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Translational Systems Biology and Voice Pathophysiology

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Abstract

Objectives/Hypothesis—Personalized medicine has been called upon to tailor healthcare to an individual's needs. Evidence-based medicine (EBM) has advocated using randomized clinical trials with large populations to evaluate treatment effects. However, due to large variations across patients, the results are likely not to apply to an individual patient. We suggest that a complementary, systems biology approach using computational modeling may help tackle biological complexity in order to improve ultimate patient care. The purpose of the article is: 1) to review the pros and cons of EBM, and 2) to discuss the alternative systems biology method and present its utility in clinical voice research.

Study Design—Tutorial

Methods—Literature review and discussion.

Results—We propose that translational systems biology can address many of the limitations of EBM pertinent to voice and other health care domains, and thus complement current health research models. In particular, recent work using mathematical modeling suggests that systems biology has the ability to quantify the highly complex biologic processes underlying voice pathophysiology. Recent data support the premise that this approach can be applied specifically in the case of phonotrauma and surgically induced vocal fold trauma, and may have particular power to address personalized medicine.

Conclusions—We propose that evidence around vocal health and disease be expanded beyond a population-based method to consider more fully issues of complexity and systems interactions, especially in implementing personalized medicine in voice care and beyond.

Keywords

Vocal folds; wound healing; systems biology; modeling

INTRODUCTION

Voice disorders, just like other forms of disease, display wide individual variability in the presentation of the pathology and response to therapy. The complexity of diseases affecting voice and other functions complicates the task of clinical decision making. For instance, pharmaceutical, surgical, and behavioral treatments have all been suggested for managing phonotrauma.¹ Clinicians and researchers have been in search of scientific evidence around putative benefits of these treatment options (for review see Pedersen et al.²). Yet currently, little evidence is available to support any of these widely used approaches. Authors of review articles repeatedly point to the importance of evidence-based medicine (EBM) practice and call for additional randomized controlled studies in the field of voice care. This approach is sensible if the underlying assumption is that the only tool available for gaining mechanistic knowledge about biological processes is reductionism.

EBM was codified within the *Journal of the American Medical Association* nearly two decades ago.³ Yet, the values, philosophical bases, and appropriate research methodologies for EBM are still hotly debated.⁴⁻⁶ In this article, we first review the values and major criticisms of EBM as it is currently conceived. Focus then turns to discussion of an alternate approach grounded in systems biology to ride the tide of a patient-specific medicine era in a way that may complement current EBM. Last, we report on work motivated by systems biology in voice care research.

EVIDENCE-BASED MEDICINE: VALUES AND CRITICISMS

The EBM paradigm has been endorsed enthusiastically in clinical practice and also within the research realm.⁷ EBM emphasizes the importance of clinical practices based on the best available scientific evidence. Within EBM, evidence is graded according to a hierarchy. Setting aside recent modifications to the EBM scheme, large-sample randomized controlled trials (RCTs) and meta-analysis are generally considered the strongest evidence regarding treatment outcomes. Controlled observational studies are considered somewhat weaker, and uncontrolled studies and expert opinions are ranked as the least reliable forms of evidence.⁸ EBM has interjected great value to the clinical endeavor by its promotion of the application of knowledge from research evidence directly to patient care. Physicians no longer need to rely completely on the sole authority of their and others' experience, and they are also prompted to seek continual updates in their knowledge base by seeking out research findings that fit defined criteria for validity. Proponents hope that the EBM movement will help to optimize treatment outcomes, promote replicable treatment, and establish standards of patient care.

If EBM is purely about medical practice based on the best available evidence, the approach can hardly be disputed at face value. However, what sets EBM apart from other approaches to clinical practice is the priority it gives to certain forms of medical evidence to determine the efficacy of interventions. As already noted, within EBM, research findings are ideally derived from large-sample RCTs, which together with subsequent meta-analyses are generally regarded as the highest level of evidence and the gold standard in clinical research methodology.⁹ At the same time, questions have been raised about whether or not these studies provide the most appropriate forms of evidence for clinical questions in all cases. Specifically, RCTs have been suggested to have the several shortcomings, including: 1) limited usefulness for predicting treatment outcomes for individual patients, 2) cost and time ineffectiveness, and 3) typical reliance on reductionist science.⁴⁻⁶ We now discuss these concerns in greater detail.

First, results from typical RCTs are limited by their direct applicability only to an average randomized patient who fulfilled the exclusion and inclusion criteria for enrollment in the

trial. Most diseases, including vocal fold pathologies, are distinctively heterogeneous across patient populations. The lack of concern for individual variability in RCTs, or the dimming of it through strict inclusion and exclusion criteria, could lead to inevitably negative consequences. The averaged results derived from a trial may provide insufficient evidence or incorrect guidance on how to approach a specific individual who may be similar to the average person in the clinical trial or not. Second, RCTs are characteristically expensive and time intensive. RCTs usually require the enrollment of many patients in search for positive yet averaged clinical outcomes that statistically indicate efficacy and safety. If wide individual variability in treatment responses is expected, prohibitively large clinical trials would be needed to establish optimized patient-specific treatments that consider all relevant biological, temporal, and contextual factors. Last, RCTs are overwhelmingly linked to a reductionist discipline. Simply put, reductionists take complex phenomena and break them down into constituent parts in order to see how they work. Unfortunately, health and disease are complex in nature. The underlying assumption of reductionism, namely that “the more details we know [about the constituent parts of a system], the more profound our knowledge is” is erroneous in biomedical research because the component parts in living organisms interact, and their interactions are often nonlinear and are nearly always dynamic.¹⁰ Information about the relationship among parts may be lost in the large-sample RCT. For example, most clinical voice studies often include a set of multidimensional outcome measures, because a single standard outcome measure generally cannot reflect the multifaceted nature of voice disorders and the behavioral treatment program. However, regardless of the phenomenon being evaluated, it is difficult to achieve statistically significant results across all outcome measures. Also, combining these multiple measures in reporting treatment effects does not actually tell us what the underlying mechanism or the active ingredients of the therapy would be.¹¹ The therapy may work on a particular level or multiple levels depending on the patient. For instance, some patients may show no changes in either psychosocial or laryngoscopic measures following therapy, whereas some patients may report improved psychosocial measures even though their vocal lesions did not show visible changes under laryngoscopic examination. Further, feedback loops among these components are expected, such as from the higher psychosocial level to the lower anatomical and/or physiological levels. Due to the highly dynamic interrelationship of the processes acting upon the disorder, RCTs are limited in their ability to represent accurately the nature of particular treatment programs and to determine a satisfactory set of outcome measures.

The proponents of EBM have become mindful of some of the limitations of EBM as originally conceived, particularly with respect to the question of personalized medicine. Since 2000, EBM has evolved in a way that clinical decisions should not be only based on the best available evidence but also on the values and preferences of an individual patient. For example, the concept of N-of-1 randomized controlled trials (N-of-1 trials) was introduced within the EBM rubric to personalize therapeutic decision making.¹² N-of-1 trials, which now stand at the top of the hierarchy of evidence treatments, are tested sequentially in a random order to identify which treatment works best for a single patient.¹² However, important limitations of N-of-1 trials remain. First, the effects of one treatment may produce irreversible results and influence another later stage of treatment. Therefore, the patient's condition is not the same in all phases of the trial for equivalent comparison purposes. Second, acute health problems are not suitable for N-of-1 trials because the condition may subside too quickly to complete the treatment cycles.

In summary, EBM is clearly of great value in promoting the systematic presentation of clinical findings and the search for the best available evidence in clinical practice. At the same time, the question can be raised as to whether or not RCTs truly represent the sole tool with which to provide the best available evidence in the consideration of patient-specific

diagnosis and treatment. As discussed below, other approaches could be used to complement EBM.

POTENTIAL UTILITY OF A SYSTEMS BIOLOGY APPROACH IN VOICE RESEARCH

A major challenge in clinical research is that large variations across patients in physical and other domains may obscure true treatment effects through data averaging. Indeed, pertinent to the case of voice disorders, experimental data have suggested that the benefits of behavioral voice exercises are patient specific and dose dependent, at least in the case of acute phonotrauma (unpublished data). As already noted, attempts to ferret out all factors pertinent to clinical results for an individual patient would be prohibitive using the large-sample RCT approach. If clinicians could identify which patients are likely to benefit from which treatment and also predict optimized treatment types and doses, patients could be spared unnecessary and costly treatments and their potentially harmful side effects.

Much contemporary thinking now emphasizes a predictive, preemptive, and personalized model of medical practice.¹³ General aims of the emerging approach—which for the moment focuses on physical aspects of disease—are to: 1) identify molecular signatures or biomarkers that are predictive of disease or treatment outcome, 2) characterize an individual's probabilistic future health history for a range of diseases and design a preemptive treatment program for the highly probable disease, and 3) prescribe personalized treatment regimes for individual patients depending on their unique probabilistic future health status. Typical EBM shares the first two emphases. However, with the great value placed on the large-sample RCT in the current EBM framework, the third falls short; the reductionist aspect of RCTs is poorly equipped to appreciate nonlinear dynamics and progression in health and disease relevant to voice and beyond.⁶ A more systems-oriented and quantitative tactic can be sought to reflect more accurately the complexity of the clinical questions in health and diseases, including those affecting voice. This novel tactic, which employs systems biology concepts, may facilitate the breakthrough of personalization in medicine and add value to population-based approaches (e.g., RCTs), without having to abandon conventional methods and techniques in clinical research.

DECIPHERING BIOCOMPLEXITY WITH SYSTEMS BIOLOGY

Physiologic systems, whether in their healthy or pathologic states, exhibit a remarkable variability of structural patterns and temporal behaviors.¹⁴ Systems biology is an emerging approach that addresses this issue by studying the dynamic interactions among the components of biological systems.¹⁵ Systems biology has been used to: 1) address the plethora of components and their interactions within complex processes simultaneously; (2) quantify interrelationships (organizational or structural) and interactions (dynamics or behavior) of genes, proteins, and metabolites; and 3) integrate this information into working models that can provide predictive hypotheses to elucidate emergent phenomena that are the outcome of complex processes.¹⁵ Although statistically based analyses can be used to extract patterns from so called “omics” (genomics, proteomics, metabolomics, etc.) datasets, mathematical and computational modeling and simulation are the essential tools for systems analysis in clinical research using the systems approach, especially in knowledge discovery (data mining) and simulation-based analysis.¹⁶ For knowledge discovery in classical systems biology, data mining is useful to conceptualize the hidden order or pattern behind the observed phenomena (e.g., inflammatory response) from large volumes of experimental data (e.g., genomic and proteomic data). For instance, knowledge discovery has been extensively applied to predict protein structure from genetic sequence. On the other hand, the use of mechanistic simulations has been advocated in order to translate biological

information into clinical use. This approach has been called translational systems biology (TSB), in which the tools of systems biology are applied to study systemic behavior seen in clinical settings (with all of their attendant complexity and variability), in a manner adapted for rapid clinical utility (details of TSB are documented in referent papers, e.g., Vodovotz et al.¹⁷). In TSB, computational models are calibrated with experimental and clinical data and used to test clinically relevant hypotheses in a framework of computer (in silico) experiments, that are in turn validated by in vivo/clinical studies. The mathematical models from TSB consist of temporal and possibly spatial components to simulate the dynamic evolution of biological systems, thereby setting TSB apart from static representations of information and relationships as in various statistical and network models.

In clinical research, simulation-based analysis can sharpen our understanding and conceptualization of a particular (patho-) physiological process of interest. Also, the dynamic property of computer simulation may allow the prediction of a range of scenarios relevant to the properties of relevant clinical or preclinical systems applied to an individual. For instance, systems-level modeling can be used to simulate and predict the responses of a whole inflammatory/wound-healing system, rather than the response of particular inflammatory mediators. Such simulations can be calibrated with data from individual patients and used for predictions of outcome, as we have done in vocal fold injury.¹⁸ Through modeling and simulation, the dynamic outcome of interactions and feedback loops among component system parts can be illustrated effectively at multiple scale levels (intracellular signaling pathways, cells, organs, and organisms).

The application of TSB to clinical practice is acknowledged in drug discovery and gene therapy to pursue the goal of predictive, preventive, and personalized clinical care.¹⁵ Also, system-level analysis has begun to be applied to study pathogenesis in various diseases, such as cancer, malaria, and acute inflammation.¹⁷ For instance, in systematic inflammatory responses, the interaction between proinflammatory (e.g., tumor necrosis factor [TNF]- α) and anti-inflammatory (e.g., interleukin [IL]-10) mediators has been shown to be time and context dependent in the establishment of a long-term equilibrium state of a system.¹⁹ This state is achieved by multiple positive and negative feedback loops among the inflammatory mediators. System-level analysis has successfully shown that the pathologic state of the inflammatory response (e.g., sepsis) results from a disruption of the interaction among the aforementioned mediators, rather than the deficiency or excess of any individual mediator. Moreover, the role of damage-associated molecular pattern (DAMP) molecules, also known as alarm/danger signals,²⁰ as integrators of the inflammatory response was demonstrated using computational modeling.

In summary, the robust systems approach, with its predictive power, can overcome the problems of carrying out prohibitively large numbers of experiments needed to: 1) collect comprehensive data in considering the plethora of relevant temporal and contextual factors for a condition, and 2) extract and integrate useful information and patterns from very large quantities of data. By direct modeling/simulating of a complex system, one can observe, manipulate, and come to understand the behavior of the whole system in an efficient way. Thus, systems-oriented simulation has the potential to improve biological insights into mechanisms within complex systems.

APPLICATION OF TRANSLATIONAL SYSTEMS BIOLOGY IN VOCAL FOLD INJURY

One of the major applications of TSB is the development of computational tools that allow clinicians and researchers to conduct in silico (in computer) clinical trials, in order to aid treatment decisions.¹⁷ A first-generation agent-based model (ABM), a form of bottom-up,

stochastic, rule-based simulation for acute phonotrauma, was reported recently with the goal of identifying individually optimized motion-based treatments.¹⁸ Detailed literature on inflammation and wound healing was reviewed to identify the essential components and rules for this model. Experimental measures of inflammatory mediators in human laryngeal secretions were used to specify the model to the setting of acute phonotraumatic injury (unpublished data). Simply put, the model of phonotrauma creates a virtual vocal fold mucosal tissue and simulates the mucosal repair response to biomechanical damage during phonation. The model includes platelets, inflammatory and repair cells (neutrophils, macrophages, and fibroblasts), cytokines (IL-1 β , TNF- α , IL-10), and a growth factor (transforming growth factor- β 1) that mediate inflammation and healing as well as a matrix component (collagen). Most importantly, the model includes a tissue stress/damage parameter that is functionally analogous to DAMP's producing positive feedback to induce further inflammation.¹⁹ This inflammation $\rightarrow$ stress/damage $\rightarrow$ inflammation cycle is a central feature of our modeling of the inflammatory response. The vocal fold model assumes that biomechanical stress during phonation causes mucosal tissue stress/damage and triggers a cascade of inflammatory and healing events as described in relevant wound healing literature. During simulation, the user can input an individual's inflammatory mediator profile (IL-1 β , TNF- α , and IL-10), add a phonotraumatic event, and then select a 4-hour behavioral treatment event (voice rest, resonant voice exercise, or spontaneous speech). In general, the agent-based model reproduced and predicted subject-specific inflammatory mediator trajectories as seen in our human data with 80% accuracy.¹⁸

Since then, ABMs of phonotrauma with expanded biomarkers and of surgically induced vocal fold trauma were developed.²¹ The models were calibrated and validated with relevant empirical data.^{22,23} Again, the models reproduced expected quantitative evolution of inflammatory and matrix markers seen in phonotrauma and surgical trauma. Also, the simulation results illustrated a spectrum of inflammatory responses to phonotrauma, surgical trauma, and behavioral treatments. Furthermore, the simulation analysis suggested that hyaluronan fragments may be an early molecular index of tissue damage (i.e., DAMPs) that are sensitive to varying stress levels, from relatively low phonatory stress to high surgical stress.

Identifying a surrogate of tissue damage is worthwhile for clinicians making decisions. For instance, when a patient comes to the clinic with an existing vocal fold lesion, current technology would not allow clinicians to accurately estimate the degree of tissue damage by looking at the appearance of the vocal folds. We assume that the ideal prescription dose for pharmaceutical or behavioral treatments will vary with tissue status. Once an in vivo measuring tool for tissue damage is available, clinicians will be able to increase their diagnostic accuracy and prescribe a tailored intervention for patients based on current tissue status. This line of work demonstrates a concrete realization of the synergistic power of modeling, integrating with clinical data to provide a new understanding of the process of acute vocal fold and other injuries with real implications for therapy.

FUTURE DIRECTIONS IN VOICE CARE

Modern voice care appears to be transitioning toward a predictive, preemptive, and personalized approach to treatment and prevention of voice and other problems. To advance toward this goal, the complexity of biological processes must be tamed. With the integration of rapidly accumulating and high-throughput genetic and molecular data within the domain of voice science, (e.g., Welham et al.²⁴ and Thibeault et al.²⁵), systems biology is a novel way for science to bridge a critical gap between the bench and bedside. Modeling/simulation, a working methodology used within systems biology, has the potential to lead us beyond a detail-filling era and progress to application of predicting notions about what will

happen. At the same time, the population-based approaches (e.g., RCTs) still play an important role in confirming if a particular patient population responds favorably to a particular treatment, providing critical information in evaluating the averaged risk-benefit of any new treatment for the population at large. We suggest that the integration of population-based and systems biology approaches will hold great promise to accelerate the advent of predictive, preemptive, and personalized care of the voice and other human functions, and ultimately improve the quality of life for patients with voice disorders.

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