Mass Spectrometry in Precision Medicine: Phenotypic Measurements Alongside Pharmacogenomics

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BACKGROUND: Precision medicine is becoming a major topic within the medical community and is gaining traction as a standard approach in many disciplines. This approach typically revolves around the use of a patient’s genetic makeup to allow the physician to choose the appropriate course of treatment. In many cases the genetic information directs the drug to be used to treat the patient. In other cases the genetic markers associated with enzyme function may inform dosage recommendations. However, there is a second way in which precision medicine can be practiced—that is, by therapeutic drug monitoring (TDM).

CONTENT: A review of the use of mass spectrometry for TDM in the arena of precision medicine is undertaken. Because the measurement of a drug or its metabolites provides the physician with a snapshot of the therapeutic exposure the patient is undergoing, these concentrations can be thought of as an actual phenotype measurement based around the patient’s genetics coupled with all of the environmental, pharmacological, and nutritional variables. The outcome of a TDM measurement by mass spectrometry provides the patient’s current phenotype vs the potential phenotype imputed by the genetics.

SUMMARY: The use of mass spectrometry can provide an understanding of how a drug is interacting with the patient, and is orthogonal to the information provided by pharmacogenomic assays. Further, the speed and relatively low expense of drug monitoring by mass spectrometry makes it an ideal test for precision medicine patient management.

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A schematic of the overall effectors of pain medication within an individual can be seen in Fig. 1.

Owing to all these factors, coupled with the cost, long turnaround time, and high complexity of genetic testing, it is often preferable to measure the therapeutic concentration of the drug along with any active metabolites in the circulating blood of the patient. This pharmacokinetic approach is often referred to as therapeutic drug monitoring (TDM) and is commonly used in many therapeutic areas such as immunosuppressants and antiepileptics (10–12). In this approach, measurement of the final drug and metabolite concentrations (the actual phenotype of that patient’s response to the therapeutic drug) takes into account all of the factors discussed above: PG, environment, dietary influences, gastrointestinal issues, body composition, and concomitant drug use. In TDM the measurement provides the clinician with the drug and metabolite exposure the individual patient is experiencing. Furthermore, the testing for TDM is typically cheaper and faster than PG assessment, with mass spectrometry being at the forefront of the phenotypic approach, alongside some automated immunoassays. However, mass spectrometry is considered to be more specific than immunoassays and allows for multiple similar metabolites to be individually measured within a single sample (13). This is highly important because structurally similar metabolites often have very different efficacies in vivo and yet often cross-react in immunoassays (14).

In this review an examination of the use of PG and TDM in the areas of chronic pain management and oncology (specifically tamoxifen treatment in breast cancer) is detailed. Example cases in which the phenotypic pharmacokinetics measurement may be superior or additive to the use of PG measurements are presented.

**Chronic Pain Management**

The most common physical symptom leading to a primary care physician visit is acute or chronic pain (15). Pain management has thus become a very important area of medicine and is growing rapidly. Part of the issue in the treatment of pain is that it is highly subjective and varies greatly from individual to individual (4, 16). Furthermore, there are clinically significant metabolic differences among patients, which lead to highly divergent responses to the therapeutic entities. In many cases the drug prescribed is really a prodrug requiring activation via the patient’s metabolic apparatus. In other cases the same enzymatic apparatus is required to metabolize the active ingredient to its metabolites and so reduce the patient’s exposure to the drug, which in turn reduces the potential for toxicities caused by accumulation of the drug within the patient. Finally, there is a need for physicians to be able to monitor compliance of their patients when it comes to chronic administration of pain medications. This helps ensure the patient is not selling the medications—an unfortunate reality that is becoming more evident as time goes by.

Over 80% of the phase 1 metabolism of drugs within a patient is accounted for by members of the CYP450 super enzyme family (17). Members of this
family are highly important in the drug concentrations observed within a patient after dosing with a therapeutic agent. Genetic polymorphisms within the CYP450 enzymes can therefore strongly affect drug and metabolite exposure. Mutations can lead to loss or gain in function, which in turn can lead to low or high levels of exposure of the active agent (17). This, coupled with the highly subjective nature of pain, can complicate determination of the appropriate dose for an individual patient (4). Thus, there has been a substantial effort to provide PG testing for the various known CYP enzymes with known change-of-function mutations (18).

One of the major players in this enzymatic function gain or loss arena is CYP2D6. More than 80 variants have been shown to cause gain or loss of function of the cytochrome P450, family 2, subfamily D, polypeptide 6 (CYP2D6) gene (19). Thus, the concentrations of drugs for which CYP2D6 is a major metabolizing enzyme will differ widely depending on the patient’s CYP2D6 profile. Traditionally, patients are broken down into 4 general phenotypes: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultrarapid metabolizers or ultrametabolizers (UM) (20). Table 1 highlights a number of the identified mutations leading to these 4 metabolizer phenotypes.

Although a patient’s genetic profile can be used to assign a metabolizer enzyme phenotype as described above, to some extent this should be considered the potential phenotype. The actual phenotype of the patient encompasses not only the phenotype of CYP2D6 due to patient genotype, but also all the external factors that affect the metabolic environment the drug will encounter within the patient. For example, comedication or over-the-counter supplements can affect other enzymes within the metabolic pathway and modify the metabolism of the drug (9). This in turn can lead to unexpectedly high or low maximal blood concentrations, increased circulating metabolites, reduced or increased half-life of the drug and its metabolites, and increased or decreased overall exposure (i.e., area under the curve). All of these factors should be taken into consideration when treating the patient. Table 2 highlights common inhibitors, inducers, and substrates of CYP2D6 and CYP3A4. In many cases efficacious dosage levels have been developed and documented for the main drug compound during the drug’s development and are reported in the package insert or online databases. These can act as a guide for the physician when determining titration of the dose on the basis of the pharmacokinetics results. There may also be information regarding the expected normal circulating as well as potentially toxic concentrations of metabolites in the same data repositories, further helping the physician to fine-tune dosing. However, it should be noted that in some cases, for which this information is not readily available, subsequent studies to determine appropriate blood concentrations might need to be completed to aid in the use of pharmacokinetics data for patient care.
An example of the problems caused by a patient’s genetic makeup coupled with drug interactions can be seen in a report by Gasche et al. (21), in which a 62-year-old man with chronic lymphocytic leukemia presented with fatigue, dyspnea, fever, and cough. Culture of a bronchoalveolar lavage specimen identified a yeast infection. The patient was treated with voriconazole and clarithromycin for the infection, as well as codeine-containing cough suppressant. After 4 days of treatment the patient’s level of consciousness suddenly and rapidly declined, and he became unresponsive. The patient was given naloxone as an opioid receptor antagonist for a suspected codeine overdose, after which he quickly improved. Follow-up testing for CYP2D6 genotype revealed the patient to be a UM because of an increase-in-function allele. This caused rapid conversion of codeine to the main active metabolite, morphine, leading to very high blood exposure. Compounding this was the fact that the major enzyme involved in the inactivation of codeine to norcodeine is 3A4. Because this CYP isoenzyme is inhibited by clarithromycin and vorcanizole, coadministration of these drugs led to higher blood concentrations of codeine and thereby even higher blood concentrations of morphine via the UM phenotype. Together, these factors caused renal failure due to the accumulation of morphine glucuronide. Although the PG would have alerted the physician to the potential for this situation, it would not have predicted the compounding factor of the medication, which was a major factor in the patient’s renal failure. In contrast, follow-up with mass spectrometry–based TDM could have quickly identified the increased production of morphine and the reduced removal of codeine, and the whole situation could have been avoided. The TDM study would require a simple blood draw, and results would be available to the physician typically within 24 h or less; in some cases a star result can be available to the physician in <12 h, allowing for withdrawal of the codeine or reduction in dose. Furthermore, the patient could have had his dosage carefully titrated over the next few days by use of mass spectrometry–generated TDM, given the low expense and rapid turnaround time of the test (22). Such titration could have taken into account the circulating metabolite concentration alongside the parent drug.

Tamoxifen

The standard of care for postsurgical treatment of breast cancers that are positive for estrogen receptor (ER) and/or progesterone receptor is 5 years of tamoxifen treatment (23). The use of this drug has seen a significant reduction in recurrence of the disease up to 10 years after beginning treatment (23). However, because of the clinically significant and unpleasant side effects, approximately 40% of women do not complete the 5 years of treatment. Furthermore, because tamoxifen is actually a prodrug that needs to be converted to the highly active endoxifen along with other active metabolites to be fully efficacious, there is potential for endogenous enzymatic variance (specifically in CYP2D6, Fig. 2) to have an impact on endoxifen exposure in individuals. Certain drugs taken for other comorbidities can also inhibit CYP2D6 (e.g., SSRIs) and lower the individual’s endoxifen exposure (9).

It has been postulated that this reduction in the endoxifen metabolite exposure reduces the efficacy of the tamoxifen treatment and increases the recurrence risk. However, several recent studies (24, 25) have contradicted this hypothesis and seem to suggest that there is more than 1 pathway to get to endoxifen even in the presence of variant CYP2D6 and tamoxifen may act through many of its metabolites, not just endoxifen.

These possibilities suggest that the current approach of using a CYP2D6 genetic test to determine if the patient carries the variant form of the enzyme may be too simplistic, and the results may in fact lead the physician to draw the wrong conclusions (26). It would therefore seem useful to develop a phenotypic test that actually measures the concentrations of the various metabolites and therefore takes that person’s enzymatic makeup into account automatically. Furthermore, the ability to measure all the major metabolites quantitatively may allow researchers to start to examine the efficacy of 1 or more of the metabolites, alone or in concert, when looking at recurrence data for patients. Some studies have already suggested cutoffs for specific metabolites (27) such as endoxifen, and more are ongoing.

As an added utility, the recent BIG1-98 (Breast International Group 1-98) study (23) suggested that some metabolites might be associated with self-reported adverse effects more than others. This finding could be highly useful when combined with data gained regarding outcomes vs metabolic profiles. In theory, a physician might be able to tailor the patient’s dose to minimize the adverse effects while still maximizing the efficacy of the regimen on the basis of the patient’s unique metabolic profiles, allowing for a personalized medicine approach.

Two studies have attempted to correlate concentrations of various tamoxifen metabolites to outcome data (27, 28). Madlensky et al. (27) measured tamoxifen and 3 major metabolites and found that patients in the lowest quintile of endoxifen concentrations had a 26% higher likelihood of a recurrence event. Kiyotani et al. (28) correlated concentrations of endoxifen and other metabolites to genotype (CYP2D6 mutations) and outcome. They found that lower concentrations of endoxifen correlated with mutations in CYP2D6 as well as increased risk of recurrence. It is not clear if it is purely the CYP2D6 status that is directly correlated to this increased risk or rather the CYP2D6 status leading to lower...
endoxifen concentrations, which then correlate to increased recurrence risk because they are directly related. However, it is clear that there is a major discussion surrounding the use of genetic testing of 2D6 in relation to the treatment of breast cancer with tamoxifen. Singh et al. (25) discussed this:

The FDA advisory committee has recommended labeling changes to indicate that postmenopausal ER-positive breast cancer patients who are taking adjuvant tamoxifen and are CYP2D6 *4/*4 (and hence lack functional CYP2D6) have significantly decreased relapse-free survival compared to CYP2D6 wt/*4 or CYP2D6 wt/wt. They have also indicated that patients taking CYP2D6 inhibitors such as paroxetine or fluoxetine have significant reductions in the plasma concentration of endoxifen relative to those taking tamoxifen alone . . . However the FDA has not recommended routine testing of all women on tamoxifen for risk stratification. Similarly the impact of CYP2D6 *5, *10, *41 and their ethnic variation has not been commented upon.

Statements such as this have caused substantial confusion in the field of breast cancer management with regard to testing. In the majority of cases, determination of tamoxifen efficacy by the ordering physician is based around self-reported side effects such as hot flashes and night sweats, because they are suggestive of the patient entering chemically induced menopause due to tamoxifen metabolite exposure (23).

A recent review by et al. (29) provided a detailed examination of the various strengths and weaknesses of the use of PG with tamoxifen treatment. Tellingly, they stated the following toward the end of their article:

An important advantage of this approach is that by measuring the endoxifen concentrations all factors influencing the generation and clearance of endoxifen are taken into account, and can be corrected for. Regarding the high interpatient variability in pharmacokinetics and the inability of monitoring efficacy and/or toxicity clinically, tamoxifen seems to be suitable for TDM . . . Accordingly, a highly sensitive and selective analytical method for the quantification of tamoxifen and its metabolites, especially (Z)-4-hydroxy-N-desmethyltamoxifen, is needed for TDM.

With this in mind, several groups, including our own, have developed LC-MS/MS methods that quantitatively measure tamoxifen and its major metabolites in a single assay (30, 31). The assay developed in our group has been vali-
dated to CLIA 1988 standards and is run in a CLIA-certified laboratory. With the commercial availability of a test covering all the major known metabolites [including the newly identified nor-endoxifen (32)], several prospective clinical trials are now using the TDM data from this assay. This will hopefully lead to the development of more nuanced cutoffs based around patient outcome data vs. tamoxifen and metabolite exposure.

Titration of tamoxifen dose could lead to the concomitant expected changes in blood concentrations within patients, potentially allowing physicians to adjust the dosage up in PM-phenotype patients and thereby increasing exposure to endoxifen concentrations (30). The TDM model would lend itself to just such dose adjustment, allowing the physician to follow each individual patient longitudinally and varying dosage over time to ensure optimal drug exposure. Furthermore, with sufficiently robust clinical cutoffs, the use of this TDM approach could allow physicians to adjust the dosage down in EM- or UM-phenotype patients to reduce their side effects while still maintaining them within the therapeutic window. Again, this is ideally suited to longitudinal TDM monitoring using a mass spectrometry assay and would not be feasible based solely on a PG result for a patient’s potential phenotype.

Summary

It is clear that PG assessments have an important place in the world of precision medicine. These assessments, for example, are invaluable in selecting the correct therapeutic agent in many oncology patients on the basis of the presence of a specific known mutation, deletion, or fusion gene. In those examples it is unlikely that a TDM measurement would benefit the physician much and would require the patient to already be on the drug before testing.

However, in cases for which PG is being used to guide therapy decisions based around the presence of a mutation in a metabolizing enzyme, things are not as clear cut. In many cases the alteration of enzyme function implied by the genetic status of the patient may have a wide range, and the PG data do not determine where in that range of activity the patient falls. Second, it is highly common to have substantial redundancy in the metabolic pathways when it comes to the CYP family. This can lead to unpredictable results for blood concentrations because the specific metabolic makeup of the patient now becomes the unknown factor. Finally, as shown earlier, a very large number of compounds, both drug compounds and those found naturally in foods, can affect the activity of CYP and other enzymes. These compounds can lead to up- or downregulation of the enzyme concentration and activity, and even inhibit them completely. This highlights the concept of the actual vs potential phenotype, wherein a patient could present as having no predicted issues with metabolism of a drug based around their PG measurement, when in reality enzyme activity is inhibited by a dietary or drug–drug interaction.

All of these myriad factors affecting the activation and deactivation of therapeutic drugs are so fluid over time that, in many cases, TDM via mass spectrometry becomes the most pragmatic and accurate way to determine the drug exposure the individual patient is experiencing. That is truly the core of precision medicine and why TDM by mass spectrometry has so much to offer in this field.

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