REVIEW ARTICLE

# THE SYSTEMATIC TRANSLATION OF BIOMARKERS RESEARCH INTO CLINICAL PRACTICE

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**Background:** The rapid gains in science and technology during the last century resulted in an epidemiological shift from infectious diseases to non-communicable diseases like cancer, diabetes mellitus, and cardiovascular diseases. However, despite the large funding put into the understanding of etiology of cancer, identification of novel diagnostic markers, and advancing cancer treatment, the translation of research findings into clinical practice leaves much to be desired for. **Methods and Results:** The translation of cancer biomarkers into clinical practice is a great challenge that needs to be promptly addressed for better cancer outcomes. This review discusses the characteristics of clinically useful cancer biomarkers, and how biomarkers identified by research can be used for the improved cancer management and patient outcomes. We also explored the underlying reasons for the less than an optimal translation of biomarkers research into clinical practice, how basic medical sciences can undertake more clinically relevant research, and provide suggestions on how to improve the clinical translation of research findings from such studies. The reason delaying the clinical translation of biomarkers are: lack of systematic analyses on existing cancer biomarkers; inadequate sample size; lack of an optimal scoring system and threshold; limited use of panels of biomarkers; technical differences between laboratories; and the need for well-designed validation studies (biomarker clinical trials). **Conclusion:** Clinical translation of biomarkers could potentially be facilitated through a systematic approach taking into account the reasons highlighted in the current study.

**Keywords:** Cancer, Biomarkers, Clinical, Translation

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## INTRODUCTION

The last century saw groundbreaking medical inventions such as isolation of penicillin, development of vaccines and improved living conditions because of public health measures.[1] Consequently, life-threatening infections could be treated and the life expectancy increased.[2] As a result of this change in epidemiologic patterns of disease, non-communicable diseases such as cancer have gained more importance and despite improvement in cancer survival during the past decades, it is still largely a killer disease.[3,4] In recent times, the focus of cancer research has shifted to a better understanding of cancer biology and improved stratification of cancers conventionally considered as a single entity.[5] Cancer cells frequently express aberrant genes or protein products not expressed by their normal counterparts. These proteins may provide survival advantage or resistance to chemotherapy to these cancer cells. Translational medicine has exploited this property of cancers for the diagnosis, risk stratification and monitoring of cancers.[6] Such proteins may be called cancer biomarkers, a classic example being alfa-fetoprotein secreted by the liver or gonadal cancer. Despite ongoing research in the field, the list of useful clinical cancer biomarkers is short.[7]

Clinical translation of basic research is a priority for both academia and industry.[8] Translational research has gained more importance from the fact that the level of investment in research is not reflected in clinical practice, and there is a concern that the benefit from the 'genetic revolution' is slow.[9]

The amount of diagnostic biomarker research in every cancer type is enormous. There is an ever-increasing number of articles published each year (Figure-1), yet the list of clinically validated biomarkers is short.[10] This review discusses the characteristics of clinically useful cancer biomarkers and how biomarkers identified by research can be used for the improvement in diagnosis and clinical outcome. In the following sections, the main factors that contribute to the hurdles in clinical translation of the candidate diagnostic biomarkers under investigation are discussed. The principles of the current review could be used for all tissue based biomarkers detected in body fluids and tissue sections using any biochemical technique.

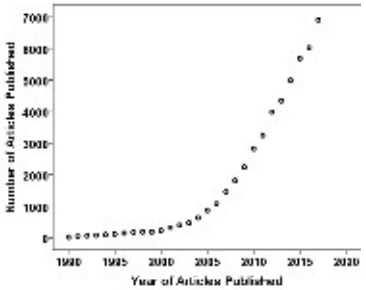


**Figure-1: Articles indexed in PubMed containing the term 'Biomarker' in title**

## METHODS AND RESULTS

Based on the literature, the following six main reasons have been identified, which delays the clinical translation of biomarkers (Figure-2).

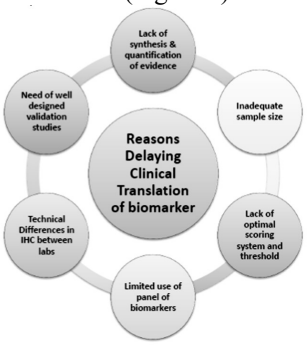


**Figure-2: Diagrammatic representation of reasons delaying clinical translation of biomarkers**

**1. Lack of systematic analyses on existing cancer biomarkers**

The most important first step is the identification of existing diagnostic biomarkers. Biomarkers have been investigated purposefully to address a clinical problem[11,12] and in some cases, they have been investigated in more than one study[13,14]. The results of some of these studies show promising results for differentiating one tumour type from another[12] or subtypes of the same tumour[11]. These studies also show the utility of biomarkers in the differentiation of benign disease from malignant disease.[15,16] Importantly, research studies on a specific biomarker may show varying, sometimes contrasting, results. For example, maspin has been shown to be a good prognostic biomarker in pancreatic cancer and a bad prognostic biomarker in breast cancer.[17] Therefore, there is a need to perform more focused systematic reviews and subsequent meta-analysis of biomarkers intended for specific clinical (diagnostic or prognostic) problems. A meta-analysis will provide the statistical evidence for the effectiveness of a particular biomarker. The clear advantage of this approach is the identification of suitable candidate biomarkers that have previously been investigated. The other important advantage of this selection process is that biomarkers investigated in different studies showing promising results are compiled for investigation in one potential validation study. This will surely provide more strength for a future validation study investigating these better biomarkers in a single setting.

**2. Inadequate sample size**

The sample size for biomarkers reported in the literature is relatively small and this is especially true for novel biomarkers assessed in pilot studies.[18,19] This is realistic because a vast tissue resource will not be available for a new biomarker under investigation. Sufficient statistical power is thus not reached in most of these pilot studies which could potentially lead to promising biomarkers being overlooked in the enormous biomarker research field. Biomarkers identified in the meta-analysis might be carefully investigated in a sufficiently powered study using a large sample size. Obviously, investigation of biomarker expression in large sample size will further elucidate the diagnostic performance (sensitivity and specificity) of biomarkers. Biomarker expression is not homogenous in tumor tissue extracted from different patients.[20,21] This Inter-tumour heterogeneity of expression of biomarkers can be shown more clearly if the sample size is large. This will help in better defining the diagnostic role of biomarkers in cancer. Biomarkers showing more inter-tumor heterogeneity are less sensitive in identifying the disease and are thus less accurate.

The other issue with sample size is the distribution of the number of samples between the two groups (diseased and normal) investigated.[18] For example, a diagnostic biomarker tested to differentiate between benign and malignant disease. The true diagnostic potential of a biomarker, in this case, can be measured if (ideally) the sample size is equally distributed between benign and malignant samples.

**3. Lack of an optimal scoring system and threshold**

Cancer tissues may over-express surface or cytoplasmic molecules which may be used as cancer biomarkers. For example, Chronic Myeloid Leukaemia cells express a novel BCR-ABL tyrosine kinase enzyme. Therefore, the presence of BCR-ABL tyrosine kinase in blood cells is diagnostic of CML. However, this is a rare example as the majority of molecules expressed in cancer cells are also expressed by normal tissue –albeit at lower levels. For example, carcinoembryonic antigen (CEA) is secreted by many cancer types (liver, gonads) but also present at very low levels in normal healthy individuals. Similarly, c-Kit/CD117 (also called Stem Cell Factor Receptor) has been used as a biomarker in gastro-intestinal stromal tumors (GISTs). Approximately 95% of GISTs show high expression of CD117. However, weak CD117 expression can also be seen in other mesenchymal cancers. Therefore, a cut-off has to be set to discriminate between benign and malignant cancers, or cancers of different origins, based on the level of expression of such proteins.

Interpretation of IHC requires a robust and comprehensive scoring system that is able to quantify the extent of biomarker expression. From this scoring scheme, then, thresholds or cut-offs can be investigated for categorizing patients into one of the two diagnostic categories (for example benign vs. malignant). There is no single uniform scoring system and researchers have used a wide array of scoring systems.[11,22–24] These scoring systems are based on staining intensity;

percentage of positively stained cells; a combination of both staining intensity and positive cells; and semi-quantitative Histoscores.[25–28] A semi-quantitative Histoscore (takes into account both intensity and proportion of staining) could possibly emerge as a standard scoring system. Histoscore quantify the expression level of biomarkers and allow for calculation of various potential cut-offs for diagnostic purposes. A commonly used histoscore is ER/PR/Her2neu in breast cancer.[29]

The list of clinically useful IHC cancer biomarkers is short. If a new biomarker is identified, a threshold or cut-off would have to be identified that can reliably discriminate between benign and malignant, or good prognosis and bad prognosis cancer. Such a cut-off has to be easy to use by practicing pathologists. An optimal cut-off should be reliable and reproducible among pathologists. A systematic way of choosing a cut-off is to perform a receiver operating characteristic (ROC) curve analysis which provides diagnostic sensitivity and specificity of biomarkers on a range of cut-offs.[30,31] This helps the researcher to select an optimal cut-off which has both diagnostic potentials and is easily scored by observers. This cut-off can then be used in future validation studies and studies involving observer variations between different scorers.

**4. Limited use of panels of biomarkers**

An ideal diagnostic biomarker should have homogenous expression within and between tumor tissues from the same cancer type. In reality, this is not always the case. Cancer being a heterogeneous disease carries significant variation both within a tumor and between same tumor types (intra-tumour and inter-tumor heterogeneity).[32] A single candidate biomarker is thus unlikely to work as a perfectly sensitive and specific biomarker[33,34] in all patients. A panel of biomarkers is thus a plausible solution to address both inter- and intra-tumor heterogeneity. Most of the researchers in reported literature have investigated biomarkers singly with a limited panel approach. The panel approach has not been reported in instances when more than one biomarker was investigated in a single study.[35]

Identification of suitable biomarkers and then exploring their diagnostic performance as a panel in a single experimental setting is a more powerful approach. The obvious strength of the panel approach is that it allows for the comparison of accuracy between biomarkers and panels of biomarkers. This comparison then determines an appropriate panel of biomarkers for future validation and clinical translational studies. Different biomarkers stain different cellular compartments and using a panel of biomarkers has this additional advantage of staining all major sub-cellular compartments. Clearly, a panel with more than one positive biomarker provides more confidence to the pathologist reporting the disease.

The most simplistic example of this panel approach is the triad of IHC markers Estrogen Receptor (ER), Progesterone Receptor (PR) and Her2/neu for the prognostic stratification of breast cancer (Figure-3). Estrogen and Progesterone receptors are expressed in healthy breast ductal cells. Stimulation by the respective hormone results in the growth of breast tissue. When expressed by the cancer cells, stimulation of these receptors by the hormone stimulates cancer cell growth. Tamoxifen, a selective estrogen receptor modulator, is used in the treatment of ER-positive breast cancers.[36] As a result, the prognosis for $ER^{+ve}$ has dramatically improved over the past few decades.[37] Similarly, breast cancers over-expressing human epidermal growth factor (HER2) are generally aggressive and carry a poor prognosis. However, with the development of a specific HER2 inhibitor, the prognosis has significantly improved.[38] The combination of expression levels in these patients divides breast cancer patients into distinct categories (Figure-3). Patients in some categories carry different prognosis and therapeutic options ($ER^{+ve}$ *vs* $ER^{-ve}$ and $ER^{+ve}/PR^{+ve}/HER2^{+ve}$ *vs* $ER^{-ve}/PR^{-ve}/HER2^{-ve}$).

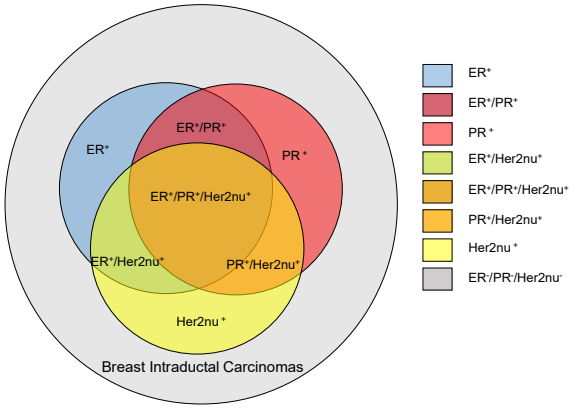


**Figure-3: Prognostic categories based on triad of ER/PR/Her2**

**5. Technical differences in IHC between laboratories**

Optimisation of IHC in biomarker research is very important to achieve appropriate staining in the tissue. The majority of IHC antibodies are available commercially from various manufacturers which provide information and suggested protocols for IHC but most research laboratories carry out their own optimization process too. The aim of optimization is to increase the strength and specificity of the signal while suppressing background signals and artefacts.[33,35,39]

Research laboratories usually employ different IHC experimental conditions including a clone of primary antibodies, antigen retrieval methods (heat induced epitope retrieval *vs* enzymatic retrieval), primary antibody dilutions and manual or automated platforms.[40] All these factors significantly contribute to the different sensitivity and specificity values reported

for a candidate biomarker investigated in different studies. Studies have addressed this issue and have compared: different clones of antibodies; different *p*H of antigen retrieval buffers (for example *p*H 6 *vs p*H 8) and dilutions of primary antibodies (for example 1/50 *vs* 1/100).[41–44] These studies provide an insight for choosing better conditions for the optimization of antibodies.

One approach to addressing the issue of technical heterogeneity is to systematically search the literature to identify IHC parameters for a biomarker that achieved an optimum combination of sensitivity and specificity. These parameters could then be used as a starting point for further optimization.

Assay development is a critical component in the qualification of the biomarker. Sometimes potential biomarkers fail to enter the list of clinically useful biomarkers not because of the underpinning science, but because of issues around assay development and a lack of validation studies.[45]

**6. The need for well-designed validation studies (biomarker clinical trials)**

Validation of potential IHC diagnostic biomarkers in independent tissue cohorts is probably the most important factor delaying clinical translation.[1] Researchers investigate promising biomarkers, publish their work and sometimes leave excellent biomarkers without designing further validation studies. More focused and aim-oriented validation studies could expedite the journey of biomarkers from bench to the clinic.[46]

Validation studies in a step-wise fashion can be as follows. Validation of biomarkers in independent laboratories and patient cohorts, using the same IHC methodology, and the same scoring system and cut-offs. The expression level and subsequent diagnostic sensitivity and specificity should broadly be similar in validation studies. This will help to establish the reproducibility of the IHC methodology and cut-offs used for diagnostic purposes. Then establishing a multi-institutional validation study group and carrying out validation studies and addressing technical and other issues.[47] For example, a study group developed in pancreatic cancer research is 'European Study Group for Pancreatic Cancer' (ESPAC).[48] The aim of this international collaboration is to carry out multinational trials which can validate the findings from pilot studies. Such groups provide an excellent platform for clinical validation of tumor biomarkers. Finally, a prospective clinical study for investigating the optimum biomarker panel will provide more confidence to translational scientists and pathologists for further validation. In fact, academic-industry collaborations can further facilitate and expedite validation studies from bench to the clinic.

Biomarker development from identification to validation and the clinical application would require: pooling of already existing data; analysis of evidence; validation of known promising biomarkers; addressing factors such as sample size, scoring systems, and cut-offs that influence the validation of biomarkers; and finally using a panel approach and best IHC methodology in well-designed and aim-oriented validation studies (Figure-4).

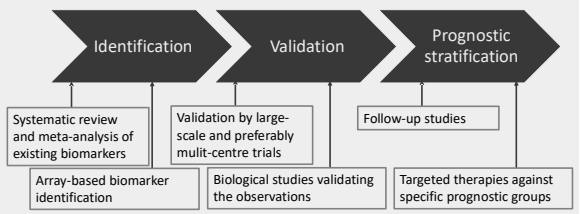


**Figure-4: Scientific methods required for establishment of a clinically validated biomarker**

## CONCLUSION

The less than an optimal translation of biomarkers research into clinical practice results from a variety of systematic and operational reasons such as the lack of pooled analysis of systematically selected and reviewed individual studies, lack of power in studies resulting from small sample sizes, lack of valid scoring systems and threshold values, the limited use of panel biomarkers, and technical differences in IHC among laboratories. These issues coupled with the scarcity of validation studies constitute the main reason why the clinical practice has not benefitted immensely from the huge funding and resulting advancements in the biomarkers field. It is therefore pertinent that future research on biomarkers takes these issues into account, for a sounder and clinically relevant and translatable research.

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