Striving towards excellence in research on biomarkers

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Risks are common in all walks of life, especially in the finance market, wherein the risk relates to the odds of losing the money, whereas in the perioperative and health sector, risk entails compromising the safety of the patient, and thereby enhancing the morbidity and mortality. The finance sector has seen wide application of algorithmic scoring methods to prevent risk and to optimise decisions.[1] Similarly, risk assessment tools (RATs) such as risk scores and risk prediction models (RPMs) have made their mark in perioperative medicine and critical care. A number of RPMs have been formulated, and perioperative risk prediction has now become an important component of the enhanced recovery after surgery (ERAS) pathway and a significant strategy to improve the perioperative quality of care.[2]

Ever since the advent of modern medicine, biomarkers have played the role of catalysts in achieving rapid progress in research methods. They are recognised as important research and clinical tools in the diagnosis and identification of severity, recurrence and progression of disease, for the development of drugs, and in the evaluation of therapeutic interventions.[3] They are used clinically either alone or sometimes along with clinical variables for the early diagnosis of conditions like sepsis in the critical care unit, as a component of RPMs for risk stratification in order to predict perioperative mortality and morbidity, to evaluate a patient's clinical course and also aid clinical decisions in the intensive care unit (ICU) by serial measurements.[4] The journey of biomarkers has been long, starting from the oldest biomarkers, namely, physiological parameters like arterial pulse and diastolic blood pressure, through the molecular biomarkers such as liver enzymes and blood glucose, imaging and histological characteristics, to the present-day biomarkers including the epigenetic signatures. Research has been carried out on various types of biomarkers including the susceptibility biomarkers, prognostic biomarkers, diagnostic biomarkers, monitoring biomarkers, response biomarkers, safety biomarkers, predictive biomarkers, composite biomarkers, omics-based biomarkers, liquid biomarkers and so on.[5] Articles related to biomarker research and RATs keep getting published, and cardiothoracic surgery has been a favourite of researchers for studies on RPMs.[6-9] However, the research interest outside this field is growing steadily, as is witnessed by three articles on risk tools in non-cardiac surgery being published in this issue of the Indian Journal of Anaesthesia (IJA).[10-12] It is exigent to make biomarker studies look more authentic and dominating in the pathway to research. Going by the evidence available from the current literature, how many such studies have really succeeded in igniting the interest of the readers and the clinicians? The popularity and citations of such studies as clinical research–related articles can be questioned anytime! Nevertheless, monetary investments and support

for biomarker research in the form of funding and research grants by biomarker consortiums, biomarker qualification programmes, the Indian Council of Medical Research (ICMR) and universities continue to pour in. Till date, biomarker researchers have not invoked such an enthusiasm even after doing quality research, as it seems that publishing of the research manuscript is more important than conveying the right message to the readers. Should this pursuit not be extended for better goals, so as to produce clinically useful biomarkers and RPMs? For this to be accomplished, it is quintessential for researchers indulging in this area of research to be well versed with the intricacies of biomarker research and RPMs and to know what drives a biomarker from the research bench to the corridors of clinical medicine.

BIOMARKER RESEARCH AND RESEARCH ON RATs

A basic understanding of the pathophysiological mechanisms involved in biomarker synthesis, its kinetic properties including its metabolism and elimination, and physiological effects can help in designing better biomarker-related studies, for example, the information as to when the tissue injury and resultant release of the biomarker takes place can help to decide the time of biomarker measurement.[13] Standard operating procedures need to be in place and religiously followed when a biomarker is applied to a new situation; nevertheless, the diagnostic accuracy of the biomarker decreases at such times.[14] In biomarker research, surrogate end points are used as a substitute for clinical end points, but necessitate extensive and robust validation.[15] It is a well-known fact that the quality of validation of the biomarker decides its potential as a prognostic/diagnostic indicator and its clinical usefulness.[16]

However, a biomarker may not work in a particular population subgroup, though it may work well in a global population. Similarly, when developing a RPM, factors like objective of the model, data quality, predictors available, statistical methodology and outcomes need to be considered. One has to weigh the pros and cons of adopting a new model or considering an updation of the existing models. Identification of the predictors for the model is important and this can be done with the help of clinical knowledge and systematic reviews.[9] The translation of prognostic and diagnostic biomarker candidates to clinical application takes time, is a costly affair and failure is commonly encountered. It is an observation that

in some published studies on biomarkers, either the clinical application is not specified or it is defined in such a way that the cost-effectiveness and the commercial value cannot be determined. This limitation entails that such research will be confined to few affluent and self-sufficient research centres only, rather than the widespread dissemination of research throughout the globe. This implies that defining the clinical application in the development process immediately after discovery of the biomarker is very important.[17] Also, simply encouraging the discovery of novel biomarkers is not enough. Their further development including analytical validation, clinical validation and qualification is very important.[3] Furthermore, if an RPM has to be useful, it must possess adequate discrimination, calibration, face validity and should exhibit clinical usefulness. A clinician will apply the RPM in clinical practice, only after thoroughly understanding its advantages and limitations.[9]

STATISTICAL ISSUES AND PITFALLS IN BIOMARKER RESEARCH AND RPMs

Biomarker studies are often presented with meagre biostatistics, methodological errors, unreliable scientific justification and incorrect interpretation of the biomarker measurements. This can drastically bring down the reliability, reproducibility and ultimately the scientific credibility of the study findings.[5,13] The sample size estimation is often not done before or, unfortunately, not done correctly. Unlike the clinical research trials which mainly depend upon clinical end points, these studies should focus on the expected sensitivity and specificity of the biomarker because they are the most important variables that determine its diagnostic/prognostic value. Other variables that are presented in biomarker research are the positive predictive value, negative predictive value, diagnostic accuracy, Youden index, negative likelihood ratio (LHR$^-$), positive likelihood ratio (LHR$^+$), diagnostic odds ratio and so on.

Area under the receiver operating characteristic curve (AUC$_{ROC}$) (C-statistic) is an important measure of discrimination for the biomarker. Biomarkers with accuracy between 0.5 and 0.75, LHR$^+$ 1–5, LHR$^-$ 0.2–1 and AUC$_{ROC}$ between 0.5 and 0.75 are said to have poor diagnostic value and discriminative property. The interpretation of the AUC$_{ROC}$ and comparison of the receiver operating characteristic (ROC) curves have to be done carefully, and parametric methods,

and sometimes resampling methods, may have to be used. The method used to choose the optimal clinical cut-off point to make a clinical discrimination assumes a lot of significance, but it is unfortunately either not reported in the published study or reported without any confidence intervals (CIs). The AUC_{ROC} has poor clinical relevance because it may not be affected by many clinically important risk factors. ROC curves do not provide information about the actual proportion of participants with high/low risk values. Risk stratification tables may have to be used to evaluate risk prediction.[13] The study by Mirakbari *et al.*[10] in this issue of the IJA compares the prognostic efficacy of three different measurements in patients with severe intoxication in the ICU: two using physiological scoring systems (Simplified Acute Physiology Score [SAPS]-II and Acute Physiology and Chronic Health Evaluation [APACHE]-II score) and one using the laboratory concentration of the well-established, cardiotoxicity-indicating biomarker: cardiac troponin I. In this study, the AUC_{ROC} and 95% CIs were used to test the discriminatory capacity, and the cut-off values were determined by analysing the best Youden index and maximal AUC_{ROC}.

The statistical techniques that are used to develop a RPM include logistic regression, linear regression, Cox regression and machine learning. The Hosmer–Lemeshow test for logistic regression is often applied for RPMs to assess model calibration (how closely the predictions of the model match the observed outcomes in the data) and how well the data fits the model. However, it has several limitations, including being influenced by the sample size, number of gaps and providing no information on the magnitude and direction of miscalibration.[9] In the multicentric study by Deo *et al.*[11] being published in this issue of the IJA, seven preoperative variables have been identified in 770 patients with chronic kidney disease (CKD) and a risk stratification tool has been developed to help predict the risk of cardiac events in high-risk CKD patients undergoing non-cardiac surgery. Logistic regression has been used to develop the RPM, estimated probabilities have been compared using ROC to get C-statistic for discrimination, and the Hosmer-Lemeshow χ^2 test has been used to assess the calibration of the model.

Apart from that, the researcher has to make use of the Standards for Reporting Diagnostic Accuracy Studies (STARD) guidelines with extensive use of CIs for accurate reporting of biomarker studies and the Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) reporting guidelines for a complete reporting of studies on RPMs.[18,19]

IMPROVING THE BIOMARKER AND RPM-RELATED RESEARCH OUTPUT

Procalcitonin is the most commonly used biomarker in clinical practice, especially in critical care. Interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-α, C-reactive protein (CRP), N-terminal pro-brain natriuretic peptide (NT-proBNP) and pro-adrenomedullin (proADM) are few other biomarkers applied in perioperative and critical care as well as in emergency medicine.[20] Recently, several studies including metanalyses have been published on the biomarkers to predict the outcomes in coronavirus disease 2019 (COVID)-19 patients.[21] Several risk scores and models related to perioperative and critical care, such as the American Society of Anesthesiologists physical status (ASA-PS), APACHE-II score and sequential organ failure assessment (SOFA) score, just to name a few, are popular among both clinicians and researchers; however, each of these has its own limitations. Nonetheless, a letter to the editor in this issue discusses how to reduce inter-user variance in the ASA-PS classification.[12] Perioperative medicine is said to be very favourable for biomarker development because data can be collected here in a controlled and closely phenotyped manner.[22] The role of perioperative biomarkers in the identification of undiagnosed comorbidities, assessment of nutritional and coagulation status, diagnosis and monitoring of organ injury, response to drugs, neuroinflammation, cognitive dysfunction and the development of chronic pain is evolving.[22,23] Biological measures such as electrophysiology in peripheral nerves and brain, complex physiological biomarkers such as facial expression, vocal characteristics and body movements in Pain Medicine and point-of-care–derived, quantitative non-invasive biomarkers are also coming up.[16]

Apart from that, machine learning–driven risk models are already being used in financial institutions for fraud protection. They have now attracted attention in analysing large and complex healthcare data. Machine learning–based models including logistic regression, treebag, support vector machine, random forest, adaboost and the neural network model show high C-statistic values and have been used to produce prediction algorithms, especially in cardiovascular risk prediction.[24]

It is an observation that most of the times, RPMs focus on preoperative predictions of postoperative mortality, though in clinical reality, postoperative morbidity and related outcomes are more common. Hence, there is a need for researchers to focus on developing RATs in this direction. The integration of preclinical data to obtain a reliable biomarker that can be measured with acceptable costs in routine perioperative and critical care practice is needed.[25] Meanwhile, the support for research on rigorous biomarker development and validation is fast growing. This includes the Human Biomarker Biospecimen and Data repositories that bank and distribute human post-mortem brain tissue and other biospecimens, Resource Glossary to clarify the terminology and uses of biomarkers, funding programmes that support the discovery, development and qualification of biomarkers, and websites hosting statistical software applications and resources with elegant algorithms to assist biomarker research.[3]

The world of biomarkers and RATs thus offers a challenging, though ever-fertile soil for research and keeps opening its doors for research opportunities. The fields of perioperative medicine and critical care can be revolutionised with novel and reliable biomarkers and RATs. It is left to the researchers to identify the gaps in the knowledge of biomarkers and to design robust studies that can provide us with findings and models that can be useful in clinical decision-making. It is highly imperative that reviewers and editors should be well-versed with the details of biomarker and RAT research, so that they can classify well-conducted and accurately reported studies worthy of publication. The authorities in charge of allotting research grants should also keep in mind that biomarker research is costly and needs monetary support, and they should support only those studies that promote rigorous biomarker identification and validation. At the moment, we should aim at conducting, identifying and publishing robust studies on biomarkers and valid, well-calibrated biomarker-based RPMs.

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