BIOMARKERS AND DIAGNOSTICS

Ms. Snehal D. Jadhav, Mr. Jeevan R. Rajguru, Mr. Vaibhav L. Narwade, Ms. Nilam S. Choudhari

1,4Department of Quality Assurance Technique, 2,3Department of Pharmaceutics
AAEM’S Delight College of Pharmacy, Koregaon Bhima, Pune

Abstract: The last decade has seen an extraordinary amount of effort devoted in biomedical research to the field of biomarkers. There have been some notable successes with novel markers being adopted into clinical practice bringing clear clinical benefit to some patients - particularly with the increasing numbers of medicines being approved with companion diagnostics. However, it is fair to say that there has not yet been the numbers of clinically valuable biomarkers brought to medical practice that the research effort would seem to warrant. This paper evaluates examples of successful biomarkers, markers which might be considered partial successes and a few problematic examples and argues that more effort spent in the validation phase of marker development, and less in the discovery phase might be a more efficient way to allocate research resources.

Keywords: biomedical, biomarkers, analytes, companion diagnostics, validation, utility

INTRODUCTION:

Biomarkers are objectively measurable indicators of biological states. Within the field of health care, biomarkers can improve our understanding of disease and can provide information on the presence of disease, or susceptibility to disease, in an individual, or predict or monitor patient response to therapeutic interventions. The use of novel molecular biomarkers within the practice of evidence based medicine may improve diagnosis or treatment of disease, improving health outcomes and reducing the social and economic impact of disease. Discovery of biomarkers is expanding at an unprecedented rate, as previous investments in genomic science are enabling improved understanding of disease mechanisms and individual patient responses to therapy. Such biomarkers are allowing early identification of disease, improved diagnoses, and safer and more efficacious treatments leading to better patient outcomes and efficient and effective public expenditure on health. Promising results from initial uses of biomarkers demonstrate that under the right conditions, their integration into evidence-based medicine may transform our approach to chronic disease and other serious diseases, changing the way disease is diagnosed and treated. Securing the right conditions for the uptake of biomarkers within health systems remains challenging, but achievable. Those countries which succeed in adopting biomarkers within their health systems stand to gain substantial improvements in the health of their citizens, and in the economic performance of their health care systems and supporting industries. A biomarker is, according to the US National Institutes of Health, “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” Biomarkers make take the form of cellular characteristics, metabolites (e.g. sugars, lipids and hormones), molecular variations, or physical features (e.g., clinical symptoms) and are assessed accordingly, via measurement, annotation, documents, and images. Increasingly, the discovery of novel biomarkers is closely associated with the advances in molecular biology techniques that can be accessed through analysis of DNA, RNA or proteins. We can discriminate four main types of molecular biomarkers:

1. Genomic biomarkers: based on the analysis of DNA (deoxyribonucleic acid) profiles, especially the analysis of SNPs (single nucleotide polymorphisms), i.e. identification of punctual variations in genomic DNA.
4. Metabolomic biomarkers: based on the analysis of metabolites

Fig: Steps from biomarkers to diagnostics
Diagnosis is about classification and how it may be used as a label to aid prediction, prognosis and treatment. The label, the diagnosis, is not an end in itself but an intermediary, a means to an end. Diagnosis is no use in itself; there must be a purpose, an objective. Tests, including the use of clinical symptoms and, signs, are the means by which a diagnosis is made so that a decision or an action can be taken. It is also the case that one can make no statement about the effectiveness of a test without knowing its purpose or objective since purpose is inherent in the formal definition of the effectiveness of a healthcare intervention. But it is not just purpose that is important in test evaluation. The nature of the disease is also important. The effectiveness, validity or utility of a test is dependent on the disease or disorder under consideration. The third factor that has a bearing on test interpretation and evaluation is population and, in particular, the effect that the disease prevalence in the studied population critically affects the test’s predictive value. The term ‘biomarker’ is often used in this context rather than diagnostics or diagnostic tests. It has been more broadly defined by the Food and Drug Administration as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. There are many reasons for carrying out a test, of which the making of a diagnosis is but one. These considerations lead to the most important conceptual insight into biomarker or diagnostics evaluation, the distinction between an ‘assay’ and a ‘test’. The assay is a method for determining the presence or quantity of a component whereas the test is its use in the context of a particular disease, in a particular population, for a particular purpose. The distinction has an important practical implication. Whereas the evaluation of an assay is reasonably straightforward and allows broadly applicable standards to be established, the evaluation of a test is more complex and inherently less susceptible to standardisation. Each test is likely to need evaluation and interpretation depending on how the test is to be used in the particular context of disorder, population and purpose.(4)

**Public Health Significance:**

Clinical medicine covers disease prevention, diagnosis and treatment. Biomarkers play a critical role in all these aspects. There are three major types of biomarkers: biomarkers of exposure, effect and susceptibility. A biomarker of exposure is an exogenous chemical or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism. Specific markers of exposure include the presence of a xenobiotic compound or its metabolites in body tissues or fluids and in excretory products. For example, blood lead concentration has been used as a marker for lead exposure; saliva cotinine (a metabolite of nicotine) level has been used as a marker in investigating adolescents’ cigarette consumption. A biomarker of effect is a measurable alteration of an endogenous factor that is shown to be linked with impairment or disease resulting from exposure to an exogenous agent. For example, the alteration in pulmonary function tests in children after exposure to environmental tobacco smoke is a biomarker of effect (1). Somatic mutations have been used as biomarkers of effect after exposure to carcinogens. A biomarker of susceptibility indicates individual factors that can affect response to environmental agents. These reflect variations between individuals in genetic structure, some of which make the individual more susceptible to health effects from environmental exposures (2). For example, skin cancer is related to excessive sun exposure, but not everyone develops skin cancer even with the same amount of exposure. Three recent studies revealed that genetic variants associated with three sections of genes were found to be linked with increased risk of skin cancer: 1) the variant of the TYR gene that encodes a R402Q amino acid substitution, previously shown to affect eye colour and tanning response, was associated with increased risk of developing cutaneous melanoma (CM) and basal cell carcinoma (BCC); 2) variations in a haplotype (set of closely associated genes) near the ASIP gene, known to affect pigmentation traits, conferred significant risk of CM and BCC; and 3) an eye colour variant in gene TYRP1 was also associated with risk of CM (3–5). There are two layers of exposure and effect biomarkers. The first represents hazardous exposures to a healthy human body that could cause negative biological effects (e.g. functional changes, somatic mutations) and eventually cause disease. Another layer indicates treatment exposures to a diseased human body that could induce positive biological effects and lead to the improvement of conditions or to the complete recovery from disease are present in every step of the process. For example, some individuals exposed to air pollutants show severe biological effects and manifest disease symptoms, while others experience little or no effect. The same discrepancy appears with drug treatment. While some patients benefit and are cured, others show no effect from treatment or develop severe side-effects or die. In clinical medicine, the first layer is more related to disease prevention and diagnosis, while the second layer is more relevant to disease treatment and recovery.

**BIOMARKERS IN DIAGNOSTICS:**

Biomarkers have been used in disease diagnosis for over a century, beginning when the ABO blood group system was first discovered and used to detect ABO haemolytic disease of the newborn (HDN) and transfusion reactions. The four basic ABO phenotypes are O, A, B and AB. After it was found that blood group A’s red blood cells (RBCs) reacted differently to a particular antibody (later called anti-A1), the blood group was divided into two phenotypes, A1 and A2. RBCs with the A1 phenotype react with anti-A1 and account for about 80% of blood type A. RBCs with the A2 phenotype do not react with anti-A1 and makeup about 20% of blood type A. HDN, caused by ABO antibodies, occurs almost exclusively in infants of blood group A or B who are born to group O mothers (22). This is because the anti-A and anti-B formed in group O individuals tends to be of the IgG type (and therefore can cross the placenta), whereas the anti-A and anti-B found in the serum of group B and A individuals, respectively, tends to be of the IgM type. Although uncommon, cases of HDN have been reported in infants born to mothers with blood group A2 (23) and blood group B (24). The most common cause of death from a blood transfusion is clerical error, in which an incompatible type of ABO blood is transfused. If a recipient who has blood group O is transfused with non-group O RBCs, the naturally occurring anti-A and anti-B in the recipient’s serum binds to their corresponding antigens on the transfused RBCs. These antibodies fix complement and cause rapid intravascular haemolysis, triggering an acute haemolytic transfusion reaction that can cause disseminated intravascular coagulation, shock, acute renal failure and death. Routine biomarker tests can confirm the diagnosis. Another important use of biomarkers in clinical medicine is the early detection and diagnosis of chromosome and single-gene disorders. Both cytogenetic and molecular genetic biomarkers have been used to accomplish this. Conditions caused by a...
change in the number (e.g. aneuploidy) or structure of chromosomes (e.g. translocation, inversion, deletion, and duplication) are known as chromosome disorders. Biomarkers used in the chromosome analysis developed in 1956 soon led to the discovery that several previously described conditions were due to an abnormality in chromosome number. For example, in Turner syndrome, only one intact X chromosome is present (45, X); all or part of the second X is deleted. Patients with Down syndrome have an extra chromosome 21 (47, XX/XY, +21). Patau syndrome is the result of trisomy 13, while trisomy 18 causes Edwards syndrome. The biomarker test in this case is assessment of chromosome numbers. Microdeletion/microduplication syndromes are a group of chromosome disorders that could be detected by biomarker copy number variation (CNV). “Micro” represents submicroscopic, meaning that these deletions, normally smaller than 3Mb, cannot be detected using a microscope. New technologies, especially array comparative genomic hybridization (array-CGH), enabled many malformations and syndromes to be recognized. Applications of new biomarkers in these disorders have generated particular interest. For example, most Angelman and Prader-Willi syndromes are related to microdeletion involving the proximal part of the long arm of chromosome 15q (15q11–12). It is now known that if the deletion occurs de novo on the paternally inherited number 15 chromosome, the child will have Prader-Willi syndrome; a deletion occurring at the same region on the maternally inherited number 15 chromosome causes Angelman syndrome. Non-deletion cases also exist and are often due to uniparental disomy (i.e. both homologues of a chromosome pair are inherited from only one of the parents), with both number 15 chromosomes being paternal in origin in Angelman syndrome and maternal in origin in Prader-Willi syndrome. This “parent of origin” effect is referred to as genomic imprinting and methylation of DNA. Here, CNV and methylation biomarkers, coupled with clinical observations, have helped identify new underlying genetic mechanisms (18). The most widely used biomarkers identified during the last few decades are for the diagnosis of single-gene disorders. More than 10 000 human diseases are believed to be caused by defects in single genes, affecting 1–2% of the population (25). The disease can be relatively trivial in its effects (e.g. colour blindness), or lethal like Tay Sachs (a fatal inherited disease of the central nervous system; babies with Tay-Sachs lack an enzyme called hexosaminidase A (hex A) which is necessary for breaking down certain fatty substances in brain and nerve cells). Other disorders, though harmful to those afflicted with them, appear to offer some advantage to carriers. For example, carriers of sickle cell anaemia and thalassemia appear to have enhanced resistance to malaria. Some other examples of single-gene diseases are cystic fibrosis, Marfan syndrome, Huntington disease and hereditary haemochromatosis.

The lack of sensitivity and specificity for single markers is not surprising given the degree of heterogeneity present in both solid tumours and the human population at large. Thus, a prevailing hypothesis is that a panel of biomarkers would cumulatively possess a higher sensitivity and specificity than any single biomarker (26).

Table 1. Concepts of biomarker validation

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Phase of biomarker research</th>
<th>Purpose of phase</th>
<th>Comments</th>
<th>References</th>
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<tbody>
<tr>
<td>1.</td>
<td>Analytical Validation</td>
<td>• Establish that the assay actually measures the intended analyte</td>
<td>Precision (repeatability)</td>
<td>[27]</td>
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<td></td>
<td></td>
<td>• Determine the accuracy and robustness of the assay</td>
<td>• Trueness (bias)</td>
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<td></td>
<td></td>
<td>• Sensitivity and specificity of the assay — clinical accuracy (in the intended patient population)</td>
<td>• Limit of Detection, limit of quantification</td>
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<td></td>
<td></td>
<td>• Assay failure rates (and reasons)</td>
<td>• Analytical specificity, interference and carry-over</td>
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<td></td>
<td></td>
<td>• Assay “no-call” rates, i.e., indeterminate results</td>
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<td>2.</td>
<td>Clinical Validation</td>
<td>• Does the assay provide medically useful information which improves patient outcomes or reduces health-care costs?</td>
<td>• Use blinded, retrospective analyses of prospectively collated samples with known outcomes</td>
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<td></td>
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<td></td>
<td>• Evaluate assay performances in different labs and in different patient populations</td>
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<td>3.</td>
<td>Clinical Utility</td>
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<td>• Use prospective randomised clinical studies to show the assay improves outcomes</td>
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[27,28,31]
Future directions and challenges:
Multiple targets, prevention and prediction, personalization and cooperation will be the future directions of biomarker applications in clinical medicine. Multiple biomarkers will be more frequently applied in clinical tests, especially for common diseases. “Multiple” could represent many markers from the same profile, or markers from different profiles, such as DNA, mRNA, microRNA or protein and gene expression. In 2007, for example, the FDA approved a gene-based breast cancer test designed to determine the likelihood of early stage breast cancer recurrence within 5–10 years after treatment. The test called MammaPrintTM (Agendia) is a DNA microarray-based diagnostic kit that measures the level of transcription of 70 genes in breast cancer tumours. The profiles are scored to determine the risk or recurrence and with it the need for adjuvant therapy (8). There is currently a great deal of research being done on multitargeted therapies, which simultaneously target some of the many signalling pathways involved in tumour development and proliferation. “Mixing cocktails,” as Charles L. Sawyers recently described it (6), will continue to grow, but should be under the appropriate molecular guidance. Preventive and predictive biomarkers will play a key role in future health care. New agents, such as antiangiogenesis/vascular targeting drugs, have moved from cancer therapy to cancer prevention. Molecular and epidemiologic studies of cancer risk and drug sensitivity and resistance began ushering in the era of personalized prevention (6,9).

Development of new treatments has increased the need for markers that predict outcome and those that direct which treatment options are most likely to be effective for a particular patient with a particular tumour (10). Personalized medicine is the use of detailed information about an individual’s inherited and/or acquired characteristics and their phenotypic data to select a preventative measure or medication that is particularly suited to that patient at the time of administration. This revolution in clinical care is predicated on the development and refinement of biomarkers, enabling disease prevention, and diagnosis and treatment of patients and populations (11). Biomarkers will be used before birth and throughout life. For example, a couple planning to have children could be tested for specific biomarkers to avoid haemolytic disease of the newborn (HDN) and some recessive diseases (carrier parents have a 25% chance of passing on the disease to the baby). Children with a family history of diabetes, heart disease or cancer may take a genetic test to adjust their lifestyle or consider preventive treatment. Therapeutic and prognostic biomarkers should be applied to all kinds of patients, especially cancer patients, to direct their treatment plans and predict the treatment outcomes. Within the foreseeable future, when the US$100 genome sequencer is developed, everybody would be able to have their whole-genome information on their ID card. In the first decade of the 21st century, the fast-growing application of omics technologies in translational research and clinical medicine have been witnessed. It has accelerated biomarker development, improved the accuracy for diagnosis/treatment, and advanced personalized medicine. One example is the application of omics in reproductive medicine, in particular in vitro fertilization (IVF) treatment, an assisted reproduction. A key step in assisted reproduction is the assessment of oocyte and embryo viability to determine the embryo(s) most likely to result in a pregnancy. Although conventional systems such as morphological characterization and cleavage rating have been successful in improving pregnancy rates, their precision is far from ideal (12,13). It was reported that two out of three IVF cycles fail to result in a pregnancy, and more than eight out of 10 embryos fail to implant (14). The presence of aneuploidy in embryos frequently causes failed implantation and pregnancy. In a recent study, CGH, a genomics approach, was used in assessment of embryo aneuploidy and achieved implantation and pregnancy rates of 68.9 and 82.2%, respectively (15). Alternatively, using microarray CGH (aCGH) and single nucleotide polymorphism microarray have the potential for further improvement in assessment of embryo aneuploidy at a higher resolution, as they can be used to detect more refined regions (less than megabases, or even less than kilobases of nucleotides) in any chromosome (16,17). Other omics have also been applied to assessing embryo viability, such as metabolomics (18,19), transcriptomics (20) and proteomics (21). These omics technologies present unique advantages as well as their own intrinsic limitations. However, a combined strategy of omics may enhance the thorough screening of gametes and embryos for their viability and reproductive potential. The applications of omics technologies in other medical fields are in different stages of development and ever expanding. It is envisioned that the biomarkers derived from those omics will realize their full potential before long in all fields of clinical medicine.

CONCLUSION:
Biomarkers have been widely used in clinical prevention, diagnostics, therapeutics, prognostics, clinical trials and drug development. With mapping of the human genome complete, rapid development of new technologies and the collaboration of different disciplines, biomarkers promise personalized medicine, though many challenges remain to be overcome.

REFERENCES: