Journal of Molecular Medicine (2026) 104:36
https://doi.org/10.1007/s00109-026-02646-0

JMolMed

COMMENTARY

Bridging gaps in mitochondrial disease diagnosis: the role of advanced biomarker discovery

Tendai Makwikwi[1] · Maryke Schoonen[2] · Izelle Smuts[3] · Francois H. van der Westhuizen[1]

Received: 2 September 2025 / Revised: 24 December 2025 / Accepted: 16 January 2026

Abbreviations

MD(s)	Mitochondrial disease(s)
OXPHOS	Oxidative phosphorylation
LMICs	Low- to middle-income countries
mtDNA	Mitochondrial DNA
nDNA	Nuclear DNA
AI	Artificial intelligence
FGF-21	Fibroblast growth factor 21
GDF-15	Growth differentiation factor 15
PMD	Primary mitochondrial disease
FDA	Food and Drug Administration
EMA	European Medicines Agency

Background

Mitochondrial disease (MD) is associated with dysfunction of the oxidative phosphorylation (OXPHOS) system and represents one of the most frequently occurring inherited neuromuscular diseases [1]. Like many rare diseases, MD is characterised by striking clinical heterogeneity, resulting from its unique bi-genomic aetiology and multi-system involvement of energy-dependent tissues. Despite four decades of genetic discoveries and the advent of omics-driven insights into genes, mutations, and phenotypes, achieving an early and accurate diagnosis remains challenging—even within advanced diagnostic settings. A reliable genetic diagnosis for MD requires specialised clinical expertise capable of recognising population-specific phenotypes, providing access to genomic diagnostic services, and interpreting local genotype–phenotype correlations. However, these resources remain unevenly distributed, limiting diagnostic yield and equity. Research output and diagnostic infrastructure for MD are disproportionately concentrated in high-income countries [2]. This imbalance persists even though ~ 84% of the global population resides in low- and middle-income countries (LMICs), where access to MD diagnostics, research infrastructure, and specialised care remains limited [3]. Recent World Health Organisation (WHO) and International Classification of Diseases (ICD-11) initiatives have acknowledged these disparities, emphasising the need for improved diagnostic access, laboratory capacity, and data-sharing mechanisms [4–7]. Nevertheless, the diagnostic capacity divide remains substantial. A major contributor to the diagnostic gap in MD is the absence of reliable biomarkers that enable early detection, disease monitoring, and assessment of therapeutic efficacy. The lack of validated biomarkers restricts both diagnostic precision and therapeutic development.

The World Health Assembly's resolution WHA76.6, adopted in May 2023, titled "Strengthening diagnostics capacity and equitable access for rare diseases," serves as a pivotal framework for advancing the integration of rare disease diagnostics into national health strategies across member states. This resolution underscores the necessity for countries to prioritise sustainable financing and procurement mechanisms, which can significantly enhance diagnostic capabilities in the face of existing disparities, particularly in LMICs. A concrete implication of this resolution for national diagnostics plans is the establishment of streamlined pathways aimed at addressing the accessibility

✉ Tendai Makwikwi
tendaimakwikwi25@gmail.com

✉ Francois H. van der Westhuizen
Francois.vanderWesthuizen@nwu.ac.za

Maryke Schoonen
mschoonen28@nwu.ac.za

Izelle Smuts
izelle.smuts@up.ac.za

[1] Mitochondria Research Group, Biomedical and Molecular Metabolism Research (BioMMet), North-West University, Potchefstroom, South Africa

[2] Centre for Human Metabolomics, Desmond Tutu School of Medicine, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa

[3] Department of Paediatrics, Steve Biko Academic Hospital, University of Pretoria, Pretoria, South Africa

Published online: 31 January 2026

of diagnostic services and ensuring that they are inclusive of diverse populations.

Affordability of assays is crucial for achieving equitable healthcare access. Mechanisms to realise this affordability include pooled procurement strategies, tiered pricing, and the development of open-source assay protocols. Pooled procurement allows collective purchasing by multiple countries or institutions to negotiate lower prices from manufacturers, effectively enhancing access for resource-limited settings. Tiered pricing involves setting different price points based on a country's income level, which can mitigate financial barriers for LMICs. Additionally, open protocols promote the use of non-proprietary technologies, reducing dependency on high-cost commercial tests and fostering innovation in test development. Establishing regional reference laboratories can further enhance diagnostic capabilities by providing centralised access to quality testing and expertise, thereby supporting local health systems in managing rare diseases more effectively.

The integration of these mechanisms into national strategies aligns with the WHO's emphasis on closing the diagnostic gaps that disproportionately affect populations in LMICs and advancing global health equity. By leveraging these approaches, countries can not only improve their diagnostic capacity but also foster a more equitable healthcare landscape. This will ultimately facilitate earlier detection, better disease management, and improved therapeutic outcomes for patients suffering from rare conditions.

The alignment of national diagnostics strategies with global health initiatives and the establishment of cost-effective procurement mechanisms are essential steps toward addressing the healthcare disparities faced by patients with rare diseases in LMICs. As the landscape of rare disease diagnostics evolves, a commitment to affordability and accessibility will be crucial in achieving meaningful health equity.

Despite recent genomic advances, the lack of reliable, accessible biomarkers remains a critical bottleneck in MD diagnosis. An important consideration in biomarker development is the balance between invasiveness and clinical applicability—features that directly influence diagnostic feasibility, especially in resource-limited settings.

This paper thus aims to address the problem at hand of high diagnostic inequity in MDs, as a direct result of the lack of validated and affordable biomarkers. The solutions are addressed in terms of non-invasive biomarkers with the assistance and integration of artificial intelligence (AI), along with the different policy mechanisms to overcome limitations. To achieve this, however, rigorous validation is necessary to make biomarkers reliable. Furthermore, we discuss the socioeconomic impact on equity and why affordability matters.

The first section explores this "biomarker gap" and the implications of invasiveness versus non-invasiveness for translational research and diagnostic equity.

The biomarker gap—invasiveness versus non-invasiveness of biomarkers

The role and classification of biomarkers

Biomarkers have been widely defined as a characteristic measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention. In the quest to improve MD diagnosis, biomarkers remain a pivotal yet underdeveloped component [8]. Biomarkers can be grouped into seven functional categories (listed in Table 1) that reflect distinct clinical use cases. These distinctions are especially important in LMICs, where diagnostic pathways must optimise limited specialist access, minimise reliance on invasive procedures, and support longitudinal care despite constrained resources.

Case example: FGF-21 and GDF-15—strengths and limitations

Among current mitochondrial biomarkers, fibroblast growth factor 21 (FGF-21) and growth differentiation factor 15 (GDF-15) have demonstrated strong correlations with mitochondrial dysfunction and metabolic stress [9], providing valuable insights into mitochondrial activity and systemic energy metabolism. These biomarkers currently align most closely with diagnostic roles and, to a limited extent, disease monitoring; however, their broader application is constrained by several confounding factors, including renal dysfunction, systemic inflammation, age-related increases, and sensitivity to pre-analytical handling [10]. The key confounders and their implications are summarised in Table 2.

These limitations underscore the critical need for rigorous analytical validation (assessing precision, matrix effects, and stability) and clinical validation across diverse ancestries and comorbidities before widespread implementation, particularly in LMIC settings. Incorporating these considerations into biomarker deployment strategies will improve diagnostic accuracy and ensure equity in resource-limited environments.

The methods used for biomarker detection differ markedly in accessibility and feasibility, largely depending on whether they are invasive or non-invasive.

Invasive biomarkers—strengths and limitations

Invasive methods, such as muscle biopsies, have been the traditional mainstay for MD diagnosis. These allow direct

Table 1 Functional categories of biomarkers and relevance to LMICs

Category	Definition	Why it matters for LMICs	FGF-21/GDF-15 relevance as an example
Diagnostic	Early case detection identifies individuals likely to have the disease	Enables earlier triage and reduces dependence on muscle biopsy where surgical expertise is scarce	Primary role: Both are elevated in MD but lack perfect specificity
Disease monitoring	Tracks disease trajectory over time	Provides non-invasive follow-up where imaging or repeat biopsies are impractical	It may reflect changes over time, but is influenced by non-MD factors
Prognostic	Predicts the likelihood of progression or severity	Helps allocate limited resources and anticipate care needs	Currently no validated prognostic markers
Predictive/theragnostic	Guides therapy choice or predicts treatment benefit	Essential where treatments are costly or limited and must be targeted	No evidence supporting predictive/theragnostic use
Pharmaco-dynamic	Measures biological response to treatment	Enables lower-cost assessment of treatment efficacy where advanced functional assays are unavailable	No validated pharmacodynamic markers in MD
Safety	A measurable indicator that predicts, detects, or monitors the presence, likelihood, or extent of toxicity	Enable early disease detection, targeted treatment strategies, enhanced patient safety during drug development and treatment, and more efficient use of limited healthcare resources	Both are reliable non-invasive indices for PMDs, often outperforming traditional biomarkers like lactate
Risk	These markers help identify people with a higher susceptibility (to cancer), allowing for preventive measures or earlier intervention	Focus on developing affordable and simple risk assessment tools for early intervention	GDF15 is a predictive biomarker for cardiovascular risk and has anti-inflammatory effects

examination of mitochondrial function and morphology, providing high-fidelity diagnostic information [4]. However, the approach has drawbacks: patient discomfort, procedural risks, and limited feasibility in LMICs where access to necessary equipment and sterile environments is limited. These constraints can delay diagnosis, hinder treatment, and adversely affect patient outcomes. Invasive biomarkers therefore predominantly serve confirmatory diagnostic and mechanistic roles, which amplifies the need for reliable non-invasive alternatives in settings where biopsy is often unavailable.

Non-invasive biomarkers—opportunities and current barriers

Non-invasive techniques, such as analyses of blood, urine, serum, or imaging data, offer a promising alternative. These products can be used for (1) metabolite assays (measurements of lactate, pyruvate, and creatine kinase) [11, 12]; (2) molecular diagnostics (analysing mtDNA variants and heteroplasmy levels) [13]; (3) imaging techniques (magnetic resonance and near-infrared spectroscopy for indirect assessment of mitochondrial function) [14] and (4) liquid biopsies (evaluation of cell-free DNA, RNA, and proteins) [15]. Each of these applications underscores the critical role of non-invasive methods in advancing our understanding of mitochondrial health and function. Despite their advantages, these techniques and approaches can be expensive, technically demanding, and sometimes insufficiently validated.

Sensitivity and specificity vary widely across populations, and no single biomarker can fully capture the heterogeneity of MD. Therefore, while non-invasive testing reduces patient risk, its cost and interpretive complexity currently limit large-scale implementation in resource-limited settings.

The way forward—integrating innovation and equity

To close the biomarker gap, investment in capacity building, technology transfer, and context-appropriate validation is essential. We therefore propose three practical steps: (1) Provide access to and encourage participation in existing national and international training programmes to improve diagnostic capabilities in LMICs; (2) promote affordability through shared procurement and open-source assay development; and (3) prioritise funding and collaborative research for validating non-invasive biomarkers in diverse populations. Some experts advocate refining invasive procedures—such as minimally invasive biopsy methods. A hybrid diagnostic model, combining invasive confirmation with non-invasive screening, may offer the most pragmatic short-term solution. Emerging computational tools, including AI-driven analysis of imaging and omics data (e.g. the

Table 2 The key confounders and their implications, using the most common markers FGF-21 and GDF-15

Biomarker confounder	Effect on FGF-21/GDF-15	Implication for LMIC implementation
Renal dysfunction	Elevates circulating levels independent of mitochondrial disease	Requires careful interpretation; may reduce specificity in populations with high CKD prevalence
Systemic inflammation	Increases GDF-15 levels	Necessitates context-specific reference ranges
Age	Age-related increases reduce discriminatory power	Reference intervals must be stratified by age
Sample matrix and handling	Serum vs plasma, processing delays, freeze–thaw cycles	Standardised protocols needed; infrastructure limitations may affect reliability

smart mitochondrial assay), may incrementally improve sensitivity and specificity. However, their utility will depend on feasibility within LMICs—requiring low-compute approaches, robust external validation, and sustainable data pipelines [16].

The discussion of invasive and non-invasive biomarkers highlights both the progress made and the barriers that persist in MD diagnosis. To bridge this gap, the field is now turning toward computational biology and data integration. By combining AI-based analytics and machine learning with multi-omics data, researchers can move beyond isolated biomarkers toward a holistic understanding of mitochondrial dysfunction—one that captures the biological complexity and heterogeneity that define these disorders.

AI and multi-omics: need for specificity and feasibility

Opportunities in AI-driven biomarker discovery

The integration of AI and machine learning with multi-omics datasets offers tangible opportunities for improving biomarker discovery in MDs. Rather than solely exploratory applications, AI approaches are increasingly applied to specific, archetypal tasks in mitochondrial disease research, including classification (e.g. distinguishing MD vs non-MD samples), anomaly detection (e.g. identifying unusual metabolomic or proteomic signatures suggestive of mitochondrial dysfunction), and risk scoring (e.g. predicting likelihood of disease progression or severity based on integrated multi-omic profiles). Supervised algorithms classify samples based on known labels, while unsupervised approaches detect novel subgroups within heterogeneous datasets. Convolutional neural networks can identify structural mitochondrial abnormalities from histology or electron microscopy, complementing traditional diagnostic workflows [17].

These AI-based approaches demonstrate substantial potential to enhance diagnostic precision. By analysing complex multi-dimensional datasets—including genomics, transcriptomics, proteomics, metabolomics, and imaging—AI models can reveal subtle disease-specific patterns not detectable with traditional statistical approaches, potentially enhancing both specificity and sensitivity of MD diagnostics [10, 18].

Training AI right: sample size and demographic diversity

These models can classify disease subtypes, predict progression, and associate biomarker profiles with clinical phenotypes. Successful implementation relies on careful consideration of adequate sample sizes, albeit a single "adequate" number does not exist, and therefore, necessary training for AI models is recommended for medical deep learning—depending on the complexity of the task (as extensively reviewed by [19]. According to [20], diversity in AI models for disease diagnosis depends on key demographic factors, including (1) race and ethnicity—this can affect disease presentation and genetic risk factors; (2) sex and gender—differences between sexes can influence disease prevalence, symptoms, and outcomes; (3) age—a broad age range is preferable; and (4) geographic location—AI trained on data from a single location may not generalise effectively to other geographic areas, which can have different disease prevalences, environmental factors, or clinical practices.

Data quality, infrastructure, and global inequities

The successful integration of AI with biomarker discovery requires access to high-quality, multi-layered datasets—including genomic variants, metabolite concentrations, and clinical metadata—all integrated within standardised and well-annotated databases. Although this is the ideal, it could introduce common pitfalls as explained by [21]. Briefly, limited data diversity and biases in training datasets can reduce model accuracy and perpetuate inequities across patient groups. Additionally, the "black box" nature of many AI models complicates interpretability, making it difficult for clinicians to understand and trust their outputs. Security and privacy of sensitive patient data must be ensured, and AI systems must comply with applicable healthcare and data protection regulations.

AI fairness and validation

Carey et al. highlight the importance of fairness in AI when it comes to healthcare [22]. Briefly, most AI training data comes from high-income Western countries, leading to algorithms that perform poorly or fail when applied to different populations. This can result in inaccurate diagnoses for certain racial, ethnic, and socioeconomic groups. Furthermore, AI developed in wealthy countries can create a new form of digital and economic dependency, where LMICs rely on externally developed technologies that may not be adapted to local needs [23]. Addressing these disparities is essential to ensure that advances in AI-driven diagnostics benefit global patient populations rather than deepening existing inequities. Furthermore, it is essential to have external validation using metrics like sensitivity, specificity, and positive/negative predictive values to measure the accuracy and reliability of AI tools. Tsopra et al. have developed a seven-step framework specifically for validating AI in genomic medicine: (1) the intended use of AI, (2) the target population, (3) the timing of AI evaluation, (4) the datasets used for evaluation, (5) the procedures used for ensuring data safety, (6) the metrics used for measuring performance, and (7) the procedures used to ensure that the AI is explainable [24]. This suggested framework and outcomes should, however, be fully validated independently with a small dataset. The biomarkers as an outcome of using AI should be validated and comprehensively described in the following section.

Feasibility of AI in LMICs

The significant healthcare challenges across LMICs when considering and integrating AI as a diagnostic tool [25]. The main challenges highlighted were the lack of proper infrastructure, such as computational power, cloud storage, and internet access. Furthermore, they highlight the hesitancy of healthcare workers to move and adapt to AI-driven diagnostics. The study concluded that collaborative efforts among stakeholders, including international organisations, governments, and nongovernmental entities, are crucial for overcoming obstacles and responsibly integrating AI. In South Africa, progress has been made by organisations such as Diplomics (https://www.diplomics.org.za/, last accessed November 2025), which provides services to African countries to support patient diagnosis and identify biomarkers in specific pathways, including those within the mitochondrion. This organisation addresses the need for sustainable computing infrastructure and pipelines and has robust data governance measures in place to ensure data confidentiality and reliability.

Socioeconomic impacts of biomarker advancements

The utility of biomarkers, particularly regarding clinical outcomes, ultimately hinges on their ability to improve healthcare delivery. Evaluating the prospective impact on time to diagnosis, reduction in invasive procedures, and overall cost-effectiveness is essential in demonstrating the clinical utility of biomarkers. By delivering quicker diagnoses, validated biomarkers can minimise the economic burden associated with prolonged diagnostic journeys, particularly in LMICs, where access to specialised testing is often limited [26]. Implementing affordable biomarker strategies is imperative to advance health equity, facilitate timely interventions, and optimise healthcare resource utilisation in economically disadvantaged regions [26].

The societal impacts of biomarker advancements underscore the profound potential to enhance healthcare access, particularly in LMICs, where the need for efficient diagnostic strategies is pressing. Ultimately, the successful translation of biomarkers from discovery to clinical use will depend on their rigorous validation processes that guarantee their analytical reliability, clinical significance across diverse populations, and demonstrable utility in improving patient outcomes.

Biomarker validation

Biomarker validation encompasses several critical phases, notably analytical, clinical, and clinical utility validation, each addressing unique aspects that shape the robustness and applicability of biomarkers within clinical settings. In the analytical validation phase, emphasis is placed on precision, accuracy, limit of detection (LoD), and limit of quantification (LoQ) to assess the reliability of biomarker assays. Furthermore, aspects such as inter-site reproducibility, matrix effects, and sample stability during pre-analytical handling are essential to ensure that the biomarker behaves consistently across varied laboratory conditions and different biological matrices [27]. Robust analytical validation helps establish the limits within which the biomarker can reliably perform, ensuring that results are reproducible across diverse settings, which is critically important for global health initiatives [28].

In the subsequent clinical validation phase, establishing reference intervals stratified by age and sex is crucial in defining the contextual significance of biomarker levels within specific populations. The identification of cut-points for clinical decision-making, as well as external validation across various ancestries, helps ensure that findings apply to diverse populations despite differences in genetic

backgrounds [29]. Comorbidity sensitivity analyses, such as evaluating the impact of conditions like chronic kidney disease (CKD) on biomarkers like GDF-15, further enhance the clinical relevance of these markers, paving the way for more tailored diagnostic and therapeutic approaches [30].

AI has demonstrated significant promise in multi-omics applications, particularly in classification, anomaly detection, and disease risk scoring. However, leveraging AI effectively necessitates several fundamental considerations regarding data requirements and anticipated challenges. For instance, effective AI approaches typically require large sample sizes and high-quality labels to ensure accurate predictions. Small cohorts can lead to unreliable outcomes, and research has highlighted the variability in biomarker detection across different populations due to insufficient sample sizes [31]. Moreover, achieving high-quality labels is crucial, as the integrity of the data directly influences model performance [32]. Various pitfalls can arise during the development of AI-driven multi-omics solutions. Batch effects and domain shifts across populations can significantly distort data, as significant variability may be introduced by differences in sample handling or patient demographics, impairing model generalisation [31]. Overfitting is another prevalent concern; models trained excessively on specific datasets may fail to perform well on unseen data, reiterating the importance of external validation processes [33]. An effective validation framework must encompass analytical, clinical, and clinical utility validations to ensure reliability and applicability across different demographics. Analytical validation focuses on measuring the reliability of biomarker assays under controlled conditions, emphasising the need for stringent standardisation in sample collection and processing protocols, particularly critical in LMICs [34, 35].

The necessity for external validation is compounded in LMICs, where factors such as computing power, data governance, and resource availability can restrict the implementation of comprehensive AI frameworks. Deploying these technologies in resource-limited settings often requires adaptations, such as federated learning models that allow multiple institutions to collaborate without directly sharing sensitive data, addressing data governance challenges. Low-compute approaches can facilitate the development of less resource-intensive pipelines, promoting sustainability in genomic analytics [32]. Moreover, biomarker validations in these contexts must prioritise feasibility, assessing whether the biomarkers can meaningfully enhance diagnostic equity. In summary, while the integration of AI within multi-omics presents a transformative opportunity, particularly for developing reliable biomarkers, careful attention must be paid to specific tasks, data requirements, and common pitfalls. Establishing a rigorous validation framework that balances analytical precision, clinical relevance, and practical feasibility is essential to translating these advancements into actionable clinical diagnostics that bridge the existing healthcare divide in mitochondrial diseases and other conditions.

Conclusion

The discovery, validation, and implementation of biomarkers present both significant challenges and transformative opportunities for improving mitochondrial diseases diagnosis. New non-invasive biomarkers, supported by advances in multi-omics technologies and computational analytics, have the potential to significantly improve diagnostic accuracy and monitoring, particularly in situations where invasive procedures are impractical.

To maximise this potential, it is crucial to conduct thorough validation in diverse genetic and geographical populations, guided by internationally recognised standards and regulatory frameworks. Moreover, accessible biomarkers can help reduce the financial challenges associated with extended diagnostic processes, make better use of limited healthcare resources, and contribute to addressing global disparities in rare disease care. We encourage policymakers, funding bodies, and research organisations to prioritise the development of affordable biomarker platforms, promote collaborative networks for validation, and integrate non-invasive diagnostics into combined care models.

Ultimately, bridging the gaps in mitochondrial disease diagnosis requires a unified global effort—one that aligns science, infrastructure, and equity to harness the full power of biomarkers in improving patient outcomes worldwide.

Author contribution FHvdW, IS, MS, and TM conceived the idea. TM and MS were responsible for drafting the manuscript; FHvdW and IS contributed to the writing and critical revision of the manuscript. All authors read and approved the manuscript.

Funding Research supported by the National Research Foundation of South Africa (SRUG210321590571) and the South African Medical Research Council (SAMRC) contributed to the knowledge used in this publication. The opinions, findings, and conclusions or recommendations expressed are those of the authors alone, and the NRF and SAMRC accept no liability whatsoever in this regard.

Data availability Not applicable

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Wen H, Deng H, Li B, Chen J, Zhu J, Zhang X et al (2025) Mitochondrial diseases: from molecular mechanisms to therapeutic advances. Signal Transduct Target Ther 10(1):9
2. Guo X, Can C, Liu W, Wei Y, Yang X, Liu J et al (2023) Mitochondrial transfer in haematological malignancies. Biomark Res 11(1):89
3. Gervasoni J, Primiano A, Cicchinelli M, Santucci L, Servidei S, Urbani A et al (2024) Mitochondrial biomarkers in the omics era: a clinical-pathophysiological perspective. Int J Mol Sci 25(9):4855
4. Aymé S, Bellet B, Rath A (2015) Rare diseases in ICD-11: making rare diseases visible in health information systems through appropriate coding. Orphanet J Rare Dis 10:35
5. World Health Organization (2023) Global report on diagnostic equity and laboratory systems. WHO, Geneva
6. World Health Organization (2024) Advancing rare disease strategies: strengthening diagnostics capacity and access. WHO, Geneva
7. World Health Organization (2022) Global genomics and health initiative: building capacity for equitable access to diagnostics. WHO, Geneva
8. Liu Y, Zhang H, Dove W, Wang Z, Zhu Z, Pickhardt P et al (2023) Quantification of serum metabolites in early colorectal adenomas using isobaric labeling mass spectrometry. J Proteome Res 22(5):1483–1491
9. Schoonen M, Fassad M, Patel K, Bisschoff M, Vorster A, Makwikwi T, Human R, Lubbe E, Nonyane M, Vorster BC, Vandrovcova J et al (2025) Biallelic variants in RYR1 and STAC3 are predominant causes of King-Denborough Syndrome in an African cohort. Eur J Hum Genet 18:1–1
10. Shayota BJ (2024) Biomarkers of mitochondrial disorders. Neurotherapeutics 21:e00325
11. Chowdhury U, Avneesh S, Kapoor P, Narang R, Gharde P, Malik V et al (2016) Short-term prognostic value of perioperative coronary sinus-derived serum cardiac troponin, creatine kinase-MB, lactate, pyruvate, and lactate–pyruvate ratio in adult patients undergoing open heart surgery. Ann Card Anaesth 19(3):439–445
12. Nickel K, Menke M, Endres D, Runge K, Tucci S, Schumann A et al (2023) Altered markers of mitochondrial function in adults with autism spectrum disorder. Autism Res 16(11):2125–2138
13. Shaham O, Slate N, Goldberger O, Xu Q, Ramanathan A, Souza A et al (2010) A plasma signature of human mitochondrial disease revealed through metabolic profiling of spent media from cultured muscle cells. Proc Natl Acad Sci U S A 107(4):1571–1575
14. Lagerwaard B, Keijer J, McCully K, Boer V, Nieuwenhuizen A (2019) In vivo assessment of muscle mitochondrial function in healthy young males in relation to parameters of aerobic fitness. Eur J Appl Physiol 119(8):1799–1808
15. Chang S, Hur J, Choi Y, Lee C, Kim W (2020) Current status and future perspectives of liquid biopsy in non-small cell lung cancer. J Pathol Transl Med 54(3):204–212
16. Kuo CW, Chen HA, Hsu RH, Wu CS, Hsu C, Lee MJ et al (2025) Machine learning to predict mitochondrial diseases by phenotypes. Mitochondrion 84:102061
17. Ali H (2023) Artificial intelligence in multi-omics data integration: advancing precision medicine, biomarker discovery and genomic-driven disease interventions. Int J Sci Res Arch 8(1):1012–1030
18. Kuo YC, Yang CC, Tsai LK (2025M) Exploring CSF biomarkers in amyotrophic lateral sclerosis: highlighting the significance of TDP-43. J Neurol Sci 15(472):123479
19. Leeflang MMG, Allerberger F (2019) Sample size calculations for diagnostic studies. Clin Microbiol Infect 25(7):777–778
20. Ahluwalia M, Sehgal S, Lee G, Agu E, Kpodonu J (2025) Disparities in artificial intelligence–based tools among diverse minority populations: biases, barriers, and solutions. JACC Adv 4(5):101742
21. Nakagawa K, Moukheiber L, Celi LA, Patel M, Mahmood F, Gondim D, Hogarth M, Levenson R (2023) AI in pathology: what could possibly go wrong? Semin Diagn Pathol 40(2):100–108
22. Carey S, Pang A, de Kamps M (2024) Fairness in AI for healthcare. Future Healthc J 11(3):100177
23. Victor A (2025) Artificial intelligence in global health: an unfair future for health in sub-Saharan Africa? Health Aff (Millwood) 3(2):qxaf023
24. Tsopra R, Fernandez X, Luchinat C et al (2021) A framework for validating AI in precision medicine: considerations from the European ITFoC consortium. BMC Med Inform Decis Mak 21:274
25. Oduoye MO, Fatima E, Muzammil MA, Dave T, Irfan H, Fariha FN, Marbell A, Ubechu SC, Scott GY, Elebesunu EE (2024) Impacts of the advancement in artificial intelligence on laboratory medicine in low- and middle-income countries: challenges and recommendations—a literature review. Health Sci Rep 7(1):e1794
26. Yun T, Koo Y, Chae Y, Lee D, Kim H, Kim S et al (2021) Neurofilament light chain as a biomarker of meningoencephalitis of unknown etiology in dogs. J Vet Intern Med 35(4):1865–1872
27. Nallagangula K, Lakshmaiah V, Chandrappa M, Deepa K, Shashidhar K (2018) A proteomic approach of biomarker candidate discovery for alcoholic liver cirrhosis. J Circ Biomark 7:184945441878841
28. Dong X, Li L, Ye Y, Zhang D, Zheng L, Jiang Y et al (2019) Surrogate analyte-based quantification of main endocannabinoids in whole blood using liquid chromatography–tandem mass spectrometry. Biomed Chromatogr 33(3):e4423
29. Ou F, Michiels S, Shyr Y, Adjei A, Oberg A (2021) Biomarker discovery and validation: statistical considerations. J Thorac Oncol 16(4):537–545
30. Motta F, Pederzani A, Carena M, Ceribelli A, Wordsworth P, De Santis M et al (2021) MicroRNAs in axial spondyloarthritis: an overview of recent progress with a focus on ankylosing spondylitis and psoriatic arthritis. Curr Rheumatol Rep 23(8):62
31. Phat N, Tien N, Anh N, Yen N, Lee Y, Trinh H et al (2023) Alterations of lipid-related genes during anti-tuberculosis treatment: insights into host immune responses and potential transcriptional biomarkers. Front Immunol 14:1210403
32. Glaab E, Rauschenberger A, Banzi R, Gerardi C, Garcia P, Demotes-Mainard J (2021) Biomarker discovery studies for patient stratification using machine learning analysis of omics data: a scoping review. BMJ Open 11(12):e053674
33. Leung J, Chen V, Hollander Z, Dai D, Tebbutt S, Aaron S et al (2016) COPD exacerbation biomarkers validated using

multiple reaction monitoring mass spectrometry. PLoS ONE 11(8):e0161129
34. Canevelli M, Bacigalupo I, Gervasi G, Lacorte E, Massari M, Mayer F et al (2019) Methodological issues in the clinical validation of biomarkers for Alzheimer's disease: the paradigmatic example of CSF. Front Aging Neurosci 11:107
35. Therriault J, Servaes S, Tissot C, Rahmouni N, Ashton N, Benedet A et al (2023) Equivalence of plasma p-tau217 with cerebrospinal fluid in the diagnosis of Alzheimer's disease. Alzheimers Dement 19(11):4967–4977