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Stratifying Diabetes: Desperately Seeking Specificity

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In 1836 William Barret Travis drew his sword and marked a line in the sand. Those willing to stay and defend the Alamo fort were invited to cross the line; in doing so, like Davy Crockett they became immortal, whereas those remaining on the other side of that line were broadly lost to history. Travis was a "splitter." Physicians, also, tend to be "splitters," designating individuals with or without disease and subdividing those with disease into groups according to key features (1). Diseases gain identity through clinical phenotype. Only in recent years have we incorporated genetic and nongenetic laboratory-based features into that identity. In doing so, we seek to encapsulate each disease as a categorical entity.

But diabetes has confounded categorization, in part because its key elements are not exclusive to the disease (1–4). Even raised blood glucose is only a proxy for loss of the homeostatic relationship between insulin secretion and insulin sensitivity—a relationship whose tipping point is designated "diabetes" when the risk of retinopathy increases. Initially characterized by the striking clinical phenotype of juvenile-onset diabetes, that classification evolved historically according to therapy (insulin-dependent diabetes) and then immunogenetic features (type 1 diabetes, T1D). The remaining cohort without these characteristic features were designated adult-onset diabetes, non–insulin-dependent diabetes, or, nowadays, type 2 diabetes (T2D). A proportion of apparent T2D patients have the immunogenetic features of T1D and are categorized as latent autoimmune diabetes in adults (LADA) (3,5). These patients with autoimmune adult-onset diabetes have diabetes-associated genes and autoantibodies, notably GAD autoantibodies (GADAb). They tend to show a more aggressive clinical course that often progresses to insulin therapy, have an increased risk of thyroid autoimmunity, and should avoid sulfonylurea therapy (1,5). However, distinguishing autoimmune diabetes from T2D cases is not easy because age at diagnosis, need for insulin treatment, metabolic syndrome, and even the GADAb assay are not categorical biomarkers.

The challenge is to define specific features of adult-onset autoimmune diabetes, and that hunt has encompassed genes, cytokines, chemokines, C-peptide (as a proxy of insulin secretion), and now metabolites. Genetic risk estimates have limited value in T2D and are confounded by the low genetic load in adult T1D (6,7). Cytokine and chemokine analyses found changes not exclusive to any clinical category, showing a graded effect across autoimmune and nonautoimmune diabetes, with an inverse relationship between adaptive and innate immunity, e.g., GADAb titers and serum interleukin-6 levels (8).

In this issue of *Diabetes*, a group from Scandinavia has sought to categorize adult-onset non–insulin-requiring diabetes by analyzing metabolites (9). Metabolites are small intermediate products of metabolism, including glucose, amino acids, fatty acids, and lactate, and have proved valuable as biomarkers for disease prediction (10–12). Importantly, recent studies identified metabolite changes in both cord blood at birth and the sera of young children before the appearance of diabetes autoantibodies and T1D (13,14). These metabolite changes included persistent decreased levels of proinflammatory lysophosphatidylcholine, most likely acquired prenatally or inherited, with particularly low levels in cord blood if the mother had had a first trimester infection (13).

In the current study, acylcarnitines, both long- and intermediate-chained, showed large differences between diabetes types. Of 13 carnitines, 12 were significantly lower in classic T1D than in both T2D and LADA—changes consistent with an imbalance in lipolysis and Krebs cycle activity (9). However, the metabolite changes were not categorically distinct and had no utility for differentiating LADA from T1D or T2D. Since lipolysis is exquisitely sensitive to insulin, a potential role of insulin (with C-peptide as a proxy) should be considered, and the authors found that plasma C-peptide levels were the strongest determinant of the metabolite profile, with a C-peptide–driven continuum in the metabolite changes

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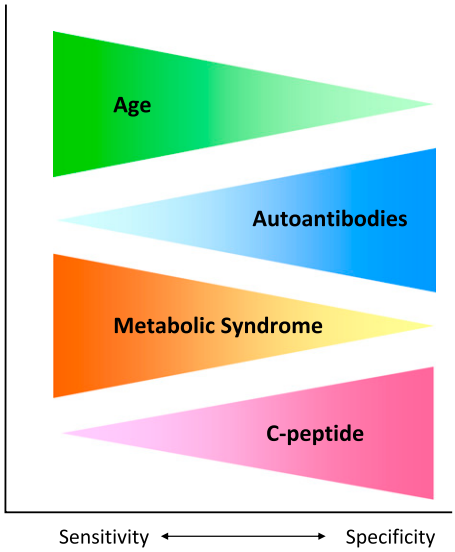

Figure 1—Collation of predictive features for diabetes endotypes can improve disease prediction because there is an inverse relationship between predictive sensitivity and specificity. As shown here, younger age at onset and absence of metabolic syndrome increases sensitivity for T1D, whereas more diabetes autoantibodies and more C-peptide increases specificity for T1D and T2D, respectively (19).

across all three clinical diabetes categories, namely, T1D, LADA, and T2D (9). This effect could be anticipated because they had not selected LADA patients by the customary three criteria: adult-onset diabetes, initially non–insulin-requiring, and GADAb positivity. Instead, they substituted—reasonably, in our opinion—the use of insulin therapy with a C-peptide level >0.3 nmol/L, that is, without an absolute insulin deficiency (15). Consequently, the C-peptide levels in LADA were similar to those in T2D. The purity of the cohort for true-positive GADAb is further compromised by the risk of false-positive GADAb in patients with true T2D. The greater the true-positive rate in the screening population for the GADAb assay and the greater the assay specificity, the lower the risk of false positives; for example, here there would have been a false GADAb-positive rate of 2% in the screening population given that the GADAb assay had 98% specificity (14). Nevertheless, those metabolite changes in LADA patients overlapping with the T1D metabolome did identify patients who progressed most rapidly to insulin treatment, despite similar GADAb titers and C-peptide levels (9). In terms of clinical utility, metabolites may yet have a role, and it is worth noting that high levels of both lysophosphatidylcholine and L-carnitine, the opposite of that found in T1D, are risk factors for atherosclerosis (10). Further, both lipid-bound choline, a major epigenetic methyl donor during pregnancy, and L-carnitine can be conflated as important products of the microbiome in early life and could be implicated in diabetes risk (16–18).

The graded loss of C-peptide levels across a range of clinical diabetes phenotypes, highlighted by the current study (9), is a consistent feature of the diabetes state (19). We could look elsewhere for definitive biomarkers to stratify diabetes, but most likely we will resolve the current uncertainty by using combinations of sensitive and specific laboratory biomarkers (12,20) (Fig. 1). In the meanwhile, clinicians should treat the individual before them, using the laboratory only as a diagnostic and therapeutic adjunct. In our current Age of Doubt, looking to emulate General Travis might be unwise; perhaps the best approach is to keep the sword firmly in its sheath.

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