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Computational Approaches for Translational Clinical Research in Disease Progression

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Abstract

Today, there is an ever-increasing amount of biological and clinical data available that could be used to enhance a systems-based understanding of disease progression through innovative computational analysis. In this paper we review a selection of published research regarding computational methodologies, primarily from systems biology, that support translational research from the molecular level to the bedside, with a focus on applications in trauma and critical care. Trauma is the leading cause of mortality in Americans under 45 years of age, and its rapid progression offers both opportunities and challenges for computational analysis of trends in molecular patterns associated with outcomes and therapeutic interventions.

This review presents methods and domain-specific examples that may inspire the development of new algorithms and computational methods that utilize both molecular and clinical data for diagnosis, prognosis and therapy in disease progression.

Keywords

Systems biology; computational methods; trauma; biomedical informatics

1. Introduction

The aim of this paper is to present a selection of computational tools, techniques, and resources from systems biology that can be used to increase understanding of the physiological mechanisms in disease progression. The intention is to show data processing and analysis methods that may be adaptable from the molecular and cellular levels to investigations at the tissue, organ, and whole body system levels, highlighting common processes and procedures that may be useful for investigative studies and personalized medicine. The scope of this paper is limited to resources for potential applications in trauma and critical care; however, the underlying methods may be useful in a wide range of translational clinical research in disease progression.

Translational clinical research has been defined as patient-oriented clinical research that focuses on the clinical aspects of a subspecialty combined with the knowledge and methods of biomedical sciences¹. The central conceptual theme of this manuscript is that systems methods can assist in translational clinical research through the identification of measurable

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entities in disease progression, such as characteristics found in critically injured trauma patients. These characteristics may point to underlying disrupted biological mechanisms that may be amenable to treatment with a resultant increase in survival²⁻⁵. The challenge is that systems biology analyzes physiology from the “bottom-up”, modeling molecules, organelles, and biological pathways within cells whereas clinical medicine treats a patient from the “top-down”, evaluating the whole body, based on observable measures from biofluids, tissues, and organs. The data from both extremes vary considerably over scales of time and space. For example, data can be baseline, measured at specific intervals, or measured continuously. Data can be numbers, words or patterns describing serum protein concentrations or heart rates.

There are vast databases with biochemical data and computational models that can offer insights into disease progression, if they can be linked to specific patterns of patient characteristics and if clinicians were enabled to judge clinically relevant factors that arose from systems biology. Specific treatments based on an individual’s underlying physiology, in addition to phenotype⁶⁻⁸, are important in the development of personalized therapies. Clearly a two-stage translational approach is required: first, clinical researchers need to identify “gold standard” data patterns that include measurable biochemical data associated with prognosis, diagnosis or treatment. Secondly, protocols and parameters that incorporate this information must be developed for clinical use. In both stages, computational methods are needed: first for discovery of likely biochemical patterns and disease associations, and secondly, to provide patient data-driven reports that include systems information to assist clinicians in assessing and directing patient care.

Because ICUs are already prepared to monitor and collect massive amounts of temporal physiological and clinical data, they are a likely candidate location for studies and applications of translational systems biology. In this manuscript, we present a selection of recent approaches and their application to research in biological processes in trauma and critical care, such as inflammatory, immune and injury responses. The emphasis is on computational methods that can be used for data-driven systems analysis of disease progression.

In the following section, we present a short overview of systems analysis and systems biology, approaches used in trauma research, and data available. In Section 3 computational methods are discussed, followed by Section 4 with selected applications. The review is summarized in Section 5.

2. Background

2.1 Systems Analysis and Systems Biology

Systems analysis is “a method of describing and understanding complex interactions among large numbers of processes or components in a generalized way. The focus is on identifying the fundamental units of a system and defining how they interact rather than the internal processes of each unit”^{9, 10}. Systems analysis can be performed to generate or test hypotheses about the systems behavior within specific assumptions and constraints. Analysis techniques may be qualitative or quantitative, static or dynamic, stochastic or deterministic, or combinations. The fundamental units (components or processes) may be nested within a hierarchy or overlapping.

Systems biology, a subcategory of computational biology, is defined by the National Library of Medicine (NLM) as the “comprehensive, methodical analysis of complex biological systems by monitoring responses to perturbations of biological processes and using the large scale, computerized collection and analysis of the data to develop and test models of

biological systems”¹¹. From its beginnings, systems biology aimed at building mathematical frameworks with some predictive abilities based on systematic organization of genomic and proteomic data¹². Since then, the scope of systems biology has expanded and spawned a number of related offshoots such as translational research¹³, translational systems biology¹⁴, translational bioinformatics¹⁵, and systems medicine^{16, 17}. Although analysis goals, abstraction levels, and scales vary widely, the fundamental units under study are usually molecules, cells, tissues, organs, and organisms within a hierarchical framework with modular control elements or related biological processes^{18–21}.

2.2 Systems approaches in trauma

Trauma refers to serious bodily injury, which, if of sufficient magnitude, may be accompanied by initiation of the systemic inflammatory response. Causes of trauma include penetrating injuries from gunshots and stab wounds, blunt injuries, such as those sustained during automotive accidents, and burns. In addition to direct tissue damage, trauma can result in injury to remote organs due to disruptions in normal physiology and underlying protective biological mechanisms. These remote injuries can be rapid in onset and potentially fatal if allowed to proceed unabated. Moreover, trauma is the leading cause of mortality in the US among individuals under 45 years of age, and the cause of death for 74% of all deaths for people ages 15–24²². In critically ill patients, normal biological processes are disrupted but the associated pathophysiology is incompletely understood^{23–25}.

Within the past decade, a number of systems approaches for analysis of trauma and critical illness have been developed^{26, 27}. Computational methods have been used to increase understanding of systemic functions such as inflammation and immune response^{28–33} and the effects of drug dosing³⁴, as well as organ specific issues such as heart rate complexity^{35, 36} and acute lung injury³⁷. Multi-scale computational models of angiogenesis, from the molecular to the organ system levels, have been integrated to improve predictive capabilities³⁸. At the molecular/cellular systems level, there are numerous computational approaches in systems biology used to study biological mechanisms such as signaling³⁹, metabolism⁴⁰, and protein interactions⁴¹ that underlie disease progression. With the advent of new technologies that make it feasible – and soon cost-effective – to capture patient’s molecular data such as mRNA expression or serum protein concentrations, translational clinical research can benefit from using computational approaches beyond classical statistical inference. A systems-wide analysis of data from the molecular to the organism level can help design evidence-based personalized therapies.

2.3 Data for systems approaches in trauma

The complex and often rapid progressions of trauma and critical illness provide a vast quantity of patient data that can be collected and evaluated through real-time monitoring in an intensive care unit (ICU) on a continuous, hourly, or daily basis. Intensive care units collect one item of documented clinical information per patient each minute⁴². In addition to monitored data, patient data includes transfusion and drug orders, microscopy, radiology and laboratory reports, nursing and clinician notes, and patient demographics. As bedside biofluid measurement devices move from prototype to practicality^{43, 44}, a patient’s molecular data such as serum proteins can also be collected in time to be of use in the ICU; currently, turnaround time for molecular assays is not practical for other than research use. The challenge today is to understand the meaning of all this data in terms of disease progression, and develop data-driven protocols that will be in place when the technology is available. For example, research has shown that specific patterns of cytokine molecules over time are associated with trauma progression^{24, 45, 46}. Cascade patterns of molecular interactions, such as those triggered by cytokines, have been identified as biological pathways – spatio-temporal networks representative of cellular functions that regulate gene

expression, metabolism and signaling⁴⁷. Because cytokines drive signaling in biological pathways, adding cytokine data may provide insight into the underlying biological mechanisms.

There are an ever-increasing number of databases with information about biological pathways. In 2001, only 18 pathway websites were active⁴⁸. Today, PathGuide.org references more than 290 pathway resources categorized by availability, data access methods, tools, organisms, network category, and contents. More than 30 million molecular interactions are accessible via the Internet⁴⁹. The Pathway Database section of KEGG (Kyoto Encyclopedia of Genes and Genomes) includes networks relating to metabolism, genetic and environmental information processing, cellular processes, human diseases and drug development⁵⁰. More than 1400 curated, experimentally determined, metabolic pathways and enzyme data for microbial, plant, and vertebrate metabolism are available from the freely accessible MetaCyc database⁵¹. There are commercial and publicly available databases of molecular interactions⁵², biological pathways^{48, 53, 54}, and genomic correlates⁵⁵. The Signal Transduction Knowledge Environment (STKE) lists 49 canonical pathways with 1084 component molecules and 33 pathways specific to a particular organism, tissue, or cell type with 718 components⁵⁶. PubMed lists more than 250,000 articles with content about signal transduction pathways; the earliest articles are from 1947 – before systems biology as such existed^{57–59}.

The question is how to integrate all this data? One approach is to use computational methods from systems biology to connect patient data with data from basic science resources in biology, chemistry and physics to develop research models that can transition to data-driven clinical trauma practice (See Figure 1). Even with computational approaches, the data translations and transformations among levels from molecule to organism and vice-versa are far from seamless. Most applications cobble together several methods to achieve their research goals. In the next section, we review some of the major computational methods that have been used to analyze biological processes related to trauma and critical illness; this is followed by Section 4, giving details of several applications. The intent is to inspire creative use of methods and data in the investigation of trauma and critical illness, with the goal of improving patient care.

3.0 Computational Methods

The biological processes in trauma and critical illness are complex and unstable. There are simultaneous and rapid changes of biological pathways across and within the entire body. Extracellular and intracellular signaling modulates systems-wide mechanisms such as inflammatory response^{60–62}, sepsis, hemorrhagic shock, and resuscitation from hemorrhagic shock^{63–65}. The choice of systems analysis and computational methods depends on several factors:

- the systems level(s) under study, from molecule to organism
- the available data
- what biological processes are under study, within what context, and for what goals.

Hypotheses about disease progression can be generated computationally in many ways: from data-driven model-free discovery to the perturbation of *in silico* models of biological processes. This section is an overview of common computational methods in use plus some general considerations for data; selected applications related to trauma and critical care will be shown in Section 4. Here we first present basic probabilistic and deterministic approaches that utilize a wide variety of fundamental tools and techniques that can be used individually,

combined, or in combination with other methods. This is followed by a selection of more specialized methods.

3.1 Basic probabilistic approaches

Classical Statistical Inference incorporates no prior information and assumes independent variables; the approach is used at all systems levels and underlies the primary tools, such as Student's *t* test, used for static analysis of injury response where there is sufficient data. In contrast, *Bayesian Statistical Inference* does incorporate prior information as well as handle interdependent variables. The Bayesian “conditional probability” approach is becoming more and more widely used in genetic data analysis⁶⁶, clinical research⁶⁷ and diagnostic medicine; complex Bayesian analyses are usually performed using Markov Chain Monte Carlo (MCMC) computational methods⁶⁸. MCMC methods use Monte Carlo random sampling to produce a Markov Chain with state transitions that converge to an invariant distribution. A Markov Chain is the simplest autonomous form of a discrete-time probabilistic state-transition Markov model where the system state is observable.

Common statistical software includes R (<http://www.r-project.org/>), Spotfire S+ (<http://spotfire.tibco.com/products/s-plus/statistical-analysis-software.aspx>}, SPSS (www.spss.com), and SAS (www.sas.com). OpenBUGS is open-source software for Bayesian analysis using MCMC methods (<http://www.openbugs.info/w/>).

3.2 Basic deterministic approaches

Deterministic approaches depend on initial states and chosen parameters. *Differential equations* are the primary methods of deterministic dynamic analysis, and are mostly used at the molecular and cellular levels because they are computationally intensive at higher levels. For example, modeling one NF κ B signaling pathway in one cell activated by one signaling TNF- α molecule requires 18 nonlinear differential equations, with 33 independent variables and 16 dependent variables in a simplified reaction kinetics model⁶⁹; scaling this method directly to the organism level is computationally intractable. Ordinary differential equations (ODEs) model dynamic changes in items, such as protein concentrations, over one independent variable whereas partial differential equations model simultaneous changes over two or more independent variables. Explicit equations are used, usually with equilibria or other constraint assumptions. In addition to experimental data, the equations require data for estimated biochemical kinetic parameters, which are usually inferred from published results. Differential equations can be solved using standard mathematical software available as open source or commercial software such as MATLAB⁷⁰ and Mathematica⁷¹.

Matrix algebra can be applied from molecular to organism levels. Stoichiometric matrices are used for flux-balance analysis (FBA) of metabolic biochemical reaction networks uses^{40, 72} to stochastically simulate chemical kinetics. Unlike differential equation approaches, FBA does not require reaction rate kinetic parameters or metabolite concentration data. Instead, the key assumptions are that the system is homeostatic with a balanced system of energy production and consumption and that the metabolites are “well stirred” so that Gillespie's Algorithm can be used⁷³. This steady-state approximation of cellular dynamics can offer insights into multi-scale snapshots of disease progression. Matrix algebra formalisms have been used to study signaling and regulatory pathways using extreme pathway analysis, an adaptation of the stoichiometric approach used for metabolic analysis^{74, 75} and to generate signaling networks from sparse time series of observed data⁷⁶. The latter computational algebra approach has potential for analysis of signaling pathways in disease progression.

Matrix decomposition methods are the basis for a wide variety of factor and component analyses in data mining and graphical analyses^{77, 78}. In addition to techniques such as singular value decomposition (SVD), new matrix approaches are evolving such as the graph-decorrelation algorithm (GraDe) that performs detailed temporal analyses on large-scale biological data using knowledge-based matrix factorization. In a recent time-course microarray experiment of mouse hepatocytes, GraDe provided a detailed separation of the time-dependent responses to IL-6 stimulation compared to standard methods⁷⁹.

Matrix algebra can be performed using software as simple as spreadsheets; more complex calculations use software such as MATLAB or Mathematica. Code for Gillespie's Algorithm is available for R (<http://cran.r-project.org/web/packages/GillespieSSA/index.html>) and for Mathematica (<http://demonstrations.wolfram.com/DeterministicVersusStochasticChemicalKinetics/>).

3.3 Graphical approaches

Cascades of molecular interactions can be represented as directed graphs and use computational methods from graph theory to explore pathways within the graph. The analysis is usually at the molecular and cellular levels, although the methods can be adapted for higher levels. Biological pathways can be abstracted as network graphs with nodes for molecules and edges for molecular interactions^{80, 81}. Patterns of molecules, such as serum cytokines, have been associated with disease progression in trauma, and graph theory methods offer ways to analyze this data. *Graph theory* is supported by extensive computational methods from mathematics and computer science that are used for analysis of static and dynamic systems ranging from computer systems to social networks. Structural properties of the graph can be measured in many ways such as counting the number of nodes and edges, number of edges per node or nodes per edge, identifying primary hubs and sub-network motifs. Computational methods are usually analysis specific. For example, the web-based Hub Objects Analyzer (Hubba-Hubba, <http://hub.iis.sinica.edu.tw/Hubba/index.php>) identifies essential hubs in a protein interaction network by using a combination of software including databases, graph generators, and topology calculators⁴¹.

A *Bayesian network* is a probabilistic graphical model constructed as a directed acyclic graph (DAG) with nodes representing variables and edges representing the conditional dependencies between the nodes. Bayesian networks are used for process modeling and diagnostic reasoning^{82, 83}. One class of Bayesian networks is based on *Hidden Markov Models (HMMs)* - Markov Chains with hidden rather than visible states but with visible state-dependent outputs. HMMs can be used to uncover an optimal sequence of state transitions. One limitation of Bayesian networks is that they must satisfy the local Markov property - each node is conditionally independent of its non-descendants⁸⁴; as a result, graphs with cycles are not supported. This limits modeling of biological pathways to small sections without loops. Recently, an extension to Bayesian network models, called *Generalized Bayesian Networks (GBN)*, has been proposed that can model cyclic networks for use in translational systems biology⁸⁵. There are number of software packages for Bayesian networks including the Python library Pebl (<http://code.google.com/p/pebl-project/>) and Hugin (<http://www.hugin.com/>).

Petri Net methods have been used to analyze Bayesian networks where the nodes are molecules and the edges represent the dependencies of the interactions between the nodes. Petri nets perform qualitative, stochastic and continuous analysis of small biochemical networks by modeling token-based transitions, such as reactions, between "places" such as proteins. Dynamic modeling is performed by incorporating differential equations to assign rate functions to transitions⁸⁶. Petri Net Toolboxes are available for MATLAB and

Mathematica, and systems biology-oriented Petri Net software called *Snoopy* is freely available (<http://www-dssz.informatik.tu-cottbus.de/snoopy.html>; <http://www.informatik.uni-hamburg.de/TGI/PetriNets/>).

Finally, *Spectral Graph Theory* incorporates both graph theory and matrix algebra to examine a network in terms of the eigenvalues and eigenvectors (spectrum) of the adjacency matrix mapped from the network graph⁸⁷. This method has been used to compare basic metabolic networks at the systems level in three organisms *Mycobacterium tuberculosis*, *Mycobacterium leprae* and *Escherichia coli*. The results found that the most highly connected biochemical reactions in an organism are not necessarily those most central to the organism's metabolism, suggesting that hubs present in mycobacterial networks that are absent in the human metabolome may be potential drug targets⁸⁸.

Pathway databases use graph theory with published biological pathway data and proprietary computational network analysis algorithms to generate specific biological pathways. For example, Biobase (BIOBASE GmbH, Germany; www.biobase-international.com) has a data analysis system called ExPlain, and the Ingenuity Knowledge Base (Ingenuity Systems, US; www.ingenuity.com) supports Ingenuity Pathway Analysis. Advantages of these combined database/algorithm systems are that the pathway/molecular interaction data are kept up-to-date, and that the algorithm is specifically designed to work well with the database to uncover the pathways associated with the input data. Although this is advantageous for the general user, it must be noted that the underlying computational methods are not amenable to modification because they are usually based on proprietary algorithms with limited documentation. In addition, access to commercial web-based pathway databases and their analysis software is by paid subscription.

3.4 Symbolic approaches

Symbolic logic is a formal qualitative modeling approach used to answer questions at various levels of abstraction. The questions usually focus on a specific intracellular function such as signaling and a model is created based on system states and rules for state changes. Symbolic models can be analyzed or run as simulations; models can be formally checked and verified. A wide variety of computational methods for symbolic systems biology have been developed⁸⁹. There are several implementations of rule-based modeling for signaling networks⁹⁰ such as *Pathway Logic*, a symbolic rewriting logic based on pi-calculus^{91, 92}.

3.5 Mechanistic approaches

Two commonly used mechanistic models are artificial neural network models and agent-based models. Both are computationally intensive and may require specialized software along with multiprocessor hardware.

An *artificial neural network* (ANN) is a mathematical model that mechanistically learns nonlinear patterns from a set of observations and then infers the optimal function that describes those observations. ANN methods evolved from the idea of simulating the human brain⁹³. Several ANN models are generated from the training input data, and the one with the best fit between predicted and observed values is considered the optimal ANN model to be used for the actual data and predictions. Optimization techniques, such as the conjugate gradient decent method^{94, 95}, may be used to optimize the model. ANNs can train classifiers, approximate functions, filter and cluster data, and direct robotics⁹⁶. ANNs are used at multiple scales from molecular (for example, predicting MHC class II peptide binding⁹⁷) to the organism level (predicting survival following traumatic brain injury⁹⁸).

Nonlinear ANN modeling has been shown to be comparable to linear logistic regression analyses when sample size is adequate. However, it has been shown that training sets for

ANN need at least 800 observations to generate an adequate model – a sample size not usually found in ICU trauma/critical care studies⁹⁹.

Standard mathematical and statistical software including MATLAB, Mathematica, SPSS and SAS have built-in algorithms or add-on modules for neural network analysis and optimization.

An *agent-based model* (ABM) is composed of autonomous fundamental units, or agents, defined at multiple scales or levels within a system along with the rules that govern the state change interactions among them. The rules may be deterministic or stochastic. No explicit equations are used and there are no equilibria assumptions as in most models. The goal is to mechanistically predict patterns of emergent behaviors that arise in complex systems from simple rules²¹. The model must be verified and validated in some way; simulations must be run many times to uncover relevant patterns. Two open-source software packages for ABM development are NetLogo¹⁰⁰ and SeSAM¹⁰¹. FLAME (Flexible Large-Scale Agent Modelling Environment <http://www.flame.ac.uk/>)^{102, 103} is a formal framework that allows a wide range of agent and non-agent models to work together within one simulation environment.

4.0 Applications

In this section, we describe computational approaches currently used, or that have potential for use, in critical care and trauma-related research. The applications are organized by research goals at levels from the organism level down to the cellular/molecular level. This is because, in order to gain a systems-based understanding of critical illness, the researcher can choose to start with methods at the highest level and move lower, or, do the reverse, building up from the cellular/molecular level. A short paragraph summarizes the goal, processes and context for the example, followed by a list of the methods and data used. An expanded table of applications organized by method category is available in the Supplement (<http://journals.lww.com/jinvestigativemed>). See Figure 2.

4.1 Organism level

The organism level includes research performed at the molecular or cellular levels that investigates systemic processes such as inflammation, immune and injury responses. In the next two subsections, we show example of process models and predictive models.

4.1.1 Organism level: Process models—The inflammatory process is a normal physiological response in acute and chronic disease, and part of the immune response to infection. However, despite numerous computational models, much work still needs to be done to automate integration of these models with data across system levels with software usable by non-mathematicians²⁸.

Abstraction: One of the earliest *agent based models* was developed by An²⁹ to create a simple abstraction to simulate the nonlinear behavior and dynamic structure of the inflammatory response. Although the model was based at the cellular level, the abstraction was used for inference of the systemic response at the organism level.

- Method: Agent Based Model using StarLogo software. (StarLogo is now available as open source OpenStarLogo at <http://education.mit.edu/starlogo/>)
- Data: Abstractions of three cell types used as agents: endothelial cells (with injury states), neutrophils, and circulating mononuclear cells, plus rules for agent interactions.

Challenge/Response: Endotoxin (LPS) and other challenges have long been used in trauma research^{65, 104, 105} to probe challenge/response relationships.

Dong³³ created an *agent based simulation* to model the host response to endotoxin using the molecular interactions involved in the NF κ B signaling pathway, coupled with the spatial orientation of various inflammation specific molecules and cell populations such as macrophages and T-helper cells.

- Method: Agent Based Model using NetLogo software (<http://ccl.northwestern.edu/netlogo/>)
- Data: Gene expression data from human subjects injected with endotoxin or a placebo. Biological data for agents (macrophages, cells and molecules) and agent rules (interaction behavior and rates).

In contrast, Vasilescu³⁰ developed an *equation based model* to evaluate whether endotoxin (LPS) tolerance is a component of the immune dysregulation in patients with trauma, severe acute pancreatitis, and diffuse peritonitis.

- Method: Differential equations
- Data: Endotoxin levels, TNF- α plasma levels, and TNF- α releasing capacity of the whole blood in patients with severe acute pancreatitis, diffuse peritonitis, and trauma.

Muller¹⁰⁶ found bistability in the early inflammatory response by using an *in vitro* model of IL-1 challenge to derive an *equation based in vivo model*. The *in vitro* model was first developed by challenging endothelial cells with IL-1; then, the expected value of IL-6 at a specific time under a specific challenge was derived. The basic mechanism of the *in vitro* model was expanded to a whole animal IL-1 challenge that modeled *in vivo* multistate inflammatory response. Of interest was the outcome that a small challenge did not lead to a response; however, a challenge above a certain threshold completely activated the endothelial cells.

- Method: Differential equations
- Data: IL-1 challenge levels and resulting IL-6 production levels in endothelial cells over time scales of minutes, hours and days.

Guthke¹⁰⁷ generated plausible models of the gene regulatory networks involved in the human peripheral blood mononuclear cells' immune response to an *Escherichia coli* infection challenge. The immune interaction networks were reconstructed by reverse engineering. First, a *statistical cluster analysis* of the scaled time profiles of the gene expression data was performed using the fuzzy C-means (FCM) algorithm¹⁰⁸, and then expression profiles of the representative genes were used to drive three dynamic models of gene regulatory networks based on *linear differential equations*, *systems of linear algebraic equations*, or *heuristic search* strategies.

- Method: Statistics, differential equations, linear algebra, heuristic search
- Data: Log-ratios of the expression intensities of more than 18,000 genes in peripheral blood mononuclear cells at five time points before and after infection by heat-killed pathogenic *Escherichia coli*.

4.1.2 Organism level: Predictive models—Probabilistic methods are used extensively in clinical research. Among the more common algorithms are the parametric *Student's t* test for normally distributed quantitative variables, the non-parametric *Mann-Whitney* test for quantitative variables without a normal distribution, and *chi-square* tests for qualitative

variables. For example, these methods were used by Pidcock¹⁰⁹ to demonstrate that the diurnal patterns of blood glucose and insulin requirements in burn ICU patients are similar to those in healthy subjects.

- Method: Means, frequency analysis, simple and cosine regressions, Student's *t* test, Mann-Whitney test, chi-square test
- Data: From 156 burn patients: total body surface area burned, injury severity score, polytrauma, age, gender, inhalation injury, glucose level (hourly), insulin dose (hourly), outcomes.

Cohen¹¹⁰ used *hierarchical clustering* to identify patterns of patients' changing physiological states that were predictive of infection, multiple organ failure and mortality. Clustering is a multidimensional analysis that identifies groups of similar variables, with the results displayed as a dendrogram tree structure. Limitations are that a variable may belong to only one cluster group, and the number of clusters may influence the result.

- Method: Hierarchical clustering, linear discriminant analysis, correlations
- Data: 45 measures of physiological, clinical, and treatment data were collected every minute from each of 17 severely injured trauma patients. Data for the cluster analysis: continuous heart monitor, ventilator, and microdialysis data over 24–72 hours (52,000 data points).

Using a *classical statistical model*, Ware³⁷ identified a combination of biologic and clinical markers in patient data that predicted acute lung injury and acute respiratory distress syndrome.

- Method: Wilcoxon rank sum test for continuous variables. Fisher's exact test for categorical variables. Spearman rank correlation coefficient. Receiver Operator Curves (ROCs). Odds ratios.
- Data: Retrospective study from NHLBI ARDS randomized controlled trial: patient baseline plasma measures of IL-6, IL-8, TNFR1, von Willebrand factor, surfactant protein D, sICAM-1, PAI-1, protein C plus baseline clinical variables such as age, cause of ALI/ARDS and APACHE III scores.

In contrast, Peelen¹¹¹ constructed three *Markov models* based on clinical data to gain insights into the probabilistic state transitions in organ failure progression in successive days of ICU stay. Peelen's models identified potential clinical patient states (number and type of failing organ systems) along with the probabilities that each state would be followed by another state, or persist over time.

- Method: Markov models with dimensionality reduction via additive logistic regression; implementation by hierarchical dynamic Bayesian networks; followed by stochastic simulations.
- Data: Temporal clinical data from a prospective study of severe sepsis patients. Patient data included SOFA scores per each of six organ systems plus total SOFA scores.

Artificial neural networks (ANNs) were constructed by Yamamura³⁴ to predict the plasma concentration of Arbekacin sulfate, an aminoglycoside, in burn patients and, from that prediction, identify patients whose Arbekacin sulfate antibiotic would be sub-therapeutic based on the patients' physiological data. ANN results were superior to multivariate logistic regression analysis in classifying patients' outcomes.

- Method: Three-layered ANN model (Statistica software <http://www.statsoft.com/>). Conjugate gradient decent method for optimization during ANN training with

training data. Leave-one-out cross-validation of predictive performance with test data. Multivariate logistic regression analysis (SPSS, JMP(SAS) and Statistica software).

- Data: Clinical physiological data from 30 burn patients, plus data for assessing burn severity. Training data for ANN model: dose, body mass index (BMI), parenteral fluid, creatinine concentration and burn severity parameters.

4.2 Organ level

Heart—Using *Multiscale Entropy* (MSE) to assess Heart Rate Complexity (HRC), Riordan³⁶ found that early loss of HRC was predictive of mortality regardless of anatomic location, severity or mechanism of injury.

- Method: HRC assessed by Multiscale Entropy (MSE)¹¹². Statistics (R software): Fisher's exact test, Wilcoxon's Rank Sum test, Kruskal-Wallis test, logistic regression, odds ratios, Receiver Operator Curves (ROCs).
- Data: MSE; continuous physiological data from the first available 6 hours plus clinical data and demographics from 2718 trauma patients.

Although HRC seems to be a useful mortality predictor in trauma, most HRC measures require a traditional 800-beat data set. In an emergency situation, such as a battlefield, this large amount of data presents a monitoring challenge. Using three entropy measures with data sets as small as 100 beats to assess HRC, Batchinsky¹¹³ found that HRC was decreased in pre-hospital trauma patients who died.

- Method: HRC assessed by approximate entropy, sample entropy and similarity of distributions. Statistics (SAS): Student's *t* test, Mann-Whitney U test, logistic regression, Receiver Operator Curves (ROCs), odds ratios, maximum likelihood, Pearson chi-square.
- Data: EKGs with 800 RRs from 31 pre-hospital trauma patients, with data sets sampled at 800, 600, 400, 200, and 100-beat data sets.

Brain—Numerous computational models have been developed to increase understanding of traumatic brain injury resulting from blast survivability in war zones with the goal of improving design of personal protective equipment. Moore¹¹⁴ used *equation based models* to study the effects of primary blasts on the central nervous system, and found that blast waves directly propagate into the brain and that stresses develop in central nervous system tissues comparable to those significant in mTBI or concussive injuries in sports.

- Method: Blast-solid interaction simulation of a full head mesh, with mesh generated from nonlinear partial differential equations in computational fluid mechanics software (ICEMCFD, <http://www.ansys.com/products/icemcfd-mesh-gen.asp>) and brain tissue modeled by nonlinear algebraic equations of state.
- Data: Peak blasts at two pressure levels; impact deceleration. High-resolution T1 MR images.

4.3 Tissue level

Adra¹¹⁵ developed a multi-scale 3D model of the human epidermis to explore the functions of TGF- β 1, a potent growth factor, during epidermal wound healing. A computational virtual epidermis was created using an *integrated agent/COPASI model*, followed by investigation of several hypotheses, including the changes in epidermal wound healing associated with different wound sizes.

- Method: COPASI (COMplex PATHway SIMulator)¹¹⁶ ordinary differential equations model for sub-cellular TGF- β 1 functions, linked to a cellular agent based model of normal human keratinocytes (NHKs) in FLAME (<http://www.flame.ac.uk>), linked to a multi-cellular layer with a mathematical solver that resolved physical issues.
- Data: Chemical reactions and coefficient factors of TGF- β 1 expression and signaling; biological rules for the behavior of normal human keratinocytes (NHK) when subjected to injury signals.

Wound healing research has also been used to investigate inflammatory response at the tissue level. The pathogenesis of vocal fold scarring in humans is not well understood despite extensive experimental and clinical temporal data from animal studies. Li³¹ developed an agent-based simulation to model the patient-specific vocal fold inflammation and wound healing following acute phonotrauma.

- Method: Agent Based Model using NetLogo software
- Data: IL-1 β , IL-6, IL-8, TNF- α , matrix metalloproteinase (MMP)-8, and IL-10 from 4 samples of laryngeal secretions from 9 human subjects.

4.4 Cellular/molecular levels

Cellular and molecular level approaches offer novel avenues for investigative research into disease progression. Computational biology has developed a wide variety of methods to model cells, molecular interactions in the form of biological pathways, and molecules at varying levels of abstraction¹¹⁷, along with extensive databases of results containing inferred and experimentally validated data. The challenge is to how to modify these methods and use these models and data for clinical insights into disease progression.

During the past ten years, signaling pathways have become the cornerstone of cancer research¹¹⁸ and an important part of translational clinical research in trauma, sepsis, and critical care^{119–121}. Signaling pathways are the primary multi-level, multi-scale communication channels within the organism that regulate physiology^{122, 123}; they clearly play important roles in disease progression. Cellular signaling pathways are initiated by extra-cellular biological entities including cytokine signaling molecules, hormones and growth factors in the blood, lymph or interstitial tissue, and biomechanical stimuli such as tissue strain^{124–126}. Because signaling triggers can be measured noninvasively in biofluids such as serum, urine or saliva, they may be useful for monitoring the rapid disease progression found in trauma and critical care. Specific patterns of signaling molecules such as cytokines have been associated with mortality in septic shock¹⁰⁴, critical illness¹²⁷, trauma⁴⁵, and multiple organ failure⁴⁶. In trauma, the signaling molecules activate a pro-inflammatory systemic response¹²⁸ across many pathways to help the body fight immediate injury; however, if the “turn off” set of signals is not received in time by the pathways in the cells – or a compensatory systemic response is too much or too little – death may ensue^{129, 130}.

Molecular signaling profiles may be one of the keys to personalized medicine; however, there is still much work to be done to make them clinically relevant. Sachs⁸⁵ created an algorithm to extend acyclic Bayesian network theory to permit loops, or cycles, in networks. The resulting *Generalized Bayesian Network* (GBN) is a Bayesian network model of nodes representing molecular data, augmented by state nodes and edges representing the statistical dependencies among the nodes. As a proof of principle, Sachs used GBN to characterize disease states and patient-specific signaling profiles in human follicular lymphoma tumors following B-cell antigen receptor stimulation¹³¹. The results showed differences in comparably diagnosed patients that might influence individual prognosis and therapy.

- Method: Generalized Bayesian Networks (GBN): Bayesian Networks model of the phospho-protein signaling pathway augmented with state nodes for patient and disease
- Data: Flow cytometry measures of six phospho-protein levels (SYK, ERK, p38, CBL, SFK, BTK) before and after B-cell antigen receptor signaling. Patient state and disease state.

Another research question is how signaling events trigger cellular responses. Signaling pathways that lead to apoptosis are of particular interest in disease progression. Using a data-driven computational technique called *Model-Breakpoint Analysis*, Janes¹³² found that the dynamic range of the molecular signals had a greater influence on predicting cytokine-induced apoptotic cellular response than either basal or maximally inducible signal strength; results were validated experimentally. The results suggest that changes in dynamic range, due to subtle molecular amino-acid changes from disease mutations, could lead to pathophysiology.

- Method: partial least-squares regression, principal component analysis, equation-based model breakpoint analysis.
- Data: Model of cytokine-induced apoptosis based on 7,980 measurements of molecular signals that are activated by combinations of the death stimulus, tumor necrosis factor (TNF), together with a survival stimuli of epidermal growth factor (EGF) or insulin.

Petri nets can represent signal transduction networks at varying abstraction levels using graphs with molecules for nodes, edges for transitions, and tokens generated by the transitions. Petri net models can be constructed from limited knowledge of the pathway behavior, with ambiguities resolved through subsequent model validation. Qualitative Petri net models can be extended to quantitative stochastic or continuous Petri net models by the addition of rate equations⁸⁶. Simulations of stochastic models can be run dynamically, and ordinary differential equation solvers can run static deterministic analyses of continuous Petri net models. Heiner¹³³ developed and validated a qualitative *Petri net model* of apoptotic pathways, using formal computer science methods to represent pathway structure and behavior. Although not directly linked to clinical data, Heiner's Petri net process model could be perturbed to gain insights into apoptosis not easily seen in other representations.

- Method: Step-wise incremental Petri net modeling with repeated analyses. Linear algebra using the incidence matrix and transition vector from the network graph.
- Data: A published schematic overview of apoptosis, comprising both extrinsic and intrinsic pathways, induced by DNA damaging and Fas signals, resulting in DNA fragmentation combined with a Fas-induced MAPK (mitogen-activated protein kinase) pathway and TNFR-1 receptor-induced pathways. Apoptosis inhibitors were not taken into account.

5. Summary

Research in trauma and critical illness is especially challenging because the effects of the original insult can be widespread across the entire body, affecting multiple organ systems. Disease progression is typically rapid, measured in hours and sometimes in minutes. At present there are growing capabilities to collect vast amounts of temporally indexed quantitative and qualitative data at multiple levels, from concentrations of biomolecules to sophisticated imaging modalities. These capabilities have the potential to support translational "bedside to bench and back" research leading to personalized therapies.

However, the current resources to integrate and interpret patient-specific data within the context of acute illness are still limited and new computational approaches are needed¹³⁴.

In this paper we have presented a selection of computational approaches and data sources primarily from systems biology that can be useful for translational clinical research in the progression of disease over time. Applications from trauma and critical illness research were shown, due to their extensive and ever-increasing use of computational methods. Of interest is the fact that a handful of methods can be used in many different ways to analyze disease progression at multiple levels. Clearly, due to the vast scope and complexity of human pathophysiology, no one methodology can be a magic bullet. We believe that judicious selection, adaptation, and application of techniques such as these can yield valuable insights into the underlying mechanisms of disease progression and help formulate effective and personalized therapies. And we hope that this selection inspires creative use of computational methods to advance clinical investigations.

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Figure 1.

Computational methods integrate systems biology methods, data, and other resources with patient data to improve understanding of disease progression.

Figure 2.
A wide variety of computational approaches have been used in disease progression research.