Host Response to Environmental Hazards: Using Literature, Bioinformatics, and Computation to Derive Candidate Biomarkers of Toxic Industrial Chemical Exposure

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ABSTRACT

It is essential to protect Service Members from toxic and hazardous chemicals and materials across the entire range of deployment missions, from humanitarian to theater-level combat. Tools that rapidly and accurately quantify exposure-induced health effects would enable Commanders to make informed decisions on return-to-duty and allow service members to thrive in environments of uncertainty and danger. Currently, U.S. Forces lack a suitable method to rapidly screen and assess risk of toxic end organ injury following chemical exposures in the field. Markers of end-organ injury and toxicity and other health effects markers, particularly those used in a clinical setting, could be integrated into a "lab-on-a-chip" fieldable detection cartridge for molecular indicators of injury following chemical exposure or for routine surveillance when operating in dangerous environments. We utilized large public repositories of drug toxicity data to infer biomarkers of toxic industrial chemicals exposure. Using a computational and relational approach we prioritized militarily relevant toxic industrial chemicals and their anticipated adverse health effects, to include potential threats related to megacities. Initially, we focused on liver and kidney toxicity because these organs are particularly susceptible to toxic injury, often used in drug toxicity studies with large amounts of publically available data, and in some cases have established FDA-qualified biomarker panels or clinical assays. We mined the literature and databases (e.g., DrugMatrix® and the Comparative Toxicogenomics Database) for candidate gene targets to make predictions about biomarkers related to toxic industrial chemicals that cause the same adverse health effects. We identified 78 and 244 gene modules associated with liver and kidney injury, respectively, and qualified some of these targets in independent animal studies. In particular, we developed panels of genes (25-50 genes per panel) detecting liver fibrosis with 70-95% sensitivity and specificity. Using this success as an exemplar, we are currently evaluating drug induced liver injury and kidney injury panels within an adverse outcome pathway framework in order to develop a multiplexed panel useful in the diagnosis of the most highly prioritized health effects caused by industrial chemicals. Integration of such efforts with physiologically based models will enhance prediction capability and contribute data to sophisticated tools for health and risk assessment and surveillance. Large drug toxicity data repositories have proven useful in making predictions about biomarkers of health effects in a manner that is useful and applicable to military-relevant threats. In doing so, we plan to integrate physiologically based models, high content screening data, and adverse outcome pathways into a new generation of improved health risk assessment and screening capabilities.

1.0 INTRODUCTION

Service members are occupationally exposed to chemicals and environmental hazards, elevating their risk for developing adverse health effects [1]. Military personnel are exposed to environmental health hazards during training exercises, deployments, and national defense. Physiological response to chemical threats varies among individuals, and is influenced by individual behavior, medical history, individual response to physiological and psychological stressors, and genetic susceptibility. In many cases, assessment of adverse health risks and effects must be made on a case-by-case basis [2-6].

During Operations Iraqi Freedom and Enduring Freedom (2001-2011), there were 585 hospitalizations and medical evacuations due to toxic substance exposure, resulting in decreased force strength and operational readiness [7]. However, it is likely that these data do not accurately reflect the real risks of exposure to service members. Acute, high-level exposures resolved onsite in field hospitals are frequently not adequately documented for inclusion in epidemiological studies, suggesting that the actual risk to deployed soldier is far greater than current estimates. Limited epidemiological data are available to adequately formulate risk assessment models for chemical and environmental exposures in theater. In most cases, quantifiable and verifiable exposure data are absent [7].

In future operations, military deployments and conflicts will likely occur in urban, industrialized megacities of greater than 10 million inhabitants. Military personnel will be at heightened risk of exposure to toxic industrial chemicals and materials (TICs/TIMs) associated with routine manufacturing and industry operations in a megacity environment [8]. Inaccurate chemical inventories and inadequate regulation by centralized regulatory agencies further increase risk to deployed personnel. Recent years have also seen an exponential advance in the development, distribution, and use of engineered nanomaterials without a commensurate assessment of potential toxicological hazard. Rapidly and accurately quantifying exposure-induced health effects will allow commanders to make informed decisions on return-to-duty, medical surveillance, or evacuation after a chemical exposure.

Currently, the military lacks standard concepts of operations for making command decisions to move personnel exposed to TICs/TIMs between medical roles I-IV. The reasons for this deficiency are multifactorial: (1) the number of potential chemical threats increases exponentially when considering mixtures in a megacity environment, making it logistically impractical to develop operational plans for every possible scenario; (2) most chemicals have no available toxicity data or standard countermeasures to guide medical decision-making; (3) sophisticated screening tools are lacking for potential occupational hazards; and (4) many chemicals including endocrine disruptors and engineered nanomaterials cause toxicity at low doses and do not have a predictable dose response relationship [9].

Ethical, financial, regulatory, and logistical considerations add to the complexity of the problem. Conducting a full battery of toxicology assessment tests would require thousands of exposure-based assays and a logistically impractical number of experimental animals, manpower hours, and resources. New approaches, applications, and technologies will be needed to provide personalized, preventative health risk assessments in the megacity operational environment. Further, detection techniques must be versatile enough to detect metabolites, nucleic acids, proteins, and other biomolecules within a single, durable platform with compact footprint.

The ideal biomarker candidate(s) will be mechanistically linked to the pathology in question. The Organization for Economic Cooperation and Development (OECD) recently launched a program to develop adverse outcome pathways (AOPs) for presenting causal relationships necessary for pathogenesis at the molecular, cellular, tissue, and organ levels. AOPs communicate and organize knowledge in a manner that is relevant for risk assessment in the regulatory arena [10]. In the AOP framework, a single molecular initiating event (MIE) triggers a cascade of key event (KE) relationships at the cellular, tissue, and finally organ levels to result in organ dysfunction and/or lethality (Figure 1) [11]. Quantitative AOP-based assessments are

gaining regulatory acceptance with the EPA [12]. Predictive algorithms that associate changes in biomolecular activity with KEs triggering adverse outcomes could be anchored to physiologically based models. For example, co-regulated gene modules and gene/protein signatures in circulating biomarkers can be correlated with KEs to provide a systems toxicology interpretation of adverse biological outcomes [13]. Further, existing databases of toxicokinetic, toxicodynamic, and toxicopathologic endpoints can be integrated into physiologically based models of intoxication, potentially providing a new powerful tool to improve health risk assessments.

This paper summarizes the current progress of the United States Army Center for Environmental Health Research (USACEHR) in (1) prioritizing militarily relevant toxicants and (2) developing appropriate computational tools to assess health effects of a wide range of TICs/TIMs in order to support medical decision-making by commanders and medical personnel in exposure scenarios. We bioinformatically mined large publically available data repositories to identify candidate biomarkers of liver fibrosis as an exemplar. We experimentally qualified these candidate biomarkers within the liver fibrosis adverse outcome pathway (AOP) framework.

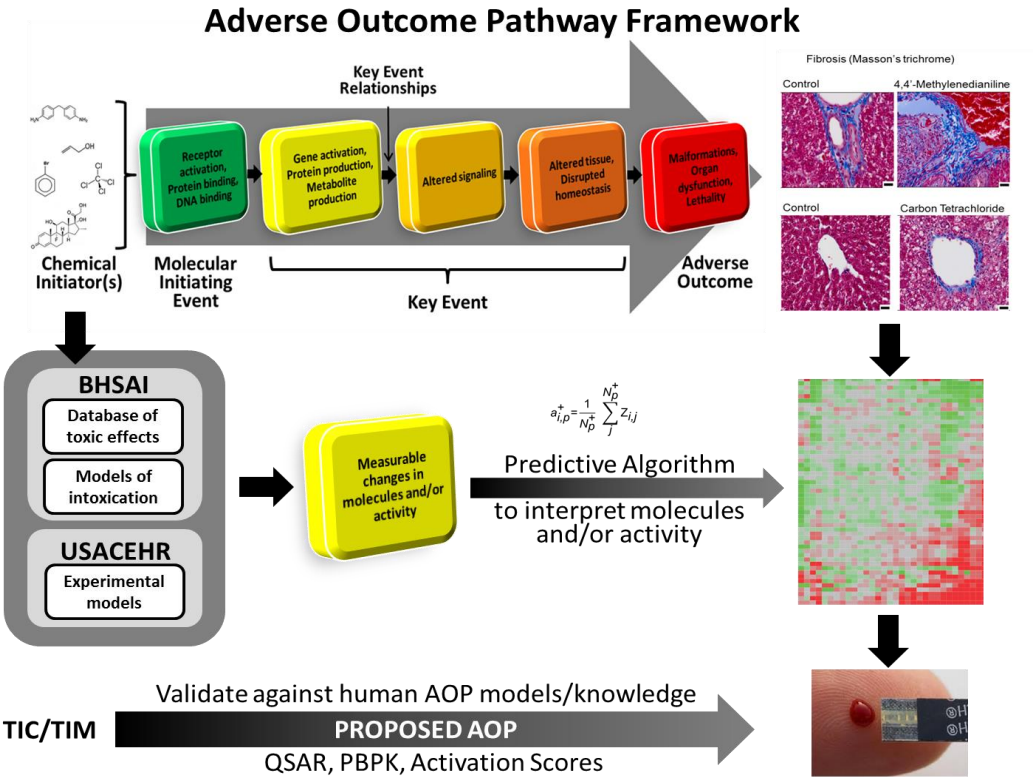

Figure 1: Strategic vision of developing materiel solutions for biomarkers of adverse health effects using the AOP framework. Developing molecular indicators linked to specific AOPs effectively broadens the tools applicable to chemical exposure scenarios. BHSAI, Biotechnology HPC Software Applications Institute.

2.0 METHODS

2.1 Prioritized list of toxic industrial chemicals and adverse health effects

To prioritize the more than 80,000 toxic industrial chemical threats, we accessed the Industrial Chemical Analysis (ICA) database compiled by the Joint Protection Manager for Individual Protection (JPM-IP) and the Naval Research Laboratories (NRL). The ICA database was prioritized in 2010 to take into account

chemical properties such as geographical abundance, reactivity, stability, and toxicity. Probability scores were calculated based on the toxic (operational) hazard scores, comprising toxicity, stability, and physical state scores. Critical and high priority sub-lists were developed based on additional scoring algorithms factoring in global geographical distribution and production and class-based, reactivity, and physical property analyses. Where possible, the Merck Index and published literature were consulted to fill in gaps in the original International Task Force (ITF) 40 ICA scoring method [14].

A relational database was constructed in a mySQL environment in order to integrate target organs of adverse health effects, chemicals of interest, and potential biomarkers of toxic chemical injury. End organ target toxicities were obtained from multiple publicly available sources. The most comprehensive, readily parsable data repository was the Comparative Toxicogenomics Database (CTD). Data from both curated and relational associations were included.

2.2 Mining DrugMatrix® and the Comparative Toxicogenomics Databases

Data for predicting gene changes associated with military threat chemicals were obtained from two public data repositories: the DrugMatrix® database and the CTD [15, 16]. Using an iterative signature algorithm (ISA) approach, we identified gene modules predicted to be associated with liver and kidney pathology. We developed a computational algorithm based on transcriptomic signatures of gene sets predictive for hepatic fibrosis, steatosis, and peroxisome proliferator activation [15, 17, 18].

Based on these predictions, prototype genomic biomarker panels were developed for experimental verification. Two computational approaches were used to down-select gene candidates from co-expression modules. First, ISA were used to group genes into co-expression modules based on similarity in expression patterns across compound-dose conditions [17, 19]. Genes in modules anchored to the liver fibrosis histopathology were further analyzed to identify genes with expression patterns closest to the average absolute activation value (i.e., z-score). These centroid genes were selected for the multiplexed panel. In the second approach, liver fibrosis-associated genes were mapped to pathways and high-confidence human protein-protein interaction networks. Rank product and hierarchical clustering determined differential expression and co-expression patterns to identify genes relevant to liver fibrosis, and the resulting genes were mapped to high confidence human protein-protein interaction networks [20-22]. Network modules representing genes with predicted common function and/or expression in a given network were extracted by Cytoscape tools (i.e., KeyPathwayMiner and Clusterviz [23]). Module genes differentially expressed in DrugMatrix® liver microarray analysis were selected for inclusion in the multiplex panel [19].

2.3 Experimental verification of biomarker panel candidates

The resulting exploratory panel of genes was tested experimentally in rodents using chemicals from both the military threat list and the DrugMatrix® database (Figure 2). Allyl alcohol and 4,4'-methylenedianiline are associated with fibrotic injury in the DrugMatrix® database after five days of daily administration. Carbon tetrachloride is a delayed-onset fibrogenic compound, resulting in fibrotic injury at 14-28 days. Dexamethasone and bromobenzene cause liver pathology but not fibrotic injury (glycogen accumulation and steatosis, respectively) [24, 25]. Male Sprague-Dawley rats were orally administered these chemicals at escalating doses for 5 days. Bioplex® technology was used to quantitatively assess changes in gene expression in liver tissue. Liver sections stained with hematoxylin and eosin and Masson's trichrome were scored for pathological changes by a certified veterinary pathologist.

Histological results were evaluated and scored for severity. Results were determined by a nonparametric Kruskal-Wallis analysis of variance by ranks with post hoc Dunnett's multiple comparison test among dose groups using GraphPad Prism (GraphPad Software, Inc.; LaJolla, CA). A p-value <0.05 was considered statistically significant. Sensitivity and specificity were determined by comparing the diagnosis predicted by the gene panel with the diagnosis made by histopathology assessment with Masson's trichrome.

3.0 RESULTS

3.1 Prioritized list of toxic industrial chemicals and adverse health effects

The NRL-JPM-IP ICA database was used as the starting point to develop a prioritized list of the physical/chemical properties and toxicology data associated with toxic industrial chemical exposure. Clinical data on acute symptoms and related biochemical mechanisms of action were incorporated into the new database. High priority industrial chemical hazard lists for ingestion, inhalation, and percutaneous exposure routes were developed and linked to basic toxicity, time-to-onset of clinical symptoms, likelihood of a chemical to manifest a toxic hazard in the operational environment, and acute onset (time to manifestation of symptoms). Merging the ingestion, percutaneous, ocular, and inhalation hazard lists resulted in a down-selected list of approximately 570 chemicals. Global distribution and production data were used to assess the prevalence and hazard posed by industrial chemicals. The top 30 megacities chemical threats were identified based on these data. The resulting list comprises a military threat list of toxic industrial chemicals and was recently submitted to the Defense Technical Information Center [26].

A relational database was constructed in a mySQL environment integrating target organs of adverse health effects, chemicals of interest, and potential biomarkers of toxic chemical injury. End organ target toxicities were obtained from the CTD, the most comprehensive, readily parsable public data repository available. Data from both curated and relational associations were parsed into the database. Approximately 54% of the chemicals on the threat list had associated CTD data. Time-to-onset was determined by manual curation. Chronic and hereditary conditions were removed and only subacute and subchronic endpoints were retained. For the remaining 46% of the chemicals, entries were manually inputted into an Excel database based on keyword searches by chemical name and/or CAS number in the TOXNET suite of toxicology databases, including the Hazardous Substances Databank [HSDB], TOXLINE, Haz-Map, and Integrated Risk Information System [IRIS] (http://toxnet.nlm.nih.gov). Data mining was supplemented with a PubMed literature review by chemical and/or CAS number. Target organs tallies from both search strategies identified the number of chemicals associated with each pathology. Toxicology data were unavailable for 87 chemicals. Of the remaining 483 chemicals, the frequencies of target organ toxicities were as follows: kidney (226), liver (275), heart (183), central nervous system (CNS) (215), lung (232), and other (60). Incidences of specific lesions were expressed as a percent of general organ injury (Figure 2).

3.2 Computational approaches identifying candidate biomarker panels

The relational database was used to identify chemicals and key health effects on the military threat list for further testing. Chemicals on the military threat list were cross-referenced with data available in publically available toxicology data repositories. Repositories accessed were (1) the National Toxicology Program's DrugMatrix® database and (2) the CTD [15, 27]. DrugMatrix® is a compilation of more than 3,200 drug and toxicant exposures in rats with accompanying clinical chemistry, histopathology, and microarray data [15-19]. CTD is a relational database associating chemical exposures with diseases and gene expression changes [16]. Militarily relevant chemicals causing liver fibrosis and change in abundance of genes in the fibrogenic co-expression modules were selected for experimental verification (see next section; [28]).

The relational database was then used to prioritize military threat chemicals with liver histopathology and differential expression of genes mechanistically linked to liver fibrosis pathogenesis (Figure 3). Using this approach, we identified gene co-expression modules anchored to periportal fibrosis.

Bioinformatics mining of DrugMatrix® and CTD identified 78 gene modules associated with liver injury and 244 gene modules associated with kidney injury. Liver fibrosis was identified as the pathology most closely correlated with predictive gene signature patterns. From the 78 gene modules associated with liver injury, we identified a 67-plex panel of presumptive fibrogenic indicator genes hypothesized to predict liver fibrosis. To experimentally test the accuracy of fibrosis prediction by gene expression alone, we conducted

rodent oral exposure studies with fibrogenic chemicals. Histopathology confirmed fibrotic injury for allyl alcohol and 4,4'-methylenedianiline. Both chemicals also caused a qualitative increase in cytoplasmic alteration, subacute inflammation, and bile duct hyperplasia, as illustrated by representative images for 4,4-methylenedianiline (Figures 3A). Severity of injury increased with dose in all categories of pathology, as illustrated by the exemplar images for 4,4'-methylenedianiline (Figure 3B).

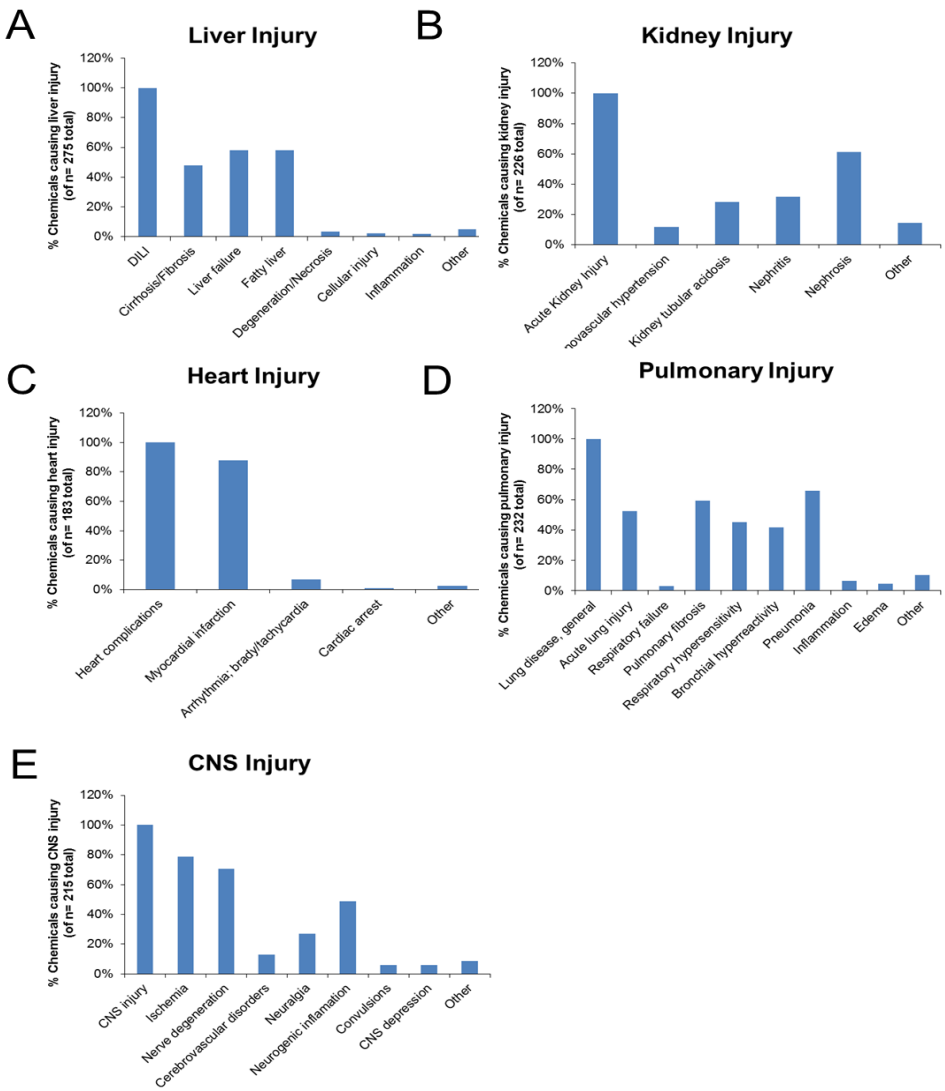

Figure 2: Target organ injury associated with 570 prioritized TICs/TIMs. Adverse health effects were determined by rank order for (A) liver, (B) kidney, (C) heart, (D) lung, and (E) CNS. The first bar in each graph represents the number of chemicals associated with generalized injury to the target organ. Subsequent bars indicate specific pathologies as a percent of total chemicals affecting each organ. Some chemicals affect multiple organs and/or induce multiple pathologies in a single organ.

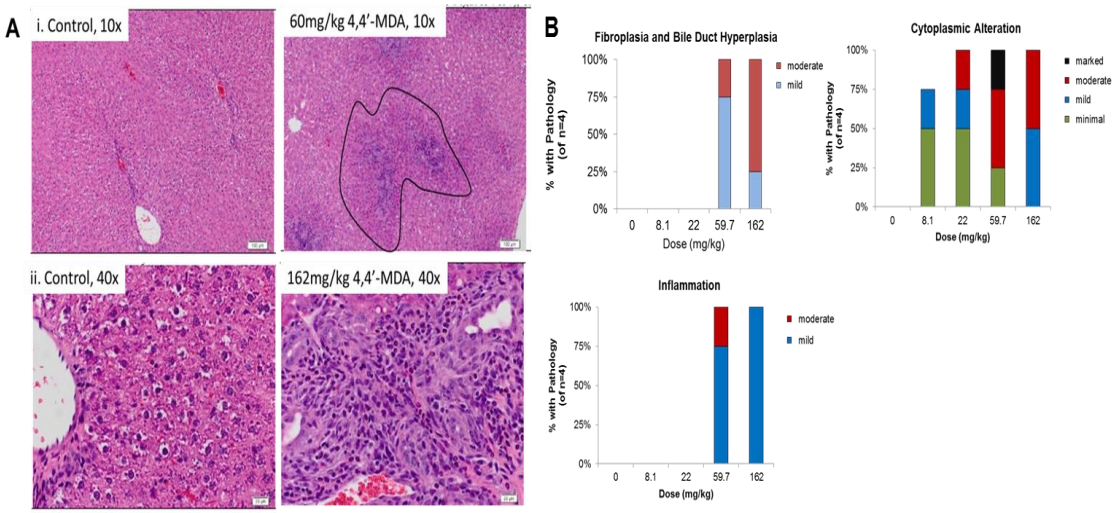

Figure 3: Liver pathology induced by 4,4'-methylenedianiline (exemplar). (A) 4,4'-methylenedianiline increased (i) cytoplasmic alteration, (i-ii) subacute inflammation, and (i-ii) bile duct hyperplasia. Comparable results were observed after allyl alcohol administration. (B) 4,4'-methylenedianiline administration caused a dose-dependent increase in liver pathology based on histopathological scoring (tissue affected: minimal, <30%; mild, >30-60%; moderate, 60-80%; marked, >80%).

Of the 67 presumptive fibrogenic genes in the test panel, 51 (76%) of the genes were ± 1.5-fold control expression in the animals with histopathological evidence of fibrosis (Figure 3). Only 33 (49%) were differentially regulated in animals without evidence of fibrosis, and 12 of the 33 differentially expressed genes showed an expression pattern which was anti-correlated with expression pattern in the fibrosis-positive cohorts. Assay sensitivity was 70-95% for detecting the progression of fibrosis.

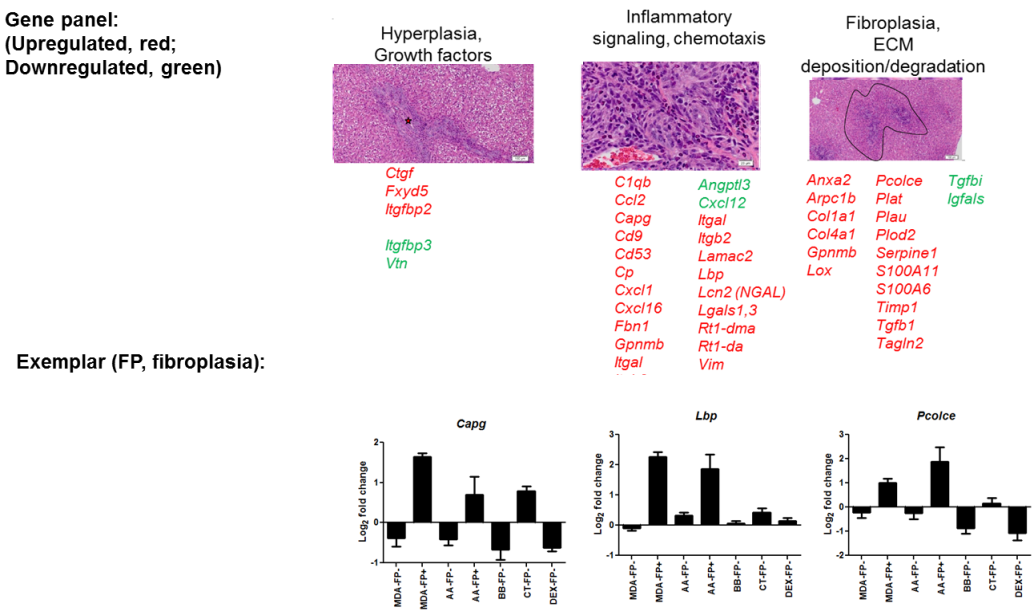

Figure 4: Dose dependent increase in liver fibrosis signature genes associated with mechanistic changes in fibrosis pathogenesis (upper panel). Gene expression data for three exemplar genes indicates fibrosis specificity (lower panel).

Differentially expressed genes were classified into mechanistic groups: hyperplasia, inflammatory signaling/chemotaxis, and fibroplasia and/or extracellular matrix remodeling (Figure 4). Termed bridging biomarkers, these markers have a literature-based association with a specific mechanism for fibrotic injury. Some of the observed differential expression patterns were previously associated with more advanced fibrotic injury. These genes may be early indicators of fibrogenesis [28].

4.0 DISCUSSION

4.1 Industrial chemical analysis

Protecting military personnel from toxic and hazardous chemicals is essential in all operational environments across the entire range of deployment missions, from humanitarian missions to combat in theater. Reducing and limiting such exposures to acceptable levels requires significant integration of environmental monitoring, biomonitoring, and biomarker assessment. The missing and/or inaccurate information in the existing ITF-40 database prompted the development of a revised threat list based on re-evaluation of the physical properties of each chemical, with emphasis placed on the potential for harm in the operational environment. USACEHR and NRL jointly integrated adverse health effects and global distribution into the database, resulting in a list of chemical threats in a megacity environment [26].

Developing independent assays for all possible military threat chemicals is neither logistically nor financially practical. However, developing assays for the top 20-25 AOPs is a more viable alternative to assess individual susceptibility and sensitivity to toxic chemical injury. AOPs could be rank ordered by the number of chemicals causing a given pathology. Our ongoing efforts aim to refine the prioritization process required for generating a toxic (operational) hazard score, incorporating additional metrics including stability scores, physical state scores, and relative probability scores derived from acute toxicology found in the material safety data sheet (MSDS), global production, and distribution data. Target organ health effects and time-to-onset metrics are integrated to evaluate health effects in time frames relevant to making return-to-duty decisions by field commanders and medics. Although oral, inhalation, and percutaneous scores were collapsed into a single score for each chemical in the initial iteration of the tool, future efforts will delineate exposure route. A computational approach will be used to assign priority scores for different field operations scenarios. A second probability scoring system will incorporate geographical distribution. Finally, time-to-onset and health effects in the subacute and subchronic range will be used to both eliminate chronic endpoints outside the concept of field operations and acute effects requiring immediate, palliative care.

4.2 Bioinfomatics mining of public data repositories for candidate liver fibrosis biomarkers

The liver and kidneys are particularly susceptible to toxic chemical injury. The liver plays a primary role in xenobiotic metabolism [29-31], while the kidney concentrates xenobiotics for excretion, increasing local concentrations at the glomeruli and renal tubules [32]. To improve sensitivity of the existing biomarker panels, publically available databases (e.g., DrugMatrix® and CTD) are routinely mined for candidate gene targets [15, 27]. Hierarchical clustering and integrated systems toxicogenomics have been used to mine the DrugMatrix® database and develop panels of candidate genes for further analysis and verification.

Clinically used biomarker panels for both liver and kidney injury have been established in the context of preclinical adverse drug reactions. Current clinical tests used routinely to diagnose liver injury and impaired liver function include laboratory tests such as ELISA (enzyme-linked immunosorbant assays) for specific biomolecules. The standard technology for measuring biomarkers of liver injury is iSTAT technology assessing clinical chemistry endpoints (e.g., alanine aminotransferase, aspartate aminotransferase, alkaline phosphate, transaminase, lactate dehydrogenase, gamma glutamyl transpeptidease, bile acids, clotting time, and protein level) [33, 34].

Late-stage liver fibrosis can be predicted by clinically used tests and biomarker panels, including hyaluronic acid and type III collagen propeptide [34]. A number of early indicators replacing the standard liver biopsy are being used in the clinic or pre-clinical trials. (1) **Fibrotest** incorporates age and clinical parameters with the following molecular indicators: α2-macroglobulin, haptoglobin, γ-glutamyl transpeptidase, bilirubin, alanine transaminase, and apolipoprotein A1. (2) **Fibrospect** includes hyaluronic acid, tissue inhibitor of metalloproteinase 1 (TIMP-1), and α2-macroglobulin. (3) **Fibroscan** is a transient **elastography** device. (4) **Actitest** is a component of Fibrotest, but lacks the clinical parameter components included in its parent test. (5) **APRI** is the aspartate aminotransferase/ platelet ratio and has been used in standard clinical batteries. (6) **αGST/Arginase-1/ALT assay** is an ELISA-based platform currently in preclinical stages of testing and includes an animal component for use in preclinical studies and is available in the MSD (Mesoscale Discovery) platform. Although all biomarker panels have been published extensively, and/or have been approved by the European regulatory agencies (e.g., ICH [International Congress on Harmonization]), there is little or no impetus for approval by the standard regulatory agencies in the United States. Fibrotest, for instance, does not require FDA approval for use in the clinic [35].

Biomarker panels for drug-induced kidney injury have already been established, validated, and approved by the ICH regulatory authorities, including the following biomolecules: blood urea nitrogen (BUN), KIM-1, albumin, total protein, β2-microglobulin, cystatin C, clusterin, and trefoil factor-3 [36]. These biomarker panels can be optimized and adapted into fieldable tests for toxic kidney injury. We are currently developing a computational approach to evaluating the 244 gene modules identified for kidney injury to develop predictive biomarker panels specific for kidney injury (AbdulHameed, in preparation).

4.3 Computational framework: biomarkers of toxic effect

Computational approaches can be used to identify biomarkers of liver and kidney injury and integrate quantitative gene and protein expression data into models bridging differential biomolecule data with histopathology for making predictions about end organ injury by sampling accessible biofluids (e.g., blood and/or urine). AOPs are a current framework for integrating biomarkers with histopathological endpoints [11]. An AOP for chemical-induced liver fibrosis has been described [11]. Briefly, the molecular initiating events of protein alkylation and covalent protein binding in the liver trigger apoptosis and other cellular hepatic injury. Activated Kupffer cells and increasing transforming growth factor β1 transition the pathology from the cellular to the tissue level (Figure 5). Stellate cells are activated, leading to inflammation and oxidative stress. The cumulative result of these changes is the accumulation of collagen and changes in extracellular matrix composition, which are the physical manifestations of the fibrosis pathology [11].

Collectively, our gene expression modules provide quantitative expression patterns of gene networks linked to mechanistic changes in the cellular events leading to the tissue pathology characteristic of fibrotic injury. The gene co-expression modules can be integrated into an adverse outcome framework. One limitation in the field of AOP development is currently the inability to incorporate network information into the current linear framework of the existing AOP structure. Our gene module approach has the potential to bridge this critical gap in the AOP field. The gene modules intrinsically incorporate network information into the cellular events anchored to the liver histopathology (Figure 5). Expression patterns of a panel of genes linked to discrete pathologies can be used to establish threshold values for predicting progression from injury to frank fibrosis. This systems-level integration of multi-omics data at the cellular and tissue level can be extended to the individual by physiologically based pharmacokinetic (PBPK) models to make biological predictions based on integrated multi-omics expression data [11-12].

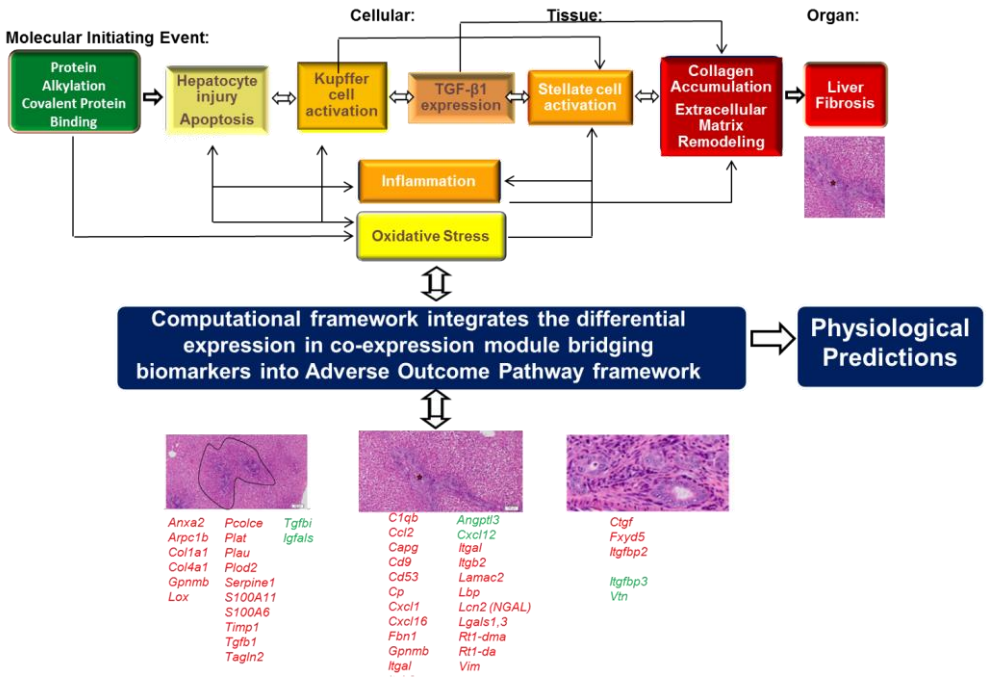

Figure 5: Basis for a computational framework to integrate differential gene expression analysis into the liver fibrosis adverse outcome pathway (AOP).

4.4 Computationally anchoring molecular responses to absorption, metabolism, distribution, and toxicity (ADME-T) parameters

Recent advancements in technology for characterizing the gene, protein, and metabolic networks underlying cellular and tissue perturbation have revolutionized toxicological science. Large-scale molecular signatures can inform dose- and time-dependent changes in cellular networks aided by computational systems biology pathway models. Different modeling approaches have been proposed for mapping biomolecular perturbations to pathological and/or physiological changes in target-organ function as a result of chemical exposure. Causal transcriptional network inference analysis identified canonical alterations of gene expression in liver parenchymal cells after activation of peroxisome proliferation activation receptors (PPAR), a class of chemicals which regulate lipid metabolism and adipogenesis in liver tissue, leading to histopathological evidence of lipid accumulation (i.e., steatosis). Multi-scale, quantitative spatial models of the human liver integrate cellular and tissue-level mechanisms. These models create a "virtual tissue" by mapping regulatory networks and dose response relationships schematically, then overlaying the network response onto a three-dimensional representation of the tissue [37]. Ongoing efforts in our laboratory are developing comparable models to bridge exposure to military threat chemicals with adverse health effects and clinical outcomes to improve diagnostic potential after exposure to toxic industrial chemicals and materials. Our laboratory recently described the first *in vivo* thermoregulation model of heat stress and integrated multi-omics data into a model predicting physiological changes after heat exposure [38-41]. Similar methods are underway for integrating physiologically based pharmacokinetic models with multi-omics molecular mechanisms and/or indicators of clinical injury after chemical exposure [42].

5.0 CONCLUSION

In conclusion, computational approaches for biomarker discovery represent a powerful tool for identifying and characterizing novel gene and protein interaction networks which can anchor gene and protein expression patterns to histopathology. Integrating biomarker panels with predictive algorithms and anchoring

the molecular responses to AOPs and physiologically-based computational models will improve hazard assessment for return-to-duty decisions in the field.

6.0 DISCLAIMER

Opinions, interpretations, conclusions, and recommendations are those of the author(s) and are not necessarily endorsed by the US Army. This research complied with the Animal Welfare Act and implementing Animal Welfare Regulations, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and adhered to the principles noted in The Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations. This research was supported in part by an appointment to the Postgraduate Research Participation Program at the USACEHR administered by the ORISE through an interagency agreement between the US DOE and USAMRMC.

7.0 ACKNOWLEDGEMENTS

We thank Drs. Mohamed Diwan AbdulHameed and Gregory Tawa (BHSAI, NIH) for the bioinformatics mining experiments leading to the identification of the kidney and liver biomarker panels. We thank Christine E. Baer (Excet, Inc.) for transcriptomics analysis and COL Thomas Timmes (USACEHR) for programmatic support. Animal exposure studies were supervised by Mr. Michael Streicker and Dr. Cheryl Hobbes, with histopathology analysis by Dr. Molly H. Boyle, DVM, MPH, DACVP (Integrated Laboratory Systems, Inc., Research Triangle Park, NC).

8.0 REFERENCES

[1] Borden Institute of the United States Army: Textbook of military medicine. Part III, Disease and the environment. Falls Church, Va.: Office of the Surgeon General, Dept. of the Army, U.S. of America. 1993.

[2] DoDD NUMBER 6490.02E: **"Health surveillance systems shall continuously capture data on occupational and environmental exposures to potential and actual health hazards and link with medical surveillance data to monitor the health … to prevent, treat, or control disease and injury** February 8, 2012.

[3] FOC-09-05 TP: **Army Health Systems "Deployed Soldiers must be protected against major endemic diseases, the increasing threat of occupational and environmental health threats, and full spectrum CBRN agents."** March 7, 2008.

[4] FOC-09-07 TP: **Army Health System Casualty Prevention "DNBI remain the largest contributor to casualty production on the battlefield and include the following factors: … occupational and environmental health threats to include chemical hazards (such as TIM), … industrial pollutants, as well as low-level chemical."** March 7, 2008.

[5] Initial Capabilities Document for Joint Force Health Protection: **"Health consequences of hostile actions and natural events are mitigated"** and **"MHS support to HD/CS to include identification of emerging health threats, protection of force health, mitigate health consequences of hostile action, achieve health preparedness and medical response."** February 24, 2010.

[6] JP-04 HSS: **"Develop medical concepts and countermeasures to mitigate disease and environmental threats."** July 26, 2012.

[7] Sharkey JM: **Hospitalization and medical evacuation of army personnel due to toxic inhalational exposure-Operations Iraqi Freedom and Enduring Freedom, 2001 through mid 2011.** US Army Med Dep J 2012:48-53.

[8] Harris M, Dixon R, Melin N, Hendrex D, Russo R, Bailey M: **Megacities and the United States Army: Preparing for a complex and uncertain future.** Chief of Staff of the Army SSG ed., 2014:28.

[9] Iavicoli I, Fontana L, Leso V, Bergamaschi A: **The effects of nanomaterials as endocrine disruptors.** Int J Mol Sci 2013, **14:**16732-16801.

[10] Groh KJ, Carvalho RN, Chipman JK, Denslow ND, Halder M, Murphy CA, Roelofs D, Rolaki A, Schirmer K, Watanabe KH: **Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: I. Challenges and research needs in ecotoxicology.** Chemosphere 2015, **120:**764-777.

[11] Vinken M: **The adverse outcome pathway concept: a pragmatic tool in toxicology.** Toxicology 2013, **312:**158-165.

[12] Wambaugh JF, Wetmore BA, Pearce R, Strope C, Goldsmith R, Sluka JP, Sedykh A, Tropsha A, Bosgra S, Shah I, et al.: **Toxicokinetic Triage for Environmental Chemicals.** Toxicol Sci 2015, pii:kfv118 (epub ahead of print) .

[13] Wilbanks MS, Gust KA, Atwa S, Sunesara I, Johnson D, Ang CY, Meyer SA, Perkins EJ: **Validation of a genomics-based hypothetical adverse outcome pathway: 2,4-dinitrotoluene perturbs PPAR signaling thus impairing energy metabolism and exercise endurance.** Toxicol Sci 2014, **141:**44-58.

[14] Williams M: Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 14 edn. Whitehouse Station/Rahway, NJ: Merck, Inc.; 2007.

[15] Ganter B, Tugendreich S, Pearson CI, Ayanoglu E, Baumhueter S, Bostian KA, Brady L, Browne LJ, Calvin JT, Day GJ, et al.: **Development of a large-scale chemogenomics database to improve drug candidate selection and to understand mechanisms of chemical toxicity and action.** J Biotechnol 2005, **119:**219-244.

[16] Davis AP, Grondin CJ, Lennon-Hopkins K, Saraceni-Richards C, Sciaky D, King BL, Wiegers TC, Mattingly CJ: **The Comparative Toxicogenomics Database's 10th year anniversary: update 2015.** Nucleic Acids Res 2015, **43:**D914-920.

[17] Ihmels J, Friedlander G, Bergmann S, Sarig O, Ziv Y, Barkai N: **Revealing modular organization in the yeast transcriptional network.** Nat Genet 2002, **31:**370-377.

[18] Tawa GJ, AbdulHameed MD, Yu X, Kumar K, Ippolito DL, Lewis JA, Stallings JD, Wallqvist A: **Characterization of chemically induced liver injuries using gene co-expression modules.** PLoS One 2014, **9:**e107230.

[19] AbdulHameed MD, Tawa GJ, Kumar K, Ippolito DL, Lewis JA, Stallings JD, Wallqvist A: **Systems level analysis and identification of pathways and networks associated with liver fibrosis.** PLoS One 2014, **9:**e112193.

[20] Breitling R, Armengaud P, Amtmann A, Herzyk P: **Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments.** FEBS Lett 2004, **573:**83-92.

[21] Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, et al.: **Bioconductor: open software development for computational biology and bioinformatics.** Genome Biol 2004, **5:**R80.

[22] Yu HY, Wang BL, Zhao J, Yao XM, Gu Y, Li Y: **Protective effect of bicyclol on tetracycline-induced fatty liver in mice.** Toxicology 2009, **261:**112-118.

[23] Alcaraz N, Friedrich T, Kotzing T, Krohmer A, Muller J, Pauling J, Baumbach J: **Efficient key pathway mining: combining networks and OMICS data.** Integr Biol (Camb) 2012, **4:**756-764.

[24] Fujii T, Fuchs BC, Yamada S, Lauwers GY, Kulu Y, Goodwin JM, Lanuti M, Tanabe KK: **Mouse model of carbon tetrachloride induced liver fibrosis: Histopathological changes and expression of CD133 and epidermal growth factor.** BMC Gastroenterol 2010, **10:**79.

[25] Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, Fukui K, Maeda N, Nishizawa H, Nagaretani H, et al.: **Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin.** Gastroenterology 2003, **125:**1796-1807.

[26] Sutto TE: **NRL Industrial Chemical Assessment for Hazard, Probability, and Biomarker Prioritization.** Defense Technical Information Center (DTIC) 2015, in press, **NRL FR 6364--14-14:**45.

[27] Davis RA, Kern F, Jr., Showalter R, Sutherland E, Sinensky M, Simon FR: **Alterations of hepatic Na+,K+-atpase and bile flow by estrogen: effects on liver surface membrane lipid structure and function.** Proc Natl Acad Sci U S A 1978, **75:**4130-4134.

[28] Ippolito DL, AbdulHameed MD, Tawa GJ, Baer CE, Wallqvist A, Lewis JA, Stallings JD: **Gene expression patterns associated with histopathology in toxic liver injury.** In Society of Toxicology, vol. Abstract #127. San Diego, CA; 2015.

[29] Reisfeld B MA: ComputationalToxicology. New York: Humana Press ; Springer; 2012.

[30] Sorrell MF, Maddrey WC: Schiff's Diseases of the Liver. 8th edn. Philadelphia: Lippincott-Raven Publishers; 1999.

[31] Arias IM: The Liver, Biology and Pathobiology. New York: Raven Press 1982.

[32] Casarett LJ DJ, Klaassen CD: Casarett and Doull's toxicology : The Basic Science of Poisons. 6th ed.; 2001.

[33] Antoine DJ, Dear JW, Lewis PS, Platt V, Coyle J, Masson M, Thanacoody RH, Gray AJ, Webb DJ, Moggs JG, et al.: **Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital.** Hepatology 2013, **58:**777-787.

[34] Yang X, Salminen WF, Schnackenberg LK: **Current and emerging biomarkers of hepatotoxicity.** Current Biomarker Findings 2012, **2:**43-55.

[35] Fuzery AK, Levin J, Chan MM, Chan DW: **Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges.** Clin Proteomics 2013, **10:**13.

[36] Vaidya VS, Ferguson MA, Bonventre JV: **Biomarkers of acute kidney injury.** Annu Rev Pharmacol Toxicol 2008, **48:**463-493.

[37] Bhattacharya S, Shoda LK, Zhang Q, Woods CG, Howell BA, Siler SQ, Woodhead JL, Yang Y, McMullen P, Watkins PB, Andersen ME: **Modeling drug- and chemical-induced hepatotoxicity with systems biology approaches.** Front Physiol 2012, **3:**462.

[38] Ippolito DL, Lewis JA, Yu C, Leon LR, Stallings JD: **Alteration in circulating metabolites during and after heat stress in the conscious rat: potential biomarkers of exposure and organ-specific injury.** BMC Physiol 2014, **14:**14.

[39] Rakesh V, Stallings JD, Helwig BG, Leon LR, Jackson DA, Reifman J: **A 3-D mathematical model to identify organ-specific risks in rats during thermal stress.** J Appl Physiol (1985) 2013, **115:**1822-1837.

[40] Rakesh V, Stallings JD, Reifman J: **A virtual rat for simulating environmental and exertional heat stress.** J Appl Physiol (1985) 2014, **117:**1278-1286.

[41] Stallings JD, Ippolito DL, Rakesh V, Baer CE, Dennis WE, Helwig BG, Jackson DA, Leon LR, Lewis JA, Reifman J: **Patterns of gene expression associated with recovery and injury in heat-stressed rats.** BMC Genomics 2014, **15:**1058.

[42] Scheff JD, Stallings JD, Reifman J, Rakesh V: **Mathematical Modeling of the Heat-Shock Response in HeLa Cells.** Biophys J 2015, **109:**182-193.