Original Article

Gene Expression Analysis and Immunohistochemistry in Evaluation of Cancer of Unknown Primary: Time for a Patient-Centered Approach

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Key Words

Gene expression analysis, immunohistochemistry, STEEEP principles

Abstract

Molecular medicine is rapidly changing the diagnosis and management of cancer of unknown primary. The science, business, and economics of the genomic revolution have moved at such a pace that coordinating practical application of all available tools, such as gene expression analysis and immunohistochemistry, often seems to clash. In fact, very little work has been done to actively coordinate use of these techniques, each of which can be very resource-intensive. The Institute of Medicine proposed the STEEEP principles, a basic set of guidelines that maintain that the best patient care is safe, timely, effective, efficient, equitable, and patient-centered. Application of these principles will help lead to a better understanding of the most appropriate use of modern diagnostic modalities. (*JNCCN* 2011;9:1415–1420)

Molecular medicine is a rapidly emerging feature in the diagnosis and treatment of disease. Enormous potential benefits for patients are developing, some of which will be truly transformational. Along the way, however, troubling questions must be answered. How will one know when to use newer strategies, and when are the older strategies "good enough"? How will the more expensive diagnostics and therapeutics be paid for in a reimbursement environment that already views medical care as too expensive? How can the legitimate business needs of developers of tests and treatments to make a profit in a capitalist environment be balanced against the legitimate, sometimes conflicting, needs of diagnosticians/clinicians to make a living? In a medical care delivery system that is largely unplanned and disjointed, how can the contributions of scientists, business people, and physicians be coordinated to produce the best possible patient outcomes?

The narrowly focused problem of diagnostic strategies in the workup of cancer of unknown primary (CUP) provides an ideal test case to examine some of these issues. Considerable tension exists between those who champion the use of older immunohistochemistry-based diagnostic strategies and those who promote newer approaches based on tumor gene expression analysis. Many unanswered questions exist regarding the strengths and weaknesses of each approach. A third approach lurks in the background, that of diagnostic nihilism—the idea that CUP remains a grim diagnosis in most cases despite the best therapeutic efforts, so that the quest to determine the site of origin in CUP is largely moot and of only academic interest, and therefore, logically, is not worth pursuing.

When faced with complex dilemmas in the care of patients, it is easy to become confused by distracting issues which, although possibly valid in themselves, can merge into a disorienting confluence. In those situations, it is always useful to step back and simplify. Sweep away all other issues and simply ask, "What is best for the patient?" Even that simple question is complex in itself, but at least it recognizes the primacy of the pa-

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tient against all other competing interests and personal points of view. The Institute of Medicine has advanced the STEEEP principles.[1] These attributes are defined as care that is:

- Safe: avoiding injuries to patients from the care that is intended to help them;
- Timely: reducing waits and sometimes harmful delays for both those who receive and those who give care;
- Effective: providing services based on scientific knowledge to all who could benefit and refraining from providing services to those not likely to benefit (avoiding underuse and overuse, respectively);
- Efficient: avoiding waste, including waste of equipment, supplies, ideas, and energy;
- Equitable: providing care that does not vary in quality because of personal characteristics such as gender, ethnicity, geographic location, and socioeconomic status; and
- Patient-centered: providing care that is respectful of and responsive to individual patient preferences, needs, and values and ensuring that patient values guide all clinical decisions.

These principles serve as an excellent template for examining the use of immunohistochemistry and gene expression analysis in the quest to define the primary site of cancer.

Defining the Problem

CUP, a clearly metastatic malignancy in which the primary neoplasm is unknown, remains a vexing clinical problem. Published statistics indicate that in the United States, approximately 2.3% of patients with cancer have a neoplasm that may be defined as CUP,[2] whereas worldwide incidence ranges from 2.3% to 4.2%.[3] A deeper analysis of how a neoplasm becomes defined as a CUP casts a clearer light on how ultimately to use diagnostic modalities such as immunohistochemistry and gene expression analysis. The ultimate usefulness of these modalities can only best be realized when considering all of the information surrounding the patient and the clinical case, including clinical history, physical findings, and radiographic studies.

From a purely pathologic point of view, all metastatic neoplasms are initially, if only briefly, definable as CUP. A pathologist, working at her microscope, is often isolated from comprehensive clinical information available to the oncologist or surgeon. Nevertheless, while examining a routine colectomy specimen from a patient with colon cancer, if she discovers metastatic mucinous adenocarcinoma when evaluating the associated lymph nodes, she will quickly and efficiently compare the histologic appearance of the primary. She almost always will conclude that the metastasis is attributable to the colon primary, thus removing it from the CUP category without even thinking about it. Is it theoretically possible that this metastasis arose from an occult neoplasm elsewhere? Yes, but no one would suggest that the pathologist embark on an expensive and time-consuming immunohistochemistry or gene expression evaluation to disprove that very remote possibility.

Another pathologist, asked by his neurosurgical colleague to evaluate an intraoperative frozen section of a large isolated brain mass, is told that the overwhelming clinical suspicion is of a glioblastoma multiforme. Under the microscope, however, the lesion is a metastatic adenocarcinoma—clearly at that point a CUP. Further examination of the clinical history reveals that the patient had a pulmonary lobectomy for lung adenocarcinoma at another institution 3 years previously. Does this CUP require an immunohistochemistry or a gene expression workup? Very likely it does not. All that is needed is a review of the slides from the lung cancer material for comparison with the metastatic material and, if they are similar, the case will no longer be considered CUP.

What is the patient-centered approach in these cases? First and foremost, without ever using immunohistochemistry or gene expression at all, patients require their cancer care team to collaborate and communicate in real time as physicians working together from a comprehensive history and physical examination to reach reasonable diagnostic conclusions; failure to do so is not safe, efficient, or effective.

If after careful clinical evaluation a patient's metastatic neoplasm truly does not yield a reasonable suggested primary, immunohistochemistry and gene expression analysis are both sensible diagnostic tools to consider. As with any tool, however, neither should be used indiscriminately or without reason. Before either tool is considered, a likelihood analysis of pretest probabilities should be performed.[4]

In this arena, as in all of clinical medicine, a shotgun approach to diagnosis is seldom effective. For example, an axillary metastasis in an adult woman is overwhelmingly likely to be of breast origin. If a small panel of breast immunohistochemistry markers does not yield a confirmatory result, perhaps moving on to a molecular tool is best, rather than engaging in a wide-ranging immunohistochemistry fishing expedition.

In a different example, if a patient has a small metastatic deposit of poorly differentiated carcinoma in a neck lymph node and a negative clinical workup of the upper airway, using the small amount of available tissue for a molecular workup may be more advisable, rather than performing any immunohistochemical stains at all. In an extreme example, an elderly patient presenting with advanced carcinomatosis probably should not have immunohistochemistry or a gene expression workup performed to determine the site of origin of the neoplasm. Ample clinical evidence exists to allow patients with CUP to be categorized into favorable (Table 1) and unfavorable (Table 2) categories[3]; clearly this evidence should be considered when use of expensive diagnostic tools is contemplated. Patient-centered care requires that physicians be thoughtful and treat each case, with its unique characteristics, as if it were one of a kind. Algorithms and pathways are useful guides, and irrational variability is wasteful, but "cookbook" medicine will never deliver the level of care that patients deserve.

Immunohistochemistry has been available for diagnostic use for decades; a literature search on the topic produces thousands of citations, and hundreds of immunohistochemical stains are available for routine clinical use. A few algorithmic or systematic approaches for the use of immunohistochemistry in the evaluation of CUP have been suggested[5,6]; however, all of these have at least 3 problems. First, essentially no clinical data confirm the effectiveness of immunohistochemistry in the management and outcomes of patients with CUP. Second, any algorithmic approach can only be a suggested guide to a given pathologist working on a given case. The decision to order one stain or another remains highly subjective and variable from pathologist to pathologist and case to case in most situations. Finally, in neoplasms that are poorly differentiated, experience shows that the usefulness of immunohistochemistry diminishes; in other words, when one needs immunohistochemistry the most, it is often of the least use. However, one study showed that patients who had CUP with a colon cancer immunohistochemical profile derived significant therapeutic benefits from specific therapies developed for colon cancer.[7] All of that having been said, the widespread clinical availability of immunohistochemistry and the very extensive arsenal of available stains make it an attractive alternative for evaluating cases of CUP in which determination of the tissue of origin is important.

Gene expression analysis, however, is a much more recently developed tool and is only available clinically through commercial specialty laboratories in the United States. A recent report thoroughly outlined and compared the characteristics of all currently available systems.[8] However, only the 3 systems clinically available in the United States are discussed here: the Pathwork Tissue of Origin Test (Pathwork Diagnostics, Sunnyvale, CA), CancerTYPE ID (bioTheranostics, San Diego, CA), and miRview mets[2] (Rosetta Genomics, Philadelphia, PA). An exhaustive scientific comparison of clini-

Table 1 Clinical Presentations of Cancers of Unknown Primary Having a More Favorable Prognosis

- Poorly differentiated carcinoma with midline distribution (extragonadal germ cell syndrome)
- Women with papillary adenocarcinoma of the peritoneal cavity
- Women with adenocarcinoma involving only axillary lymph nodes
- Squamous cell carcinoma involving cervical lymph nodes
- Isolated inguinal adenopathy (squamous carcinoma)
- Poorly differentiated neuroendocrine carcinomas
- Men with blastic bone metastases and elevated prostate-specific antigen (adenocarcinoma)
- Patients with a single, small, potentially resectable tumor

Table 2 Clinical Presentations of Cancers of Unknown Primary Having a Less Favorable Prognosis

- Adenocarcinoma metastatic to the liver or other organs
- Nonpapillary malignant ascites (adenocarcinoma)
- Multiple cerebral metastases (adenocarcinoma or squamous carcinoma)
- Multiple lung/pleural metastases (adenocarcinoma)
- Multiple metastatic bone disease (adenocarcinoma)

cally available systems is beyond the scope of this article, and a search of the literature fails to reveal any published comprehensive comparison of their performance. Table 3 outlines some comparative characteristics, most of which are taken from each company's marketing materials. Weaknesses in each assay are suggested: the Pathwork Tissue of Origin Test does not evaluate for neuroendocrine carcinoma, which represents a significant omission given the potential for neuroendocrine neoplasms to present as a CUP; CancerTYPE ID includes several primary sites that have little or no relevance as originators of CUP; and miRview mets[2] also determines some irrelevant primary sites and is unable to discriminate metastatic prostatic carcinoma. Clinical evaluation studies have been published for each system,[9–14] and company-sponsored studies of clinical efficacy have been published for the Pathwork Tissue of Origin Test,[15–18] CancerTYPE ID,[19–21] and miRview mets[2].[22] No currently reported study directly compares actual clinical performance characteristics of these tests. More importantly, published efficacy reports on each of these systems have been largely anecdotal and retrospective. Finally, and most important to the question at hand, no studies of the interface between the use of immunohistochemistry and gene expression tests in the diagnosis of CUP have been published; it is highly unlikely that the ultimate answer to the question of which of these strategies should be used will suggest only immunohistochemistry or gene expression. Both approaches may yield valuable information, but without performance data, deciding which to use when will be difficult. A patient-centered approach should ignore marketing strategies and financial imperatives for any testing system, whether new or previously established; independent evidence of efficacy and efficiency will be key.

Pathway to Patient-Centered Care in CUP Laboratory Diagnosis

What do patients with CUP need from oncologists, surgeons, radiologists, radiotherapists, pathologists, nurses, pharmacists, scientists, administrators, and all the other myriad of professionals who impact their lives? Most of all, they need answers to their questions. What is it that

Table 3 Comparison of Gene Expression Tests Currently Available in the United States for Analysis of Cancer of Unknown Primary

	Pathwork Tissue of Origin Test	bioTheranostics CancerTYPE ID	Rosetta miRview mets[2]
Clinically available	Yes	Yes	Yes
Turnaround time	7–11 d	5 d	10 d
FDA-cleared	Yes	Yes	No (available as a "laboratory-developed test")
Analytical platform	2000-gene microarray	92-gene quantitative RT-PCR	48 microRNAs by quantitative RT-PCR
Sensitivity	89%	85%	85%–90%
Specificity	99%	> 99%	99%
Tissue type	FFPE	FFPE, FNA	FFPE
Decalcified bone	No	Yes	Yes
Tissue requirements	Three to four 10-mcg unstained slides and one H&E slide	Three to four 7-mcg unstained slides and one H&E slide; whole FFPE block preferred	Twelve 5-mcg or six 10-mcg unstained slides; paraffin cuttings; paraffin block
Origin sites identified	15 (includes 58 tumor subtypes, not specifically diagnosed)	30 (includes 54 tumor subtypes, specifically diagnosed)	26 (includes 43 tumor subtypes, specifically diagnosed)
Weaknesses	Does not include neuroendocrine carcinomas	Includes categories such as "brain," meningioma, squamous and basal cell carcinoma of skin, thymus	Includes astrocytoma, oligodendroglioma, thymus, skin squamous; does not identify prostate metastasis reliably

Abbreviations: FFPE, formalin-fixed paraffin-embedded; FNA, fine-needle aspiration; H&E, hematoxylin-eosin; RT-PCR, reverse-transcription polymerase chain reaction.

I have? Why and how is it different from other cancers? How can it be treated? How good are the treatments? What are the side effects? How long will I be able to enjoy living? How long will I live? They need answers to the questions that are known, and they need to realize what is not known. Evaluation of most of these questions is far beyond the scope of this article; however, through examining each of the STEEEP principles as they impact the challenges in laboratory diagnosis of CUP, valuable insight may be gained.

Safety

Safe care may be defined simply as not harming the patient. In the context of CUP diagnosis, however, the definition is not so simple. Should a goal of absolute genomic and proteomic perfection be pursued in laboratory testing, to the exclusion of the use of common sense and good stewardship of resources? Absolutely not. Targeted strategies must be developed for integrating all testing modalities, to be used only as necessary to arrive at a diagnosis that is useful for treating the patient, given the best current clinical information. In some patients, that will mean using neither immunohistochemistry nor gene expression studies, because, given the particular clinical circumstance, no clinically useful information is likely to result. Patients can be harmed beyond just physical damage from a missed diagnosis. Expensive resources are often used carelessly and with little potential for benefit, costing patients and their families thousands of dollars with very little potential benefit.

Timeliness

In the usual sense, *timeliness* in medical care generally refers to not making patients or their caregivers wait inappropriately for care. Certainly the tissue workup of a patient with a difficult-to-diagnose CUP may take many days or even weeks, especially if gene expression studies are only undertaken after immunohistochemistry avenues have been exhausted. Again, a coordinated and thoughtful diagnostic approach can limit this delay. It may be wiser, for example, to limit or completely eliminate use of immunohistochemistry when very limited diagnostic material is available and when gene expression studies may be likely to yield more clinically useful information. Timeliness also has a more overarching meaning in the discussion of the CUP, in which protein expression (immunohistochemistry), gene expression, and molecular-based oncology therapies all converge. All aspects of molecular medicine are advancing rapidly, with new and important information available almost daily. Patients need their practitioners to follow these advancements and be able to modify their approaches rapidly and in a timely manner; specifically, the right workup of a particular subset of CUP today may not be correct tomorrow, depending on advances in immunohistochemistry, gene expression analysis, or therapy.

Effectiveness

The root of effectiveness in medical care is the concept of doing the right thing for the right patient in the right way—that is, getting the right answer, based on the best science available. This is the area in which the question of molecular assays and immunohistochemistry in the diagnosis of CUP falls farthest short. Not a lot of science is published about when, how, and why to use each technique, either separately or in a coordinated way. Several reasons exist for this: nonindustry funding is not really available to answer practical clinical questions, such as when to use immunohistochemistry alone or in concert with molecular assays; in fact, little is known about how pathologists choose a series of stains to workup a puzzling or difficult case. In this environment, it is not surprising that the proponents of immunohistochemistry perform their industry-based studies, and the proponents of molecular assays perform likewise, and nothing is known about the interface between the two. Given the huge amount of money spent on diagnostics and therapeutics in oncology and, more importantly, the high personal stakes that may be involved for patients, practitioners can and must do better.

Efficiency

Efficiency has much more to do with appropriate use of resources; no more is known about resource use than is known about effectiveness in the debate regarding immunohistochemistry versus molecular assays. An immunohistochemistry workup in a CUP case may cost thousands of dollars, and a molecular workup always costs thousands of dollars. When is one more appropriate? In what clinical settings is it most efficient to skip an immunohistochemistry workup altogether and go straight to molecular assay? In what clinical settings are neither indicated? These and many other similar questions are never going to be answered without well-thought-out collaborative investigations and discussions. Practitioners should not and must not engage in cookbook automatism in which they develop an entirely rule-

based approach to the workup of CUP, but frankly much of what is now known is entirely disorganized, wasteful, and therefore inappropriate.

Equitability

As is obvious, cancer diagnosis and treatment are expensive undertakings; they are becoming more so as advances in diagnosis and therapy continue. Equitability, the assurance that comparable care is available across all strata of society, is perhaps the most difficult of the STEEEP principles to attain in the fragmented and often bewildering health care system. A comprehensive review of health care economics and how they affect the disadvantaged is far beyond the range of this article; however, one comment can be made. If effective and efficient strategies are developed for the diagnostic workup of CUP, less money will be wasted in that process, and the hope is that more resources will be available to provide equitable services to patients who cannot provide for themselves. However, that may go against the commercial grain of those who develop and market molecular and immunohistochemistry tests, and it may go against the economic interests of pathologists who (like most physicians) are generally paid more for doing more tests. Providers can all take a higher road for all of their patients.

Patient-Centeredness

As should now be obvious, all of the attributes of the STEEEP principles are wrapped up in the unifying concept of patient-centeredness. To derive a patient-centered approach, providers have much to do in furthering the understanding of the workup of patients with CUP. Testing strategies have been developed in a vacuum, and sometimes without any focus at all. Increasing focus on the actual applicability of test results to specific patients, accompanying a more global and coordinated approach based on sound research and enrichment of knowledge in this interesting and difficult area of oncologic diagnostics, is greatly needed. This will take hard work, abandonment of short-term financial self-interests, and collaboration, but it can be done.

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