ECRONICON OPEN ACCESS

EC GYNAECOLOGY
Review Article

The Development for Interpretation and Application of Biomarkers in Female Hormone Replacement Therapy (HRT)

Nicholas A Kerna[1,2]*, Hilary M Holets[3,4], Kevin D Pruitt[5,6], ND Victor Carsrud[7], Rashad Roberson[8], Jerome Adadzi[9], Shain Waugh[10], Raymond Nomel[11], Uzoamaka Nwokorie[12], Jamie Hujan[13] and John V Flores[3,4]

[1]*SMC–Medical Research, Thailand*

[2]*First InterHealth Group, Thailand*

[3]*Beverly Hills Wellness Surgical Institute, USA*

[4]*Orange Partners Surgicenter, USA*

[5]*Kemet Medical Consultants, USA*

[6]*PBJ Medical Associates, LLC, USA*

[7]*Lakeline Wellness Center, USA*

[8]*Georgetown American University, College of Medicine, Guyana*

[9]*Sam's Club Pharmacy, USA*

[10]*Fettle Path, USA*

[11]*All Saints University, College of Medicine, St. Vincent and the Grenadines*

[12]*University of Washington, USA*

[13]*Windsor University School of Medicine at St. Kitts and Nevis*

***Corresponding Author:** Nicholas A Kerna, (mailing address) POB47 Phatphong, Suriwongse Road, Bangkok, Thailand 10500. Contact: medpublab+drkerna@gmail.com

Received: September 30, 2021; **Published:** December 31, 2021

DOI: 10.31080/ecgy.2022.11.00706

Abstract

The research and application for treatment determination and monitoring of biomarkers in hormone replacement therapy is a comparably novel domain. Currently, regulations are limited and insufficient, but are evolving. Nevertheless, there is decisive anticipation of what future biomarker research will reveal and apply more specifically to negative symptoms and adverse disorders, particularly affecting females—with the end goal of improving and enhancing their quality of life.

Considering multiple biological outcomes, hormone replacement therapy (HRT) performs a vital role in determining treatment recommendations and regimes for many menopausal symptoms and conditions: cancer, diabetes, cognitive decline, osteoporosis, and autoimmune disorders.

Targeted treatments are challenging to achieve for some female hormone-related conditions and disorders. Nevertheless, physicians frequently order biomarker analyses. Conventional biomarkers, such as blood glucose, blood pressure, hormone levels, and mineral levels, can be identified in in-clinic labs. However, reference laboratories are typically utilized to develop biomarker panels, especially for rare or novel biomarker analysis, thus ensuring appropriate therapeutic regimes.

Biomarkers, measured in the body, influence or predict the incidence of outcome or disease. Biomarkers are more characterized explicitly as biomarkers of risk stratification, disease detection, diagnosis, prognosis, and predictive of a disease or disorder.

Biochemical markers comprise lipids, inflammatory (high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and leukocyte count), adipokines, endothelial (E-selectin, P-selectin, ICAM, and VCAM), glucose tolerance (fasting glucose, insulin, HOMA-IR, IGF-1, and metabolic syndrome), hemostatic (D-dimer, factor VIII, von Willebrand factor, homocysteine, fibrinogen, tissue factor pathway inhibitor, and acquired activated protein C resistance), matrix metalloproteinases, and sex steroid hormones (such as globulin-binding sex hormone).

Genetic markers cover factor V Leiden, glycoprotein IIIa leu33pro, gene variants (related to sex hormone biosynthesis, metabolism, and signaling), genome-wide association studies (GWAS), and exome sequencing (for gene discovery), gene variants (in the ABO blood group), and polymorphisms (of estrogen and progesterone receptors).

Various and distinct technologies are utilized to measure specific body fluids, cellular content, tissue-based composition, and other physiological measures; also, imaging techniques are employed. Biomarkers may be hormones, enzymes, glycoproteins, oncofetal antigens, receptors, or changes in tumors (such as mutations, amplifications, or translocations). Specific biomarkers are identified in special studies for the same or similar diseases or disorders.

Keywords: *Blood Panels; Biopsy; Hormones; Risk Stratification; Specimen; Validity*

Abbreviations

GWAS: Genome-Wide Association Studies; HRT: Hormone Replacement Therapy; PSA: prostate-specific antigen; WHI: Women's Health Initiative; WHO: World Health Organization

Introduction

Various signs and symptoms manifest during the progression of diseases and disorders. Biomarkers are molecules, characteristics, or genes by which these diseases or disorders can be better identified. These are measurements that assess biological processes (physiological, pathophysiological, and pharmacological). Clinical biomarkers are broad subcategories of clinical signs that can be used as objective indicators of health or disease processes. They can be measured reliably and accurately. A biomarker is also defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention by the National Institutes of Health Biomarkers Definitions Working Group in 1998 [1].

The International Program on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labour Organization, also defined a biomarker as any substance, structure, or process that can be measured in the body or its products that influence or predict the incidence or outcome of a disease [2].

From blood and pulse pressure to physical characteristics, such as chemistry, to more complex tests of tissues in the laboratory, are considered biomarkers. Modern laboratory science has made it possible to measure reproducibly and objectively quantifiable medical signs that can help medical professionals plan optimal care. Since the early 1990s, biomarkers have been used in the diagnosis of diseases. Since then, various biomarkers have been discovered to detect diseases, diagnose them, provide prognoses, predict treatment response, and monitor disease progression—classified as follows.

- **Biomarkers of risk stratification:** Measurement of risk factors associated with disease in patients; for example, smoking for lung cancer, a high-carbohydrate diet for diabetes.
- **Biomarkers for disease detection:** Used to detect diseases before symptoms manifest, when therapy is more likely to succeed. (e.g. blood pressure for hypertension).
- **Diagnostic biomarkers:** Used to detect the presence of diseases (e.g. glomerular filtration rate to identify patients with chronic kidney disease).
- **Prognostic biomarkers:** Provide information on general expected clinical outcomes, regardless of the selection of therapy or treatment (e.g. prostate-specific antigen (PSA) level at the time of a prostate cancer diagnosis).
- **Predictive biomarkers:** Evaluate the expected overall clinical outcome based on treatment decisions in only biomarker-defined patients; for example, a GFR mutation predicts an erlotinib response [3].

Measurement of biomarkers

A biomarker can be any biological indicator that can be measured. For example, biomarkers can be cellular or molecular (DNA, RNA, protein, and metabolites). They are measured from a tissue biopsy or a liquid biopsy (blood, urine, and saliva). As these molecules are sometimes released into the circulation, some biomarkers can be detected by blood-based assays, while others require tissue biopsies. Thus, several methods are employed to technologies measure fluid, cellular, tissue-based, imaging, and other physiological measures [4].

Biomarker characterization

A biomarker may be hormones, enzymes, glycoproteins, oncofetal antigens, receptors, or changes in tumors, such as mutations, amplifications, or translocations [5,6]. The ideal properties of biomarker characterization techniques should satisfy the following criteria (Table 1) [3].

Have a binary (present or absent) or quantifiable measure
Be adaptable to routine clinical practice, having the ability to promptly produce results (i.e. in a matter of days rather than weeks)
Be sensitive and specific
Be detected from easily accessible specimens

Table 1: *Ideal properties of biomarkers*

Establishing biomarkers in all areas of disease treatment and assessment begins with the discovery and validation of biomarkers. The evolutionary steps of biomarkers include discovery, assay development, analytical validation, clinical validation, and clinical implementation [7]. The process of biomarker development includes evaluating the sensitivity, sensibility, reliability, and precision of the assay (through optimization) in the laboratory [8]. Also, a thorough evaluation is necessary for aspects related to biomarker variabilities, such as relevant interactions, confounders, choice of surrogate tissue, acceptability of sampling, and protocol variability [9].

Biomarker discovery

Biomarker discoveries are primarily based on monitoring and measuring samples, such as body fluids related to disease or treatment mechanisms. It is also fundamental to determine which biological process, pathogen, or pharmacological response should be studied. Different types of biomarkers are identified in distinct studies for the same or similar diseases or disorders. Biomarkers for risk stratification and screening are identified in the target population and should be detected early in the development process [10].

Before conducting biomarker discovery studies, several factors must be considered, including the patient population, the number of samples and events, disease prevalence, analytical validity of the biomarker test, and a pre-planned analysis [11].

Identifying prognostic biomarkers requires well-conducted retrospective studies, such as case-control studies and single-arm trials with biospecimens taken from a cohort representing the target screening population [3]. An association between prognostic biomarkers and results is identified by the primary effect test [12]. Predictive biomarkers can be identified through secondary analyses of the results of randomized clinical trials, using an interaction test between treatment and biomarker [3].

Randomization and blinding studies are two of the most significant tools for avoiding bias in studies designed to discover biomarkers. To prevent batch effects, experiments must be performed to control for nonbiological effects due to changes in reagents, technician inconsistencies, and machine drift [13].

To ensure that cases and controls are distributed equally, random assignment of specimens from controls and cases into arrays, testing plates, or batches are recommended [14]. The process of generating biomarker data should be randomized and blinded whenever possible and be utilized at every study stage.

Biomarker validation

Validating each biomarker candidate is crucial before it can advance to clinical trials. Developing assays to monitor specific biomarkers requires high sensitivity and selectivity [15]. A biomarker's validity is based, in part, on epidemiologic studies to determine whether its

prevalence is correlated with the associated disease. In this case, the cohort approach helps avoid deviations caused by reverse causality—the effect of the disease on the marker) [16].

Biomarkers are internally validated against the data from which they were developed [17]. During external validation, the performance of a biomarker is verified against an independent test set. Thus, data from different periods, institutions, or regions must be used [3].

Furthermore, the verification of biomarkers involves both analytical and clinical validation. Analytical validation of biomarkers involves precise and accurate measurement, while clinical validation concerns interpreting measurements for a particular disease [18]. A vital quality of all validation studies is collecting specimens from prospectively selected individuals who are unaware of any previous results.

Analytical validation of biomarkers

Through analytical validation, the performance characteristics of a biomarker are evaluated, such as its sensitivity, specificity, precision, reproducibility, and reproducibility between laboratories [19]. Biomarkers are tested to determine their technical performance rather than their value. In analytical validation studies, the hypotheses tested and the success criteria must be defined. It is essential to control multiple analogs when evaluating multiple biomarkers, considering both the sample size and clinical context when determining how to combine them best [20].

Clinical validation of biomarkers

Clinical validation of biomarkers establishes an association between the biomarker and clinical validity, indicating the clinical usefulness of the biomarker (sensitivity, specificity, and accuracy) [21]. Validation of biomarkers clinically relies on external validation, which can be performed retrospectively or prospectively using trial data. Using a biomarker to guide patient care will lead to improved health outcomes only if a prospective clinical trial, a form of external validation, is conducted to demonstrate its clinical usefulness. It is possible to perform a retrospective, blinded screening, diagnostic, and prognostic biomarker evaluation of collected samples [22].

Clinical application of biomarkers

Clinical biomarkers that indicate each stage of a disease over time can be longitudinally tested in a well-defined patient population compared to gold-standard clinical indicators [23]. An intervention biomarker must be validated simultaneously with a candidate therapeutic drug, for example, to assess its interventional effect [23].

To be relevant to both the drug mechanism of action and pathophysiology of the disease, surrogate endpoint biomarkers must reflect both [23]. Protein and expression arrays, high-throughput immunoassays, and epidemiological and statistical analyses are essential tools and technologies needed for such validation [23].

Physician's consideration of biomarkers for treatment decision-making

A recent approach to the treatment of disease is more specifically determining those patients who are likely to benefit. Biomarkers provide significant determinants in identfying those patients likely to benefit from treatment [24]. In the coming years, molecular subtyping will play an increasingly compelling role in treatment decisions. To determine the most likely therapy to be effective, patients must understand that biomarker testing may provide vital information. The effectiveness of targeted therapy varies depending on the disease conditions, but currently, only a tenth of patients experience benefits.

Targeted therapies are complicated to accomplish, but testing is increasing as physicians increasingly request biomarker analysis. Common biomarkers, such as blood glucose, blood pressure, hormone level, and mineral level, can be identified in in-clinic laboratories. However, for the development of biomarker panels, reference laboratories may be preferred for rare or new biomarker analysis to ensure appropriate therapeutic regimes.

Discussion

Role of biomarkers for hormone replacement therapy (HRT) in women

Despite its complex biological effects, hormone replacement therapy (HRT) plays a vital role in treating vasomotor and other menopausal symptoms [25]. Several studies, including the Women's Health Initiative (WHI), suggest that some women may be more suitable for HRT than others, depending on their clinical and biological characteristics [26].

Recent studies suggest that it may be possible to identify women with biomarkers, indicating that they might experience fewer adverse events. For example, lower LDL cholesterol and lower LDL/HDL ratios are strongly predictive of more favorable cardiovascular disease outcomes with HRT [27].

Other biomarkers predictive of the risk of cardiovascular disease—reported in WHI—include increased interleukin 6, matrix metalloproteinase 9, D-dimer, factor VIII, von Willebrand factor, leukocyte count, homocysteine, and fasting insulin [28].

In addition to biomarkers already being developed to assess the risks of cancer, diabetes, cognitive decline, and autoimmune disorders, various and distinct biomarkers are being investigated—some of them are listed in Table 2. The effectiveness of these biomarkers will be determined in the future.

Biochemical Markers
• Lipids: serum LDL cholesterol, LDL/HDL ratios, triglyceride levels, Lp (a), 27-OH-cholesterol, and apolipoprotein levels
• Inflammatory markers: high-sensitivity C-reactive protein (hsCRP), interleukin-6, tumor necrosis factor-alpha, and leukocyte count
• Adipokines (adiponectin, leptin, and retinol-binding protein-4 (RBP4)
• Endothelial P-selectin, ICAM, and VCAM
• Glucose tolerance markers: fasting glucose, insulin, HOMA-IR, IGF-1, and biomarkers of metabolic syndrome
• Matrix metalloproteinases
• Hemostatic markers: D-dimer, factor VIII, von Willebrand factor, homocysteine, fibrinogen, tissue factor pathway inhibitor, or acquired activated protein C resistance
• Sex hormone markers: steroid hormone levels and globulin-binding sex hormone level
Genetic Markers
• Factor V Leiden
• Glycoprotein IIIa leu33pro
• Gene variants related to sex hormone biosynthesis, metabolism, and signaling
• Genome-wide association studies (GWAS) and exome sequencing for gene discovery
• Gene variants in the ABO blood group
• Polymorphisms of estrogen and progesterone receptors

Table 2: *Selected biomarkers under consideration and investigation for HRT decision-making. Adapted from Manson, 2013 [25].*

Future of biomarkers: interpretation and application

Anticipation, yet uncertainty, surrounds the future of biomarkers. It is challenging to identify a single biomarker for a specific pathological condition out of a field of hundreds and thousands of factors that interact with each other, even though biomarkers are vital in many areas of medicine [29]. The complexity of data analysis results in challenges when only a single biomarker is associated with a specific disease. A closer look at the data and identifying correlations between biomarkers, associated diseases, and outcomes in patient populations over time may produce effective identification and results. Conditionally dependent biomarkers can be compounded into a composite score and might prove to be better indicators than single biomarkers.

Those researchers and physicians actively pursuing the discovery of biomarkers are motivated by new developments in analytical technology and disease treatment, which keeps medical research striving to find the next "best" biomarker. However, the field is relatively new, and there are few regulations and industry standards in place to drive forthcoming validation and regulations.

The fact that biomarkers cannot be designed and applied in a one-size-fits-all manner makes establishing regulations more complicated than with more standardized bioanalyses. For the benefit of patients and the healthcare system at large, the scientific community must develop and implement uniform standards, guidelines, and protocols.

Innovations and technological advances drive the evolutions of biomarker research. In this field, technologies—such as multiplexed systems, ultrasensitive platforms, and miniaturized, automated systems—have proven invaluable [30]. Digital biomarkers have become an important tool to improve precision medicine and support clinical trials. Digital biomarkers are objective, quantifiable, physiological, and behavioral measures collected using digital devices that are portable, implantable, or digestible [31]. Health-related outcomes can be explained, influenced, or predicted using these specific and individualized health measures.

Specific technologies enable digital biomarkers to be measured longitudinally and continuously, while sharing the same clinical goals as traditional biomarkers. It is typically less invasive or non-invasive to measure digital biomarkers. Also, they are modular and cheaper, producing qualitative and quantitative measurements. The growing demand for data encourages the development of digital biomarkers that can provide information in real-time.

Biomarker research needs should address the challenge of integrating data from various interdisciplinary platforms rapidly and reliably [32,33]. It is currently unconfirmed whether a biomarker panel that includes genes/transcripts, proteins, and metabolites is relevant. Thus, it is crucial to establish composite panels' scientific and economic values, genes, and transcripts, compared to individual panels of genes, proteins, or metabolites. In the future, gene panels will be compared against protein panels and metabolite panels.

Conclusion

Biomarkers are substances, structures, or processes measured in the body, influencing or predicting the incidence of outcome or disease. Biomarkers are more categorized explicitly as biomarkers of risk stratification, biomarkers for disease detection, diagnostic biomarkers, prognostic biomarkers, and predictive biomarkers.

Various and distinct technologies are employed to measure specific body fluids, cellular contents, tissue-based compositions, and other physiological measures; also, imaging techniques are applied. A biomarker may be a hormone, enzyme, glycoprotein, oncofetal antigen, receptor, or tumor change (such as a mutation, amplification, or translocation). Different biomarkers are identified in particular studies for the same or similar diseases or disorders.

Prognostic biomarker identification requires well-designed and executed retrospective studies—such as case-control studies and single-arm trials-with biospecimens taken prospectively from a cohort representing the target screening group. Clinical validation of

biomarkers confirms an association between the biomarker and its clinical validity. The purpose is to determine the clinical usefulness of the biomarkers, such as their sensitivity, specificity, and accuracy.

Targeted therapies are challenging to fulfill. Thus, clincians are increasingly requesting biomarker analyses to aid in more efficacious therapy choices. Common biomarkers, such as blood glucose, blood pressure, hormone level, and mineral level, can be identified by in-office, in-clinic, or in-hospital laboratories. However, reference laboratories are typically used to generate biomarker panels, especially for rare or innovative biomarker analysis, thus assuring appropriate therapeutic regimes.

Despite its complex biological effects, hormone replacement therapy plays a vital role in determining treatment recommendations and regimes for many menopausal symptoms and conditions, such as cancer, diabetes, cognitive decline, and autoimmune disorders.

Biochemical markers include lipids, inflammatory (high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and leukocyte count), adipokines, endothelial (E-selectin, P-selectin, ICAM, and VCAM), glucose tolerance (fasting glucose, insulin, HOMA-IR, IGF-1, and metabolic syndrome), hemostatic (D-dimer, factor VIII, von Willebrand factor, homocysteine, fibrinogen, tissue factor pathway inhibitor, and acquired activated protein C resistance), matrix metalloproteinases, and sex steroid hormone levels (such as globulin-binding sex hormone).

Genetic markers include factor V Leiden, glycoprotein IIIa leu33pro, gene variants (related to sex hormone biosynthesis, metabolism, and signaling), genome-wide association studies (GWAS), and exome sequencing (for gene discovery), gene variants (in the ABO blood group), and polymorphisms (of estrogen and progesterone receptors).

The field of biomarker research, interpretation, and application is relatively recent. Currently, there are limited but emerging regulations. Nevertheless, there is a positive expectation of what future biomarker research will unveil and implement more specifically for troubling symptoms and disorders particularly affecting females—the end goal of improving their health and enhancing their quality of life.

Conflict of Interest Statement

The authors declare that this paper was written without any commercial or financial relationship that could be construed as a potential conflict of interest.

References

1. Biomarkers Definition Working Group. "Biomarkers and surrogate endpoints: preferred definitions and conceptual framework". *Clinical Pharmacology and Therapeutics* 69 (2001): 89-95. https://pubmed.ncbi.nlm.nih.gov/11240971/

2. WHO International Programme on Chemical Safety. Biomarkers in Risk Assessment: Validity and Validation (2001). https://apps.who.int/iris/handle/10665/42363

3. Ou FS., *et al*. "Biomarker Discovery and Validation: Statistical Considerations". *Journal of Thoracic Oncology* 16.4 (2021): 537-545. https://pubmed.ncbi.nlm.nih.gov/33545385/

4. Amaravadi L. "Biomarker measurements: how far have we come and where are we heading?" *Bioanalysis* 8.23 (2016): 2383-2386. https://www.future-science.com/doi/10.4155/bio-2016-4987

5. Duffy MJ. "Clinical use of tumor biomarkers: An overview". *Klinická Biochemie a Metabolismus* 25 (2017): 157-161. http://www.cskb.cz/res/file/KBM-pdf/2017/2017-4/KBM-2017-4-Duffy-157.pdf

6. Lech G., *et al*. "Colorectal cancer tumour markers and biomarkers: Recent therapeutic advances". *World Journal of Gastroenterology* 22 (2016): 1745-1755. https://pubmed.ncbi.nlm.nih.gov/26855534/

7. Goossens N., *et al*. "Cancer biomarker discovery and validation". *Cancer Research* 4.3 (2015): 256-269. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4511498/

8. Zerhouni E Medicine. "The NIH Roadmap". *Science* 302 (2003): 63-72. https://pubmed.ncbi.nlm.nih.gov/14526066/

9. Micheel CM and Ball JR. "The Biomarker Evaluation Process. In. Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease. Washington (DC): National Academies Press (US) (2010). https://www.ncbi.nlm.nih.gov/books/NBK220297/

10. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and Other Tools). Resource. Silver Spring, MD: Food and Drug Administration (2016). https://www.ncbi.nlm.nih.gov/books/NBK326791/

11. Simon RM., *et al*. "Use of archived specimens in evaluation of prognostic and predictive biomarkers". *Journal of the National Cancer Institute* 101 (2009): 1446-1452. https://pubmed.ncbi.nlm.nih.gov/19815849/

12. Pécuchet N., *et al*. "Different prognostic impact of STK11 mutations in nonsquamous non-small-cell lung cancer". *Oncotarget* 8 (2017): 23831-23840. https://pubmed.ncbi.nlm.nih.gov/26625312/

13. Leek JT., *et al*. "Tackling the widespread and critical impact of batch effects in high-throughput data". *Nature Reviews Genetics* 11 (2010): 733-739. https://www.nature.com/articles/nrg2825

14. Qin LX., *et al*. "Blocking and randomization to improve molecular biomarker discovery". *Clinical Cancer Research* 20 (2014): 3371-3378. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4079727/

15. Rifai N., *et al*. "Protein biomarker discovery and validation: the long and uncertain path to clinical utility". *Nature Biotechnology* 24 (2006): 971-983. https://www.nature.com/articles/nbt1235

16. McDermott JE., *et al*. "Challenges in biomarker discovery: combining expert insights with statistical analysis of complex omics data". *Expert Opinion on Medical Diagnostics* 7.1 (2013): 37-51. https://pubmed.ncbi.nlm.nih.gov/23335946/

17. Harrell FE Jr. "Regression modeling strategies with applications to linear models, logistic and ordinal regression, and survival analysis". 2nd edition. Berlin, Switzerland: Springer (2015). https://www.springer.com/gp/book/9783319194240

18. Hunter DJ., *et al*. "A Pathway and Approach to Biomarker Validation and Qualification for Osteoarthritis Clinical Trials". *Current Drug Targets* 11.5 (2010): 536-545. https://pubmed.ncbi.nlm.nih.gov/20199395/

19. Ohtsu Y., *et al*. "Analytical method validation for biomarkers as a drug development tool: points to consider". *Bioanalysis* 13.18 (2021): 1379-1389. https://www.future-science.com/doi/full/10.4155/bio-2021-0173

20. Storey JD and Tibshirani R. "Statistical significance for genome-wide studies". *Proceedings of the National Academy of Sciences of the United States of America* 100 (2003): 9440-9445. https://www.pnas.org/content/100/16/9440

21. Teutsch SM., *et al*. "The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative: methods of the EGAPP working group". *Genetics in Medicine* 11 (2009): 3-14. https://pubmed.ncbi.nlm.nih.gov/18813139/

22. Pepe MS., *et al*. "Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design". *Journal of the National Cancer Institute* 100 (2008): 1432-1438. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2567415/

23. Naylor S. "Biomarkers: current perspectives and future prospects". *Expert Review of Molecular Diagnostics* 3.5 (2003): 525-529. https://pubmed.ncbi.nlm.nih.gov/14510173/

24. Bossuyt PM and Parvin T. "Evaluating Biomarkers for Guiding Treatment Decisions". *The electronic Journal of the IFCC* 26.1 (2015): 63-70. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4975224/

25. Manson JE. "The role of personalized medicine in identifying appropriate candidates for menopausal estrogen therapy". *Metabolism* 62.1 (2013): S15-19. https://pubmed.ncbi.nlm.nih.gov/23018143/

26. Bassuk SS and Manson JE. "Menopausal hormone therapy and cardiovascular disease risk: utility of biomarkers and clinical factors for risk stratification". *Clinical Chemistry* 60.1 (2014): 68-77. https://pubmed.ncbi.nlm.nih.gov/24379312/

27. Bray PF., *et al*. "Usefulness of baseline lipids and C-reactive protein in women receiving menopausal hormone therapy as predictors of treatment-related coronary events". *The American Journal of Cardiology* 101 (2008): 1599-1605. https://pubmed.ncbi.nlm.nih.gov/18489937/

28. Rossouw JE., *et al*. "Inflammatory, lipid, thrombotic, and genetic markers of coronary heart disease risk in the Women's Health Initiative trials of hormone therapy". *Archives of Internal Medicine* 168 (2008): 2245-2253. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2726792/

29. Vincent JL., *et al*. "The Future of Biomarkers". *Critical Care Clinics* 36.1 (2020): 177-187. https://pubmed.ncbi.nlm.nih.gov/31733679/

30. Babrak LM., *et al*. "Traditional and Digital Biomarkers: Two Worlds Apart?" *Digit Biomark* 3.2 (2019): 92-102. https://www.karger.com/Article/Fulltext/502000

31. Dorsey ER. Digital biomarkers. Basel: Karger. https://www.karger.com/Journal/Home/271954

32. Poste G. "Bring on the biomarkers". *Nature* 469 (2011): 156-157. https://www.nature.com/articles/469156a

33. Elefsinioti A., *et al*. "Key factors for successful data integration in biomarker research". *Nature Reviews Drug Discovery* 15.6 (2016): 369-370. https://pubmed.ncbi.nlm.nih.gov/27173942/

Volume 11 Issue 1 January 2022