Evolution of the biomarker concept

The search for biomarkers in body fluids is evolving into a broader quest for molecular phenotyping of tissue and disease reclassification. The original biomarker concept was too limited, failing to recognize that the interpretation of the molecular changes in body fluids requires a molecular understanding of the diseased tissue.

A molecular biomarker in nephrology implies a molecule that can be measured in body fluids as an indicator of a pathologic state in kidney tissue, perhaps avoiding biopsies by providing similar information. This demands that the biomarker is superior to current laboratory assessments such as creatinine and conventional urinalysis.

The problem with this concept is that the biomarker must perform in one dimension, perhaps even dichotomized by a cutoff, to deliver actionable new information. This expectation is flawed: molecular changes in human disease states do not operate in one dimension. Disease states have diagnosis, activity, stage, and prognostic information, and are imposed on aging and pre-existing diseases in the kidney or other organs. For example, in acute kidney injury (AKI), age, injury, and underlying diseases may all influence the levels of a biomarker, since many of the changes of AKI are also induced by chronic diseases (1). In some cases, a higher level of a biomarker is better than a lower level. If the marker indicates injury-repair, and the tissue has been injured, elevated levels of injury-repair molecules will indicate normal healing and their absence would be abnormal, like a wound that is not healing.

Molecular changes must be quantitative, and inter- and intralaboratory variation can create major problems. Molecular measurements delivered as laboratory-developed tests are difficult to normalize and standardize. Ideally, they should be measured centrally and normalized against a reference set.

Emerging lessons in how to use “big data”

An assessment of a disease state using laboratory tests is in fact a prediction of the unknowable true disease state. Molecular phenotyping adds a new dimension to increase the accuracy of this prediction (Figure 1).

Our approach is guided by new thinking that show “big data” should be used to create accurate predictions (2). As dramatized in the movie “Moneyball,” the use of rich baseball databases added a statistical dimension to decision making in that sport, which had previously been based on expert opinion. The key was that the database included hard outcomes on which to train predictive equations—“Ws and Ls,” wins and losses. Predictions from big databases in cancer are emerging using the same principle, capturing hard outcomes to build predictive equations using high dimensionality molecular platforms. Such predictions of the true disease state should be: 1) Bayesian, acknowledging prior probabilities and biases; 2) probabilistic, with estimates of potential for error; 3) updatable with new knowledge; and 4) consensus-seeking, including expert opinion.

The challenge is to assemble these pieces of information into an understanding for the individual patient (Figure 2).

Developing the Molecular Microscope

Using these principles, we developed a system for kidney transplant biopsy assessment, as recently reviewed (3). Our stepwise analysis is outlined in Table 1.

Our project related molecular changes in kidney biopsies to histologic changes, clinical phenotype (function, proteinuria, etc.), and outcomes, as well as specific diseases. We used indication biopsies as the centerpiece for disease understanding and reclassification. The project has captured more than 1000 indication biopsies from kidney transplant recipients and defined relationships among function, histologic, outcome, and molecular changes measured by microarrays. Our understanding was helped by characterizing gene sets associated with biological changes in mouse models, permitting a sketch of the underlying biology—infiltration and activation of macrophages and effector T cells, tubulo-interstitial injury, and microcirculation injury, the types of diffuse changes that can be detected in a core biopsy.

We found that the prevailing classifications of diseases in transplants had major errors, for example interpretation of staining for complement factor C4d (4). We reclassified the diseases states based on conventional and molecular assessments. These biopsies became our reference set against which new biopsies can be assessed. We developed equations to turn microarray results into estimates of the diseases and the degree of tissue injury, and validated and calibrated the readouts. We integrate this with conventional assessments to create an overall view, which we envisage as that assembled by the clinician, not the pathologist.

The result is the Molecular Microscope system of equations, which currently provides estimates of the probability of: 1) T cell-mediated rejection (TCMR) score; 2) antibody-mediated rejection (ABMR) score; 3) atrophy-scarring score—extent of chronic damage; 4) acute kidney injury score—extent of recent parenchymal injury and ongoing injury repair; and 5) prognosis—the risk of progression to failure.

Lessons

The molecular phenotype of a biopsy is a reproducible dimension of the biopsy, but time and experience will be needed to define its full meaning. The molecular changes correlate with histologic and clinical phenotypes, but do not necessarily “agree” with them and are generally superior to histology or clinical parameters in predicting prognosis. The combined values of the molecular, histologic, and clinical assessments represent an opportunity for a consensus, not competition. Our goal for each biopsy and for each patient is to assign numerical values in multidimensional space, where N=1, a step toward precision medicine, and can be compared to her nearest neighbors in the reference set.

For example, the TCMR score is virtually always abnormal in typical TCMR, but there is considerable disagreement, which includes false negative histologic assessment of scarred tissue, confusing situations such as polymavirus with TCMR, and false positive histologic diagnoses caused by sharing of lesions with other diseases. The ABMR score, on the other hand, must assess a much more complex and pleomorphic phenotype. ABMR has a large dynamic range, from fulminant to indolent to inactive, and accrues time-dependent changes in the microcirculation. The ABMR molecular score may not be positive in patients who have relatively inactive ABMR. This is a new phenotype—histologic ABMR with low molecular activity. The histologic, clinical, and molecular states together create new disease classes, as has already happened in cancer, where complex multidimensional phenotypes are emerging as molecular measurements become standard of care.

The implication for biomarkers in body fluids is not necessarily bleak. They cannot provide the richness of phenotypic detail needed for disease reclassification and creation of new phenotypes, and as measurements in one dimension are unlikely to answer the unmet need for precision medicine. However, biomarkers in body fluids can be re-examined as useful additions to new multidimensional disease classifications to see how they contribute to care in the clinic, for example monitoring after biopsy.

Philip F. Halloran, MD, PhD, is affiliated with the University of Alberta and the Alberta Transplant Applied Genomics Centre in Edmonton, AB, Canada.

References