Review Article

Biomarkers in Clinical Medicine: Perspective from a Clinical Biochemistry Laboratory

Basant Joshi[1], Sangeeta Singh[2], Sankha Simlai[3], Sumeru Samanta[4*]

[1]Assistant Professor, Dept. of Biochemistry, RMCH, Bareilly, Uttar Pradesh, India
[2]Associate Professor, Dept. of Biochemistry, SSJGIMSR, Almora, Uttarakhand, India
[3]Associate Professor, Dept. of Biochemistry, SIMC, Junwani, Chattisgarh, India
[4]Associate Professor, Dept. of Biochemistry, RMCH, Bareilly, Uttar Pradesh, India

Received: 30-11-2021 / Revised: 29-12-2021 / Accepted: 03-01-2022

Abstract

Biomarkers have been used in clinical medicine for decades. With the rise of genomics and other advances in molecular biology, biomarkers studies have entered a whole new era and hold promise for early diagnosis and effective treatment of many diseases. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to therapeutic intervention. There is increasing pressure to provide cost-effective healthcare based on "best practice." Consequently, new biomarkers are only likely to be introduced into routine clinical biochemistry departments if they are supported by a strong evidence base and if the results will improve patient management and outcome. This requires convincing evidence of the benefits of introducing the new test, ideally reflected in fewer hospital admissions, fewer additional investigations and/or fewer clinic visits. Carefully designed audit and cost-benefit studies in relevant patient groups must demonstrate that introducing the biomarker delivers an improved and more effective clinical pathway. From the laboratory perspective, preanalytical requirements must be thoroughly investigated at an early stage. Good stability of the biomarker in relevant physiological matrices is essential to avoid the need for special processing. This article will focus on how these biomarkers have been used in preventive medicine-diagnosis therapeutics and prognostics as well as public health and their current status in d practice. This article also describes the major uses of biomarkers in clinical investigation. Careful assessment of the validity of biomarkers is required with respect to the stage of disease. Causes of variability in the measurement of biomarkers range from the individual laboratory. Issues that affect the analysis of biomarkers are discussed along recommendations on how to deal with bias and confounding.

Key Words:-Biomarkers, Variability & Validity

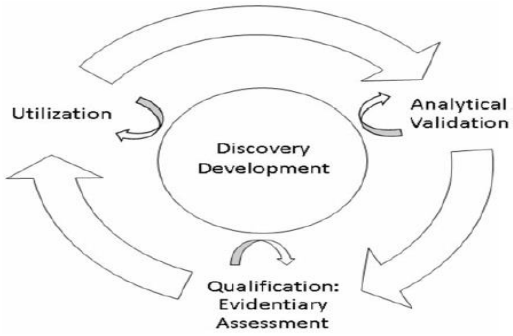
Introduction

The strengthening of the robustness of discovery technologies, particularly in genomics, proteomics and metabolomics, has been followed by intense discussions on establishing well-defined evaluation procedures for the identified biomarker to ultimately allow the clinical validation and then the clinical use of some of these biomarkers[1].

Biomarkers are critical to the rational development of drugs and medical devices. But despite their tremendous value, there is significant confusion about the fundamental definitions and concepts involved in their use in research and clinical practice. Further, the complexity of biomarkers has been identified as a limitation to understanding chronic disease and nutrition[2,3].

Biomarker definitions recently established by the U.S. Food and Drug Administration and the National Institutes of Health as part of their joint Biomarkers, EndpointS, and other Tools (BEST) resource. These definitions are placed in context of their respective uses in patient care, clinical research, or therapeutic development. The basic definition of a biomarker is deceptively simple: "A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention"[4].

*Correspondence
Dr. Sumeru Samanta
Associate Professor, Dept. of Biochemistry, RMCH, Bareilly, Uttar Pradesh, India
E-mail: bjoshi86@gmail.com



Steps in the evaluation framework for biomarkers

Biological markers (biomarkers) have been defined as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids." More recently, the definition has been broadened to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. In practice, biomarkers include tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease[5].

This broad definition encompasses therapeutic interventions and can be derived from molecular, histologic, radiographic, or physiologic characteristics. For the sake of clarity, biomarkers should be distinct from direct measures of how a person feels, functions, or survives—a category of measure known as a clinical outcome assessment (COA). This difference between biomarkers and COAs is important, because

COAs measure outcomes that are directly important to the patients and can be used to meet standards for regulatory approval of therapeutics, whereas biomarkers serve a variety of purposes, one of which is to link a measurement to a prediction of COAs[4].

Steps in the evaluation framework for biomarkers

A number of subtypes of biomarkers have been defined according to their putative applications. Importantly, a single biomarker may meet multiple criteria for different uses, but it is important to develop evidence for each definition. Thus, while definitions may overlap, they also have clear distinguishing features that specify particular uses.

The ability of biomarkers to improve treatment and reduce healthcare costs is potentially greater than in any other area of current medical research. For example, the American Society of Clinical Oncology estimates that routinely testing people with colon cancer for mutations in the K-RAS oncogene would save at least US $600 million a year. On the other side, thousand of papers in the course of biomarker discovery projects have been written, but only few clinically useful biomarkers have been successful validated for routine clinical practice[6].

The following are the major pitfalls in the translation from biomarker discovery to clinical utility:

1. Lack of making different selections before initiating the discovery phase.
2. Lack in biomarker characterization/validation strategies.
3. Robustness of analysis techniques used in clinical trials.

The Biomarkers and Surrogate End Point Working Group (7) has defined a classification system that can be used for biomarkers (8).

1. Type 0 consists of disease natural history biomarkers that correlate with clinical indices;
2. Type I tracks the effects of intervention associated with drug mechanism of action;
3. Type II consists of surrogate end points that predict clinical benefit.

Types of biomarkers

Susceptibility/risk biomarkers

A biomarker that indicates the potential for developing a disease or medical condition in an individual who does not currently have clinically apparent disease or the medical condition is classified as a susceptibility/risk biomarker. The concept is similar to prognostic biomarkers, except that the key issue is the association with the development of a disease rather than prognosis after one already has the diagnosis. These types of biomarkers are foundational for the conduct of epidemiological studies about risk of disease.

Diagnostic biomarkers

A diagnostic biomarker detects or confirms the presence of a disease or condition of interest, or identifies an individual with a subtype of the disease. Such biomarkers may be used not only to identify people with a disease, but to redefine the classification of the disease. For example, the detection of cancer is moving rapidly toward a molecular and imaging-based classification rather than a largely organ-based classification scheme[3].

Monitoring biomarkers

When a biomarker can be measured serially to assess the status of a disease or medical condition for evidence of exposure to a medical product or environmental agent, or to detect an effect of a medical product or biological agent, it is a monitoring biomarker. Monitoring is a broad concept, so there is overlap with other categories of biomarkers.

Monitoring biomarkers have important applications in clinical care. When blood pressure is treated or low-density lipoprotein (LDL) cholesterol-lowering drugs are used, blood pressure or LDL cholesterol levels are monitored. Similarly, when HIV infection is treated, CD4 counts are monitored[9].

Monitoring biomarkers are also important in ensuring the safety of human research participants. For example, the safety threshold for drugs with possible liver toxicity is monitored through serial measurement of liver function tests, and cardiovascular events are measured through the use of serial troponins. Monitoring biomarkers are also useful for measuring pharmacodynamic effects, to detect early evidence of a therapeutic response, and to detect complications of a disease or therapy. International normalized ratio (INR) is a classical pharmacodynamic measure used to titrate the dose of warfarin anticoagulation. Similarly, when blood pressure is treated, a reduction in the measure of blood pressure provides evidence that the therapy is working[10].

Prognostic biomarkers

A prognostic biomarker is used to identify the likelihood of a clinical event, disease recurrence, or disease progression in patients with a disease or medical condition of interest. Although this distinction is not uniformly accepted, the BEST working groups concluded that prognostic biomarkers should be differentiated from susceptibility/risk biomarkers, which deal with association with the transition from healthy state to disease. Furthermore, they are distinguished from predictive biomarkers, which identify factors associated with the effect of intervention or exposure.

Predictive biomarkers

A predictive biomarker is defined by the finding that the presence or change in the biomarker predicts an individual or group of individuals more likely to experience a favorable or unfavorable effect (loin the

exposure to a medical product or environmental agent. Proving that a biomarker is useful for this purpose requires a rigorous approach to clinical studies. Ideally. patients with or without the biomarker are randomized to one of two or more treatments (or is placebo comparator) and differences in outcome as function of treatment are significantly related to the difference in presence, absence, or level of the biomarker. Proof of a reliable predictive biomarker thus represents a "high hurdle" to clear.

Predictive biomarkers arc important for enrichment strategies in the design and conduct of clinical trials. Especially in the pre-registration phase of development, focusing enrollment on participants with elevated levels of a predictive biomarker enables a clearer signal that the treatment actually has an effect by enrolling people in whom the treatment is likely to "work: Using predictive biomarkers for enrichment is a more targeted approach than using prognostic biomarkers, which can be used to increase event rates but not to select specific patients who are more likely to respond or not respond to therapy.

Pharmaco dynamic/response biomarkers

When the level of a biomarker changes in response to exposure to a medical product or an environmental agent, it can be called a pharmacodynamic/response biomarker. This type of biomarker is extraordinarily useful both in clinical practice and early therapeutic development. If one is treating hypertension or diabetes and no reduction in blood pressure or glucose occurs with a therapy, there is good reason to eschew that intervention and pursue another. Similarly, a candidate drug for a condition that does not alter the key parameter of that biomarker in phase I trials would hardly be worth pursuing. A special circumstance is phase I studies of normal individuals. It would be unexpected for a disease-related biomarker to show a major change (for example, blood pressure) in persons with normal baseline values. In this circumstance, the main focus is on developing preliminary evidence that the drug will be safe to use in individuals with the target disease. For many drugs, dosing is determined by measured change in a phannacodynamic/ response biomarker when a therapy is given.

Safety biomarkers

A safety biomarker is measured before or after an exposure to a medical intervention or environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse event. For many therapies, monitoring for hepatic, renal, or cardiovascular toxicity is critical to assuring that a given therapy can be safely sustained. Safety biomarkers arc useful for identifying patients who are experiencing adverse effects from a treatment. When antiarrhythmic drugs are prescribed, prolongation of the QT interval on the electrocardiogram is used as a safety biomarker because it predicts the risk of developing the lethal arrhythmia torsades de pointes and can be used to identify patients in need of countermeasures for effective therapy[11].

BEST (Biomarkers, EndpointS, and other Tools) Classification: Treatment-focused biomarkers[12]

Susceptibility / risk biomarker	**Examples:-** • BMI or 2 hr post-meal glucose for diabetes risk • Apo E genotype risk for Alzheimer's disease **Key uses**: • Define population for more efficient prevention trials
Diagnostic biomarker:	**Examples:** • Blood pressure in hypertension • FEV I for COPD **Key uses:** • Define disease population for study
Monitoring biomarker:	**Examples:** • HCV-RNA • PSA in prostate cancer **Key uses**: • Monitor patient status in trials
Prognostic biomarker:	**Examples:** • Gleason score in prostate cancer • Total kidney volume in AD-PCKD **Key uses**: • Define higher risk disease population, enhancing trial efficiency
Predictive biomarker:	**Examples:** • Cystic fibrosis genotypes response to ivacaftor • Microsatellite-high predicts response to pembrolizumab **Key uses:** • Trial enrichment - improves efficiency, reduces sample size, increases response to treatment
Predictive biomarker:	Examples: • Cystic fibrosis genotypes response to ivacaftor • Microsatellite-high predicts response to pembrolizumab **Key uses:** • Trial enrichment -improves efficiency. reduces sample size, increases response to treatment
Pharmacodynamic/Response biomarker:	**Examples:** • Blood pressure in hypertension • FEV I or 6 minute walk test • LDL-C Key uses: • Demonstrating drug-target engagement, dose-ranging • Surrogate endpoints (validated or reasonably-likely)
Safety biomarker:	**Examples:** • ALT, creatinine/eGFR • Urinary kidney injury biomarkers (KIM-I, etc.) **Key uses:** • Detecting / assessing drug toxicity

Contributions of Valid Biomarkers to Clinical Research

- Delineation of events between exposure and disease
- Establishment of dose-response
- Identification of early events in the natural history
- Identification of mechanisms by which exposure and disease are related
- Reduction in misclassification of exposures or risk factors and disease
- Establishment of variability and effect modification
- Enhanced individual and group risk assessments

Variability

Although biomarkers have numerous advantages, variability is a major concern. Variability applies regardless of whether the biomarker represents an exposure or effect modifier, a surrogate of the disease, or an indication of susceptibility. Interindividual variability can result from the amount of an external exposure or from the way a putative toxin is metabolized. For example, individuals exposed to the same chemical might differ in their ability (or inability) to metabolize the agent, or they may have experienced different types of exposures (in the field as compared with in the office). Intraindividual variability is usually related to laboratory errors or other conditions, or exposures unique to the individual. Group variability is also encountered, but this is often the desired outcome of a study.

While measurement error is always a concern with biomarkers, other important factors may explain individual or group variability. Some workers may always wear protective equipment whereas others may not. Interaction with other exposures, drugs, or effect modifiers can increase or decrease the effect of the biomarker under consideration as an exposure or as a measure of susceptibility. Variability can also be attributed to the effects of factors such as individual diet or other personal characteristics. The amount of dietary fat can influence the biological measurement of lipid-soluble vitamins as well as toxic chemicals. These individual factors must be considered by the investigator to fully establish the major causes of variability in these investigations[13].

Biomarker Validation

Validation is "a process to establish that the performance of a test, tool, or instrument is acceptable for its intended purpose"[14]. Internal validation establishes a biomarker's performance in the data in which the biomarker was developed and should be assessed by means of resampling methods, such as bootstrapping or cross-validation, to provide realistic expectations[15]. External validation establishes a biomarker's performance in a completely independent data set not used during development; it must be established using data from different time frames, institutions, or geographic regions which we discuss in subsequent paragraphs. Analytical validation and clinical validation are two distinct aspects of biomarker validation.

Analytical validation aims to establish the performance characteristics of a biomarker including sensitivity, specificity, accuracy, precision, interlaboratory reproducibility, and other relevant performance characteristics following a prespecified protocol. Clinical validation aims to establish an association between the biomarker and the end point of interest (i.e., clinical validity per Teutsch et at and to reveal the usefulness of the biomarker (i.e., clinical use per Teutsch et a/. [16]. Clinical validation relies on external validation and can be done by retrospective use of clinical trial data or by prospective clinical trials.

Precise numbers are enticing, but they are prone to the same problems as any variable. Reliability, validity, sensitivity, specificity, ascertainment bias, and interpretation of data using biomarkers should be reviewed just as carefully as any other variable. These problems remain whether the biomarker is being used as a variable in a clinical trial or in an epidcmiologic study. Reliability or repeatability is crucial. Laboratory errors can lead to misclassification of exposures or disease if the biomarker is not reliable. Pilot studies should be performed to establish areasonable degree of reliability. Changes in laboratory personnel, laboratory methods, storage. and transport procedures may all affect the reliability of the biomarkers used in any investigation. Kappa statistics for binary or dichotomous data and intraclass correlation coefficients should be used to assess test--retest agreement and consistency. The evaluation of the validity of a biomarker is complex. Schulte and Perera suggest three aspects of measurement validity:

1. Content validity, which shows the deuce to which a biomarker reflects the biological phenomenon studied.
2. Construct validity, which pertains to other relevant characteristics of the disease or trait, for example other biomarkers or disease manifestations, and
3. Criterion validity, which shows the extent to which the biomarker correlates with the specific disease and is usually measured by sensitivity, specificity and predictive power.

Metrics Useful for Evaluating Biomarker Performance[17]

Metrics	Description
Sensitivity	The proportion of cases that test positive
Specificity	The proportion of controls that test negative
Positive predictive value	Proportion of test-positive patients who actually have the disease; is function of disease prevalence
Negative predictive value	Proportion of test-negative patients who truly do not have the disease; is a function of disease prevalence
Receiver operating characteristic (ROC) Curve	Plot of sensitivity (true positive rate) versus I specificity (false-positive rate), with a data point calculated for every value of the marker in the data set
Discrimination	How well the marker distinguishes cases from controls; often measured by the area under the ROC curve; ranges from 0 to 1, with 0.5 indicating performance equivalent to a coin flip and 1 corresponds to perfect ability to distinguish
Calibration	How well a marker estimates the risk of disease or of the event of interest

Advantages and Disadvantages of Biomarkers[13]

Advantages	Disadvantages
Objective assessment	Timing is critical
Precision of measurement	Expensive (costs for analyses)
Reliable; validity can be established	Storage (longevity of samples)
Less biased than questionnaires	Laboratory errors
Disease mechanisms often studied	Normal range difficult to establish
Homogeneity of risk or disease	Ethical responsibility

Transition if a new biomarker from research to routine

It is immediately apparent after even superficial review of the relevant literature that many more biomarkers arc identified than ever reach routine practice. For the relatively few that do so, the time frame is often years. The tumour marker now known as prostate-specific antigen (PSA) was Identified in 1970, but it was not until the late 1980s that the first definitive study investigating its clinical utility in prostate cancer was published and another decade later until establishment of the 1" International Standard for PSA. The example of PSA illustrates very well some of the challenges likely to be encountered during the introduction of a new diagnostic test into routine practice. The appropriate clinical application and interpretation of PSA measurements remain controversial even after many years of clinical use of this test[18].

Key points

1. Taking a new biomarker from the research laboratory into the routine clinical laboratory requires proactive three-way collaboration involving the research laboratory, the diagnostics industry and the clinical laboratory.
2. Some tests may be most appropriately offered in specialist laboratories.
3. Rigorous investigation of pre-analytical requirements of a new biomarker is essential at the earliest possible stage of evaluation.
4. Analytical performance must be documented in detail.
5. Well-documented evidence of clinical utility and cost effectiveness in populations representative of those which will be encountered in routine practice is essential for a new biomarker.
6. Evidence is required of the additional diagnostic or predictive information provided by the biomarker when used together with or when replacing other clinical or biochemical tests, i.e. its likely beneficial effect on the patient pathway.
7. Appropriate regulatory requirements must be fulfilled.

Practical considerations and concerns

Pre-analytical considerations in the laboratory

Numerous different types of specimen-primarily blood and urine but also cerebrospinal or pancreatic fluids, semen, microbiological swabs and others - arrive at the laboratory reception desk, where are they sorted according to the test requirements for processing and storage. Specimens are usually bar coded during the booking-in process. At which time patient details and the tests required are entered into the laboratory computer. Increasingly, many of these processes are at least partially automated. Although specimens from within the hospital may be delivered by porters or through pneumatic tube systems from ward or clinic to the laboratory, those from other hospitals or general practice arrive by van and hence the delay from time of sampling to processing may exceed 16 h[19].

Analytical considerations in the laboratory

As a consequence of the high workload and perceived need for rapid turn-around time in routine clinical biochemistry laboratories, assay automation is essential for almost every test. Robust internal quality control procedures must also be in place. EQA is not likely to be available until a reasonable number of laboratories (often a minimum of ten) offer the test. In the absence of an EQA or proficiency testing scheme, informal exchange of samples among laboratories offering the test can provide some assurance that results are similar and may highlight potential difficulties at an early stage, when it is relatively easy to address them. Exchanging information about possible clinically relevant interferences and other aspects of best practice — including appropriate reference intervals, decision limits and interpretation — is also very helpful[20].

Post-analytical considerations in the laboratory

Appropriate reference interval data should be readily available from the laboratory together with clear guidance about clinical interpretation of results in relevant patient groups. This is particularly important for a newly introduced biomarker since clinical staff will not be familiar with the new test and its limitations. Laboratory staff can play a major role in collecting audit data required to assess test performance in routine clinical practice. Recording any unexpected or atypical results and discussing these at an early stage with clinical colleagues is also highly desirable. Effective clinical audit studies should also be conducted to evaluate whether introduction of the new test has met expectations and to identify any problems at an early stage.

Measurement errors

Imperfect measurement of the biomarker would naturally lead to deceased validity of the relation to the disease. However, there are numerous types of measurement errors other than those errors that occur in the laboratory. Problems with the collection equipment or in the transportation of specimens to the laboratory can affect the measurement of the biomarker. Improper storage of samples or changes in storage environment can also affect measurement of biomarkers. Technicians are the handlers of most specimens and so appropriate training of new personnel is essential. Finally, receipt and control errors such as in the transcription of identification numbers if done by hand can always be source of error. A well organized procedures manual outlining the details for documentation, storage, monitoring of specimens and maintaining records, can alleviate many of these issues. Most laboratories and large-scale studies institute a quality assurance and quality-control program to reduce measurement errors.

Bias

Bias occurs in any study including those with biomarkers. When biases occur without regard to the outcome, so-called non differential bias, the effects on the study are less serious but favor the null hypothesis of no association. Problems arise when availability of the biomarker is differentially related to either the disease or the exposure or when the specimen acquisition, storage measurement, or ascertainment procedures differ in those with the disease compared to those without the dice or outcome of interest. Differential biases to favor an association in either direction, which may not be the true relationship between the true relationship between the biomarker and the disease.

To reduce such biases, a high response rate from all cases and controls should be maintained and the investigators should have an objective review board review and monitor the conduct of the study, observing possible biases in subject participation or specimen ascertainment.

Confounding

The most important source of confounding is the failure to identify factors that may alter the measurement of the biomarker. These can be internal, such as the weight of the subject, or external, such as the batch of laboratory kits used. Individual properties of biomarkers should influence the choice and interpretation for its inclusion in any investigation. The effects of potential confounders such as age, gender, diet, and other metabolic factors should be investigated before initiating the investigation.

Cost

The choice of the biomarker for research should be guided by the scientific question and by the financial resources. Cost is always a concern. In a small clinical trial this may be important; if an epidemiologic study includes thousands of subjects the cost can be quite high unless the laboratory procedure is automated and relatively simple. In fact, for some investigations larger sample sizes can bring down the cost per subject. This generally implies that the biomarker is readily available and its inclusion in the study is feasible.

Conclusions

Many studies using biomarkers never achieve their full potential because of the failure to adhere to the same rules that would apply for

the use of variables that are not biological. The development of any biomarker should precede or go in parallel with the standard design of any epidemiological project or clinical trial. In forming the laboratory component, pilot studies must be completed to determine accuracy, reliability, interpretability, and feasibility.

Future Prospects

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.

New biomarkers can be taken from research into routine practice provided there is sound evidence of clinical utility, funding can be assured, mechanisms are in place to ensure that the test is done only for those likely to benefit, analytical procedures are simple and robust, and quality is verified through internal quality control and EQA/proficiency testing procedures. For these requirements to be met in a timely manner for a specific biomarker, it is necessary to learn from past mistakes and perhaps to think differently in the future[21].

Acknowledgement

I thank all the participants of this review, who welcomed, assisted, and provided me with the information that I needed and without whose cooperation this review work would not have been successful. I express my sincere gratitude to Department of Medical Education for their valuable information and support.

References

1. Javle M, Hsueh CT: Recent advances in gastrointestinal oncology—updates and insights from the 2009 annual meeting of the American society of clinical oncology. J Hematol Oncol 2010 2009, 23(3):11–23.
2. Robb MA, McInnes PM, Califf RM. Biomarkers and surrogate endpoints: developing common terminology and definitions. JAMA 2016;315:1107–8.
3. Institute of Medicine. Evaluation of biomarkers and surrogate endpoints in chronic disease. Washington, D.C.: National Academies Press, 2010, www.nationalacademies.org/hmd/Reports/2010/Evaluation-of-Biomarkers-and-Surrogate-Endpoints-in-Chronic-Disease.aspx (accessed 22 September 2017).
4. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD): Food and Drug Administration (US); Bethesda (MD): National Institutes of Health (US), www.ncbi.nlm.nih.gov/books/NBK326791/ (2016, accessed 22 September 2017).
5. Naylor S. Biomarkers: current perspectives and future prospects. *Expert Rev Mol Diagn* 3:525–529, 2003.
6. Poste G: Bring on the biomarkers. Nature 2011, 469:156–157.
7. Atkinson AJ, Colburn WA, DeGruttola VG: Biomarkers and surrogate end points: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001, 69(3):89–95.
8. Frank R, Hargreaves R: Clinical biomarkers in drug discovery and development. Nat Rev Drug Discov 2003, 2(7):566–580.
9. The SPRINT Research Group,Wright JT Jr,Williamson JD, Whelton PK, Snyder JK, Sink KM, Rocco MV, Reboussin DM, Rahman M, Oparil S, Lewis CE, Kimmel PL, Johnson KC, Goff DC Jr, Fine LJ, Cutler JA, Cushman WC, Cheung AK, Ambrosius WT. A randomized trial of intensive versus standard blood-pressure control. N Engl J Med 2015;373:2103–16.
10. Writing C, Lloyd-Jones DM, Morris PB, Ballantyne CM, Birtcher KK, Daly DD, Jr, DePalma SM, Minissian MB, Orringer CE, Smith SC. Jr., 2017 Focused update of the 2016 ACC expert consensus decision pathway on the role of non-statin therapies for LDL-cholesterol lowering in the management of atherosclerotic cardiovascular disease risk: a report of the American College Of Cardiology task force on expert consensus decision pathways. J Am Coll Cardiol 2017;70: 1785–1822.
11. Sager PT, Gintant G, Turner JR, Pettit S, Stockbridge N. Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium. Am Heart J 2014;167:292–300.
12. http://www.ncbi.nlm.nih.gov/books/NBK326791/
13. Richard Mayeux, Biomarkers: Potential Uses and Limitations. *The Journal of the American Society for Experimental NeuroTherapeutics. Vol. 1, No. 2, 2004,182-188.*
14. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and Other Tools). Resource. Silver Spring, MD: Food and Drug Administration; 2016.
15. Teutsch SM, Bradley LA, Palomaki GE, et al. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative: methods of the EGAPP working group.Genet Med. 2009;11:3–14.
16. Harrell FE Jr. Regression Modeling Strategies With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis. 2nd ed. Berlin, Switzerland: Springer; 2015.
17. Fang-Shu Ou *et al.* Biomarker Discovery and Validation: Statistical Considerations. Journal of Thoracic Oncology Vol. 16 No. 4: 537-545.
18. Ablin, R. J., Soanes, W. A., Bronson, P., Witebsky, E., Precipitating antigens of the normal human prostate. *J. Reprod. Fertil.* 1970, *22*, 573–574.
19. Hutton, H. D., Drummond, H. S., Fryer, A. A., The rise and fall of C-reactive protein: managing demand within clinical biochemistry. *Ann. Clin. Biochem.* 2009, *46*, 155–158.
20. Khatami, Z., Hill, R., Sturgeon, C. M., Kearney, E. *et al.*, Measurement verification in the clinical laboratory [Association for Clinical Biochemistry]. UK Association for Clinical Biochemistry. 2005, http://www.acb.org.uk/An%20ver/Measurement%20verification_final_090608.pdf.
21. Rajappan, K., Murphy, E., Amber, V., Meakin, F. *et al.*, Usage of troponin in the real world: a lesson for the introduction of biochemical assays. *Q. J. Med.* 2005, *98*, 337–342.