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Screening for Cancer with Molecular Markers: Progress comes with Potential Problems

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Abstract

Recent research has raised hopes for impressively accurate screening for cancer with molecular biomarkers. These molecular markers will probably be more sensitive and specific than older screening modalities, as well easier to use. In this Perspective article, I argue that these sensitive screening tests may be clinically valuable – but will present unique issues in implementation and interpretation. These are likely to affect the way clinicians conduct screening and the way they make diagnoses in individuals who screen positive.

Introduction

Newer proteomic and genetic technologies have invigorated research into cancer screening, but the use of molecular biomarkers for early detection of cancer is not new. Testing stool for heme and measuring levels of serum prostate specific antigen (PSA) are established screening modalities for bowel and prostate cancer, respectively, each relying on the detection of particular molecular entities. Carcinoembryonic antigen (CEA) and cancer antigen 125 (CA-125) are other familiar molecular biomarkers that were found to be ineffective for screening, but are currently used for follow-up in patients with colorectal and ovarian cancer, respectively.[1–3]

In developed countries, the general public has high expectations for cancer screening that seemingly extend beyond the evidence for its efficacy.[4, 5] Potentially, screening with molecular biomarkers could help meet these expectations, but recent reports of striking accuracy for some molecular screens[6, 7] have not been confirmed.[8, 9] Nonetheless, there is considerable on-going research, and new molecular cancer markers seem likely to reach the clinic, such as DNA-based stool testing for colorectal cancer.[10] This Perspective considers the uses and interpretation of molecular biomarkers for detecting cancer as part of a screening process, taking advantage of the knowledge gained from more traditional screening tests and the experience to date with familiar molecular markers, such as PSA and stool heme testing.

Molecular and anatomical screening

Many established screening tests are explicitly designed to detect tumor masses, and in this sense are ‘anatomical’. This is clearly the case for virtually all radiographic screening (mammography and chest X-rays, for example), physical examination (breast and pelvic examinations) and endoscopy. To the extent that benign masses look like cancer, there are false positive anatomical screens. For example, lung cancer screening with computed tomography detects small nodules that are often benign remnants of infections or inflammatory processes, but that need to be followed-up to clarify whether they are actually

malignant or benign.[11] Other common screening tests (such as cervical PAP smears) are anatomical in the sense that they assess the microscopic anatomy of whole cells.

Molecular biomarkers, on the other hand are independent of the presence of a detectable tumor mass or even the detection of intact transformed cells. Instead, they represent detection at a distance, using molecular signals in blood or excretia to indicate the presence of a cancer or pre-invasive lesion. These molecular biomarkers fall into four groups (Table 1). Some are products of the neoplastic process that are shed by the tumors, such as mutated or hypermethylated DNA ('carcinogenesis markers'). Others are molecular species generated by the host response to the cancer ('response biomarkers'). Examples include antibodies, protein degradation products,[12] and acute phase reactants.[13] A third group of biomarkers, like blood in stool or PSA in serum, are released in abnormal amounts as a result of the anatomical or metabolic disruption associated with a tumor ('released biomarkers'). The final group of molecular markers comprises factors associated with or supporting the underlying carcinogenesis ('risk biomarkers'). Examples are high estradiol levels in relation to breast cancer or markers of human papilloma virus in relation to cervical cancer.

These classes of biomarkers will probably behave differently in early detection. Carcinogenesis markers are likely to be relatively specific for invasive or pre-invasive neoplasia, as they are essentially found only in on-going carcinogenesis. Testing for PSA or blood in stool has already shown that released biomarkers can be non-specific: pathology other than cancer often leads to their release into blood and stool respectively. Risk biomarkers are often abnormal in individuals without cancer; these are really risk factors, markers of cancer risk rather than markers of cancer itself. Typically only a minority of individuals with the risk factors actually develop the associated disease.

Thus, just as benign masses can mimic tumors or obscure cancers in anatomical screening, pathological and metabolic processes will affect the specificity of molecular screening, especially if it is not based on carcinogenesis markers. PSA provides many examples: hemodilution of PSA levels in obese men,[14, 15] distortion of levels by medication,[14] and increases in levels from prostatitis.[16] Inflammation – a risk factor for cancer in many organs -- may be a particular problem as it shares molecular mediators with carcinogenesis.[17, 18]

Validation of molecular tests including controls with various benign inflammatory and acute conditions will help avoid these potential problems. However, they may become recognized only as the screen is widely used outside the often-narrow populations tested in validation research. Consequently, phase 4 post-approval studies may be needed to fully understand some molecular-based screening tests. These studies will enable us to define the metabolic limitations of such a screening test, as radiologists have learned to deal with anatomical factors that impede a clear assessment of visualized masses.

Advantages of molecular screens

In formal terms, screening is "the examination of asymptomatic people in order to classify them as likely or unlikely to have the disease that is the object of screening."[19] In a subsequent, diagnostic step, individuals who screen positive are then assessed further to see whether or not they have the target disorder. A molecular screen in itself cannot diagnose cancer. That requires anatomical evidence: confirmation and histological characterization of the cancer and localization of the tumor in the involved organ. Successful molecular screens will serve as high-quality triage, separating patients who can forgo anatomical assessment from those who are more likely to harbor a tumor and need further anatomical investigation.

It is natural that the accuracy of molecular screening is the focus of discussion of early detection biomarkers as they are being developed, but these screening tests will probably bring advantages independent of their accuracy. One is that the tests are convenient and safe – typically requiring only the donation of blood, urine or stool. Measuring these molecular biomarkers does not involve tests that deliver radiation, a visit to the clinic, or unpleasant procedures as is needed for colonoscopy. Thus, the use of molecular biomarkers is likely to improve the uptake of screening by the general population and make repeated testing practical and affordable. This would increase the sensitivity of the screening process, and correspondingly increase the chances of detecting early cancers. However, it would also increase the potential for false positives, with the adverse downstream consequence of unnecessary diagnostic follow-ups.

Another advantage of molecular biomarkers is that they can easily be combined into panels using mathematical techniques such as logistic models or recursive partitioning to enhance sensitivity and specificity. Such combinations might be less susceptible to measurement artifacts than the individual markers. Also, the quantitative nature of many molecular markers means that they can potentially be personalized, using age, sex and race-specific norms, for example.[20]

Sensitivity: the yin and the yang of molecular screening

It is expected that molecular screens will be more sensitive and specific than existing molecular screens. High specificity will bring clear benefit and has no downside: false positive screening results will be minimized. Sensitivity is another story. While the ability to detect early cancers, perhaps even before there is much of a tumor mass present, is a defining characteristic of early cancer diagnosis with molecular biomarkers, it is also central to some potential problems.

Overdiagnosis

As molecular screening followed by early diagnosis becomes more sensitive, it will find malignancies that – even if untreated – would progress to clinically relevant cancers only slowly and perhaps not at all during a patient's natural lifespan.[21–23] PSA screening for prostate cancer is a now well-known example. In the Prostate Prevention Trial, 24% of biopsies were positive for prostate cancer in men randomized to placebo,[24] a far higher proportion than would have been diagnosed with clinical cancer during their remaining lives. The overdiagnosis associated with PSA screening led to a dramatic increase in the recorded incidence of prostate cancer in the U.S.[25] Most of the 'extra' cases would not have had any clinical consequence even if they had not been discovered and treated. Thyroid and breast tissue also frequently contain nests of what are likely to be 'pseudocancer'[26–28] that could be detected with sufficiently sensitive screening. Although the magnitude of overdiagnosis in breast cancer is controversial, the high estimates are above 50% for tumors detected by mammography.[29]

For any cancer with a subclinical reservoir of cases, one can anticipate an increase in the observed incidence after the institution of molecular screening, made up of both clinically relevant tumors and those that would never harm the patient if left untreated. As molecular cancer screening becomes more widely used, the need to separate truly aggressive lesions from those that are unlikely to be clinically troublesome will grow correspondingly.

Detected cancers that can't be found

A novel problem of molecular screening may well be the detection of minute invasive cancers that cannot be found by anatomical techniques, such as endoscopy and radiography (Box 1). Clinically, a patient with such a lesion would appear to have a false positive

molecular screen. Methylated gene markers for prostate cancer[30] have clearly illustrated this point. Cancers that cannot be located could be a serious issue for patients with a positive molecular screen in the bronchial tree, in Barrett's esophagus or in ulcerative colitis, where discrete lesions are often not evident on anatomical imaging. This problem is rare after an anatomical screen, as these locate a tumor mass that can be biopsied to ascertain the diagnosis.

The definitive treatment of most epithelial cancers is surgical removal. But it is impossible to treat a cancer surgically if it cannot be located. This need to locate lesions that have been 'detected' by screening means that the full potential of molecular screening might not be realized until it is coupled to enhanced imaging techniques such as fluorescence endoscopy and PET scanning.[31–34] However, 'mini-cancers' detected in this way may have a different behavior than their more familiar counterparts detected through current means. It is possible that some of these may not be clinically significant, even in organ sites not currently recognized as having a reservoir of asymptomatic cases.

A related problem may arise because different cancers share molecular features, creating cross-reactivity in carcinogenesis or response biomarkers. This was an issue for CEA, as it is expressed in several gastrointestinal cancers.[35] Individual newer molecular markers may suffer from the same problem. Methylated *APC* in serum has been found in lung cancer patients [36] as well as in some patients with esophageal cancer.[37] Mutated *KRAS* may be found in serum from patients with diverse cancer types.[38] Methylated RAS association domain family protein 2 (*RASSF2*) and secreted frizzled related protein 2 (*SFRP2*) have been seen in stool samples from patients with either stomach or colorectal cancer.[39] As long as the positivity is limited to a few cancer sites, the follow-up of a screen positive subject might be straightforward, but it will probably not be helpful to have a screen that is positive for cancer 'somewhere.' It should be possible to address this issue with multi-marker panels and careful validation of the early detection markers. Ideally, validation would include checking results in patients with other malignancies.

Other implications of molecular screening for cancer

Pre-malignant lesions

Since the molecular defects of early cancer are often similar to those of intraepithelial neoplasia,[40] molecular screening for cancers will likely identify substantial numbers of pre-invasive lesions. In organs such as the colorectum and cervix, these can be removed relatively easily to reduce risk of future cancer. Excision of pre-invasive lesions identified in less accessible tissues, such as the pancreas, entails considerable morbidity. It may not be clear what *should* be done to address the increased risk of invasive cancer, particularly as the natural history of these screen-detected lesions may not be well characterized. Increased surveillance presumably would be recommended, and conceivably chemoprevention, but the latter has not been shown to be effective outside the colorectum and breast.[41, 42] Such clinical dilemmas need to be considered before the introduction of molecular screens that may detect early disease for which there is no effective treatment.

Persistent markers

After a tumour has been resected, carcinogenesis biomakers and release biomarkers would presumably revert to normal and signal successful treatment. However, some risk biomarkers and some reaction biomarkers (such as antibodies) might remain in the abnormal range even after the responsible lesions are completely removed. For example, long-term hormonal patterns that promoted carcinogenesis presumably would continue, and some antibodies generated by a tumor may persist. Markers that do revert to normal after successful treatment could be used to follow disease recurrence and progression, as PSA (for

prostate cancer) CEA (for colorectal cancer) and CA-125 (for ovarian cancer) are used now. Here again, advanced anatomical detection may aid the molecular screen to locate recurrent neoplasia that is not otherwise evident. Ideally, the validation of molecular screening markers would include study of their behavior as markers of disease progression after excision of the tumors that are detected.

Conclusions

Because of its independence from tumor masses, screening for cancer with molecular biomarkers has different properties than anatomical screening. The tests are convenient and cheap in comparison to anatomical modalities, and more user-friendly. We can hope that molecular screens will be more accurate than older screens. However, the sensitivity of the screens has predictable implications, inevitably shifting our recognition of cancer toward the less severe end of the carcinogenesis spectrum. In some organs, it may even be a challenge to find the cancers indicated by molecular biomarkers. In any case, improved anatomical imaging will probably be needed to take full advantage of the more sensitive detection.

The consequences of the shift to the detection of very early cancers have been suggested by our experience with older molecular markers, such as PSA. Some of the cancers detected may not need to be treated, and being able to identify this subset will be an important issue for the clinical application of molecular screening. More generally, studies to document the cost-effectiveness of the screening will be needed, as will research into the proper treatment of the early lesions found. As for any new screening modality, the benefits of molecular screening will need to be weighed against the potential limitations.

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Box 1 Cancer that cannot be found

Molecular detection of cancers that cannot be found has already been documented. Hubert Humphrey, U.S. vice president (1964–1968), died from bladder cancer in 1978, having been diagnosed with microinvasive bladder cancer in 1973 and frankly invasive disease in 1976.[43] He had had hematuria since at least 1967. Using archived specimens, Hruban and colleagues demonstrated that urine cytology specimens from 1967 contained the same p53 mutation as the cancer diagnosed years later – and raised the prospect that earlier treatment might have been an option had molecular screening been available. However, biopsies in 1967 were given conflicting readings, and regular cystoscopies every six months apparently did not lead to a diagnosis until 6 years later. Would the molecular findings have led to a different course of action in 1967, in the absence of anatomic confirmation of the diagnosis?

Table 1

Modalities of Cancer Diagnosis and Screening

	Detects	**Examples**
Anatomical screen		
Macrosopic	Tumor masses	Endoscopy, radiology and physical examination
Microscopic	Abnormal cells	Cytology and histology
Molecular biomarker screen		
Carcinogenesis biomarkers	Abnormal molecular species generated by carcinogenesis	Mutated DNA and aberrantly methylated DNA
Released biomarkers	Physiological molecules released in abnormal amounts as a result of the anatomical or metabolic disruptions of carcinogenesis	PSA and stool heme
Response biomarkers	Molecular species generated in reaction to the presence of a tumor	Antibodies and protein degradation products
Risk biomarkers	Molecular species associated with, or supporting, carcinogenesis	Increased hormone levels and the presence of HPV antigens

HPV, human papilloma virus; PSA, prostate serum antigen;.