Chapter 29

BIOLOGICAL MONITORING

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INTRODUCTION

Biological monitoring, also termed biomonitoring, is the use of blood, urine, or other human samples to assess an individual's state of health, responses to therapeutics, and exposure to chemicals, or other environmental agents of concern.[1] Biomonitoring is of considerable potential value to assess military exposures and possible contributions to health outcomes.[2,3] This chapter provides a review of biomonitoring for military purposes and a perspective for research and surveillance opportunities for biomonitoring both within and outside the military. It also addresses the potential to develop partnerships to examine deployment health-related questions. Further, the chapter will define the exposome; describe several types of currently available biomonitoring; discuss biomonitoring detection methods, sample media, and "omics" technologies available to examine biomarkers; and address the considerable range of information provided by these tools. The chapter's last section discusses new directions in biomonitoring and big data analysis.

The chapter also addresses how the Department of Defense (DoD) Serum Repository (DoDSR) can continue providing data for biomonitoring and ultimately improve health protection of service members while also contributing to advancing research in the field. Through the adoption of improved quality assurance practices and the addition of capabilities to handle varied sample types, the repository will be able to maintain state-of-the-art capabilities to support biomonitoring in the future.[4,5]

DEFINITION AND TYPES OF BIOMARKERS

What Are Biomarkers?

The National Institutes of Health defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention."[6] A biomarker could be any chemical produced by the body, an environmental chemical or its metabolites, or a measurement of physiological or cognitive function that reflects the effect of chemicals on the body. In all cases, the measured biomarker reflects the unique genetic interaction of the host with the environmental chemical and the metabolism of the chemical in the body. Everyone's susceptibility to environmental chemicals is affected by their nutritional status and how well the body metabolizes and excretes the metabolic breakdown products.[7] Figure 29-1 diagrams a completed pathway of exposure from external dose to potential health outcome.

Certain biomarkers can be used to assess internal dose of exposure, while others can be used to assess physiological effects, such as pulmonary function test results. Biomarkers must be stable in the blood or body fluid in which the chemical or metabolite is found. The biomarker is useless if it is metabolized and excreted before the sample media can be collected.[8] Biomarkers for military use must be reliably measured; sample collection must be minimally invasive or noninvasive and the sample feasible to collect while deployed; and the test must be economical and logistically feasible to analyze in the deployed environment.[9]

Types of Biomarkers

Biomarkers of Exposure

Chemicals that enter the body can be measured directly as the unchanged compound; they also can be measured as the metabolic breakdown product of the chemical; or they can be measured as the product of their interaction with tissues in the body.[10] The identification and quantification of chemicals, or their metabolites, in biological sample media can provide accurate assessments of systemic exposures and total dose. Baseline and periodic assessments of chemical substances in the blood or urine of deployed service members may support health risk assessments related to deployment exposures.

In recent decades civilian and military scientists have been enhancing medical surveillance programs with biomonitoring applications. Since the 1990s, the Centers for Disease Control and Prevention have measured biomarkers of exposure for more than 300 environmental chemicals and nutritional indicators in non-occupationally exposed populations in the United States as part of the National Health and Nutrition Evaluation Survey (NHANES).[11] The chemicals

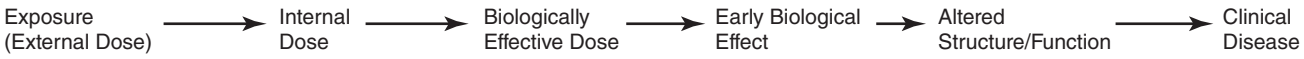

Figure 29-1. Pathway from exposure to disease.

selected for measurement are found in air pollution, pesticides, plastics (such as bisphenol A), and flame retardants. Trends observed over time allow for assessment of regional differences and demographic differences. NHANES measured 308 chemicals in a cross-section of the US population, but it is estimated there are between 25,000 and 85,000 chemicals in production today.[11] Thus, the ability to assess biologically relevant dose may not be possible for most chemicals in production today. However, with newly advanced chemical profiling techniques, it is possible to identify and monitor environmental exposures in deployed troops.

In 2001, as part of the Military Deployment Human Exposure Assessment Study, researchers obtained air and personal monitoring samples for a variety of chemicals commonly encountered during deployments.[12,13] The air and personal monitoring was done during and after deployment. Predeployment serum was collected but the serum was not analyzed. The capabilities exist to retrospectively examine serum for specific biomarkers of chemical exposure, but this is not routinely done because the costs are prohibitive. After exposure, a retrospective look-back at the predeployment serum is possible for selected hazards in small numbers of troops to monitor changes in internal dose over time after consideration of other confounders that affect exposure.

The use of biomarkers of exposure in deployed military populations has been recommended in the past when personal breathing zone air sampling data was not available. This data is necessary to gain an understanding of the complex exposure situations that exist during deployment, for example, when personnel encounter smoke from open-pit burning of trash.[14,15] The biological specimen must be collected soon after exposure, before the chemical is metabolized or excreted. Understanding the toxicokinetics of the chemical in the body will permit selection of the most appropriate exposure biomarker so that problems of specificity can be minimized. Information on smoking status is also helpful to control for metal and volatile organic chemical exposures found in cigarettes. It is also important to note that any biomarker detected may have little to no relationship with recorded health outcomes.[8] Exposure biomarkers approved for use by the DoD currently include blood lead and the 24-hour urine-depleted uranium bioassay for troops who report exposures on the Post Deployment Health Risk Assessment.[16] Additionally, red blood cell cholinesterase is used to monitor occupational exposure to nerve agents in explosive ordinance handlers and pesticide applicators.

In the occupational setting, exposure biomonitoring is most frequently done as part of established medical surveillance programs. Workplace chemicals are sampled in the blood or urine to evaluate whether exposures have exceeded acceptable limits.[17] DoD Instruction 6055.05M, *Occupational Medical Examinations and Surveillance Manual*, recommends sampling for a few specific hazards.[18] DoD pesticide applicators and technical escort personnel who handle explosive ordinance have medical monitoring requirements.

At least two highly publicized incidents of specific chemical exposures have occurred after which the individuals involved were offered biological monitoring. In one incident, National Guard members providing security around a water treatment plant in Iraq were exposed to sodium dichromate powder that was dispersed when thieves stole drums storing the powder. In another incident, at Camp War Eagle in Iraq, off-camp burning by local nationals exposed troops to high lead concentrations.[19,20]

Surrogate biomarkers can be considered a type of exposure biomarker when the biomarker is used to substitute for a clinical endpoint. For example, cotinine levels are elevated in smokers.[21,22] Methemoglobin levels have been used as a marker of cyanide exposure, but clinicians should be aware that other chemical exposures will also raise methemoglobin levels.[23]

Biomarkers of Effect

When the chemical agent or its metabolite causes a measurable change in a biochemical process or an alteration in a structure or body function, the biomarker reflecting the measurable change is a biomarker of effect. The change may be due to a specific airborne hazard in the deployed environment; for example, pulmonary function testing results may be altered when service personnel experience high silica exposures during sand storms in Iraq and Afghanistan. Exposure to these inhalation hazards may impact pulmonary function as much as, if not more than, personal habits such as smoking.[24,25] Workers exposed to nerve agents will show a drop in their red blood cell cholinesterase immediately after exposure.

Biomarkers of Susceptibility

Biomarkers of susceptibility reflect an individual potential risk of developing disease, genotypic and phenotypic changes, or physiological changes in response to environmental exposures. Asthma and other respiratory conditions, cardiorespiratory disease, and other diseases produce changes in metabolomic, immunologic, and genetic and epigenetic biomarkers

that are clues to changes in disease susceptibility. Although several molecules, including proteins and ribonucleic acid (RNA), can serve as susceptibility markers, genome polymorphisms are particularly well suited as indicators of susceptibility. Genetic susceptibility to air pollution has been studied in asthma patients. Genetic variation has been shown to increase susceptibility to environmental tobacco smoke and diesel exhaust; variation in metabolizing genes may also increase susceptibility to pollutant-related cellular damage.[26,27] Toxicology studies have led to new genetic screening tests for susceptibility to specific exposures. For example, a mutation on the HLA-B69 increases workers' risk of becoming sensitized to beryllium and developing chronic beryllium lung disease, and coal workers who developed silicosis were found to have a polymorphism in tumor necrosis factor that increases their risk of developing silicosis.[28]

Comparison of Biomarker Types

An environmental chemical is taken into the body through inhalation, ingestion, or skin absorption. When dose response information is known for a given chemical, it may be possible to predict health effects if the internal dose is known. Exposure biomarkers detect the agent or its metabolic breakdown products, which is considered to be a measure of internal dose closer to the targeted site of action than the external dose.[14] Biological markers of effect may identify subclinical changes caused by various exposures. More recently, biomarkers in the pathophysiology of tissue injury or inflammation, including chemokines, cytokines, and immunoglobulins, are being used to examine specific tissue injuries.

Limitations on Use of Biomarkers

Advancements in analytical chemistry have permitted the identification of biomarkers of exposure at extremely low levels, so it is imperative that detection of these biomarkers in a given sample be sensitive and specific. Low detection limits for these biomarkers make correlation with clinical findings very difficult, and only subclinical effects are expected. In order for the biomarker to be used, there must be a population reference value that defines the background levels in the unexposed general population, so that sample results can be compared to the reference population values. Further, the toxicology of the chemical should be worked out so that at a given exposure level, the biomarker sample results should correspond to the degree of severity of the response based on pathology studies.[9] Pre-validation and validation studies are also necessary prior to clinical or biomonitoring use so that reference values can be provided and the results can be explained to the affected individuals.

Lastly, use of biomarkers to study associations between environmental exposure and health outcomes is difficult to do in a scientifically rigorous way because of the lack of breathing zone sampling data, often poorly understood toxicodynamics, variability in nutritional status, and genetic variability among individuals.

DEPARTMENT OF DEFENSE NEEDS FOR EXPOSURE ASSESSMENT AND BIOMARKERS

The Exposome

The "exposome" is the cumulative measure of environmental influences and biological responses throughout a lifespan.[29,30] The exposome includes all environmental, dietary, microbiome, behavioral, therapeutic, and endogenous processes experienced cumulatively throughout life. Military personnel experience a broad array of exposures, and advances in this area will enhance the interpretation and utility of biomonitoring for military exposures.[31] New developments in this area are also helping to drive advances in personalized, or precision, medicine.[32]

Airborne Hazards and Military Deployment

This section briefly presents an overview of prior deployment exposures and associated studies to illustrate the need for real-time breathing zone sampling and expansion of the DoDSR to increase the specimen types that are collected and stored there. Service members who fought in Vietnam were potentially exposed to the dioxin-containing herbicide Agent Orange; many filed compensation claims because they believed Agent Orange exposure caused their health problems.[33,34] No area sampling data or breathing zone sampling data was performed documenting exposure to Agent Orange. Research on Agent Orange exposure showed few differences in health outcomes between the exposed and unexposed groups and supported the conclusion that most troops were not heavily exposed to Agent Orange.[34]

However, because uncertainty regarding actual exposure levels remained, the US Department of Veterans Affairs (VA) presumptively awarded compensation to veterans who deployed to Vietnam, regardless of exposure status, if they developed any health conditions linked to Agent Orange exposure.[33,34] Unfortunately, no

predeployment serum samples were drawn that could be used to assess baseline dioxin levels for comparison with the postdeployment laboratory analysis of serum dioxin levels. If DoD had collected predeployment and postdeployment serum dioxin levels, and collected blood for monitoring changes in deoxyribonucleic acid (DNA) to identify genetic susceptibility for the conditions linked to Agent Orange exposure, the health risks and disability compensation determinations may have been more evidence based.

Similarly, many service members who fought in the first Gulf War developed Gulf War illness. They reported poorly characterized symptoms following exposure to smoke from oil well fires, pesticides, depleted uranium, mustard and nerve chemical warfare agents, vaccinations for anthrax and smallpox, and the nerve agent antidote pyridostigmine bromine. Again, there was no breathing zone sampling data to document exposures. Over a third of Gulf War veterans still report nonspecific symptoms. Deployment exposures were thought to be the cause of several conditions that were collectively referred to as "Gulf War syndrome," but no exposures were definitively established as the cause.[35]

In response to public concerns and congressional direction to obtain better deployment exposure information, DoD Instruction 6490.03, *Deployment Health*,[36] was revised in 2006 to require the military services to perform deployment health risk assessments that included baseline, routine, and incident-related exposure monitoring and to document any deployment-related exposures in the individual medical record.[1] Routine exposure monitoring was recommended for chronic low-level exposures.

During the early years of Operation Enduring Freedom and Operation Iraqi Freedom, service members were exposed to high levels of silica-containing particulate matter that exceeded the US Environmental Protection Agency's national ambient air quality standard by a factor of six during dust storms.[37] Some personnel were also exposed to smoke from open-pit burning operations, other personnel were exposed to hexavalent chromium dust at a water treatment plant, and still others were exposed to sulfur dioxide while fighting a sulfur mine fire.[37]

Personnel who deployed to Iraq were potentially exposed to smoke from burn pits that were used to destroy plastics, metals, rubber, paints, solvents, munitions, wood, and medical waste.[38–45] Very high levels of particulate matter were recorded in the vicinity of the burn pits, in addition to low levels of volatile organic compounds, polycyclic aromatic hydrocarbons (PAHs), and heavy metals.[38–41] Burn pit operations were conducted at Joint Base Balad, Iraq, until incinerators were installed in 2010. During the peak of burn pit operations in 2007, the base housed 25,000 troops, who generated 250 tons of waste that was burned daily.[39–42] Ambient air monitoring stations were set up to monitor particulate levels that year.[39–41]

In 2010, the Institute of Medicine, part of the National Research Council (NRC), was asked to examine health effects of exposure to open-pit burning during deployments.[43] The resulting 2011 report, "Long Term Health Consequence of Exposure to Burn Pits in Iraq and Afghanistan," noted that DoD did not collect breathing zone exposure data on individual service members.[43] Further, it observed that linking ambient air pollution measurements to individuals and their health outcomes was not possible because the air sampling was not carefully designed, nor was it representative of an individual's exposure. The report recommended that DoD collect breathing zone samples, conduct long-term studies to examine the health outcomes of deployed troops, and address service members' concerns about perceived health risks.[42] The NRC recommended identification of a cohort who had deployed and a control cohort whose serum could be obtained from the DoDSR and compared. However, as the NRC recognized, this comparison had considerable uncertainty because the time the service members had actually spent at Joint Base Balad, their proximity to the burn pits, and other variables were unknown. Individual breathing zone sampling and bio-specimens taken at known intervals before, during, and after deployment would have supported a more rigorous, scientifically based health outcome study.[42]

Assessing deployment-related health outcomes with little or no breathing zone sampling data has challenged military epidemiologists.[43–46] Recent epidemiologic studies have investigated the association between deployment environmental exposures and postdeployment chronic illness, including chronic respiratory conditions, among service members and veterans.[43–46] These studies compared deployed with nondeployed personnel and produced a range of findings, from no association to evidence of increased symptoms and specific lung conditions.[43–46] Sharkey et al reported that deployment to Afghanistan was associated with an elevated risk of postdeployment respiratory symptoms and new onset asthma.[46]

Environmental sampling can capture the external dose using real-time breathing zone sampling, which is the gold standard for exposure assessment, but this is often difficult to achieve in the deployed setting.[42,47] Operational commanders give their attention to managing the greatest risks first, such as protecting the troops from enemy fire. In this scenario, the logistics involved in conducting breathing zone sampling could

hinder other force health protection measures needed by deployed service members to survive in combat. A careful balance must be struck between methods designed to monitor for possible exposures that could lead to long-term health effects and the immediate risks of combat.

Efforts that rely upon traditional exposure assessment, such as breathing zone sampling collectors and pumps worn by the individual, limit mobility, have short battery lives, and require oversight by an individual trained in their operation. Further, the specimen collected must be analyzed in a laboratory equipped with gas chromatography and mass spectrometry. Ideally, the results should be reviewed by a healthcare provider and explained to the service member before being placed in the medical record. Unfortunately, the analyses may take weeks to months to complete, and often the service member has moved or redeployed out of theater, so the results do not make it into the medical record.

For these reasons, breathing zone sampling in combat situations with the available equipment was thought to be impractical.[42,47] However, breathing zone sampling was conducted by the US Air Force at Bagram Air Field, Afghanistan, from November 2011 until March 2012, to assess potential exposures from open-pit burning. Blasch et al collected breathing zone sampling data (without interfering with combat readiness) and noted several PAHs and metals in low concentrations in the breathing zone of a cohort of deployers that often exceeded concentrations in ambient sampling data.[48]

The DoD has focused efforts on the areas of health risk assessment and identification, analysis, and prevention of exposures since the start of Operation Enduring Freedom and Operation Iraqi Freedom.[1–3,49,50] However, lack of data as well as conflicting data indicates a need for alternative ways to characterize exposures that are both valid and reliable. In the absence of breathing zone air sampling data, exposure biomarkers may provide measures of internal doses that allow the DoD to demonstrate exposures to health hazards and any associations between exposure and health outcomes that require further investigation and research.

In 2012, the NRC published a report called "Exposure Science in the 21st Century: A Vision and Strategy," which was developed to guide research on developing exposure information for large segments of the population to relate human health and the environment.[14] The authors noted that a key research need is to develop advanced analytic tools for measuring internal dose, identifying bio-signatures of exposure, and measuring biochemical modifiers of internal dose to complement the individualized biomedical profiles available from genotyping.[14] The use of "omics" technologies, including metabolomics, proteomics, metallomics, transcriptomics, immunomics, genomics, and methylomics, provides many of the measures recommended by the NRC, including use of exposure susceptibility and biological response biomarkers, biomarkers of effective dose, and markers of health outcomes.

The emergence of new laboratory omics technologies offers a potential means of dealing with exposures to assess internal dose. Integration of metabolomics with traditional exposure assessments and a central platform that links exposure to internal dose, biological response, and health outcomes may yield useful measures for the study of long-term adverse health consequences in the future. Many of these platforms are now sufficiently developed to support analyses of stored biological specimens. Applying this approach to DoDSR samples can greatly improve an individual's exposure characterization. The large number of samples, diversity of the population, and extensive demographic and health information in the DoDSR-associated Defense Medical Surveillance System (DMSS) makes the DoDSR an invaluable resource for DoD exposure assessment and precision medicine research.[3]

The US military has been unsuccessful in identifying and assessing potentially hazardous exposures in deployed service members in real time or near real time to give commanders recommendations for preventing environmental exposures. Many area environmental samples have been collected, but few efforts have been made to collect individual breathing zone samples, nor has there been much effort to link results of the sample analysis to the health record of a particular person or group deployed to a specific location at a specific point in time. Among many reasons for this gap are limitations in the training of personnel collecting the samples, limitations in sampling equipment, and limitations in the information technology that prevent sample results from being linked to an individual's medical record.

The DoD is engaged in a significant effort to improve training of personnel to oversee the proper collection of airborne sampling data. DoD efforts are also directed at decreasing the size of sample collection equipment so it does not add weight to service members' equipment or interfere with warfighting capabilities. Improvements are needed so that service members' laboratory results are linked to their medical records, and to ensure results are reviewed by healthcare providers and discussed with the service members.

Department of Defense Serum Repository

The DoDSR was established in 1989 to store serum collected when mandatory HIV testing was performed on active, Guard, and reserve service members,[51,52] and samples have been collected from military personnel every 2 years since. The registry currently contains 61 million serum samples that are linked to individual service members and their health data.[51,52] Service members provide predeployment and postdeployment serum samples, which may be used to address questions related to deployment exposures. DoDSR studies of specimens have usually addressed biological exposures for which antibodies could be measured.[51,52] The associated DMSS database includes service member medical outcome data and predeployment and postdeployment health questionnaire information, which is linked to the DoDSR by Social Security numbers.[51,52]

To ensure future pre-event and post-event evaluation of exposure biomarkers, the Armed Forces Health Surveillance Center (AFHSC) asked, "What could be done better with the serum?" and "What other biospecimens should be collected?" AFHSC identified gaps in current capabilities along with areas for improvement to ensure that serum quality was maintained. Adoption of a quality assurance program that involved testing of the serum against quality control standards was one advance.[4] Other enhancements, including expanding the types of specimens stored and lowering the temperature from -30°C to -80°C, would allow the DoDSR to remain a state-of-the-art bio-repository.[5]

The serum samples stored in the DoDSR have been used for a variety of studies.[53] These studies range from analyses of antibody levels against various infectious agents to analyses for biomarkers possibly associated with physiological changes that could be linked to combat, such as posttraumatic stress disorder. However, serum has not been found useful for whole genome sequencing or RNA analysis. Serum samples are linked to demographic, medical encounter, military occupation, and deployment data. This data provides a powerful epidemiologic resource to investigate deployment burn pit exposures, serum biomarkers, and potential for specific health outcomes.[54] Further, a large cohort of deployers to Iraq and Afghanistan who provided security around burn pits had their predeployment and postdeployment serum examined for PAHs, dioxins, cotinine levels, microRNA (miRNA) levels, metabolic breakdown products, and inflammatory biomarkers. The study noted that the serum was of excellent quality and all those biomarkers could be detected in it. Thus, the DoDSR should be considered a resource for future studies of biomarkers of exposure.[54–61]

Recent studies using new laboratory technologies have confirmed optimism about the quality of serum specimens currently in the DoDSR. The serum specimens were consistently found to contain glucose and amino acids in the normal physiological range.[61] The current DoDSR has considerable utility, but its utility could be expanded if the latest technological advances and laboratory management practices were incorporated into daily operations. Serious consideration must be given to upgrading the capabilities of the DoDSR to allow for the collection and storage of state-of-the-art bio-repository specimens that are not currently collected, including urine, fecal, and whole blood samples or blood spots.[3–5]

To expand the DoDSR's capabilities, its storage equipment must be upgraded to permit colder storage, at -80°C. In addition, standardized procedures should be put in place to ensure specimens are collected, processed, shipped, received, and stored properly, with routine quality assurance procedures to allow a statement of assurance to be issued to specimen users. Presently there is variability in sample collection and processing. Use of state-of-the-art collection tubes may minimize threats to specimen quality.[3]

Although possibly of little value in general health surveillance, the new laboratory technologies could be of great value for examining individual exposures. Military leaders and preventive medicine personnel must be informed of the need to rapidly identify and respond to actual or possible exposures, and to be prepared to collect the specimens and data needed to meaningfully assess the health risk.

BIOMARKER DISCOVERY AND APPLICATIONS

Many different molecular methods are available to study exposure biomarkers. Potentially useful applications of each method are being identified and evaluated.[62,63] For example, a recent study of ultrafine particulate levels and inflammatory biomarkers, including C-reactive protein, demonstrated cardiovascular effects in a group of exposed individuals compared with controls.[62] The different omics techniques (Exhibit 29-1) have been recognized for their potential power in precision medicine, epidemiology, and exposome research.[64–66] The following is a brief description of what each of these techniques analyzes and the benefit that they might provide to military medicine.

EXHIBIT 29-1

"OMICS" TECHNOLOGIES

Metabolomics. High-resolution metabolomics studies provide evidence of thousands of unidentified chemicals in human plasma. The human metabolome is defined as the chemical profile of all low-molecular-weight compounds in a biological specimen and includes endogenous metabolites, chemicals from human-environment interaction, and reactants arising from interaction of these compounds with enzymatic and bacterial processes occurring within the body.

Genomics. Genomics is the analysis of DNA sequencing and requires high-quality DNA. Studies of service members are underway to look for single nucleotide polymorphisms that identify genetic predispositions to diseases caused by environmental exposures and induced mutations caused by mutagenic agents.

Transcriptomics. Transcriptomics is the analysis of messenger RNA (mRNA) transcription as expressed genes in cells. It requires high-quality RNA, which is difficult to obtain because it degrades easily. Transcription analysis can detect metabolic changes due to exposure to infectious agents or toxins that are controlled at the mRNA level.

Serum cytokines and proteins. Biomarkers of inflammation and cardiovascular risk are measured in serum using bead-based multiplex technology from Luminex Corporation (Austin, TX). Immunologic and cardiovascular changes can be detected. The cytokine panel includes 22 cytokines and chemokines associated with inflammation. The cardiovascular panel includes 10 markers including β_2-microglobulin, C-reactive protein, and a number of other serum markers.

Serum microRNA. MicroRNAs (miRNAs) are a class of small, endogenous regulatory RNA molecules that are essential in physiologic processes and regulating stress responses, inflammation, and immunity. Unlike RNAs, miRNA are stable, exist at high levels in serum, and can serve as biomarkers of exposure.

Serum IgE. A sentinel of allergy and asthma, serum IgE is increased by exposure to environmental chemicals, including diesel exhaust and other hydrocarbon pollutants that promote allergic sensitization. Serum IgE levels can be measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit.

Serum cotinine. Cotinine is a metabolic product of nicotine and a sensitive indicator of tobacco smoking status. Tobacco smoke contains polyaromatic hydrocarbons (PAHs) and is a potential confounder in exposure assessments. Cotinine can be measured in serum by testing using a commercial ELISA kit.

Polyaromatic hydrocarbons and dioxin. PAHs and dioxins can be measured in serum by cloud-point extraction. The resulting nonpolar core micelles are isolated, extracted with hexane, and analyzed by gas chromatography and mass spectrometry. Benzo[a]pyrene is converted by cellular enzymes to benzo[a]pyrene diol epoxide (BDPE), which forms adducts with DNA and proteins. BDPE-protein adducts can be detected in serum using a commercial ELISA.

Proteomics. Proteomics is the study of proteins in the host examining molecular events after transcription has occurred. The Department of Defense force health protection program uses proteomics to identify biomarkers and metabolic changes associated with diseases. Environmental chemicals may bind to serum proteins when reactive electrophiles bind to protein carriers in the blood and serum, forming protein adducts.

Epigenomics. Epigenomics is the study of heritable changes not directly encoded in DNA sequences. These changes do not alter the genome and are reversible. The most common epigenetic effects are histone modification and DNA methylation. These DNA modifications are involved in gene regulation and affect gene transcription and expression. Epigenomic analysis allows for the study of pre-transcriptional changes that effect gene regulation before mRNA is produced.

Genomics

Genomics is the analysis of data derived from DNA sequencing. It requires high-quality DNA material. The DoD has invested in genomics as a force health protection tool.[3] The DoD and VA have undertaken genomics studies of service members to look for single nucleotide polymorphisms that may identify individuals with genetic predispositions to diseases caused by various environmental exposures. Also, DNA sequence analyses in exposed personnel might identify induced mutations related to mutagenic agent exposures.[67–69] Additionally, genome-wide association studies, coupled with epidemiologic analysis, may help identify specific alleles and their associated risk of disease.

Transcriptomics

Transcriptomics is the analysis of messenger RNA (mRNA) transcription levels as expressed genes in the cell. This technique requires high-quality RNA, which is much more difficult to generate experimentally than DNA because RNA is prone to degradation. Transcription analysis can be used to detect metabolic changes resulting from exposure to infectious agents or toxins that might be controlled at the mRNA level.[70–72]

Transcriptomics has also been used to predict health outcomes following influenza exposure in clinical studies.[73]

Proteomics

Proteomics is the study of proteins in the host to examine molecular events after transcription has occurred.[3] Proteomics techniques require non-degraded protein, and recent studies have noted that serum samples in the current DoDSR are suitable for analyzing circulating protein, but not cellular or cell-associated protein. The DoD force health protection program could use proteomics to identify biomarkers associated with specific disease states and to detect metabolic changes associated with these diseases.[3] Proteomics may also detect specific biomarkers that represent changes in serum and cellular proteins.[72,74,75] Environmental chemicals may bind to serum proteins when reactive electrophiles bind to protein carriers in the blood and serum to form protein adducts.[76] Quantification of DNA adducts in humans is challenging, even with the use of targeted methods.[77] Protein adducts have been detected by targeting the sulfhydryl group of human serum albumin using high-resolution mass spectrometry up to 30 days after exposure.[78] Redox proteomics, which aims to measure redox-based changes in the proteome, has also been used to identify exposure-response changes.[79]

Metabolomics

Metabolomics has been extensively developed but not yet extensively used for biomonitoring of military exposures. Importantly, high-resolution metabolomics studies have provided evidence of thousands of unidentified chemicals in human plasma. Low-level exposures present a challenge for reliably measuring chemical residues. The human metabolome is defined as the chemical profile of all low-molecular-weight compounds in a biological specimen and includes endogenous metabolites, chemicals from human-environment interaction, and reactants arising from the interaction of these compounds with enzymatic and bacterial processes occurring within the body.[80] Biomarkers in human specimens can therefore provide measures of metabolites from core biochemical processes, lipids, microbiome-related metabolites, dietary chemicals, pharmaceuticals, and chemicals from environmental sources and commercial products.[81]

Various platforms are in use to measure the metabolome, including nuclear magnetic resonance and mass spectrometry. Due to recent advances in instrumentation and computational techniques, applications using liquid chromatography with ultra-high resolution mass spectrometers provide the greatest capabilities for exposome and precision medicine research.[82] Feasibility studies suggest it is possible to measure nearly a million mass-to-charge (m/z) signals. This analytical framework has been successfully applied to identify the metabolic phenotype of chronic diseases,[83–90] aging,[91] infectious diseases,[92] and inflammation[93]; it has also been used as a central platform linking exposure to internal dose and biological response through a metabolome-wide association study framework.[94]

Recent studies have shown DoDSR serum samples are of suitable quality for profiling by high-resolution metabolomics. Initial characterization of 30 samples obtained from the DoDSR showed these samples provided measures of common metabolites consistent with expected ranges, and a detectable biological response to benzo[a]pyrene (a PAH and ubiquitous environmental pollutant) exposure was present.[61] Additional characterization by Liu et al using complementary analytical strategies for high-resolution metabolomics could routinely detect over 20,000 m/z features, including over 7,000 matches to metabolites present in the Kyoto Encyclopedia of Genes and Genomes.[95]

Another study examined cotinine levels, tobacco use, and common health indicators. Its results provided further evidence of DoDSR sample quality and suitability for metabolomic profiling.[96] Based on these results and the available technology, high-resolution metabolomics is sufficiently developed to allow implementation on a test basis for ongoing deployment surveillance.[55] The measures available from this platform include metabolic indicators of nutrition, renal function, and liver function, as well as other indicators. If adapted, chemical profiling of samples obtained before and after deployment, and over the course of service, can be evaluated for exposure biomarkers, effects, and health outcomes. This may improve identification of individuals with risk for environment-associated disease. Implementation is cost effective, and including these capabilities into the DoDSR structure may facilitate hazard identification and improved management of health risks associated with troop deployments.

New methods of monitoring exposure such as silicone wrist bracelets and badges, which are inexpensive and require no external power source, show promise.[97,98] Coupled with untargeted chemical profiling by high-resolution mass spectrometry, these monitors have the potential to improve characterization of exposure to both known and unknown chemical agents. However, there are still challenges with linking external exposure to internal dose, measuring biological relevance, and developing a system for widespread distribution to troops. Thus, biological samples collected for the DoDSR represent a key resource for monitoring troop exposures.[52]

Metallomics

Heavy metal exposure and its associated health effects are an ongoing concern in the military. Chemical profiling by metabolomics can measure organic metal compounds. Metallomics, which uses inductively coupled plasma mass spectrometry (ICP-MS), can be applied to measure the total and speciated metal levels in biological specimens.[3] Biomonitoring studies have shown that the sensitivity of the ICP-MS method is superior to other analytical methods and is more cost effective than graphite furnace atomic absorption spectroscopy. Some recent studies have assessed the micronutrient status and background levels of exposure to toxic metals, but there are limitations with this approach, including contamination during sample collection and suitability of a matrix for metal species.[3]

Epigenomics

Epigenomics is the study of heritable changes that are not directly encoded in the cell's DNA sequences.[3] Epigenetic changes do not modify the sequence of the genome and are reversible. The two most common epigenetic effects are histone modification and DNA methylation. These two DNA modifications are involved in gene regulation in that they affect gene transcription and gene expression.[3] Epigenomic analysis allows for the study of pretranscriptional processes that effect gene regulation before the production of mRNA. The bio-sample most suitable to support this technique is high-quality genomic DNA, although methylation studies have been done on DNA recovered from dried blood spots. Epigenomic analysis has been used to predict disease outcome, especially in the areas of cancer and neurological disorders.[85,87,89] One preliminary study suggests that DNA methylation states may be associated with posttraumatic stress disorder.[88]

Immunomics

Immunomics is the analysis of information pertaining to the immune system, particularly associated with adaptive immunity.[3] This includes the study of the immune response to pathogens as well as to environmental chemicals. To do a full and complete immunomic analysis, white blood cells must be stored and analyzed for immunological markers. Analysis of host immune cells can provide information about the vaccination status and the health of the person's immune system following potential toxic exposures. Immunomic technology has been used to analyze physiological processes, such as autoantibody response, and to develop personalized medical treatments.[3]

MicroRNA

In contrast to the above categories, miRNAs (see below for more detail) are encoded by the genome and are strong regulators of gene expression, but they are not translated into protein and thus do not fit in the transcriptomic or epigenomic categories. However, they provide an important measure of responses to environmental exposures and have been associated with a number of human diseases including Alzheimer disease.[89,91] Recent studies have shown that miRNAs are both upregulated and downregulated in response to environmental chemical exposures.[1] Environmental exposures may interfere with miRNA-regulated translation of proteins; for example, PAHs were found to alter circulating levels of miRNAs in the serum.[63]

Serum IgE

Serum IgE, a sentinel of allergy and asthma, is increased during exposure to environmental toxicants, including diesel exhaust and other hydrocarbon pollutants that promote allergic sensitization. Serum IgE levels can be measured in small volumes of serum using a commercial enzyme-linked immunosorbent assay (ELISA) kit. An analysis of predeployment and postdeployment serum noted that deployment exposures contributed to allergic sensitization and pulmonary symptoms.[1]

Serum Cotinine

Cotinine is a metabolic product of nicotine and a sensitive indicator of tobacco smoking. Tobacco smoke contains PAHs, and tobacco smoke PAHs must be accounted for when examining individuals for exposure to PAHs in the deployed environment. The DoD examined cotinine levels to control for smoking by testing serum using a commercially available ELISA kit in a cohort of deployed individuals who were exposed to PAHs from open-pit burning of trash.[1]

PHYSIOLOGICAL TEST MATRICES

Historically, patients have been monitored with conventional physiological measurements such as height, weight, blood pressure, pulse, oxygen saturation, audiometric testing, and pulmonary function testing. With the advancement of biological monitoring, whole blood, serum, plasma, urine, saliva, and cerebrospinal

fluid began to be used to measure the body's changes in response to internal and external stimuli.[99] Of these, blood and urine are the most commonly collected media to study biomarkers. Biomarkers of internal dose, either the chemical itself or its breakdown product in blood, may indicate recent chemical exposure or mobilization from stores from prior exposure. Urinary biomarkers can also indicate recent exposure, but there is a slight lag time compared to when chemicals show up in the blood. Some chemicals may be sequestered in fat, bone, or other tissue and not show up in blood or urine. Operational commanders and service members prefer the least invasive way of assessing troops for exposure, including sample media collections, if the required quality of information can be obtained.

Nasal Lavage Analysis

Nasal lavage is increasingly gaining attention in allergen testing and as a test matrix for lung injury biomarkers.[93] Nasal lavage has been found to be a useful, noninvasive method in biomarker studies of asthma patients to examine eosinophil-derived neurotoxin for evidence of eosinophilic inflammation.[86]

Exhaled Breath Condensate

Exhaled breath contains microscopic droplets of lung lining fluid that contain biomolecules present in the lung. Exhaled breath can be condensed and collected, and the resulting fluid, exhaled breath condensate (EBC), can be assayed for biomarkers of interest. Portable EBC collecting units have been developed that consist of a collection tube with mouthpiece and integrated sample vial. The collecting tube is placed in a metal sleeve that has been pre-chilled in a freezer, and the donor breathes through the mouthpiece for 10 minutes to collect 1 to 2 mL of EBC. The sample vial is capped and stored for later analysis, the sleeve is returned to the freezer for subsequent collections, and the remainder of the unit is disposable. The process is fast, simple, and noninvasive.[100,101]

EBC samples the lung lining fluid, and numerous analytes have been found in EBC, including proteins, fatty acids, byproducts of metabolism, and host- and pathogen-derived DNA and RNA.[102] Analytical methods include ELISA and similar multiplex methods, polymerase chain reaction (PCR), and mass spectrometry, depending on the analytes to be measured. EBC has been used to investigate DNA mutations in lung cancer, surfactant proteins in chronic obstructive pulmonary disease, and proinflammatory cytokines in asthma.[103] In the field of occupational and environmental medicine, EBC has been used to study exposures to traffic air pollution, ozone, foundry dust, metal nanoparticles, welding fumes, and industrial chemical exposures. EBC has been evaluated as a screening tool for asthma in military recruits, and used to demonstrate increased proinflammatory markers in sailors after an 8-hour duty period on board small diesel-powered coast guard patrol boats.[104,105] As analytical techniques grow ever faster, more sensitive, and less expensive, EBC is poised to become a major sampling site for biomonitoring of pulmonary and systemic health, disease, and occupational exposures.

Saliva

Saliva is an excellent medium, better than urine or blood, for exposure biomonitoring. It can be quickly collected by noninvasive methods, which makes the collection process easy and acceptable in deploying troops. Saliva biomarkers can be used to monitor health and conduct disease surveillance.[92] Biomarkers for therapeutic interventions, hormonal and immunological changes, and toxic chemicals and their metabolic intermediates can be detected in saliva. For example, thiocyanate can be detected in the saliva of smokers.[106]

NEW DEVELOPMENTS IN BIOMARKERS

Exposure Memory

Although still an evolving concept, evidence suggests multiple exposure memory systems exist with the potential to provide long-term markers of underlying injury arising from exposure.[55,107] These systems can be used as nonspecific indicators of exposure, and comparing predeployment and postdeployment indicators provides a means of identifying changes in these systems. Current approaches to measure exposure memory include redox-proteomics, epigenomics, and metabolomics. In one study, metabolic changes were observed in individuals exposed to an extremely high dose of dioxin. The study, conducted in 2011, examined biomarkers that were obtained from healthy controls and eleven workers exposed to dioxin residues during the 1960s. The data showed these metabolic alterations were still present in the workers relative to the healthy controls, and included changes in expression of cytochrome P-450, hepatotoxicity, bile acid biosynthesis, and oxidative stress.[108] The data showed that changes in the metabolome due to a recent, acute dioxin exposure could still be detected in exposed individuals 40 years later.

Exposure memory is a key component of the exposome, and continued characterization of multiple systems is ongoing through efforts integrating different omics platforms and health outcomes.[109]

Serum Cytokines and Protein Biomarkers

Biomarkers of inflammation and cardiovascular risk can be measured in serum using bead-based multiplex technology from Luminex Corporation (Austin, TX), which allows detection of 42 different molecules in a single 0.025-mL sample. The cytokine panel includes 22 cytokines and chemokines associated with inflammation. The cardiovascular panel includes 10 markers including β_2-microglobulin, C-reactive protein, and a number of other serum markers that can be assayed in 0.05 mL of serum to simultaneously measure 32 biomarkers. A recent study utilized two panels of biomarkers to detect immunologic and cardiovascular changes associated with service-related exposures.[56,60]

Biomarkers of Pulmonary Injury

The goal in the study of pulmonary injury is to develop omics technologies that can provide molecular signatures indicative of lung injury.[110] Recent studies demonstrate the potential utility of several types of biomarker molecules in different media.[111] Many new biomarkers of volatile organic compounds have been detected and quantified in EBC.[112] These biomarkers provide a measure of airway inflammation and offer a complementary tool to assess airway disease and asthma.[113]

Development of new analytical platforms, including GC Orbitrap (Thermo Scientific, Carlsbad, CA) high-resolution mass spectrometers, has greatly improved the capability to measure low-molecular-weight volatile organic compounds in small volumes of biological samples.[114] The use of high-resolution mass spectrometry greatly improved the detection of ethyl-thiocyanate generated from thiocyanate present in EBC, resulting in low nanomolar detection limits. The improved detection limits were a direct result of the ability to extract accurate mass ion chromatograms, which is not possible using traditional instrumentation due to noise at low m/z levels.

Additionally, biomarkers of oxidative stress and inflammation including proteins have been measured in bronchoalveolar lavage fluid. The study of chronic obstructive pulmonary disease and lung function impairment has led to discoveries through transcriptomic analyses of gene expression in the bronchial airway epithelium and lung parenchyma cells. Condition-specific genes and altered molecular pathways were noted to be associated with cigarette smoking.[115,116] Further, RNA expression studies were used to identify gene expression signatures for particular types of pulmonary injury or disease. In addition, recent findings in asthma transcriptomics studies noted that T-cell type 2–mediated inflammation symptoms correlated well with biomarker levels of interleukin 13 and interleukin 14–induced genes.[117] While noninvasive testing is preferred, bronchial lavage and brushing can provide researchers with biomarkers that may one day enhance the understanding of molecular disease mechanisms caused by the inhalation of airborne hazards.

GENETIC AND EPIGENETIC BIOMARKERS

In the past 10 years, epigenetics has expanded through technological advancements in the laboratory including the use of high-throughput multiplex methodologies.[118] For environmental exposures, research has focused on how these exposures affect posttranslational modification of proteins.[119] Researchers continue to focus on how exposures and other environmental factors (eg, demographic, exercise, diet) alter the normal epigenetic processing of proteins and other molecules.[120] Recent research has suggested that environmentally induced epigenomics changes may alter gene expression in such a way that specific pathway alterations may become permanent.[121–123] More research is needed to confirm that the observed epigenetic changes are causally related and not just correlated with long-term changes.

Non-Coding RNAs

Non-coding RNAs (RNAs that do not encode for protein) have recently emerged as powerful candidates for biomarker discovery. The most widely described are the miRNAs, a class of small, endogenous regulatory RNAs (20–25 nucleotides in length) that are essential in normal physiology and disease processes, as well as the regulation of stress responses, inflammation, and immunity. A single miRNA can regulate expression in literally hundreds of distinct target genes, and thus one miRNA can have a profound impact on gene expression and physiology. Changes in miRNA levels lead to altered gene expression and the development or promotion of disease states. To date, about 1,500 different miRNA species are encoded in the human genome.

Emerging evidence supports miRNA expression profiling as a crucial diagnostic tool. Highly abundant in human serum and plasma samples, miRNAs are much more stable than other classes of RNAs due to their small size, sequestration into small particles called exosomes, and binding proteins that shield them from the abundant RNA-degrading enzymes found in most biological fluids.[123] The remarkable stability of some miRNAs has been revealed by several studies of miRNA levels in stored serum and other biofluids over extended time periods.[124,125] For example, one study showed that even at -20°C, many serum miRNAs are stable for up to 8 years, with minimal impact on detection. Interestingly, differential miRNA levels have been detected in blood (serum, plasma), urine, EBC, and tissue biopsies from patients with disease compared to unaffected individuals.

Another advantage of utilizing miRNAs as biomarkers is the relative ease and universal method of their detection. Levels of miRNAs can be accurately quantified from biological fluids using PCR, a laboratory technique that uses selective amplification steps to reliably measure the level of nucleic acids (in this case specific miRNA). The ability of PCR to dramatically amplify the level of the analyte means many miRNAs can be detected in small biological samples (up to 120–150 different miRNAs can readily be detected in only 0.1–0.2 mL of human serum). Specific miRNA levels can also be used to determine biological sample quality. For example, miRNA expression by quantitative PCR can be used to determine if certain serum samples are affected by hemolysis (red blood cell lysis) due to the presence or absence of specific miRNAs found almost exclusively in red blood cells.[126]

In addition to being utilized as biomarkers of disease state, miRNAs are being tested as markers of exposure. Recent work has linked environmental PAH exposures with altered plasma miRNA levels.[63] Serum samples from the DoDSR were used to identify specific miRNAs that show a strong correlation with smoking status and serum markers including inflammatory cytokines.[56] Another study used DoDSR serum samples to show that certain miRNAs change expression after personnel deployment to military bases with active open-air burn pits.[60] Thus, miRNAs may provide an ideal biomarker for exposure, disease, or pathological state.

While miRNAs are currently the most widely used non-coding RNA class for biomarkers, other RNA species are increasingly being investigated as novel biomarkers. Long non-coding RNAs (lncRNAs) are typically over 200 nucleotides in length and have been related to multiple functions, including activating or repressing gene expression, recruiting various protein cofactors, and inducing methylation-dependent epigenetics,[127] and they are also involved in gene imprinting and X-chromosome inactivation. Recently, differences in certain lncRNAs have been detected in atherosclerosis and certain cancers. However, given the lower stability of lncRNAs compared to miRNAs, and the current lack of studies on lncRNAs in various biological fluids readily obtainable for biomarker analyses, the usefulness of lncRNAs in biomarker discovery requires more study.

Another class of non-coding RNA is the piwi-interacting RNAs (piRNAs), which form RNA-protein complexes with the piwi proteins. Like miRNAs, piRNAs regulate gene expression through epigenetic and posttranscriptional gene silencing. Compared to miRNAs, piRNAs are slightly larger and less stable, and piRNAs are unique in that they are abundantly expressed in germ line cells (sex cells), and thus can be directly inherited by offspring. Recently, piRNAs have been measured in human blood samples, and differences in piRNA expression were observed in certain cancers, suggesting they may be potential biomarkers.[128] Whether piRNAs are altered by various environmental exposures is currently unknown and future studies are needed.

Genetics in Pulmonary Injury Research

Genome-wide association studies have permitted the discovery of gene mutations associated with increased susceptibility to environmental hazards. Currently, whole genome sequencing studies in large military cohorts are planned that will link alterations in DNA sequences to health outcomes and exposures (phenotypes).[129,130] Genetic research may also yield information on biomarkers and specific pathways altered due to specific environmental exposures and dosages. Lung disease studies of gene–environment interactions have identified a number of genes that predispose people to higher injury risk.[131]

Polymorphisms caused by oxidative stress have been found to make individuals more susceptible to environmental exposures to particulate matter and ozone.[132] Research in nutrition and gene interaction in the presence of pollution has shown that ϖ-3 fatty acids, antioxidants, and methyl nutrients are protective.[27,133,134] Susceptibility studies in silicosis patients have noted a cellular DNA polymorphism associated with increased risk.[135] Researchers also examined biomarkers of susceptibility in animals following exposure to inhaled silicon dioxide nanoparticles, noting that toxicity varied with age and suggesting that there may be different biomarkers of susceptibility at different stages of development.[29]

Gene–environment interactions is another area of ongoing research. In one study, individuals were exposed to airborne endotoxins and genotype signatures were examined. Carriers of three single nucleotide polymorphisms in the CD14 gene and one in the MD2 gene were associated with increased risk of asthma symptoms following endotoxin exposure, compared with the allele homozygotes.[136] Genetic research in the military is helpful in understanding lung injury under specific conditions. However, its use for susceptibility screening may be of limited value. The test will only be cost effective when the prevalence of the genetic mutation is moderately high and susceptibility cannot be determined. In the end, change in the genotype linked to a health decrement caused by exposures is the gold standard for proving causation. Once the evidence is established, exposure prevention countermeasures become necessary to avoid adverse health outcomes.

The military has an ongoing screening program for personnel who are at risk for a genetically linked glucose-6-phosphate dehydrogenase (G6PD) deficiency.[137] The services screen for G6PD deficiency prior to deployment to regions of the world where malaria is endemic. Personnel who have the G6PD deficiency can have adverse blood reactions to the antimalaria drug primaquine. The G6PD deficiency occurs in 6% of individuals worldwide, but the prevalence is lower in US military personnel, where 2.5% of males and 1.6% of females have the deficiency, usually African American, Asian, and Hispanic individuals.

BIG DATA ANALYSIS

Informed and appropriate "big data" collection and storage, with support from experienced analysts, will allow public health practitioners and researchers to integrate results from a multitude of diverse laboratory tests with clinical, demographic, and exposure data. Presently, big data is used mainly for susceptibility studies.[137] In the future, big data will have increased use in support of environment-wide associated studies, which provide a hypothesis-free approach that examines associations among multiple variables. This approach links biomarker discovery studies by specifically integrating metabolomics data with results of studies in many other areas, such as gene expression, microbiomics, and redox proteomics. Most current efforts to monitor and minimize exposures to toxic chemicals and other hazards use a public health approach. This has been the most cost-effective way to provide maximum benefit to populations because it prioritizes risks according to those with the greatest hazard and likelihood of exposure. The public health approach has also yielded precise analytical measurement and extensive population data on a relatively small number of known hazards. However, existing exposure surveillance methods to monitor and protect against hazardous exposures in civilian life differ from military exposure surveillance because the range, intensity, and complexity of hazardous exposures in the deployed environment exceed those encountered by civilians and cannot be fully anticipated.

Recent analytical developments have created an opportunity for a different approach to exposure surveillance based on ultra-high resolution mass spectrometry and new strategies in biomonitoring.[138] Application of either liquid chromatography or gas chromatography with ultra-high resolution mass spectrometry and advanced data extraction techniques now routinely provide relative quantification of up to 20,000 chemicals. These approaches provide for the measurement of environmental chemicals in human samples at five to seven orders of magnitude lower than many endogenous metabolites, and hundreds of environmental chemicals have been detected at low levels using these methods without prior knowledge of the chemicals' existence.[139]

Development of computational metabolomics has mirrored advances in analytical chemistry.[2] An important advance is the use of multiple technical replicates to enhance reliability of detection of low-abundance chemicals. Bioinformatics methods are now available to link multiple metabolic byproducts derived from a single chemical precursor.[139]

Additionally, advances in high-throughput technologies have enabled large-scale and high-resolution measurements of various molecular signatures of exposure (eg, in the genome, transcriptome, proteome, and metabolome) to assess the health status of military personnel. Figure 29-2 is an overview of how big data analytic methods are used to integrate omics results with clinical symptoms. The observed health status and clinical symptoms are related to exposures to chemicals measured by environmental profiling techniques described above. The observed clinical symptoms are an outcome of a complex interplay between the exposure, the genotype, and alterations in structure or function noted by omics technologies. Indeed, medicine must rely on information captured at increasingly greater depth. This requires new tools to enable the use of big data for presymptomatic detection of disease and clinical decision-making.

The goal of big data analysis is to identify molecular signatures using data generated from omics analysis that results from environmental exposures and

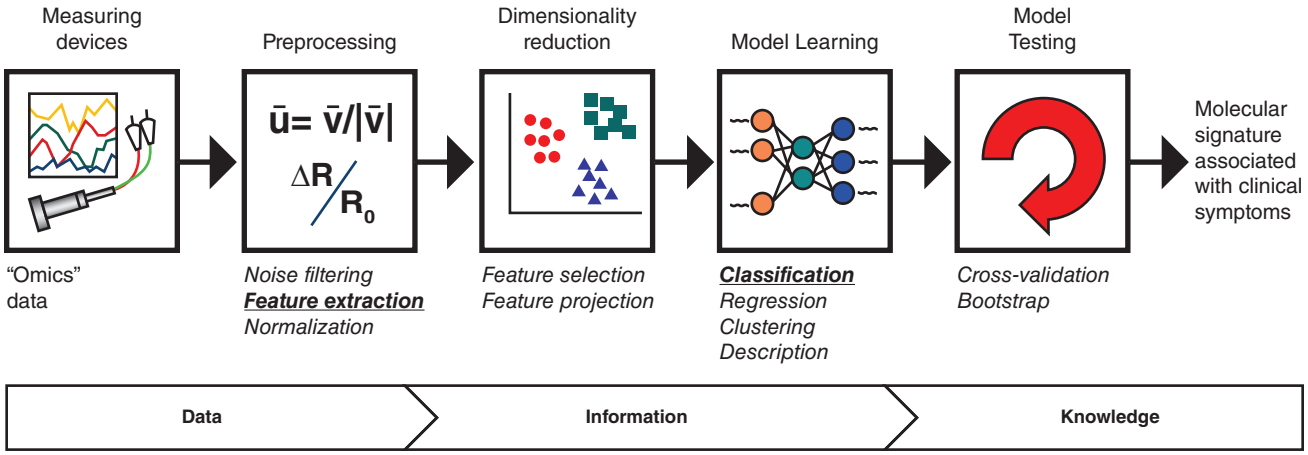

Figure 29-2. "Big data" analytic methods to integrate "omics" results with clinical symptoms.

correlates with observed clinical symptoms. Statistical distributions of the data generated by omics analysis is the first step, followed by data processing and normalization.[140–142] Data processing evaluates signal-to-noise ratios so that low signal fields can be thrown out. Data normalization is done to reduce systematic biases prior to any downstream quantitative analysis. Several technology-specific parametric and nonparametric statistical methods are currently available for this analysis.[143] Graphical representation of sample results and variables facilitates interpretation and identification of the most informative data features. Principal component analysis and other methods permit visualization of data where there is considerable noise. To identify molecular signatures that correlate with clinical symptoms, group machine learning approaches can be used with *International Classification of Diseases, Ninth Revision* codes to find variables and integrate the data.[144,145] Thus, molecular signatures associated with the clinical symptoms of military service members can be investigated for use as new biomarkers. These can then be experimentally tested in animal models or clinical studies in humans.[146] Bioinformatic tools such as gene set analysis methods, also called pathway analysis methods, allow contextualization of high-throughput data with a priori biological knowledge about interaction and coexpression of genes, which maximizes use of big data.[147–150] For example, the miRNAs and metabolic pathways associated with deployment exposure may be used to develop interventions to reduce service member exposures.[151,152]

Finally, clinical decision-making enabled by data-intensive technologies will test the limits of the current information technology infrastructure in terms of physical storage, database management, data processing, and data mining. Complex data management systems like the US Army Medical Research and Materiel Command's SysBioCube and data integration tools are needed to offer scalable and collaborative solutions to explore and contextualize data.[153,154] Beyond technological developments, concerns about information privacy and security must be addressed.

SUMMARY

Although the DoD has made great progress in using the DoDSR to address questions related to infectious agent and other environmental exposures of military service members, there are opportunities to do more by studying the serum of deploying service members using available omics technologies. Most of the medical information gained from omics-based studies is research oriented, but the search for specific associations with disease will facilitate acceptance of this new technology. The most advanced of these technologies is genomics and genome-wide association studies coupled with mass spectrometry-based proteomic analysis.[155] However, these technologies are only part of a complicated systems biology approach that likely will pay benefits in the future. As more scientific information is gained, it may become possible to screen service members for the propensity to develop specific medical outcomes in response to environmental exposures. Future research may provide information that supports the notion that service members' medical conditions are associated with specific exposures such as fires, dust, or aerial spraying, to name a few. These are critical questions that DoD must address.

Improvements in exposure science are needed to better understand the effects of deployment exposures on service member health.[42,156] Exposure information that can be linked to an individual is needed for conducting rigorous epidemiologic studies designed to compare health outcomes in the exposed and non-exposed populations,[4] leading to better force health protection measures such as troop relocation and use of personal protective equipment.[19] Biological monitoring will add to the DoD's tools for assessing the internal dose of exposure. Refinements in biomarker science may permit the linkage of exposure with health outcomes to identify populations at higher risk from exposures. High-throughput omics technologies are driving analyses of the exposome and enabling analyses of the entire internal biochemical environment. Omics in the future may identify the absorbed dose of chemicals and provide insight into how the metabolic pathways and gene and protein expressions are altered.

The DoD must develop the infrastructure needed to appropriately and effectively use available modern technologies. Considerable work has been done to identify bio-repository needs for the future and the options available.[3–5] However, this information has not yet been put into a plan to secure funding. The large quantity of data generated from omics studies will require big data analysis capabilities that do not currently exist. Improvements could be achieved by developing in-house capability; forming civilian partnerships, possibly with universities already doing this work; or a combination of both. Lastly, the DoD must consider all new and emerging technological capabilities and decide which suites of tools will be employed in various scenarios to assess actual or possible exposures. Clearly defined protocols for exposure assessment using biological monitoring and analysis of the results is critical to achieving informative and cost-effective health risk assessments.

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