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Issues and updates: evaluating estrogen receptor-α, progesterone receptor, and HER2 in breast cancer

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There are currently three prognostic/predictive biomarkers used in routine clinical management of patients with breast cancer, and their assessment is mandatory. They include estrogen receptor-alpha (ERα), progesterone receptor (PgR), and the HER2 oncogene/oncoprotein. This paper briefly reviews the assessment of ERα, PgR, and HER2 in breast cancer, emphasizing recent progress and persistent controversies.
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Immunohistochemistry (IHC) has an important role in the assessment of prognostic and predictive factors in invasive breast cancer (IBC) today. Prognostic factors are defined as clinical, pathological, and biological features associated with the innate aggressiveness of untreated IBCs and, if adverse enough, usually result in the use of additional (ie, adjuvant) therapies following surgery. Predictive factors, in contrast, are defined as features that predict the likelihood of responding to specific types of adjuvant therapies. Many features have both prognostic and predictive significance to varying degrees. Although a large number of potentially useful factors have been identified,[1–4] only three are currently used in routine clinical practice and their assessment is mandatory. These include the estrogen receptor-alpha (ERα), the progesterone receptor (PgR), and the HER2 oncogene/oncoprotein. IHC is the most commonly used method of assessing these factors, although fluorescent *in situ* hybridization (FISH) also has a prominent role in HER2 testing.[5–8] This presentation briefly reviews the assessment of these biomarkers in breast cancer, with special emphasis on standardization, validation, and other issues of importance during the past 5 years (such as new clinical applications, testing error rates, testing guidelines, and new methodologies). HER2 is further along than hormone receptors on many of these issues, and will be discussed first.

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HER2 oncogene/oncoprotein

HER2 (also referred to as *HER2/neu* and *erbB2*) is a proto-oncogene located on chromosome 17.[9] It encodes a tyrosine-kinase receptor residing on the surface membrane of breast epithelial cells.[10] HER2 forms complexes with similar proteins (such as *erbB1, erbB3,* and *erbB4*), which act as receptors for several ligands (such as epidermal growth factor, heregulin, and amphiregulin), which regulate many normal cellular functions, including proliferation, survival, and apoptosis.[11–13] Many studies during the past 25 years have shown that the HER2 gene is amplified in up to 30% IBCs, and that amplification is highly correlated with overexpression of the protein.[11,12] The rate is closer to 15% today, which is probably because of screening mammography detecting early-stage tumors before amplification has occurred.

The relationship between HER2 status and clinical outcome is complex, and varies with the setting. There is a weak but significant association between poor outcome and 'positive' (ie, amplified and/or overexpressed) HER2 in patients receiving no additional therapy after initial surgery, which represent a small minority of patients today.[14,15] Most patients receive some type of adjuvant therapy, and the association between HER2 status and outcome seems to depend on the type of therapy.[14–22] For

example, many studies suggest that HER2-positive IBCs are resistant to certain types of cytotoxic chemotherapies (eg, the combination of cytoxan-methotrexate-5-fluoracil) but sensitive to others (eg, anthracyclines and taxanes). Other studies suggest that positive HER2 status may be associated with resistance to hormonal therapies, although not all agree and this issue remains somewhat controversial.[21,23] The most promising and useful findings come from recent studies showing that HER2-positive tumors respond favorably to new antibody-based therapies, which specifically target the HER-2 protein, such as trastuzumab,[22,24] and the main reason for assessing HER-2 status today is to identify candidates for targeted therapy. Although trastuzumab was originally demonstrated as being effective in HER2-positive metastatic disease, more recent clinical trials have demonstrated significant benefit as adjuvant therapy for women with less advanced HER2-positive breast cancer.[25–28] For example, the NSABP-B31 clinical trial, which randomized patients with HER2-positive breast cancer to adjuvant chemotherapy ± trastuzumab, showed a 52% improvement in disease-free survival with trastuzumab, which is remarkable.

There has been a long and persistent controversy about whether it is best to evaluate HER2 status by measuring protein expression by IHC or gene amplification by FISH. Although there are vocal proponents of both methods, many studies have shown that, when properly performed, there is a very strong correlation between IHC and FISH,[8,15,29,30] and that they are equivalent (and sometimes complimentary) in clinical utility.

Approximately 70% of breast cancers show little or no protein expression, a normal gene copy number, and do not respond to trastuzumab. Another roughly 15% show low-to-intermediate levels of protein expression, the gene is amplified (usually at low levels) in about a third of these cases, and there is still uncertainty regarding how well this group responds. The remaining 15% of cases show very strong membrane staining, indicating high levels of protein expression, the gene is almost always amplified in these tumors, and they show the best response in any setting to trastuzumab, as well as newer and more effective therapies targeting HER2.[16,31]

A particularly notable recent issue regarding HER2 testing is the joint publication of guidelines for HER2 testing by the American Society of Clinical Oncologists (ASCO) and College of American Pathologists (CAP).[8] They were developed to improve substantial inaccuracies in HER2, which were revealed primarily in association with large clinical trials in which results from laboratories of enrolling institutions were compared with testing by expert central laboratories. They consisted of false-negative and false-positive IHC results up to 20%,[32,33] and false-positive FISH results up to 15%,[33] which are all unacceptable. Although the guidelines were implemented only 2 years ago, studies are beginning to show that they have resulted in substantial improvement of testing accuracy.[34] Figure 1 highlights the history, assays, clinical utility, problems, and solutions represented by the ASCO/CAP testing guidelines for HER2 testing.

Estrogen receptor-α

ERα is as a nuclear transcription factor activated by estrogen to regulate growth and differentiation of normal breast epithelial cells.[35–37] These pathways remain operative to varying degrees in IBCs, including estrogen-stimulated growth of tumor cells expressing ERα, which is detrimental.[36–38] ERα expression has been measured in IBCs for almost 40 years. During the first 20–25 years, it was measured by radiolabeled biochemical ligand (ie, estrogen)-binding assays (LBAs) on whole tissue extracts prepared from fresh-frozen tumor samples, which was costly and difficult. Many studies using LBAs in large randomized clinical trials showed that ERα was a relatively weak prognostic factor but a very strong predictive factor for response to hormonal therapies, such as tamoxifen.[38] Tamoxifen, which binds ERα and blocks estrogen-stimulated growth, has been shown to significantly reduce disease recurrence and prolong life in patients with ERα-positive IBCs.[38,39] The clinical response to newer types of hormonal therapies, such as the aromatase inhibitors, which suppress the production of estrogen, is also dependent on the status of ERα, and only positive tumors benefit.[40–42] The primary reason for assessing ERα is its ability to predict response to these hormonal therapies.

Although the clinical utility of assessing ERα was initially based almost entirely on studies using technically standardized LBAs, beginning in the early 1990s, laboratories around the world abandoned LBAs in favor of IHC, which is used for nearly all testing today.

There are advantages to using IHC over LBAs, especially its ability to measure ERα on routine formalin-fixed paraffin-embedded samples, eliminating the need for fresh-frozen samples and the onerous infrastructure required to provide it. Other advantages include lower cost, better safety, as well as superior sensitivity and specificity in the sense that the assessment of ERα is restricted to tumor cells under direct microscopic visualization, independent of tumor cellularity or the presence of benign epithelium, which is problematic for LBAs. For all these reasons and more, IHC was approved by the CAP and ASCO for routine clinical use.[5,6] However, despite these approvals, there are significant problems with IHC that persist today, including the widespread use of diverse staining procedures of unequal quality and varied often arbitrary methods of interpreting results, resulting in error rates as high as 20% overall (primarily false negatives).[43–49] There

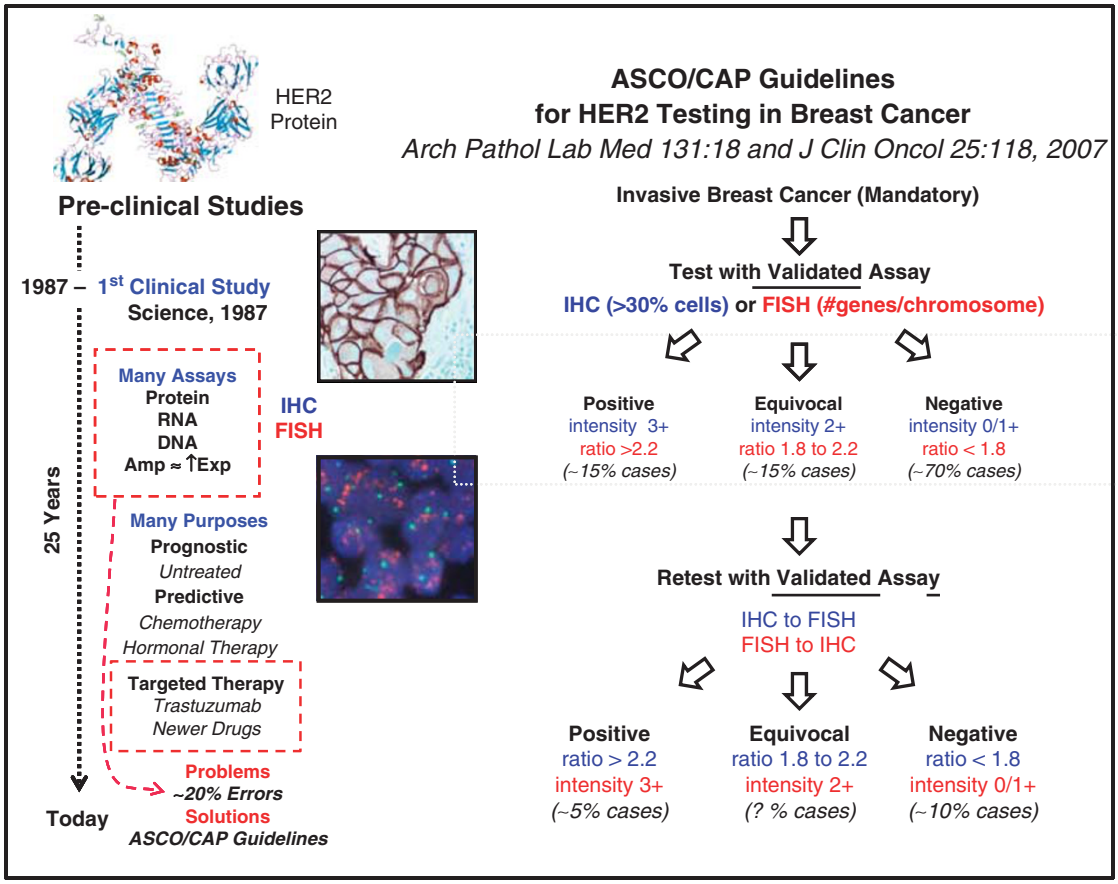

Figure 1 Overview of the history, assays, and clinical utility of HER2 in breast cancer, as well as problems with testing accuracy and solutions provided by the ASCO/CAP testing guidelines for HER2 testing.

are currently no widely accepted solutions to these problems, but most can be avoided by following general guidelines which have been published for assessing prognostic and predictive factors[6,50,51] These guidelines all agree that tests used in routine clinical practice should be based on sensitive and specific reagents, standardized laboratory procedures and, especially, calibrated to relevant clinical outcome in a comprehensive manner.

There are arguably no tests for any prognostic or predictive biomarkers in breast or any other types of cancers, which entirely satisfy these guidelines, but several strategies have been published for assessing ERα by IHC which come very close.[41,52–58] Collectively, these studies show that ~75% of IBCs express ERα, that it is almost entirely nuclear in location, and that there is tremendous variation between ERα-expressing tumors on a continuum ranging from 0 to nearly 100% positive cells.[59] More importantly, they show a direct correlation between the likelihood of clinical response to hormonal therapies and the level of ERα expression.[53] Although there is a gradient of increasing response with increasing levels of ERα, the gradient is skewed such that tumors expressing even very low levels (eg, between 1 and 10% positive cells) show a significant benefit far above that of ERα-negative tumors, which are essentially unresponsive. This evidence provides support for laboratories adopting ≥1% positive staining for tumor cells as the definition of 'ERα-positive' clinically and setting the threshold higher may deny hormonal therapy to some patients who might benefit, and a 1% cutoff has now been clinically validated in several comprehensive studies.[41,52–58] In head-to-head comparisons, many studies have also shown that assessing ERα by IHC provides equivalent or even stronger correlations with response to hormonal therapy than LBAs,[53,60] which is comforting as IHC replaced LBA before such proof was available.

A few recent studies have suggested that the distribution of ERα assessed by IHC in IBC is essentially bimodal (either entirely negative or strongly positive), leading the authors to recommend reporting results as either positive or negative without further quantification.[61,62] However, the studies reporting bimodal ERα are not an accurate representation of the true biological continuum of expression, and may be too sensitive, resulting in saturated signals.[63] It is important to provide quantitative ERα results for many reasons. Foremost among them, most patients want to know their

predicted outcome as precisely as possible and their physicians use quantitative information in making therapeutic decisions. For example, recent results from clinical trials suggest that most postmenopausal patients with tumors expressing very high levels of ERα can be optimally treated with adjuvant hormonal therapy alone, and can safely forego the rigors of chemotherapy, which is an important recent improvement in medical care.[64,65]

Assessing ERα by IHC may also be useful in patients with ductal carcinoma *in situ* (DCIS). Results from a large randomized clinical trial (NSABP-B24) showed that, in patients with DCIS managed by lumpectomy and postoperative radiation, the use of tamoxifen resulted in an additional 50% relative reduction in local recurrence in ERα-positive disease, and assessing ERα by IHC in DCIS is now routine in many centers.[66,67]

The most notable current issue related to ERα testing by IHC is the soon-to-be published guidelines by ASCO/CAP to improve accuracy.[58] These guidelines are conceptually modeled after the recently published guidelines for HER2 testing by ASCO/CAP,[8] which have already shown a positive impact on quality.[34] Hopefully, the new guidelines for ERα (and PgR) testing by IHC will be as helpful, and following them will be mandatory for laboratories conducting the tests under CAP certification. Figure 2 outlines essential general elements of accurate testing for ERα (and PgR) in breast cancer by IHC.

Another outcome partially motivated by problematic IHC testing is the development and ongoing validation of newer technologies to assess ERα and other clinically relevant gene products simultaneously, including qRT-PCR (eg, OncotypeDX)[46,68] and gene-expression microarrays.[69] Eventually, these multigene prognostic and predictive signatures will replace ERα testing by IHC, because the response to hormonal therapies is biologically too complex to be accurately predicted by measuring a single gene, regardless of how it is performed. However, these new tests are still being validated and are not mature enough to be used in routine clinical practice; therefore, testing for ERα by IHC will be with us for a while longer (perhaps a decade).

Progesterone receptor

PgR is also routinely assessed by IHC in IBCs. ERα regulates the expression of PgR; hence, the presence of PgR usually indicates that the estrogen-ERα pathway is intact and functional.[35,38,70,71] Once expressed, PgR is activated by the hormone progesterone to help regulate several important normal cellular functions, including proliferation which, of course, is detrimental in breast cancers.[35,38,70,71] Most of the discussion above regarding the historical assessment of ERα in IBCs also applies to PgR. It was measured by standardized LBAs for nearly two decades and shown to be a weak prognostic factor but a relatively strong predictive factor for response to hormonal therapy. LBAs for PgR were replaced by IHC beginning in the mid-1990s, and IHC was eventually approved by the CAP and ASCO for routine clinical use despite persistent shortcomings.[5–7]

Compared with ERα, there are fewer studies in the medical literature standardizing and validating IHC assays for PgR.[54,56,57,60,72] Those available show that PgR is expressed in the nuclei of 60–70% of IBCs, that expression varies on a continuum ranging from 0 to nearly 100% positive cells, that there is a direct correlation between PgR levels and response to hormonal therapies, and that tumors with even very low levels of PgR-positive cells ($\geq$1%) have a significant chance of responding.[54,72] Preliminary studies suggest that, similar to ERα, PgR expression is also associated with reduced local recurrence in patients with DCIS treated with lumpectomy and radiation followed by hormonal therapy.[66,67]

Although the expression of PgR is highly correlated with ERα, the correlation is imperfect, resulting in four possible phenotypes of combined expression, each with significantly different rates of response to hormonal therapy, which would not be apparent measuring one or the other alone. For example, in a recent comparison of patients receiving adjuvant tamoxifen, the relative risk of disease recurrence was 28% higher in patients with ERα-positive/PR-negative than ERα-positive/PgR-positive tumors.[73,74] Distinguishing these significantly different outcomes is the primary reason that both ERα and PgR are measured in routine clinical practice.

Recent studies[75–80] have suggested that functional ERα, which is predominately nuclear in location in most IBCs, may also reside at the outer cell membrane in a subset of tumors, especially those that are HER2 positive. The majority of HER-positive IBCs are also PgR negative, suggesting that nuclear ERα may be nonfunctional in these cases and, thus, possibly unresponsive to the antagonistic effects of tamoxifen. However, membrane ERα appears to remain functional and promotes tumor cell proliferation in cooperation with overexpressed HER2. To further complicate the story, there is also evidence that tamoxifen has a stimulatory or agonist affect on membrane ERα, leading to the speculation that aromatase inhibitors may remain effective in this setting as they inhibit the upstream production of estrogen, which is the ligand for both nuclear and membrane ERα. If these preliminary studies are confirmed, then the quantitative assessment of PgR may take on added importance, especially in the ERα/erbB2-positive subset of IBCs.

As with ERα, the most notable current issue for assessing PgR by IHC is the increasing alarm about problems with accuracy and the impending ASCO/

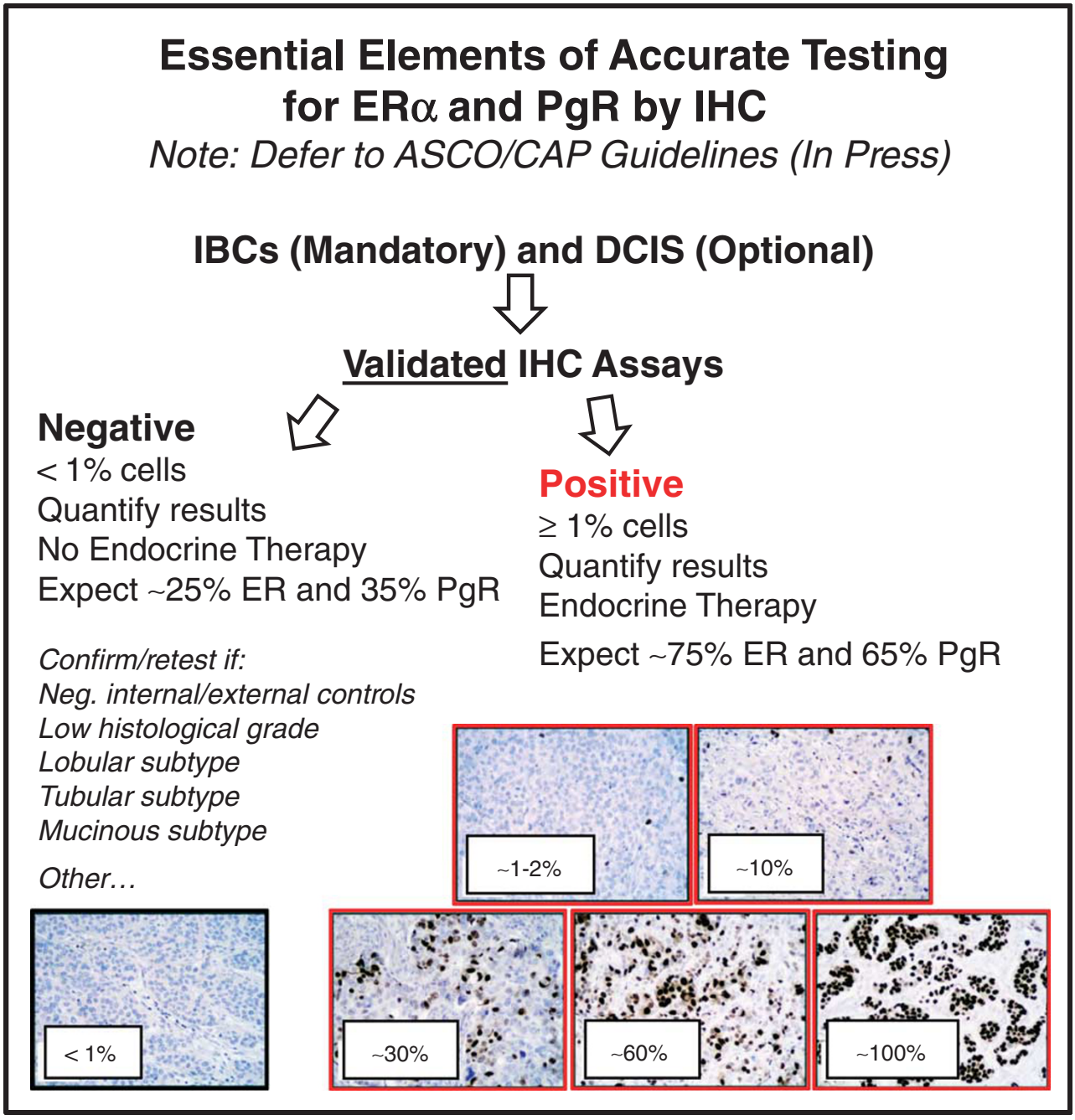

Figure 2 Overview of essential elements required for accurate testing of ERα and PgR status in breast cancer by immunohistochemistry. Similar to HER2 testing, guidelines have recently been developed by the ASCO/CAP to reduce the error rate associated with testing (in press; *Arch Pathol Lab Med* and *J Clin Oncol*), estimated to be as high as 20% overall.

CAP guidelines intended to improve it. Alternative methods for assessing PgR are also emerging, including qRT-PCR (eg, Oncotype DX). However, measuring PgR by IHC will also be with us for several years; therefore, improving accuracy is essential.

Summary

The assessment of ERα, PgR, and HER2 are mandatory in the routine care of all patients with breast cancer. All are targets and/or indicators of response to highly effective therapies in many clinical settings, so accurate assessment is essential. However, accurate testing has been problematic (with error rates of ≥20% with all of them), and there are several recent and ongoing efforts to improve it, such as the recently published ASCO/CAP guidelines for HER2 testing, and imminent similar guidelines for ERα and PgR.[58] It is the responsibility of every pathologist evaluating these biomarkers to be aware of these issues, to possess appropriate

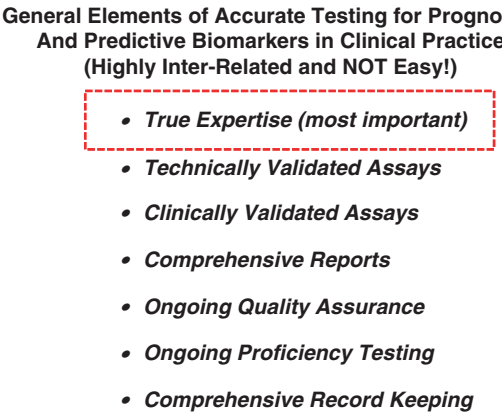

Figure 3 General requirements to ensure accurate testing for any prognostic or predictive biomarkers in routine clinical practice.

expertise, and to use accurate assays which have been validated in a comprehensive and ongoing manner. Figure 3 outlines general elements of accurate testing for prognostic and predictive biomarkers of any type in routine clinical practice.

Disclosure/conflict of interest

The author declares no conflict of interest.

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