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Biomarkers for Early Cancer Detection – Methodological Aspects

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Key Words
Biomarkers for early cancer detection · Breast cancer · Analytical validation · Clinical validation

Summary
The development of a new breast cancer biomarker for early detection is a process that begins with biomarker discovery, followed by a rigorous definition and evaluation of the whole process of biomarker determination (analytical validation). It terminates with the assessment of the impact of the biomarker on clinical practice (clinical validation). A 4-phase scheme for the analytical validation process of the biomarkers for early diagnosis has recently been proposed with the aim of covering the need for standardized operating procedures as well as the need for monitoring and maintaining their quality. As far as clinical validation of biomarkers for early diagnosis is concerned, however, a well established phased approach exists, and guidelines are available for both planning studies and reporting results. Although analytical and clinical validation should be logically linked, often this is not the case in real-word practice, especially in the early phases of biomarker development. This is also the case with breast cancer biomarkers for early detection.

Schlüsselwörter
Biomarker für die Früherkennung · Brustkrebs · Analytische Validierung · Klinische Validierung

Zusammenfassung
Der Prozess der Entwicklung neuer Biomarker für die Brustkrebsfrüherkennung beginnt mit der Entdeckung des Biomarkers, gefolgt von einer strengen Definition und Bewertung des Gesamtprozesses der Biomarker-Ermittlung (analytische Validierung). Der Vorgang endet mit der Einschätzung der Auswirkung des Biomarkers auf die klinische Praxis (klinische Validierung). Vor kurzem ist ein 4-Phasen-Schema für die analytische Validierung von Biomarkern für die Früherkennung vorgeschlagen worden, mit dem Ziel, den Bedarf an standardisierten Operating Procedures zu decken und deren Überwachung und langfristige Qualität zu gewährleisten. Für die klinische Validierung von Biomarkern für die Frühdiagnose existiert dagegen bereits ein etabliertes schrittweises Verfahren, und es sind Richtlinien für sowohl die Studienplanung als auch das Berichten von Ergebnissen vorhanden. Die analytische und die klinische Validierung sollten in logischer Folge miteinander gekoppelt sein, aber in der Praxis ist dies oft nicht der Fall, insbesondere in den frühen Phasen der Biomarkerentwicklung. Dies trifft auch auch die Biomarker für die Brustkrebsfrüherkennung zu.

Introduction

The need for a standardized process for biomarker validation in oncology has become increasingly relevant in the last years given the great number of promising biomarkers continuously proposed in the literature. The development of a new cancer biomarker is a process that begins with biomarker discovery, followed by a rigorous definition and evaluation of the whole process of biomarker determination (analytical validation). It terminates with the assessment of the impact of the biomarker

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Table 1. Phases of biomarker analytical validation

Phase		Description
I	operating procedures – setting-up	definition of operating procedures for biomarker determination
II	operating procedures – standardization	validation of the operating procedures in terms of precision and accuracy according to the standards
III	internal quality control	evaluation of the validated standards within laboratory
IV	external quality assessment	between laboratories – comparison and assessment of their accuracy

Table 2. Phases of biomarker clinical validation

Phase		Objective	Study design
I	preclinical exploratory	identify promising directions	case-control
II	clinical assay and validation	determine if a clinical assay detects a specific disease	case-control (population-based)
III	retrospective longitudinal	verify if the biomarker is able to detect disease before it becomes clinical	nested case-control in a population cohort
IV	prospective screening	determine extent and characteristics of disease detected by the test	cross-sectional cohort of people
V	cancer control	impact of screening on reducing the burden of disease on the population	randomized trial (ideally)

on clinical practice (clinical validation). The two latter steps are needed to confirm the usefulness of a novel biomarker as diagnostic tool, and they are logically linked so that the first one must precede any other kind of investigation moving to the clinical setting. This article will discuss some methodological aspects related to the identification of biomarkers for early Cancer detection.

Analytical Validation of Biomarkers for Early Cancer Detection

Analytical validation is the process of assessing the biomarker assay, its performance characteristics, and the optimal analytical setting to guarantee a satisfactory level of reproducibility and accuracy. The ultimate goal of this process should be reducing the number of promising biomarkers that fail in the clinical setting as a result of a lacking robust analytical validation. So far, a coherent and comprehensive set of guidelines for analytical validation of new biomarkers has not yet been delineated. A 4-phase scheme (table 1) has recently been proposed for the analytical validation process of biomarkers for early cancer diagnosis [1]. This scheme tries to cover the need for standardized operating procedures (SOPs) for the whole analytical process involved in early cancer biomarker determination as well as the need for monitoring and maintaining their quality. Both these needs have been recently stressed by some authors [2–5] as a prerequisite for clinical validation. In this context, assays for early cancer biomarkers should be analytically validated in external quality assurance (EQA) schemes before their effective implementation into routine laboratory testing in order to generate clinically useful information. EQA programs devoted to directly evaluating the performance of biomarkers for early diagnosis have recently been proposed by different authors as an alternative approach to specific target-oriented EQA [6]. It should be considered that the reliability and validity of these cancer biomarkers are influenced by many factors, the impact of which may vary for different biomarkers in different types of specimens. For this reasons, it is crucial in EQA planning to have a thorough understanding of the assays employed by the participating laboratories in order to decide upon the most appropriate source material for challenge specimens. Another important aspect related to EQA implementation regards the choice of reference value that ideally should correspond to the true biomarker value. If a reference value is available, an EQA program can answer the question 'Can the results of a laboratory be deemed accurate?' On the other hand, in the absence of this value, an EQA program can only answer the more humble question 'May the results of a laboratory be deemed consistent with the majority (i.e. 95%) of the results provided by all the participating laboratories?' For any EQA program based on cancer biomarkers, the latter is the most common situation. In this case, the most suitable reference value should be estimated by applying appropriate statistical procedures such as those based on parametric [7], robust [8], or distribution-free [9] approaches to data analysis.

Clinical Validation of Biomarkers for Early Cancer Detection

As far as clinical validation of biomarkers for early diagnosis is concerned, a well established multi-phased approach exists [10], with guidelines available for both planning studies [11]

Table 3. Statistical guidelines for nested case-control studies

Item	Description
1	For the clearest interpretation, statistics should be based on false- and true-positive rates, not odds ratios or relative risks.
2	To avoid overdiagnosis bias, cases should be diagnosed as a result of symptoms rather than screening.
3	To minimize selection bias, the spectrum of control conditions should be the same in study and target screening populations.
4	To extract additional information, criteria for a positive test should be based on combinations of individual markers and changes in marker levels over time.
5	To avoid overfitting, the criteria for a positive marker combination developed in a training sample should be evaluated in a random test sample from the same study and, if possible, a validation sample from another study.
6	To identify biomarkers with true- and false-positive rates similar to mammography, the training, test, and validation samples should each include at least 110 randomly selected subjects without cancer and 70 subjects with cancer.

and reporting results [12]. In general, biomarker evaluation should follow an orderly multi-phased process (table 2) in which phase 1 studies are exploratory and often based on high throughput technology that produce high dimensional data for biomarker discovery. An important effect of the application of these 'omics' technologies on tissue samples and body fluids is the availability of complicated data in which the number of parameters, and thus the complexity of the model, is increasingly greater than the number of samples. As a consequence, the model fits the original data but fails when used to predict disease in an independent data set (overfitting). Two approaches are available to avoid this phenomenon of overfitting data: cross-validation and validation of independent datasets. Feng et al. [13] provided a detailed discussion on the most relevant statistical tools related to the use of high-dimensional biomarkers for early detection. In phase 2 studies, biomarker values in cases (individuals with cancer) and controls (individuals without cancer) are directly compared. Phase 3 studies imply evaluation of the biomarker in a case-control study to assess its capability to detect sub-clinical disease. A phase 3 study should ideally be designed as a nested case-control study that involves prospective collection of specimens before outcome ascertainment from a study cohort that is relevant to the clinical application. An excellent guideline in statistical design and analysis of nested-case control study [14], available from the 4th report of the Early Detection Research Network (EDRN), is schematically shown in table 3. In a phase 4 study, the biomarker is detected prospectively as a screening test in a population. Finally, a phase 5 study consists essentially of a cancer screening study carried out to evaluate the utility of the biomarker as an indicator for early intervention. Baker et al. [15] reviewed key statistical methods applicable to each of the above-mentioned phases of biomarker clinical validation. Pepe et al. [10] have recently discussed how this original 5-phase approach could be slightly modified for situations in which preclinical specimens are available. Detailed description of the methodological issues related to each phase is beyond the scope of this note. The references provided should serve as a first step toward a more in-depth research to the interested reader.

Table 4. Phases of development of breast cancer biomarkers for early detection

Biomarker	AV phase	CV phase
Circulating nucleic acids	II	II
Methylation-based DNA in serum	I/II	I/II
High mobility group A proteins	I	I
Na^+/H^+ exchanger regulatory factor 1	I	I

AV = Analytical validation; CV = clinical validation.

Conclusion

Table 4 summarizes the development status of the different breast cancer biomarkers for early detection [16–19] according to the above-mentioned validation process. We consider as promising biomarkers those whose performance as a diagnostic tool should still be assessed or is being assessed. Interestingly, and by moving from a theoretical to a practical point of view, it seems that for all the biomarkers discussed in this issue, analytical and clinical validation proceed not as logically desirable. However, this trend is very common in cancer research, especially for potentially promising biomarkers the determination of which is based on the application of highly complex and quickly evolving technologies. The real-word validation process in such cases often consists of an overlap of the early phases of both validation processes. From this point of view, among the considered biomarkers, circulating nucleic acids appear to be the most developed, followed by methylation-based DNA biomarkers detected in serum. Specifically, as far as the analytical validation of circulating nucleic acids is

concerned [16], different pre-analytical sources of variation have been described as related principally to the biological material (substrate) used for the assay and to the methods (in-house or commercial kit) adopted for their purification. Overall, it seems that none of the different analytical approaches currently available can be used as standard until their validity within and between laboratory programs is not assessed. A direct consequence of this is the lack of prospective studies carried out to evaluate the clinical performance of circulating nucleic acids in detecting sub-clinical disease in asymptomatic subjects. Similar considerations seem to be compelling also for methylation-based DNA biomarkers [17]. Their development level appears to be appreciably slightly lower in comparison with the circulating nucleic acids as a result of poor assay performance (underperformance). Both analytical and clinical validation of most recent biomarkers based on the expression of high mobility group A proteins [18] and Na^+/H^+ exchanger regulatory factor expression [19] present a stimulating challenge in finding more effective interventions for the prevention and treatment of breast cancer. However, their analytical reliability is still influenced by many factors the impact of which should be more deeply investigated in order to develop shared operating procedures. This calls for a standardization of experimental protocols before clinically assessing the performance for classifying subjects into those with and without breast cancer.

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Conflict of Interest

The authors declare no conflict of interest.

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