Predictive Values for Molecular Diagnostics: Converting Unknown Unknowns to Known Unknowns

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1 Introduction

While molecular diagnostics are a relatively recent development in the field of oncology, the utility of this limb of pathology is undeniable. Prototypic companion diagnostics treatments are widely described in the fields of oncology and pathology; for example, *KRAS* mutation testing prior to anti-epidermal growth factor receptor (EGFR) monoclonal antibody therapy (cetuximab, panitumumab) for metastatic colorectal cancer (mCRC), or *EGFR* mutation testing in stage IIIB and stage IV non-small-cell lung cancer (NSCLC) to establish suitability for gefitinib and erlotinib therapy.

In the case of predictive mutation testing for oncology, the results of a single set of examinations (molecular markers) decide a patient's eligibility for therapy. This places on the treating oncologist an absolute dependence on a single set of investigations to decide a patient's

treatment options. However, as some authors have already demonstrated, our intuitive grasp of probabilities can lead to a false level of confidence in the veracity of medical results [1], and predictive mutation assays are no exception to this rule. As Gigerenzer and colleagues have demonstrated, this false confidence can stem from a lack of information on, or understanding of, the positive or negative predictive value of an assay. This problem may be compounded where the same mutation may be found in multiple disorders and may predict favourable response to therapy for one cancer type and may contra-indicate it for another.

2 The Mathematical Basis of Predictive Values

To illustrate the difference between sensitivity and specificity and the predictive value of an investigation, we will first discuss a theoretical mutation detection assay with a sensitivity of 0.90 and a specificity of 0.95, where a positive mutation result is required to institute a therapeutic option.

While the above sensitivity and specificity values may appear acceptable, calculation of the predictive values of the assay tell a different story. If one imagines that the mutation being detected by the theoretical assay described above is quite rare in the population being tested, 1 in 100 for example, the positive predictive value (PPV) can be calculated as follows: with a prevalence in our tested population of 1 in 100, for every 1,000 patients tested, 10 will have the mutation of interest and 990 will not. With a sensitivity of 90 % it is likely that 9 of the 10 true positives will be detected. However, the remaining 990 mutation-negative patients will also be tested, what of their results? Quite simply, our theoretical assay with a specificity of

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0.95 will have a false positive rate of 0.05 (5 %). This will result in approximately 50 false positives (990×0.05). Thus, for every 1,000 patients screened for this mutation with this assay, we will obtain 59 positive results. True positives will account for 9 of the positive results and the remaining 50 positive results will be false positives (Fig. 1). This can be calculated to give a PPV of 15.25 %. Or, to put it another way, a positive result is likely to be indicative of a true patient positive in 15.25 % of cases. Calculation of the negative predictive value (NPV) would yield a more acceptable 99.9 % (see Fig. 1). From the figures above, it can be clearly seen that the prevalence of the disease can directly affect the probability that the patient's result does correspond to a true positive. From the intuitive presumption of a positive result being 90 % likely to indicate a true positive result, we are reduced to the comparably poor likelihood of 15.25 %.

3 Relevance of Predictive Values in a Diagnostic Setting

While the previous example is one of a theoretical assay and disease, clinical scenarios already exist where these principles are of direct relevance, as is the case with *BRAF* mutation testing. Testing for mutations of the *BRAF* gene is a pre-requisite for prescription of vemurafinib for the treatment of malignant melanoma. This serine-threonine kinase inhibitor has been shown to extend the lifespan of stage III and stage IV malignant melanoma patients, and has recently been approved by the European Medicines Agency (EMA) for prescription in un-resectable or metastatic melanoma where the tumour exhibits a mutation at codon 600 of the *BRAF* gene [2]. Thus, once a patient's tumour has been shown to contain an activating mutation of the *BRAF* gene, specifically a p.V600X-generating mutation, it is likely that treatment with vemurafinib will

Fig. 1 Illustration of the combined effect of disease prevalence and assay sensitivity and specificity on the positive and negative predictive value of an assay. The figure illustrates a starting population of 1,000 patients; 10 patients harbour the mutation being examined by the assay, the remaining 990 patients do not. Based on an assay specificity of 95 %, 940 patients will be correctly classified as not carrying the mutation (A), 50 patients will be incorrectly classified as carrying the mutation (C). The assay sensitivity of 90 % means that 1 of the 10 mutation-carrying patients will be incorrectly classified as not carrying the mutation (B), while the remaining 9 patients will be correctly classified as carrying the mutation (D)

induce regression of the tumour by inhibiting the overactive BRAF protein.

The opposite of the above case is noted in mCRC, where the presence of an activating mutation of the *BRAF* gene has been shown to be a negative prognostic factor and also may predict a poor response to therapy with the monoclonal antibody (mAb)-based cetuximab [3–8]. Cetuximab, an anti-EGFR monoclonal antibody, operates by competing with the EGFR ligands to prevent signalling via this pro-inflammatory, pro-oncogenic pathway. However, as a downstream signalling molecule in the EGFR signalling pathway, an overactive BRAF protein may render a tumour resistant to mAb treatment targeting the EGFR.

In the context of malignant melanoma, *BRAF* mutation testing is now standard of care, whereas in the context of mCRC it is not. However, for mCRC, it is likely that more emphasis may be placed on less frequently observed molecular aberrations, such as *BRAF* mutation status, as evidence accumulates to describe additional causes of anti-EGFR therapy resistance. Additionally, from a medical genetics perspective, *BRAF* mutation status is widely used to differentiate between spontaneous CRC and Lynch syndrome [9]. Furthermore, the abundance of more efficient sequencing technologies may encourage the use of multi-marker panels and the increased use of off-label molecular testing to fine tune treatment decisions. This multi-marker approach to oncology brings with it many advantages, but as a corollary, presents many potential pitfalls not only for the oncologist but also for the laboratory.

To help us illustrate the context-specific predictive value of these assays we will use the sensitivity and specificity figures presented to us in the package insert of one CE-IVD marked assay in routine use for predictive testing of *BRAF* mutations for the treatment of malignant melanoma. We are informed that these figures have a sensitivity of 99.6 % and a specificity of 87.9 %. If an approximate *BRAF* p.V600X mutation rate of 50 % is assumed for malignant melanoma based on published reports [10–12], this would equate to a PPV of 89.2 % and a NPV of 99.5 %. Thus, a prescribing oncologist could be reasonably certain that approximately 9 in 10 patients who are prescribed vemurafenib actually harbour the BRAF p.V600X mutation in the tested tumour sample. Depending on the side effects of the therapy, this may be considered a respectably low risk level. Similarly, the NPV for this assay in malignant melanoma (99.5 %) should allow the oncologist to be confident that any patient who is refused therapy on the basis of a negative *BRAF* mutation test is highly unlikely to have been given a false negative result.

The opposite situation to malignant melanoma applies if *BRAF* mutation testing using this same assay is applied to pre-testing for cetuximab or panitumumab therapy in

mCRC; that is, a positive *BRAF* mutation result would contraindicate therapy. With an estimated prevalence of 8 % in mCRC tumours [4, 7, 13–15], *BRAF* mutations are approximately 7-fold less prevalent in mCRC than malignant melanoma. In this case, the clinical utility of the assay is noticeably altered; the PPV can be calculated to be 41.4 % and the NPV 99.9 % for the same assay used for a different condition. Thus, a clinician could only be confident that a positive result truly reflected a treatment-resistant tumour sample in approximately four out of every ten positive results. While, the NPV may give one reason to be confident that those given therapy are likely to be *BRAF* mutation negative, one could not reliably contraindicate therapy in the case of a positive result, which would render the assay far less useful if implemented in this context. So, the implementation of *BRAF* mutation testing in CRC would not be a simple case of using the same assay on a different tumour type. Likely, a new or re-optimised assay dedicated for use in CRC would be required.

4 Conclusions

While BRAF mutation status is but a single molecular target, the implications of blindly applying an assay to a novel clinical scenario are obvious. It is important that assays are selected and optimised based on clinical rather than purely analytical properties. To this end, calculation of positive and negative predictive value in the correct clinical context may eliminate misconceptions regarding the clinical applicability of an assay. A further point that can be inferred from the above discussion is that testing of populations with low mutation prevalence rates may increase the likelihood of false positive results; thus, judicious use of molecular testing is necessary to reduce this avoidable form of error.

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