PERSPECTIVES

CANCER DETECTION - TURNING POINT

CANCER

Deploying blood-based cancer screening

AI-based risk assessment may enable personalized blood-based multicancer screening

By **Douglas S. Micalizzi[1,2], Lecia V. Sequist[2], Daniel A. Haber[1,2,3]**

The past 20 years have witnessed transformative advances in molecularly targeted and immunological treatments for advanced cancer, providing many patients with prolonged survival and quality of life. However, the main determinant of cure across diverse cancers remains the stage at diagnosis. Finding an invasive cancer while it is still localized and without clinically detectable metastatic spread provides the best chance at eradicating the primary tumor through surgery and/or radiation and killing any disseminated microscopic cells through therapeutic drugs. The recent development of blood-based multicancer detection (MCD) assays, together with advances in imaging and artificial intelligence (AI) algorithms, have the potential to transform early cancer detection. But these innovations are not without health and financial risk, and their increasing availability raises both opportunities and challenges, which are evident as clinics dedicated to early cancer detection are launched.

Take the case of a 55-year-old woman who has recently lost a close relative to cancer and is concerned about her own risk. Her family history does not fit a known cancer genetic susceptibility syndrome. She has a history of tobacco use, exercises routinely, maintains a normal body mass index, and drinks alcohol in moderation. She is up to date on current recommendations for cancer screening, including Pap smear, mammography, and colonoscopy. She decides to pay for a multicancer detection test (Galleri), which is currently available for purchase in the US but without US Food and Drug Administration (FDA) approval or insurance reimbursement. The test indicates a "cancer signal detected" with ovary as a top predicted tissue of origin, yet clinical work-up, including high-resolution imaging and the ovarian cancer antigen 125 (CA-125) blood marker, is negative. How should a patient who appears healthy but has a positive cancer signal on a blood test be counseled, and how common is such a scenario likely to be as MCD screening becomes increasingly available?

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Several MCD assays are at various stages of development, with the Galleri test from GRAIL being the most advanced in clinical studies, and in negotiations for approval by US and UK regulators (*1*). Galleri uses 40 ml of blood to extract free DNA in the plasma, a fraction of which may be derived from tumor cells if cancer is present (i.e., circulating tumor DNA, ctDNA). Given the large number of DNA methylation changes at CpG dinucleotides throughout the cancer genome, the test applies bisulfite sequencing to annotate over 100,000 genomic loci, using algorithms to identify a potential cancer signal and a likely tissue of origin, admixed with normal tissue-derived DNA in the blood. Other emerging blood-based cancer assays rely on the altered size distribution of cancer-derived ctDNA (DELFI) (*2*) or the presence of recurrent mutations and abnormal protein markers (CancerSEEK) (*3*). Beyond these and other ctDNA-derived assays, cancer-associated blood analytes include high-throughput proteomics, circulating tumor cells, exosomes, platelet-associated RNA, and circulating free RNA.

The argument for developing a single blood-based test to screen for multiple cancers, rather than a tumor type–specific test, is that shared molecular features of all cancers can be leveraged in this way, providing a "one test for all" clinical paradigm that could be readily implemented across asymptomatic populations. The caveat is that test performance and predictive power depend on the prevalence of the cancer under screening, and different cancers have distinct risk populations, as well as variable patterns in the time to progress from a single cell to an invasive cancer shedding ctDNA into the blood. A major unanswered question is whether the most lethal cancers that currently lack screening tests (e.g., pancreatic and ovarian cancers) exhibit a sufficient window of opportunity between plasma detectability and tumor metastasis to deploy curative surgery.

How effective are MCD screening tests at uncovering early-stage, potentially curable cancers? Initial studies (*1*) compared patients known to have different types of cancer with healthy individuals, reporting an overall sensitivity (correctly identifying a patient with cancer) for Galleri of 16.8% for stage I and 40.4% for stage II cancer, when the assay parameters were set at a threshold of 99.5% specificity (correctly identifying a patient without cancer). In the PATHFINDER trial, a population-based study of 6621 apparently healthy individuals over age 50, 1.4% had a positive cancer signal on Galleri testing; of these, cancer (of any stage) was ultimately confirmed in 38%, whereas 62% appeared to be false-positives. Such false signals may require costly imaging and invasive tests to rule out the presence of cancer and can cause unnecessary anxiety (*4*). Previously unsuspected stage I or stage II cancer was present in 14 of the 36 cases that were correctly identified by Galleri as having cancer, i.e., 0.2% of the initially screened population was discovered to have a potentially curable early-stage cancer. A major population-based trial is ongoing through the National Health Service (NHS) in the UK, involving randomization of 140,000 asymptomatic individuals between ages 50 and 75 to either standard clinical cancer screening protocols plus annual Galleri testing for 3 years, versus clinical screening alone. The primary end point for this study is earlier stage at cancer diagnosis within the MCD-tested cohort, rather than a reduction in overall cancer-related survival. This end point will deliver a more expedient trial readout but lacks the ability to assess for important confounders such as lead-time bias, when cancers are discovered earlier in their course owing to study intervention but not early enough to alter their curability.

Perhaps the most critical question regarding the implementation of MCD screening tests is whether they are best applied to all persons above a certain age, or whether advances in AI will enable more individualized risk-based screening strategies, thereby raising the baseline prevalence and hence predictive value of testing. Cancer risk increases by age, and in Western countries, the annual incidence is estimated to be 0.5% at age 50 and 1.5% at age 65. The positive predictive value (PPV) of a screening test, meaning the chance that a positive test result corre-

sponds to a true cancer case, combines assay-inherent specificity and sensitivity with the cancer prevalence in the tested population. Thus, a hypothetical test with 99% sensitivity at 99% specificity, when applied to a population with only a 1% cancer prevalence, will produce one false-positive result for every true-positive result (i.e., PPV 50%). However, if the cancer prevalence in the population rises to 5%, the PPV for the same test jumps to 84% (i.e., fewer false-positives).

Current-generation cancer risk calculators typically focus on a single cancer type [e.g., Tyrer-Cuzick for breast cancer; the prostate, lung, colorectal, and ovarian cancer screening trial (PLCO); and the colorectal cancer risk assessment tool (CCRAT)], and they use a limited number of static risk factors as input, generating validated risk predictions that can be used to select at-risk patients for classic cancer screening tests [e.g., mammogram, low-dose chest computed tomography (CT), and colonoscopy]. Similarly, there are well-established algorithms for cancer screening in individuals carrying highly penetrant inherited genetic mutations that confer susceptibility to melanoma, breast, ovarian, colon, renal, and endocrine cancers. There are, however, multiple risk modifiers that may only be accessible through complex algorithms. AI-driven approaches to cancer risk assessment may integrate traditional risk factors with new or harder-to-assess factors, including lower-penetrance genetic variants, diverse environmental exposures, and other health indicators. This approach was illustrated in a recent retrospective study using machine learning–based analysis of clinical records to predict risk at specific time intervals for pancreatic cancer (*5*), a tumor for which a validated risk calculator is not currently available. Additionally, radiology images of noncancerous tissue may now be analyzed to help predict an individual's future risk of breast or lung cancer, by using AI-powered techniques that are distinct from traditional clinical radiology assessments of current lesions (*6*, *7*). Thus, the evolution of individualized cancer risk assessment may enable more effective targeting of blood-based MCD screening to populations with an increased cancer prevalence, which would in turn improve PPV.

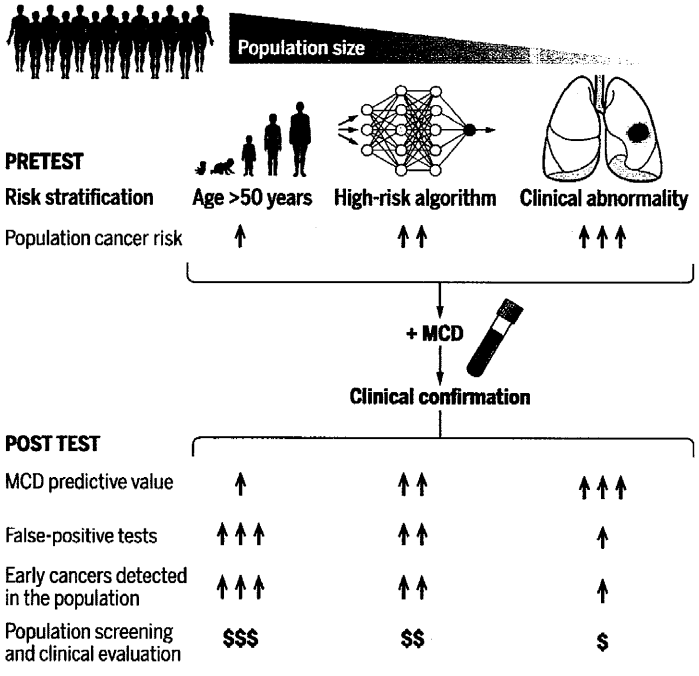
Beyond selection criteria for MCD testing, the clinical evaluation of patients with a blood-based cancer signal is critical to their deployment. For the Galleri test, DNA methylation patterns give an initial clue about the tissue of origin, providing a formula to begin clinical workup, but if this is unrevealing, the subsequent evaluation is unclear. Whole-body imaging [e.g., positron emission tomography (PET) scan and whole-body magnetic resonance imaging (MRI)] is a consideration, but it is fraught with poor sensitivity, incidental findings, and high cost. Notably, in the PATHFINDER study, 44 of the 90 patients with a positive Galleri test underwent an invasive diagnostic procedure to determine the presence or absence of cancer. GRAIL currently offers free repeat Galleri testing in 3 to 6 months if no cancer diagnosis is made after an initial positive test. It is also possible that routine application of orthogonal blood-based validation assays may play a role in reducing the fraction of false-positive results at initial screening. Such second-line assays could include high-sensitivity detection of cancer-associated DNA mutations or circulating tumor cells in the blood (*8*), or molecular probes coupled with high-sensitivity imaging analyses. However, without clinical confirmation, a positive cancer signal from a blood test represents either a false-positive result or a yet-undetectable malignancy that warrants ongoing vigilance, an unresolved dilemma that may be the source of profound anxiety.

There are additional clinical scenarios in which MCD testing may contribute to early cancer detection. Clinical medicine is replete with sophisticated imaging for diverse indications, increasingly yielding radiographic lesions of unknown significance. Examples include indeterminate lung nodules identified in 18% of individuals undergoing chest CT scoring of coronary calcium deposits for cardiac risk assessment (*9*) and incidentally discovered premalignant intraductal papillary mucinous neoplastic cysts in the pancreas of 10% of individuals over age 70 (*10*). Blood-based MCD tests might play a role in the evaluation of such incidental lesions, helping to assess the need for invasive biopsy or surgery. Additionally, MCD testing could be useful in individuals presenting with signs or symptoms that are consistent with but not diagnostic for cancer. Indeed, in such a high-risk population, a UK study of 5461 patients reported a PPV of 75% for the Galleri test among patients suspected of having cancer before a definitive clinical diagnosis (*11*) (see the figure).

As MCD-based cancer screening evolves, the history of cancer screening for prostate and lung cancers offers some distinct lessons about implementation. Age-based screening for prostate cancer using the blood protein marker prostate-specific antigen (PSA) is no longer routinely recommended after overambitious implementation in the 1990s highlighted its low PPV for invasive disease, to the extent that for every life saved by population-based PSA testing, another was lost through a biopsy or surgery-related complication (*12*). PSA testing for men aged 55 to 69 is currently left to the discretion of individual patients and their physicians (*13*). By contrast, in lung cancer, randomized controlled trials clearly demonstrated a 20% reduction in cancer mortality after low-dose chest CT screening among heavy smokers (*14*). Yet fewer than 10% of eligible patients undergo lung screening, owing to the lack of comprehensive implementation strategies, nihilism about lung cancer outcomes, and stigma about smoking (*15*). Furthermore, for both prostate and lung cancer screening, associated risk factors and availability

Multicancer screening tests according to risk

Population-based screening using age as the sole risk factor may have greatest benefit for the total number of early cancers detected in the population, but with a considerable number of false positives given the low disease prevalence, and at high cost. Risk stratification, potentially using AI-based risk calculators, may increase population prevalence, thereby improving positive predictive value (PPV) of the test. Applying multicancer detection (MCD) testing for evaluation of radiographic lesions of uncertain significance may be another relevant clinical application with high PPV.



GRAPHIC: K. HOLOSKI/*SCIENCE*

of sophisticated diagnostics are unequal across diverse communities in the US. Compared with white people, Black people suffer higher rates and worse outcomes for both prostate and lung cancers and, despite efforts to improve access, remain less likely to qualify for lung cancer screening (*15*). Just, equitable, and affordable deployment of cancer screening is a major concern that should be actively addressed in MCD test deployment. In this regard, cost-effectiveness analysis of MCD testing should be evaluated at all stages of implementation, including the downstream costs of clinical confirmation and their combination with standard screening approaches.

Most importantly, individual perception of personal risk for cancer is often difficult to quantify, but it underlies many patient preferences and decisions. MCD screening is not dissimilar from existing cancer screening tests in having imperfect sensitivity and a high false-positive rate. It differs perhaps in the public perception that molecular tests have a diagnostic level of certainty, whereas radiographic abnormalities tend to be understood as being preliminary until confirmed by definitive biopsy. Furthermore, organ-based cancer screening is more amenable to clinical confirmation than a multicancer signal in the blood, whose origin may elude immediate validation.

The role of MCD screening as a new tool within the spectrum of clinical care thus presents both an unprecedented opportunity and a major challenge. Coupled with such potent cancer detection technologies, the enhanced ability to objectively assess personalized cancer risk is probably the most important element in a rational cancer screening strategy, maximizing predictive power while minimizing unnecessary anxiety and medical workups. ■

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An ant–plant relationship is vital to a food web that includes the predation of zebra by lions in a Kenyan savanna.

ECOLOGY

A big-headed problem drives an ecological chain reaction

Disruption of key species interactions reverberates across an African savanna

By **Kaitlyn M. Gaynor**

Human activity is driving the rapid loss of global biodiversity, through declines in individual species and the wholesale destruction of ecosystems (*1*). This loss can arise from myriad forms of anthropogenic disturbance that include land conversion, hunting, pollution, resource extraction, and climate change (*2*). Although it is often straightforward to document the direct effects of disturbance on species and habitats, these impacts can ripple throughout food webs by altering interactions among species. These indirect effects may have far-reaching consequences that are not immediately apparent, but could fundamentally alter ecosystems. On page 433 of this issue, Kamaru *et al.* (*3*) describe how one disturbance—the introduction of an invasive species—disrupted an interaction between trees and ants, and traced its consequences through an African savanna landscape.

Species interactions are essential to the functioning of healthy ecosystems. Regardless of whether they benefit both species (mutualism), one species (predation), or neither (competition), species interactions can stabilize the composition of communities and the state of an ecosystem. Some interactions play a particularly outsized role in maintaining ecological dynamics by shaping the physical environment, cycling nutrients or energy, or limiting the populations of other species. These interactions may involve numerically abundant species (foundational interactions) or rare but important species (keystone interactions) (*4*). Given their central role, the disruption of such interactions by human disturbance can have reverberating and transformative ecological effects.

Humans have been characterized as a higher-order hyperkeystone species, given that human activities can radically alter interaction chains (*5*). However, it is often difficult to disentangle the pathways linking the fate of one species to another as disturbance cascades throughout complex ecosystems, even if these pathways involve foundational or keystone interactions. When an ecosystem is confronted with multiple anthropogenic pressures that have differential effects across species, it can be nearly impossible to attribute an observed system-wide change to a particular link in the chain. Studies often

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