When does a fingerprint constitute a diagnostic?

Diagnosis of disease via biomarkers in circulating blood depends on several factors. Markers must be detectable during the preliminary stages of disease, or at least when successful treatment is possible. Tests should be cost effective and readily available at point-of-care, such that the turnaround time for diagnosis is minimised. Preferably, diagnosis should be minimally invasive and, most importantly, must be absolutely specific to the disease to minimise misguided or inadequate therapy. In today’s Lancet, Dan Agranoff and colleagues describe the use of surface-enhanced laser desorption-ionisation time-of-flight mass spectrometry (SELDI-TOF) to identify serum biomarkers from patients with advanced Mycobacterium tuberculosis infections.\(^9\)

Serum proteomics with this technique provides a pattern-based diagnosis, because multiple peptide and protein signals are detected simultaneously in a single mass spectrum. Because the detector is based on well-established matrix-assisted (MALDI-TOF) mass-spectrometry methods, there is a bias toward lower mass signals (1–10 kDa), and the approach is often termed peptidomics.\(^2\) Furthermore, disease states seem to be characterised by raised levels of cleavage products of abundant serum proteins.\(^3,4\) Alternatively, MALDI-TOF mass-spectrometry profiling, which provides spectra of higher resolution, allows for the improved acquisition of higher mass ranges, circumventing the limitations of low mass profiling (figure). In the earliest iterations of

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SELDI-TOF mass spectrometry for biomarker discovery, identification of the individual components within the signature was not possible, although later studies overcame this difficulty with much better algorithmic interpretation of the resulting peptide masses and concurrent use of tandem mass spectrometry for amino-acid sequencing. The capital cost of equipment and assays are such that SELDI-TOF mass spectrometry is unlikely to be routinely and, therefore, identification of a suite of markers from within the signature is needed to provide a test amenable to frequent low-cost use.

The advantage of SELDI-TOF mass spectrometry is that it does not rely on evidence of a gold-standard biomarker, but rather on combinations of peptide signals. The disease signature given by the serum sample in the mass spectrometer, and compared against a database of controls and test samples, is the potential diagnostic at the preclinical stage. Mass spectrometry is a bioanalytical technique with almost unparalleled sensitivity, and thus provides an ability to mine substantially deeper into the serum or plasma proteomes than has previously been possible. Unfortunately, substantial caveats remain (table). The use of serum or plasma is challenging technically. More than 3000 proteins are contained within the plasma protein set of the Human Proteome Organisation (HUPO). However, the dynamic range is such that fewer than 20 of these represent more than 98% of total protein mass. A major flaw in studies of SELDI-TOF mass spectrometry is the attempt to find biomarkers from among only the 25–50 most abundant serum or plasma proteins, with only limited prefractionation with selective binding to specific target surfaces with low capacity and subsequent competition for binding sites. Therefore the same proteins, and their breakdown products, are identified as markers in diseases with different underlying pathological processes. For example, serum amyloid A (a common marker of inflammation and identified by Agranoff as a major marker in tuberculosis patients) has been identified in ovarian cancer and stroke in plasma, and in renal, prostate, and nasopharyngeal cancers in serum. The question of marker specificity is therefore obvious. Combination with other potential markers (as in Agranoff’s study) provides better specificity.

At this point, however, we should be cautious against over-interpretation of the diagnostic capabilities of individual proteins identified within a disease signature until more data are obtained from a wider number of patients. A fair argument would be that the absolute diagnostic specificity depends on a marker being raised in one disease alone. Substantial debate also remains about sample collection, preparation, and interpretation of data for SELDI-TOF mass spectrometry. Even the relative merits of serum over plasma have been argued, because of the raised protease activity seen after clotting in serum. HUPO are attempting to provide operating procedures for serum and plasma proteomics that would provide better standardisation and reproducibility needed to move this technology beyond an analytical tool.

Tuberculosis is a disease in need of a rapid, sensitive, and specific diagnostic test, replacing slow culturing of M tuberculosis (2–6 weeks for a positive assay), poorly sensitive microscopy-based detection in patients’ sputum, and non-specific serological analyses in which substantial cross-reactivity with other mycobacteria (including previous immunisation with BCG) reduces specificity. Agranoff and colleagues’ study highlights two important facts. First, that microbial infections caused by one organism might specifically alter the serum proteome profile differently from infection with another pathogen, and this is the first study, to our knowledge, to investigate serum responsiveness to a bacterial pathogen with serum

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* Complex protein samples contain more than 2000 discrete proteins/peptides, needing instrumentation with higher resolution and dynamic range capabilities. Simple samples will contain fewer proteins/peptides. † High abundance proteins/peptides suppress and mask signal resulting from low abundance proteins/peptides. ‡ Variation in ionisation efficiency affects total ion current due to differences in sample components (eg, salts).
peptide profiling. Second, a disease in which early diagnosis and therapy is an important prognosticator of clinical outcome can be detected rapidly with this approach. Further evidence of the absolute specificity of these tuberculosis markers, and generation of mass-spectral serum patterns from patients in the early stages of disease, could eventually have a huge effect on the clinical outcomes of tuberculosis infection worldwide by reducing the time between infection, diagnosis, and onset of therapy.

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Compliance with osteoporosis therapy is the weakest link

The lifetime risk for fragility fractures due to osteoporosis after the age of 50 years is about 50% in women and 20% in men. The resultant high morbidity, mortality, and economic costs for elderly people are well recognised, and have stimulated the development of effective interventions to reduce fracture risk. Despite these advances, however, two challenges have hindered reduction of this public-health burden. We need to better identify individuals with a high risk of fracture to target treatment more cost-effectively. We also need to ensure that patients take their treatment as prescribed (compliance) and for the recommended duration (persistence).

Compliance and persistence are poor in at least 50% of patients during the first year of treatment for osteoporosis, and in 80% after 3 years. Most instances of inadequate compliance and persistence occur within 3 months of the start of treatment, and are associated with higher rates of fracture than those observed when compliance and persistence are good. In over 35 000 women on bisphosphonates, fracture rates in those who did not comply or persist with treatment were 20–30% higher than those persisting with medication as prescribed at 24 months. Higher rates of prescription refills were also associated with lower fracture rates. The stage at which inadequate compliance and persistence compromise effectiveness is unknown. However, fracture rates increase when compliance falls below 50%. Extra costs from such fractures probably outweigh savings in drug costs and adversely affect the overall cost-effectiveness of fracture prevention, especially in high-risk populations.

The problem of poor compliance with treatment for chronic diseases has several puzzling aspects. In trials for prevention of coronary heart disease, individuals who complied poorly with placebo had worse health outcomes than those who were compliant, suggesting that inherent characteristics of poor compliers increase morbidity. Differences in lifestyle might partly explain this finding, but did not account for all of the observed increase in mortality. The causes of poor compliance and persistence are poorly understood—putative causal factors such as age, previous history of fracture, use of multiple medications, and comorbidities explain less than 10% of variability in compliance. We need to know the relative contribution of other potential factors,