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Abstract

Measuring the abundance of biological molecules and their chemical modifications in blood and tissues has been the cornerstone of research and medical diagnoses for decades. Although the number and variety of molecules that can be measured have expanded exponentially, the blood biomarkers routinely assessed in medical practice remain limited to a few dozen, which have not substantially changed over the last 30-40 years. The discovery of novel biomarkers would allow, for example, risk stratification or monitoring of disease progression or the effectiveness of treatments and interventions, improving clinical practice in myriad ways. In this review, we combine the biomarker discovery concept with geroscience. Geroscience bridges aging research and translation to clinical applications by combining the framework of medical gerontology with high-technology medical research. With the development of geroscience and the rise of blood biomarkers, there has been a paradigm shift from disease prevention and cure to promoting health and healthy aging. New -omic technologies have played a role in the development of blood biomarkers, including epigenetic, proteomic, metabolomic, and lipidomic markers, which have emerged as correlates or predictors of health status, from disease and exceptional health.

Geroscience and the rise of blood biomarkers

The shift of attention from disease prevention and cure to promotion of health and, especially, healthy aging represents a fundamental change of direction in medical research, with important implications for the future of medical practice. This transition has arisen from both conceptual and technological advances over the past 15 years. From a conceptual perspective, mounting evidence indicates that the phenotypic and functional manifestations associated with aging reflect biological processes that are both causal and consequences of chronic diseases highly prevalent in older individuals. The Geroscience Initiative takes this concept and posits that "since aging physiology plays a major role in many — if not all — chronic diseases, therapeutically addressing aging physiology will directly prevent the onset or mitigate the severity of multiple chronic diseases" [1]. This conceptualization of geroscience implies that diseases are not physiological anomalies that evolve through separate trajectories but rather a state of accelerated aging, and as such, it should be possible to define a metric of disease susceptibility that would place an individual on an evolving continuum of health, accumulation of pathology and frailty. This notion breaks from the traditional idea that the study of aging addresses changes that occur unavoidably in organisms independent of disease development and embraces an overarching approach for a mechanistic interpretation of all physiologic and functional changes over the lifespan. Indeed, the idea of a strong connection between aging and chronic disease is not a new formulation, as it emerged in the 1950s [2] when the concept of extending healthspan through aging intervention evolved [3, 4]. More recently, the concept has been formally stated [5, 6] offering Geroscience as an approach to investigating links between aging biology and susceptibility to aging-related chronic diseases [7]. The growth of the geroscience concept creates a bridge between aging research and its translation to clinical applications, which is becoming increasingly feasible mostly due to revolutionary advances in technology over the last few years. Measuring the abundance of biological molecules and their chemical modifications in blood and tissues has been the cornerstone of research and medical diagnoses for decades. Although the number and variety of molecules that can be measured have expanded exponentially, the blood biomarkers routinely assessed in medical practice remain limited to a few dozen, which have not substantially changed over the last 30-40 years. Rapid discovery of novel biomarkers could improve clinical medicine in several ways ranging from risk stratification to monitoring disease progression and the effectiveness of treatments and interventions.

The critical role of new technology

A key limitation to more extensive use of biomarkers in medical research has been the requirement to measure them one at a time, which is expensive and labor intensive, and requires a large amount of biological material. However, these limitations have been largely overcome by new technologies that yield thousands of biomarkers from just a few drops of biological material, although the use of such technology is still mostly limited to research. The motivation to use an expansive set of biomarkers reflects a growing awareness that different diseases and conditions may produce pathology-specific profiles in plasma or other biological fluids detectable at an early subclinical phase when classic symptoms are not manifest due to compensatory and/or resilience mechanisms. Technological advances that facilitate the assessment of different layers of molecular markers are outlined below. With advances in sequencing technology, gene expression can be assessed at unprecedented depth with long-read RNA sequencing permitting identification of splicing variants that increase the number of gene products that can be identified beyond the estimated 20,000 human proteins. Indeed, RNAseq easily quantifies more than 60,000 gene transcripts that include not only the protein-coding mRNA but also a large number of non-coding RNAs that drive essential biological mechanisms, many of which are important for aging and disease. Epigenetic biomarkers, especially but not exclusively DNA methylation, have garnered substantial attention in the study of aging and are fast approaching clinical application. Although the biological mechanisms that drive DNA methylation remain unclear, initial attempts to identify methylation patterns associated with specific diseases have shown promise [8, 9]. One exciting advancement in DNA methylation in aging research has been the development of various "epigenetic clocks" proposed to capture the pace of aging. Using range of machine learning and data reduction methods tuned to chronological age as well as other phenotypic manifestations of aging, these DNA methylation clocks have been found to predict disease outcomes and mortality. Conceptually, the deviation between DNA methylation predicted age and chronological age harbors information on biological age. Those predicted to be older than their biological age from methylation data are considered to be aging faster than those predicted to be younger. This suggests that measuring DNA methylation at multiple time points may be particularly important for measuring the rate of aging in observational studies and possibly clinical trials [10, 11].

Several proteomics methods and assays have been developed over the years for the discovery of blood biomarkers (Shown in Table1). Perhaps the discovery/innovation with the greatest potential for clinical application encompasses technical advances in systems that combine Liquid Chromatography (LC) and Mass Spectrometry (LC-MS) to enable measurement of thousands of proteins in tissue and plasma specimens, including dozens of post-translational modifications (PTMs). Untargeted proteomic analyses of plasma and serum, the two most useful matrixes for clinical application, have been challenging because of the wide dynamic range of proteins in these biological fluids and interference of a handful of highly abundant proteins, including albumin (constitutes ~ 55% of plasma protein, on the order of 30 g/L) IgGs, transferrin, etc. [12]. Although many challenges continue to exist [13, 14], development of new approaches that address issues with reproducibility and quantitative accuracy [15], increased throughput [16-18], depth [16, 18, 19], and reducing interference [16] have greatly improved the utility of LC-MS based plasma proteomics as a clinically useful tool. Huge leaps in standardization have been made in increasing the adoption of data-independent acquisition (DIA/SWATH) protocols, which have been validated in clinical cohorts [20] and large multi-site consortium studies [15]. These and other targeted assays, including DIA, selected reaction monitoring (SRM) and parallel reaction monitoring (PRM/MRM) mass spectrometry assays, are now widely adopted and studied as clinical biomarkers [21, 22]. The Clinical Proteomic Tumor Analysis Consortium (CPTAC), in particular, has driven improvements in the standardization and technological application of mass spectrometry-based assays for clinical use, with the establishment of a proteomic assay repository with standardization criteria that include a response curve, validation of repeatability, selectivity, stability, and reproducibility (https://proteomics.cancer.gov/assay-portal). Two recent technological advancements substantially improve the depth of proteins measured in biological fluids without the need for the high-level expertise required for LC-MS. The SomaScan Assay (SOMALOGIC®) uses Slow Off-Rate Modified Aptamers (called SOMAmer reagents) to yield over 7,000 highly reproducible measurements of circulating proteins from a single sample of plasma, serum, or urine [23]. The Olink® platforms, use a Proximity Extension Assay (PEA) technology to recognize two protein-specific antigens located at a pre-specified distance and use a multiplexed DNA- sequencing methodology to identify up to 3000 proteins in a very small sample of biological fluids [24]. Extensive validation of specific proteins is still a work in progress, but both technologies have shown high reproducibility, and both have been used in large population studies where clear proteomic signatures of aging have been described in cohorts from different geographic locations [25, 26].

Through these studies, several robust proteomic biomarkers of aging have been identified, including sclerostin (SOST), ADP ribosylation factor interacting protein 2 (ARFIP2) and growth differentiation factor 15 (GDF15) [26, 27]. Many age-associated proteins predict age-related conditions such as multimorbidity and mortality [27]. As an example, relatively high IL-6, TNFAR2, IL-1RA and low DHEAS have been associated with greater co-morbidity (15 candidate chronic conditions) in 1018 participants aged 60 years or older in the InCHIANTI study [28]. Tanaka et al. showed 247 aging protein biomarkers predicted multimorbidity and mortality. Of these, 17 proteins are core SASP factors including GDF15, MMP1, and STC1 – and other extensively reported classic SASP factors such as IGFBP2, 4, 5, and 7, TIMP1 and TIMP2. In community-dwelling men aged ≥65 years, 56 peptides (31 proteins) with absolute fold change >1.2 for 5-year all-cause mortality have been shown as biomarkers [29]. As knowledge/discovery of blood biomarkers continues to expand, new resources such as MortalityPredictors.org which provides a comprehensive list of published biomarkers of human all-cause mortality risk become increasingly essential. The mortality predictor database can also be used to compare aging-related all-cause mortality biomarkers, perform meta-analyses, and serve as a central resource for mortality and aging biomarker analyses. As of now, this manually curated database is derived from 589 all-cause mortality publications, with 1,576 biomarker associations, involving 471 distinct biomarkers (including 365 blood type publications and 165 blood biomarkers) [30]. Comparison of results from studies of aging proteome has identified over 200 proteins consistently associated with age across different assessment methods and in different tissues (ie: blood vs muscle) [31-33]. These studies collectively support proteins as promising clinical biomarkers of aging that could be translated into clinical practice.

An emerging area of interest for the development and future application of proteomics in aging is the identification of PTMs and proteoforms related to aging. There is little doubt that specific post-translationally modified or splice variants of proteins have functional differences that likely correlate with aging and disease changes. However, proteoforms are not quantified by most large-scale proteomic approaches, which generally provide an aggregate measurement of multiple variants of each protein measured. An advantage of MS-based assays in this respect is the ability to identify new PTM sites, peptidoforms, and proteoforms using existing or modified workflows specialized for PTM and proteoform detection [34, 35]. Also, MS-based assays have the

ability to inspect and confirm the peptide sequence information for any signal that is detected, enabling the localization of PTM sites and amino acid variants along the peptide/protein sequence, eliminating the need for developing new affinity reagents for new targets [36, 37]. Going forward, it will be important to expand the capabilities of all available proteomic technologies for detecting proteoforms, which will undoubtedly provide more sensitive and specific biomarkers than current approaches.

Metabolomics technologies are extensively used in research to comprehensively quantify hundreds to thousands of metabolites, including amino acids, carbohydrates, nucleotides, and lipids that reflect metabolic profiles that may lead to, underlie or reflect disease or aging processes. Clinical applications of metabolomics in precision medicine have recently emerged [38, 39]. Because small metabolites have highly variable physical and biochemical properties, metabolomic platforms typically divide the metabolome into subsets of metabolites—often based on compound polarity, common functional properties, or structural similarities. Detailed protocols for sample preparation and analytical procedures are optimized for each subset and then aggregated into a unique database. Lipidomics is a subfield of metabolomics dedicated to quantifying thousands of lipids from multiple classes. Despite substantial research in this field, especially in the area of neurodegenerative diseases, lipidomics remains exclusively used as a research tool. Aggregate clusters or indexes derived from metabolomics and lipidomic analyses have been strongly associated with prevalent chronic conditions as well as predictive of incident conditions and events including diabetes [40], cardiovascular disease [41, 42], Alzheimer's disease [43], cancer, inflammatory bowel disease (IBD) [44] and obesity [45]. In addition, metabolomic and lipidomic scores have been found associated with aging and similar to lipids and metabolites, relevant molecular patterns appear to differ between men and women [46-48]. Often, elucidating the relationship between metabolite profiles and diseases and biomarker identification requires prolonged and intricate analysis and reliable analytical platforms for isolating and characterizing metabolites/lipids. These studies provide an essential baseline for defining the metabolome and the main sources of variation as a measurable indicator of normal biological processes, as well as response to therapeutic interventions.

Blood biomarkers as correlates or predictors of disease

Results from several seminal studies highlight the potential for clinical applications of high throughput biomarkers. For example, blood transcriptomics has been associated with sporadic Alzheimer's disease [49], coronary artery disease [50, 51] and cancer [52]. Of note, associations typically are not limited to protein-coding transcripts but to thousands of human non-coding RNAs (ncRNAs), including long ncRNAs (lncRNAs), microRNAs (miRNAs) and other ncRNA species that exert regulatory functions on protein expression, either through direct interaction with DNA or proteins or with other mRNAs. While transcriptomic analysis is a particularly powerful tool, several hurdles exist before clinical application becomes practical. Some issues to be addressed include inconsistency of transcriptomic data that leads to variable results with respect to identifying global biomarkers of chronic disease and aging. For example, Peters et al. generated transcriptomic aging clocks using RNA extracted from human peripheral blood in eight different cohorts which yielded a wide range of R-square from 0.121 to 0.599 [53].

As noted above, DNA methylation has mostly been used to produce "epigenetic clocks", that estimate the pace of aging and, at least theoretically, identify persons aging "faster" or "slower" than the general population. However, as some studies have found the pattern of DNA methylation associated with chronic pulmonary diseases [54], DNA methylation may also reflect pathology. The first generation of epigenetic clocks was generated using chronological age as the primary predictor (Horvath and others). However, second-generation clocks tuned on age-related risk factors have improved prediction of aging outcomes. For example, "PhenoAge" is tuned on a composite clinical measure of phenotypic age previously associated with lifespan, while "GrimAge" was constructed in part from DNAm-based estimators of plasma proteins considered risk factors for cardiovascular disease, including plasminogen activator inhibitor 1 (PAI-1) and growth differentiation factor 15 [10]. Several efforts to build more accurate predictive clocks are ongoing, with the Dunedin pace of aging methylation (DunedinPACE) based on trajectories of aging traits as a leading example [11]. Lastly, mounting evidence indicates epigenetic scores for the circulating proteome show promise as tools for disease prediction [8].

Perhaps the most powerful biomarkers associated with chronic diseases are circulating proteins. Studies involving large populations have identified several circulating protein profiles associated with chronic disease [55], especially neurodegenerative diseases [56-58], cardiovascular disease, cardiorespiratory fitness, fatty liver disease and insulin resistance [59-61]. Specific circulating proteins and protein patterns have been found to predict the accelerated accumulation of multimorbidity [28, 27] as well as all-cause mortality and healthy life expectancy

[27]. A number of “proteomic clocks” have been developed that predict age and phenotypes of accelerated aging with similar or better accuracy than the epigenetic clocks published so far. Additionally, senescence-associated proteins are secreted into circulation and thus may have utility as predictors of age and other clinical outcomes. A subset of the senescence-associated secretory phenotype (SASP) biomarkers has been proposed as biomarker candidates for aging, multimorbidity, mortality, medical risk, and other clinical outcomes in proteomic and epidemiological studies [25, 62-64, 27]. Some of the most promising SASP biomarkers include GDF15, stanniocalcin 1 (STC1), matrix metallopeptidase 1 (MMP1), Inhibin Subunit Beta A (INHBA, also known as ACTIVIN A), TNF receptor superfamily member 1A (TNFRSF1A), and PAI-1 (also known as Serpine1). A unique strength of proteomics versus other -omics, including epigenetic clocks, is the high likelihood that select proteins more directly reflect or impact basic and/or essential mechanisms of biological aging. We anticipate this connection will become progressively more clear as data from multiple cohorts becomes available to facilitate large meta-analyses [25-27].

From disease prediction to exceptional health biomarkers

We have provided a few examples of the rapidly growing literature demonstrating the potential of biomarkers for the diagnosis and tracking of pathological conditions. The potential for commercial application of these results motivates this line of inquiry; however, high throughput biomarkers have potential beyond the development of new disease diagnostics and therapeutics. As noted above, aggregate measures of biomarkers have the potential to track the rate or progression of damage accumulation as a function of age and/or pathologic processes. Clearly, several challenges remain before these molecular clocks can be applied in non-research settings. Currently, although the predictive associations of several clocks are statistically robust, the added value to conventional approaches remains modest. Yet, with near-constant and rapid updates of algorithms to improve clock performance, clinical applications may not be too far off. For example, while the reliability of epigenetic clocks has been challenged, new methods of estimation based on factor analysis have substantially improved their psychometric properties [65].

As described, rapid expansion of -omics research in aging has led to the development of different -omics clocks. Despite similar performance in predicting aging and age-related adverse health outcomes, the metrics that estimate the pace of aging or, more accurately, deviation of predicted from observed age have low to moderate correlation, both within -omic and across -omics platforms [66, 67, 11, 27]. This suggests that different -omics capture different dimensions of the pace of aging and implies that combining different -omics layers may provide a more comprehensive metric that is more powerful, predictive, and potentially translatable than a single -omics approach. While many “clocks” have been tuned on age or physiological parameters assessed cross-sectionally, a new generation of clocks tuned on trajectories of phenotypic and functional manifestations of aging will likely yield higher predictive validity and potential for clinical utilization [66, 11]. Although it is exciting for the scientific community to have these tools for help us better understand the aging process, we are also cautious about how these tools could be used commercially, and the social and ethical implications of aging/predictive biomarkers [68-70].

Importantly, most age-omics metrics are strongly correlated with aging and adverse health outcomes even in young- and middle-aged adults, who are largely free of chronic disease [71]. This suggests these tools have the potential to assess “health” status prior to the presentation of clinical symptoms and/or abnormal traditional clinical indicators but when pathology is already accumulating. With further development and validation of omics-based clocks, we can begin to envision a new chapter of precision medicine where the pace of aging is regularly monitored over time. Early signs of “accelerated” aging and other information derived from a multi-omics evaluation may reveal susceptibilities that can be addressed before they manifest into a health outcome as well as the effectiveness of specific interventions aimed at “slowing” the aging process. Progress in research on circulating biomarkers and new technologies that drastically reduce the cost of measuring several -omics biomarkers remain important limiting factors to the broad application of this new revolution in health care.

Conflict of Interest Statement

The authors have no conflicts of interest to declare

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Author Contributions

LF conceptualized the article. LF, CU, TT, RM, ZM, QT, NB and ES prepared the draft, wrote sections and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Proteomic Biomarker Methods/Assays						
Proteomic Biomarker Tools/Assay	Method	# Measurable Blood Biomarkers	Quantitative	Analytical Measurements	Advantages/ Disadvantages	Level of Expertise
LC-MS based Proteomics (Untargeted)	Data Dependent Acquisition (DDA)	300-3500[18]	Relative	AUC, Spectral count, peak intensity	High throughput but low dynamic range (linear range of 2-3 order magnitude) [16]	High
LC-MS based Proteomics (Targeted)	Data Independent Acquisition (DIA) Swath	300-3000	Relative	AUC, Spectral count, peak intensity	High accuracy, high throughput, medium dynamic range (linear range of 3-4 order magnitude) [16-17]	High
LC-MS based Proteomics, (Targeted)	Targeted Dependent Acquisition (TDA) MRM/SRM/PRM	1-100	Quantitative	molar	High sensitivity, high precision, high accuracy, and large dynamic range [20-21]	High
SomaScan Assay (Targeted)	Aptamers	7,000	Relative	RFU	High precision, high reproducibility, scalability, and dynamic range [23]	Low
Proximity Extension Assay (PEA) (Targeted)	Oligonucleotide antibody-pairs	3,073	Relative	NPX/RFU	High precision, high reproducibility, scalability, high specificity, and dynamic range [18]	Low

Table 1. Current proteomic methods and assays for measuring blood biomarkers.