Chapter 28

CONSIDERATIONS REGARDING BIOMONITORING

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INTRODUCTION

Determining Military Exposures

Reliable estimates of acute or chronic exposures of large military populations in constant flux in theater have been difficult, with multiple assessments reporting minimal or no health associations.[1–4] Therefore, the study of exposure/health outcome associations in service members following deployment has been limited by a lack of individual exposure information, as discussed elsewhere in this book. In addition, individual variability, including susceptibility characteristics (eg, genetics and epigenetics, preexisting health conditions, and psychosocial stress) plays a role in the overall risk and development of disease. Strategies to improve exposure monitoring and risk assessment have been suggested in other chapters, including localized or personal detection devices for monitoring of airborne and chemical hazards, optimization of current techniques (eg, spirometry), and new epidemiology approaches. In addition to these strategies, biomonitoring has been proposed as another approach.[5,6] Past utility of biomonitor techniques focused on the determination of the body burden of environmental chemicals (xenobiotics) of interest. For exposures with sufficient dose–response information, the internal dose as determined by this method may correlate with potential health effects. However, a newer and more encompassing definition of biological monitoring includes the quantitative detection of the molecular changes that occur in the body on exposure.[7]

The Exposome

With the described difficulties establishing accurate individual exposure levels using traditional methods, more recent efforts have been focused on determining the body's own molecular signatures to indicate types and levels of exposures.[8–10] The term *exposome*, coined by Dr Christopher Wild in 2005,[11] has come to signify alternations in the body that occur on acute and chronic environmental exposures over a lifetime, as well as individual social determinants that also play a role in outcome.[12] In effect, Wild has suggested that a person's exposome data could be used to track a lifetime of environmental exposures, and used to identify *individualized* risk and disease outcome. Such data could be seen as the exposure ancillary to the personalized medicine thrusts in both military[13] and civilian[14] populations.

WHAT ARE BIOMARKERS?

Definition of the term *biomarker* varies depending on scientific focus and use, but the formal definition recognized by the National Institutes of Health is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention."[15] With this broad definition, a biomarker would include any molecular entity produced by the body, a xenobiotic or its metabolites inside the body, or even measurement of physical or cognitive attributes. In each case, however, the biomarker will reflect, in a quantitative manner, the interaction between a biological system and an exposure.[16] Thus, within the National Institutes of Health definition, the utility of biomarkers spans a continuum from exposure to physiological effect, as well as susceptibility to exposures or outcomes (Figure 28-1). Desirable biomarkers are accurate, minimally or noninvasive, economical, easily repeated, and accessible.[17] Biomarkers should also be stable with a relatively long half-life in the

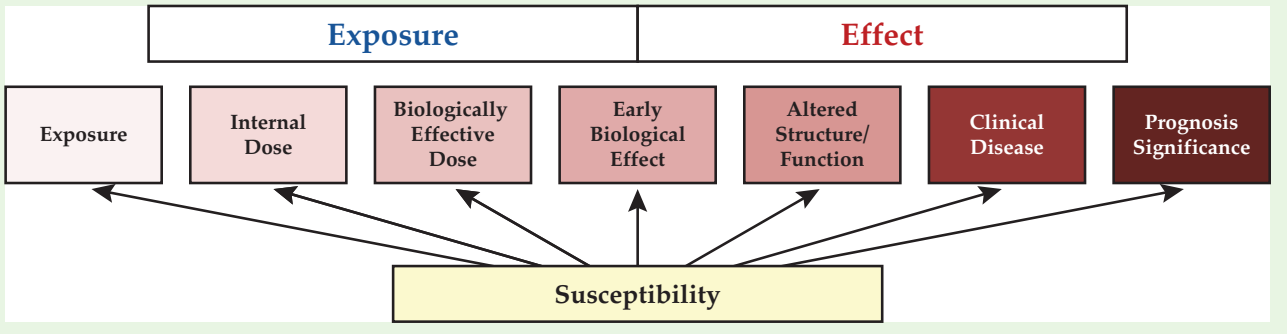

Figure 28-1. Schema of events between causal exposure and resultant disease.

test sample. Optimally, a biomarker should be specific to the exposure or stressor of interest and not confounded by other exposures, although such nonspecificity for molecular markers may be overcome by the use of a test panel rather than usage of a single marker. Biomarkers should be translatable, that is, bridge preclinical research (most likely done in animal models) to clinical results. Desirable biomarkers that indicate susceptibility to a particular condition or exposure should have sufficient prevalence in the population of interest to justify screening or targeted screening, as well as enough data to prompt an action. Finally, in order to utilize biomarkers, there must be a robust technological infrastructure and expertise available. Unlike physiological biomarker testing, detection of specific molecular changes for monitoring may allow early detection of subtle, subclinical changes that occur upon even acute exposures, as well as the possibility of the identification of exposure level/type long after such an event.

TYPES OF BIOMARKERS

Biomarkers of Exposure

Biomarkers of exposure are measured as the unchanged, parent chemical substance; its metabolite; or the product of its interaction with a target within the body.[18] The quantitation of a specific chemical, or a metabolic derivative of that chemical, within a biological sample can provide accurate assessments of systemic exposures for internal or external doses. The merits of baseline and periodic assessment of levels of chemical substances in blood and/or urine have been considered for service members as a group. In 2001, the Military Deployment Human Exposure Assessment compared exposure levels to a variety of chemicals with air and personal monitoring during a deployment.[19,20] Although there were no detections at actionable levels, such measurements could serve as a baseline to detect changes over time, after consideration of other confounding exposures. For the past three decades, the Centers for Disease Control and Prevention (Atlanta, GA) have been measuring biomarkers of exposure for more than 200 chemicals in nonoccupationally exposed populations in the United States as part of the National Health and Nutrition Evaluation Survey.[21] Currently, measured chemicals are associated with pollution in the air, pesticide use, and chemicals that are based on human activities, such as phthalates (found in plastics such as bottles and cups), bisphenyl A, and flame retardants. Although these measured levels usually have no prognostic value, they are assessed to evaluate trends over time and to look at regional or age differences.

Biomarkers of exposure in deployed military populations have been recommended and considered as an approach to understanding complex exposure situations, such as smoke from burning trash, although there are a number of limitations.[22,23] These include a brief window of opportunity for sample collection from rapid metabolism or excretion, a lack of specificity as to the source (ambient air vs smoking, for example, for some metals and volatile organic compounds), and the general lack of prognostic value.[17] The US Department of Defense (DoD) policy addressing the use of exposure biomarkers related to deployment includes the testing of blood for lead levels (when appropriate), as well as a 24-hour urine-depleted uranium bioassay for those considered at high risk based on responses to a screening questionnaire.[24]

Exposure biomonitoring is most frequently done in occupational settings for established medical surveillance programs. In workers with specific exposures due to their work operations, exposure biomarkers, largely in blood or urine, can be useful to evaluate whether exposures to these specific hazards exceed acceptable limits following a shift or at the end of the work week.[25] Currently, DoD occupational medicine guidance recommends hazard-directed medical surveillance, with few occupation-specific requirements.[26] DoD pesticide applicators and technical escort personnel (those who deal with explosive ordinance) are two occupations with specific biomonitoring requirements to address potential deployment-related exposures. Measurement of specific exposures in deployment may follow specific events or incidents. Two examples include (1) the assessment of whole blood chromium levels following a potential exposure to sodium dichromate powder dispersed at a vandalized water treatment plant in Iraq; and (2) lead and zinc protoporphyrin levels following exposure to elevated lead levels in air, potentially from the burning of batteries by local Iraqis outside a base camp.[27,28]

Depending on how biomarkers are categorized, *surrogate biomarkers* or *surrogate endpoint biomarkers* can be considered a type of exposure biomarker.[29] Surrogate biomarkers are used to substitute for a clinical endpoint and can be either intrinsic (blood pressure, protein isoforms) or extrinsic (cigarette consumption).[30] In the DoD, methemoglobin has been used as a surrogate biomarker to indicate cyanide intoxication levels, providing a level of protection by linking physiological/toxicological effects of cyanide to methemoglobin levels.[31]

Biomarkers of Effect

Biomarkers of effect include any measurable biochemical, functional, or structural change associated with exposure to and interaction with an agent. The effect may not be specific

to a given exposure or agent. Pulmonary function testing is a familiar example; exposure to inhalation hazards may impact pulmonary function, as can personal habits such as smoking.[6] Application of pulmonary testing as a biomarker of effect in specific cohorts and the relative merits and issues associated with pulmonary function testing are discussed in other chapters in this book.[32]

Biomarkers of Susceptibility

Biomarkers of susceptibility reflect inherent or acquired modifications in the response to exposures or other stressors.[33] Susceptibilities can be disease states, genotypic and phenotypic variants, or the corresponding physiological states. Asthma and other respiratory conditions, cardiorespiratory disease, and other physiological changes associated with disease states are known to be associated with susceptibility to adverse outcomes of exposures. Although several types of molecules (protein, ribonucleic acid [RNA], etc) can serve as susceptibility markers, genome polymorphisms are particularly well suited as indicators of susceptibility. Toxicogenetic studies can lead to the development of new genetic screening tests for susceptibility to specific exposures. An example of such research is the identification of a variant allele in the tumor necrosis factor-α gene. While still in the research phase, studies examining the genotypes of coal workers who have developed silicosis have identified a polymorphism in the tumor necrosis factor-a that seems to be linked to susceptibility in the development of silicosis.[34]

Comparison of Biomarker Types

For exposures with sufficient dose–response information, the internal dose may correlate with potential health effects. *Exposure* biomarkers can integrate and quantify the dose internalized by inhalation, ingestion, and skin absorption routes of exposure. Internal measures of exposure to stressors are closer than external measures are to the targeted site of action for biological effects, potentially reducing confounding factors and consequently strengthening the ability to determine whether exposure correlates with the biological effects.[22] Markers of *effect* may identify subclinical changes from various exposures with the same target organ effect. Biomarkers of *susceptibility* may identify individuals at higher risk for adverse effects, although the prevalence of such susceptibility may be insufficient for screening to be an appropriate use of limited resources. Less frequently used classifications include the role of the biomarker in the pathophysiology of tissue injury (eg, inflammation) or activation of coagulation or fibrosis.

BIOMARKER DISCOVERY AND APPLICATIONS

Omics Technologies

The uses of high-throughput methodologies to examine the global (entire) expression of a given molecule are usually described as "Omics" technologies. These research areas include the following:

- proteomics (proteins),
- metabolomics (metabolites),
- genomics/transcriptomics (deoxyribonucleic acid [DNA] or RNA),
- adductomics (DNA adducts),
- lipidomics (lipids),
- epigenomics (epigenetic changes), and many others.

The ability to examine large sample numbers using analytic methods linked with bioinformatics has led to substantial gains in biomarker discovery in the last 10 years. Omics studies permit an unbiased examination of the expression of a given molecular population under different conditions (exposures, exposure routes, dosages). This *bottom up* approach allows identification of heretofore unlinked and unknown pathways. Although not within the scope of this chapter, excellent reviews on each of these technologies can be found elsewhere. Most of the primary Omics (proteomics/genomics/metabolomics) are currently used in biomarker discovery related to pulmonary diseases.[35]

Test Matrices

Traditional biological sources used for discovery and biomonitoring include whole blood, serum, plasma, urine, saliva (historical, now reemerging), among others (Figure 28-2). Additionally, physiological measurements—including pulmonary function testing, blood pressure, and pulse—can be used.[36,37] All other things being equal, the least invasive of these is preferable if the same quality of information can be obtained from the sample. Of these, blood and urine are commonly examined. Measurements of molecular components or chemicals in blood may indicate exposures, mobilization from stores, or cellular damage. Urinary biomarkers may be indicative of the same, although the response may slightly lag in time compared with those found in blood. Neither may provide information about the whole-body burden or internal dose sequestered in bone, fat, or other tissue.

Saliva is another medium of potential interest for exposure biomonitoring. It is readily accessible by noninvasive methods. Saliva biomarkers produced by healthy or diseased individuals are "sentinel molecules that could be used to scrutinize health and disease surveillance."[36] Levels of therapeutic, hormonal, immunological, or toxicological molecules are reflected in the molecular composition of saliva.

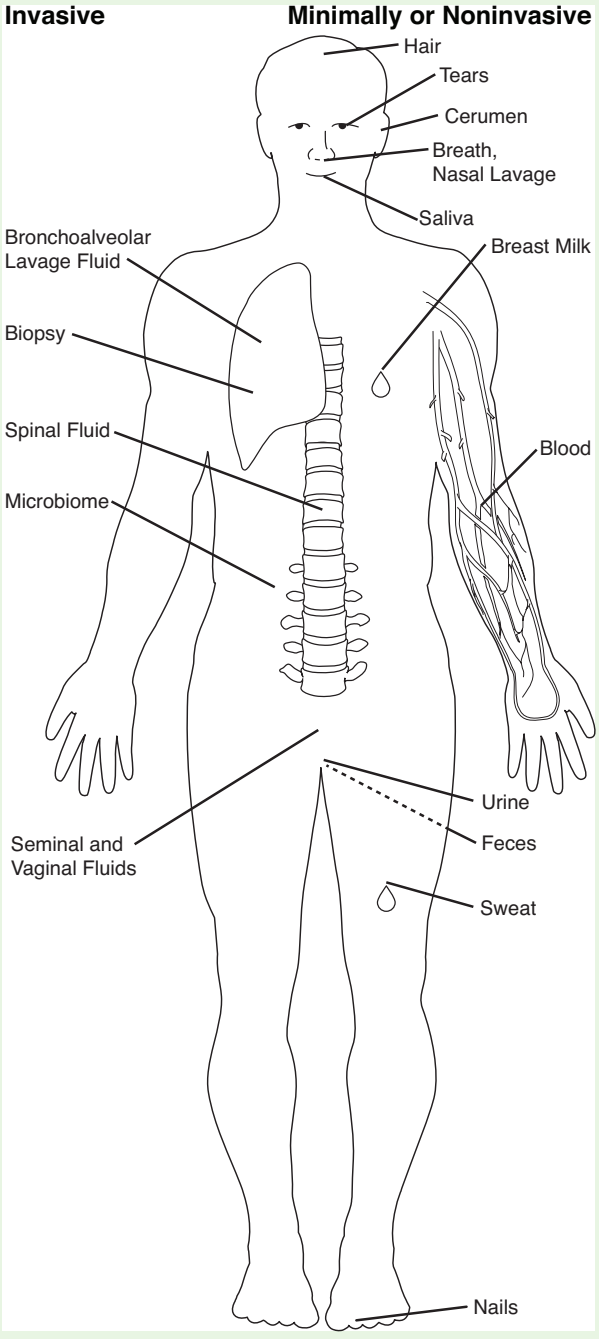

Figure 28-2. Test matrices for biomarker discovery and monitoring.

An example of the use of saliva as a biomarker of exposure uses levels of thiocyanate ions to differentiate smokers from nonsmokers.[38] Compared with urine and blood, saliva was found to be the most sensitive. Currently, saliva analysis for chemical exposures of interest is not common.

Hair or nails are most useful if there is a desire to assess internal dose over time, and the compound is known to be found within the sample. In recent years, the number of sources has expanded to include saliva, induced sputum (cellular or acellular), bronchoalveolar lavage fluid, and others.

Biomarker Detection

Assays for the selected biomarker must be sensitive (few false negatives) because detection levels are most likely low (picogram for proteins) for the marker of choice in the given sample. Such low levels of biomarker detection, for molecular signals, are highly probable given that most acute or chronic exposures will display only subclinical effects. The measurement should have a reference standard in unexposed or healthy populations, provide prognostic information, and be predictive (proportional to the degree of severity of the pathology).[17,36] Once a biomarker has been identified and appropriate assays and baselines developed, a stringent set of prevalidation and validation studies are necessary prior to clinical or biomonitoring uses.[39]

Current Pulmonary Biomarker Discovery Research

The application of Omics technologies for a new molecular signature indicative of lung injury or disease is still relatively recent when compared with analogous studies in kidney and liver diseases.[40] However, several recent studies demonstrate the potential utility of several types of molecules in a wide range of text matrices.[41]

There is a growing inventory of potential biomarkers that can be detected and quantified in exhaled air or exhaled condensate.[42] Breath analysis of exhaled volatile organic compounds may be a quantitative, noninvasive, simple, and safe method of measuring airway inflammation that provides a complementary tool to other methods of assessing airway disease, including asthma.[43] Volatile compounds can be measured directly in expired air, and are currently under examination for the detection of lung inflammation and asthma. Other nonvolatile compounds have been measured almost exclusively in bronchoalveolar lavage fluid. A less invasive approach uses exhaled breath condensate, which is formed by cooling expired air to assess nonvolatile compounds. These include biomarkers of oxidative stress and inflammation, often proteins.

The exhaled breath condensate has also been used as biomarker of exposure to some heavy metals and mineral compounds.[44]

Examination of serum and urine for pulmonary disease biomarkers has identified a number of potential markers in the past few years.[40] Clara cell proteins and surfactant protein A in serum have been examined in several studies for associations with lung injury. Studies of samples taken from multiple cohorts with smoke inhalation exposures indicate that Clara cell proteins and surfactant protein A seem to increase in serum approximately 24 hours postexposure.[45–47] Metabolomic analyses of urine and plasma in chronic obstructive pulmonary disease have identified trigonelline and formate as potential urinary metabolite biomarkers for this condition.[48] The success seen in this study suggests that metabolomic approaches may be successful for other pulmonary diseases.[49]

A large number of transcriptomic analyses of gene expression in the bronchial airway epithelium and lung parenchyma cells has permitted identification of condition-specific genes and molecular pathways modulated by cigarette smoking or as a result of lung function impairment in chronic obstructive pulmonary disease.[50,51] RNA expression studies such as these are used to develop panels of gene expression signatures specific to a given pulmonary injury or disease. In asthma transcriptomics studies, T-cell type 2–mediated inflammation symptoms can be followed by a set of interleukin-13 and interleukin-14–induced genes.[52] In addition, while sample collection via bronchial brushing is invasive, data collected from such studies would be invaluable in gaining a comprehensive understanding of the molecular disease mechanisms initiated by inhalation of airborne hazards.

NEW DIRECTIONS IN PULMONARY INJURY BIOMARKER RESEARCH

Epigenetic Biomarkers

In the past 10 years, the field of epigenetics has expanded with the development of high-throughput multiplex discovery methodologies.[53] For environmental exposures, applicability of epigenetics to environmental exposures is particularly relevant.[54] Indeed, the idea of a "longitudinal epigenome-wide association study" approach has been suggested to allow the examination of influences of various exposures and environmental factors (eg, exercise, diet) on the epigenetic signature.[55] This signature (or the epitype) can and does change during its lifetime, altering gene expression to incur specific pathway alterations.[56,57]

Nasal Lavage Analysis

Although not unknown in allergen testing,[58] the use of nasal lavage as a test matrix for lung injury biomarkers may be a relatively new idea. Biomarker studies in asthma have indicated that monitoring eosinophil-derived neurotoxin in nasal lavage is a useful and noninvasive method to monitor eosinophilic inflammation.[59]

CURRENT USES OF BIOMARKERS IN MONITORING PULMONARY INJURY

Currently, there are few pulmonary biomarkers routinely used in clinical applications. Discussions of the use of spirometry and its limitations in the measurement of lung function are examined in Chapter 8 (Pulmonary Function Testing—Spirometry Testing for Population Surveillance), Chapter 9 (Discussion Summary: Recommendation for Surveillance Spirometry in Military Personnel), and Chapter 10 (Spirometry Monitoring and Prevention Using Spirola Software). One reason for the limited number of clinical biomarkers is the lack of reference values and standardization of known markers. As such, further research, development, and refinement are imperative for pulmonary disease biomarkers to become an integral part of clinical practice. In addition, new pulmonary biomarkers, as previously described, are still in the prevalidation stages or early validation stages, and have yet to be fully evaluated and approved for clinical uses. One exception to the lack of clinical applications is the measurement of fractional exhaled nitric oxide in the evaluation of patients with obstructive airways disease.[42] Fractional exhaled nitric oxide has been shown to reflect an increase in eosinophilic inflammation in the lung, although its use may be limited to specific lung diseases (eg, asthma).[60]

GENETICS IN PULMONARY INJURY RESEARCH

The availability of the Human Genome Sequence, high-throughput capabilities of multiplex, chip-based, genome-wide association screens, and bioinformatics has accelerated discovery of new gene–gene mutations associated with susceptibility to environmental hazards. It is projected that with the increased affordability of whole genome sequencing,[61,62]

large cohort studies will link DNA sequence (genotype) to health outcomes and exposures (phenotypes). Genetic research will yield information on new potential biomarkers and, more importantly, specific pathways altered upon specific exposures and dosages. Studies of gene–environment interactions in lung disease have already identified a number of genes that predispose individuals to a higher risk of injury.[63]

Several genes have been shown to act as modifiers of response to exposures making an individual more susceptible. For example, polymorphisms in oxidative stress genes (*GSTM1*, *GSTP1*, *NQ01*) have been shown to modify response to particulate matter and ozone.[64] Diet and genes influencing metabolism have been shown to influence closing volume response to pollution, thus indicating protective effects for n-3 fatty acids, antioxidants, and methyl nutrients.[65–67] Another polymorphism of cellular DNA has been associated with susceptibility to silicosis.[68] Response to inhaled silicon dioxide nanoparticles in animal studies demonstrated that toxicity varied with age, and different biomarkers of susceptibility may exist at different stages.[11]

Current research strategies are exploring gene–environment interactions. One research strategy studied subjects exposed to airborne endotoxins and potential genotype signatures. It was seen that three single nucleotide polymorphisms in the *CD14* gene and one in the *MD2* gene could modify asthma symptoms generated in response to endotoxin exposure, indicating that carriers of these major allele variants were at greater risk than homozygotes.[69]

Although genetic research is helpful in understanding lung injury, its use for susceptibility screening in the military may be of limited value and only under specific conditions. Such tests may only be cost-effective when the prevalence of the genetic mutation is moderately high and the susceptibility difficult or impossible to ascertain otherwise. In addition, validated linkage of genotype to health decrement caused by exposures is essential, with a clear plan of action to advert adverse outcomes with intervention.

An example of a current military screening program for genetic susceptibility is the genetically based test for glucose-6-phosphate dehydrogenase (G6PD) deficiency.[70] The Army screens for G6PD deficiency prior to deployment to malarious regions, as deficiency is associated with adverse reactions to some medications, one of which is the antimalarial primaquine. Worldwide, G6PD deficiency occurs in approximately 1 in 16 individuals (6.25%). In US military personnel, prevalence was found to be 2.5% in males and 1.6% in females, and overall most common in African Americans, Asian, and Hispanic individuals. Screening for this susceptibility is considered clinically warranted due to the prevalence rate and the fact that the finding is actionable because specific drugs cannot be administered to these individuals.

SUMMARY

It has been broadly recognized that to understand the potential implications of deployment exposures on service member health, improvements in exposure science are needed.[71,72] Accurate exposure information is critical in epidemiology studies to compare outcomes in populations that have different exposure levels,[27] and it has been seen that questionnaires have known limitations and do not accurately access past exposure levels. Better exposure data can provide more precise risk estimates that may lead to public health actions. Biological monitoring is one tool for improved exposure assessment and has the potential for accurate, on-site, real-time monitoring (Figure 28-3). It can also identify effects of exposure and populations at potentially higher risk from exposures. The concept of the exposome has been proposed as a research challenge equivalent to the Human Genome Project.[11] Using a combination of high-throughput Omics technologies, efforts are now being directed at the evaluation of the exposome, which would evaluate the entire internal biochemical environment, including natural and disease processes and absorbed doses from xenobiotics and their effects on metabolism, gene and protein expression, and damage to biological molecules.

One approach might use high-resolution mass spectroscopy or ^{1}H-NMR (proton nuclear magnetic resonance) to identify chemicals and their metabolic signatures in multiple test matrices. This may detect low levels of exposure to anticipated and unanticipated exposures, identify changes in normal cellular metabolism, or identify trends over time and space. Proteomics could also be used to perform global scans for alterations in protein levels of pre- and

Figure 28-3. Conceptual field monitoring for exposures. Photograph: Reproduced from *Air Force Research Laboratory Technical Report,* AFRL-RH-WP-TR-2009-0106.

postdeployment samples. Such analyses could examine expression changes in immunoglobulins and other proteins known to modulate on exposure to allergens, for example, or even determine unique protein "signature" patterns indicative of specific chemical exposures or specific organ effects. Beyond the use of serum, whole blood and other specimens may allow the detection of changes in cells, genetic material, and transcription products. Additionally, changes following exposure to vaccinations or stress may be identified beyond changes associated with exposure to toxic compounds.

The DoD is assessing the progress of this field and hopes to initiate at least one pilot project to assess the feasibility of Omics techniques on serum from the DOD Serum Repository (DoDSR) to increase understanding of the environment–effect relationship, particularly as it relates to deployment. In 1989, the DoDSR was established for storing the serum that remained from mandatory human immunodeficiency virus testing.[73] Serum may be utilized to evaluate exposures by examination of a number of potential molecular marker patterns. The DoDSR has expanded to include the storage of operational deployment specimens, and now contains more than 50 million specimens representing pre- and postdeployment specimens, as well as serum remaining from human immunodeficiency virus testing. It has serial specimens on active and reserve components of Army, Navy, Air Force, and Marines, as well as the ability to link to demographic and health outcome data.

Significant challenges remain in the field of exposomics.[11,64,74] The exposome can vary over time for poorly understood reasons, such as aging and general environmental conditions. Therefore, large cohort processing of a large number of specimens is needed to eliminate experimental noise and pick up the low level signals of interest. If DoD supports efforts to move the suggested initiatives forward, it will likely be some time before the efforts yield practical information and clinical utility. Many of the biomarkers of the past were limited by difficulties in their interpretability to identify changes early enough to initiate preventive measures, as well as provide limited prognostic information. Even new biomarkers have limitations in their use.[75] However, the full scope of benefits from new biomarker discovery efforts will not be revealed for several years. The question of exposure and effect is a very old one that continues to be asked, but new technologies in biomonitoring may contribute to the answer.

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