of disease progression has not yet made any significant impact on clinical practice or research, and thus points out the difficulties of developing useful biomarkers in ADPKD. As a reminder, HtTKV was first shown to predict disease progression in ADPKD approximately 12 years ago, and approval of this tool for clinical trial enrichment took more than a decade to come to fruition. The lessons learned during the TKV validation (choosing the most reproducible imaging tool to measure TKV, combining collaborative efforts from large multinational registries, using longitudinal predictive power rather than cross-sectional correlations, etc.) will need to be applied to these biomarkers to accelerate their validation.

Several issues need to be resolved to help in the validation process. Establishing a robust reference range for normal values (in the general population, chronic kidney disease patients, and ADPKD patients), using a standardized assay and reproducible assay with known coefficients of variation will be important. Published data include different methodologies, different biological fluids (serum vs. plasma), and different assay-to-assay variability in different cohorts. Given the paucity of results in chronic kidney disease (CKD) or ADPKD cohorts, direct comparisons between those levels are not available to determine the direct applicability to ADPKD. Reported mean values of plasma Endocan in this study (4.85 μ g/dL using the Cusabio assay) are 7-fold higher than the previously published serum levels in a Turkish cohort (control group for a cohort of patients with Behçet disease; mean 0.7 μ g/dL using the Bio-Tek Synergy HT assay; Balta et al. [9]) but similar to the plasma levels in an Egyptian cohort (4.12 ± 2.9 μ g/dL using enzyme-linked immunosorbent assay, LUNGINNOV Systems assay) [10]. Whether the observed differences are simply due to cohort differences, biological sample differences, laboratory methodology, or assay-to-assay variations is unclear. Furthermore, the factors that lead to day-to-day variations and loss of reproducibility need to be identified and adjusted for.

Clinically what level of incremental changes in concentration associates with and/or is predictive of relevant outcomes (doubling of serum creatinine levels, progression to CKD-stages III–IV, time to onset of end-stage renal disease, or death) remains to be determined. This paper shows a significant but moderate correlation between

eGFR and biomarkers and more needs to be done. Also, any future prognostic model that includes several parameters with strong correlation to each other (ADMA vs. HIF-1 α , Angiopoietin 1 and endocan) should be carefully investigated for collinearity and for a more excitingly true biological relevance.

The field of biomarker development will need to address the equipoise of current knowledge and will be useful only if they enhance the robustness of existing biomarkers, or if they are less expensive. To illustrate this point, the Consortium for Radiologic Imaging Studies in PKD looking at independent prognostic value of HtTKV vs. PKD genotype or mutation type showed that both PKD genotype and mutation type and HtTKV-predicted CKD outcomes, PKD genotype, or mutation type was not

an independent prognostic factor after adjusting for HtTKV [11].

The exciting additional discovery of new biomarkers in ADPKD that sheds light on potential new mechanistic pathways involved in cyst proliferation, fluid secretion, and renal injury opens new doors to test new interventions. Raptis et al. [7] targeted endothelial dysfunction, angiogenesis, and renal hypoxia. These efforts will potentially bring new molecules into clinical trials and lead to new promising therapeutic interventions for ADPKD.

Disclosure Statement

Both authors declared that they have no conflicts of interest to disclose.

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