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Research and Technology in Neurocritical Care

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For the First Neurocritical Care Research Conference Investigators

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This study was conducted for the First Neurocritical Care Research Conference Investigators.

The list of investigators who participated in this study are given in Appendix.

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Abstract

The daily practice of neurointensivists focuses on the recognition of subtle changes in the neurological examination, interactions between the brain and systemic derangements, and brain physiology. Common alterations such as fever, hyperglycemia, and hypotension have different consequences in patients with brain insults compared with patients of general medical illness. Various technologies have become available or are currently being developed. The session on “research and technology” of the first neurocritical care research conference held in Houston in September of 2009 was devoted to the discussion of the current status, and the research role of state-of-the art technologies in neurocritical patients including multi-modality neuromonitoring, biomarkers, neuroimaging, and “omics” research (proteomix, genomics, and metabolomics). We have summarized the topics discussed in this session. We have provided a brief overview of the current status of these technologies, and put forward recommendations for future research applications in the field of neurocritical care.

Keywords

Neurocritical care; Neuromonitoring; Genomics; Neuroimaging; Biomarkers

The session on “research and technology” of the first neurocritical care research conference held in Houston in September of 2009 was devoted to the discussion of the current status, and research role of state-of-the art technologies in neurocritical patients including multi-modality neuromonitoring, biomarkers, neuroimaging, and “omics” research (proteomix, genomics, and metabolomics). This statement summarizes the topics presented and discussed in this session. It provides a brief overview of the current status of these technologies, and puts forward recommendations for future research applications in the field of neurocritical care. The initial draft of each subsection of this statement was composed by the presenting author.

Continuous Multi-Modality Neuromonitoring (Presenter: Paul Vespa)

Introduction

Multi-modality monitoring refers to the tracking of several parameters of brain physiology and function that can be affected by direct medical or surgical intervention. These parameters include intracranial pressure, brain electrical activity (continuous electroencephalography—cEEG), brain oxygenation (jugular venous, brain tissue oxygen pressure—PbtO₂, near infrared spectroscopy), neurochemistry (cerebral microdialysis), and cerebral blood flow monitoring (transcranial Doppler ultrasound, thermal diffusion probe monitor). These techniques have now been used in selected neurointensive care units around the world for the past 20 years, and a considerable body of knowledge exists concerning the nature of the information gleaned from each monitor, and a general sense of clinical utility. However, there exists a rich potential for fundamental and applied research for each of these instruments. The following will be a summary of the research potential for neuromonitoring as a whole, followed by an assessment for the most commonly used monitors.

Multi-modality neuromonitoring has a number of advantageous features for research. First, these techniques permit a direct biomarker of brain function in patients with injury, and can be applied to large areas of the brain (EEG), or very focal regions of the brain (microdialysis, PbtO₂). The effects of a systemic treatment or study protocol upon the brain can be directly measured. This is important, since, outcomes based research is limited by poor predictability and multisystem illness that may overshadow the direct effects of treatment upon the brain. Second, these techniques permit high-resolution monitoring with

continuous datastreams of longitudinal information. This enables within-subject designed experiments which enhance our power to determine a treatment effect. Third, these techniques are applicable to a wide range of neurologic injury. Fourth, the techniques permit tissue level assessments of ongoing activity and enable hypothesis generation. This latter point is critical since information from animal models of neurocritical care diseases is often limited due to species differences, as well as differences in timing, duration, and intensity of the pathological processes. For example, human cEEG monitoring was the first to find seizures after traumatic brain injury, whereas, animal models did not account or recognize post-traumatic seizures as an important pathological process before the human discovery. Careful consideration for statistical analysis of data stemming from multi-modality monitoring is necessary, since the data series must be assessed for interactions and multiple covariates, and are not necessarily simply streams of independent observations.

In the paragraphs below, several prominent monitoring techniques and their potential for use in neurocritical care research will be highlighted. The techniques herein have been selected based on several criteria: (1) Feasibility: Is the technique feasible for use in the ICU? (2) Relevance to biological pathophysiological processes: Does the monitor provide information that enables us to understand the biology of the disease? (3) Ability to be used in outcomes research: Does the monitor provide information that, when available, could reasonably be expected to alter neurologic outcome or mortality? (4) Generalizability: Can the technique be done in the same fashion across multiple centers? (5) Low risk profile. (6) Statistical feasibility: Does the technique provide information that can be used in a statistical analysis process that is unbiased and accurate? For each monitor, a short list of potential research questions will be listed.

Intracranial Pressure (ICP) Monitoring

Intracranial pressure monitoring is the mainstay of neurocritical care. It is feasible, relevant to the pathological processes, has the ability to enable outcomes research, and is generalizable. The risk profile is appropriate and the technique lends itself well to statistical analysis. One limitation is that with current methods, it is invasive and may not be available for all patients. Importantly, advanced signal processing and appropriate time series analysis enables its use in novel ways that go beyond traditional ways of analysis, making it more appealing for research [1–3]. Potential research projects for ICP would include the following: (1) Outcomes research project to determine if ICP control per se leads to better outcome in traumatic brain injury. To date, studies have targeted the intention to treat arm, but have not studied ICP control as the main outcome determinant. (2) ICP as the biomarker outcome variable by which to gauge the success of a standardized intervention. There is ample precedence for making use of physiologically or clinically meaningful biomarkers as primary outcomes. One example is the use of neuropsychologic function to gauge Alzheimer's disease progress. (3) Advanced processing of ICP to predict progressive brain swelling as an early warning system [4]. The technology to do this is being developed, and requires a development phase. One could envision a treatment trial based on advanced ICP monitoring [3, 5] as a selection criterion.

Electroencephalography Monitoring (cEEG)

Electroencephalography monitoring is a very common [6, 7], but not yet a universal mode of monitoring in neurocritical care. cEEG is feasible, relevant to pathological processes, and is generalizable. cEEG has been correlated extensively with neurologic outcome in critically ill patients [6]. The risk profile is very low. cEEG is labor intensive, and requires expertise that not all neurointensivists have, but potentially could have. The reading and scoring of EEG in the injured brain is complex and calls for objective strategies to measure EEG. Quantitative EEG is one potential method to make EEG more objective. Furthermore, networking among

cEEG capable units would be useful to facilitate research. To date, studies have documented the high incidence of nonconvulsive seizures in various neurocritical care patient populations [6], and have documented potential prognostic usefulness in patients who are in a coma [8]. Potential research projects for cEEG would include the following: (1) Prospective multicenter monitoring for the incidence of seizures in specific disease states. (2) Randomized trial of cEEG for specific disease states to determine the effect of cEEG on clinical outcome. (3) Use of cEEG in randomized trials of therapies for a specific diagnosis (i.e. TBI INTREPID study, hypothermia trial, or a status epilepticus study). (4) Observational studies to develop the best predictive monitoring algorithms for early detection of brain ischemia and neurologic prognosis.

Microdialysis

Microdialysis is a novel, and somewhat uncommonly performed brain monitoring technique that has yielded important information in neurocritical care [9]. Microdialysis is feasible, although somewhat more difficult than ICP monitoring due to the need for an analysis system and equipment. Microdialysis has the ability to enable outcomes research. The risk profile is appropriate and the technique lends itself well to statistical analysis. There is some controversy about the generalizability of very focal measurements upon the general brain function, and considerable differences exist between injured and noninjured brain regions. Some of the most novel hypotheses in brain metabolism with significant treatment implications have resulted from observational monitoring studies in microdialysis. For example, disturbances in brain glucose and excitatory amino acid levels have been found, which suggests that prolonged disturbances of metabolism, including ischemia, may occur in normal appearing brain tissue for many days after an injury [10–12]. Potential research projects for microdialysis include the following: (1) Multicenter randomized controlled trial of glycemic control using microdialysis glucose values as the target, (2) Multicenter phase 2 trials of novel therapeutics using microdialysis metabolic measurements as the biomarkers, (3) Multicenter observational trials of sequential changes in microdialysis metabolic or proteomic [10] profiles, during various interventions for primary diseases in neurocritical care. (4) Multicenter studies of multi-modality monitoring using microdialysis as a tissue biomarker.

Brain Tissue Oxygen (PbtO₂)

Brain tissue oxygen monitoring is minimally invasive, commonly performed, feasible to use [13], relevant to pathological processes, and generalizable. The risk profile is appropriate and the technique lends itself well to statistical analysis. Important observational data about low levels of PbtO₂ have been documented in the literature [14, 15], raising the concern about the lack of oxygen diffusion [16] to brain tissue. Observational studies have resulted in controversy about the role of therapeutic oxygen for common neurocritical care illnesses [17]. Potential research projects for PbtO₂ include: (1) Multicenter randomized controlled trial of targeted PbtO₂ therapy in traumatic brain injury or subarachnoid hemorrhage. (2) Multicenter observational trial of the time course and duration of PbtO₂ values in hypoxic ischemic coma after cardiac arrest. (3) Multi-modality monitoring multicenter study of the influence of PbtO₂ levels on selected biomarkers of disease, including neuroimaging or measurements obtained with other brain monitors.

Near Infrared Spectroscopy (NIRS)

Near infrared spectroscopy has been performed in operating rooms and intensive care units for many years. Pediatric ICUs seem to have done most of the work. While NIRS is feasible [18], the validity of the monitoring values has been questioned. There is less enthusiasm for this technique than all of the others for the intensive care unit in adults. Potential research projects for NIRS include: (1) Observational NIRS monitoring and validation using imaging

or other multi-modality monitoring techniques in pediatric neurocritical care. (2) Randomized trial of NIRS in the neuroanesthesia setting for the prevention of intra-operative ischemic events.

Thermal Diffusion Cerebral Blood Flow Monitor (tdCBF)

The thermal diffusion CBF monitor is a new and somewhat untested regional device that is inserted similarly to the PbtO₂ device. The tdCBF has been validated in a small series of patients and recently gained FDA clearance. Little is known about the performance of this device. This device does not appear ready to be used for multicenter research, but is ready to be evaluated by sites with expertise in other brain monitoring techniques.

Standardizing Multi-Modality Monitoring

To standardize a research approach for various brain monitoring techniques, it is critical to propose the research hypothesis and base the standardization criteria upon the needs of the scientific question. The hypothesis and scientific approach would need to address, whether, a basic biologic process is being considered, or a variety of processes or clinical outcome is being addressed. One could envision a variety of approaches based in large part on the scientific question. There are many important areas of standardization of multi-modality monitoring for the purposes of multicenter studies. (1) The types and numbers of monitors that address the scientific question. (2) The sampling rate and frequency of sampling. This would require an outline of data storage capacity and location. (3) The location of the probes to be used. (4) The total duration of monitoring including the early start window and the ending date. (5) The specific methods of each technique, including the microdialysis perfusion rate and fluid, EEG electrode array, and similar information. (6) The prospective measurements that are to be made. (7) The proposed analysis plans for the data. There have been previous documents written about these techniques, but it would be up to each study to clearly enumerate these criteria. Outlining information technology support for these projects would be necessary. Existing groups with expertise in database construction and networking exist, and would likely need to be hired to facilitate standardization and data sharing.

Biomarkers in Clinical Trials (Presenter: Wendy Ziai)

A biomarker is a measurement which correlates with a clinically meaningful endpoint [19]. From a research standpoint, the development of biomarkers is a useful endeavor to provide surrogate markers for diagnosis, clinical status, change in behavioral status over time, and efficacy of an intervention or recovery. Properly employed, the use of biomarkers has the potential to make phase I and II studies more efficient in terms of sample size and protocol organization.

Biomarkers are particularly useful in Phase I and II clinical trials. Important uses include:

- Investigation of the biological activity of a proposed therapy—for example, determination of cytokine levels in cerebrospinal fluid after traumatic brain injury in rats, to study the effect of hypothermia on multiple mechanistic cascades [20].
- Guiding dose selection.
- Determining whether or not to proceed to a Phase III trial—for example, the use of intracerebral hemorrhage (ICH) volume determination, to evaluate the efficacy of recombinant activated Factor VII in decreasing ICH expansion [21] as a surrogate for improving neurologic outcomes; also the use of intraventricular hemorrhage (IVH) clot lysis rates determined on serial CT scans to assess the potential efficacy of intraventricular fibrinolytics (rt-PA) for improving neurologic recovery from severe IVH.

- Defining target populations—for example, the use of diffusion–perfusion mismatch as a useful marker of salvageable penumbra, to permit selection of acute stroke patients suitable for recanalization or specific neuroprotective strategies [22].
- Providing early determination of therapeutic efficacy—for example, use of functional MRI to predict gains in stroke patients undergoing motor related therapy [23].
- Determining the validity of the assumed underlying biological mechanisms—biomarkers have an important role in informing us, of which of the many competing mechanisms are important in the pathways of primary and secondary neurologic injury.

Choosing an appropriate biomarker requires attention to multiple factors. In comparison to the behavioral and other difficult measurable endpoints that they substitute for, biomarkers are intended to save time, money, and should be relatively easy to measure and standardize across observers (although this is not always the case). It is important to choose surrogate endpoints that lie in the causal pathway of disease, which are directly affected by the therapy, and capture the full intended effect of the intervention on clinical outcome [24]. Capacity for extended testing is important for behavioral surrogates and these biomarkers should be robust against the numerous influences of medical and demographic variability and other covariates.

Suggested steps in employing a biomarker to assist in trial design:

1. Find a simple accurate quantitative surrogate measure of important biologic phenomena under investigation.
2. Link the biomarker to your Phases II and III hypotheses.
3. Make sure the biomarker allows you to answer critical questions with small cohort sizes by performing a power analysis.

Finally, although often taken for granted with many validated measurement tools, determining sensitivity, specificity, validity, and reliability of a biomarker in controlled clinical trials is an essential step in the development of new biomarkers, and in bringing these to the point of clinical utility [24]. In the central nervous system, we currently lack a wide choice of serum markers with adequate sensitivity and specificity for routine clinical use. Equally important, the snapshot biomarker profile at a single time point may not predict events over longer intervals, which involve multiple genetic and environmental interactions. The importance of serial physiologic profiling to develop a longitudinal picture of the biomarker and the disease cannot be underestimated in these situations.

Advances in Neuroimaging (Presenter: Stelios Smirnakis)

Introduction

State-of-the art neuroimaging modalities allow anatomical and physiological monitoring of neuro-vascular function at high spatio-temporal resolution, and are paramount for the management of the critically ill neurological and neuro-surgical patient. In reviewing the current status of neuroimaging techniques and their relevance to neurocritical care research, we will keep in perspective the clinical questions that neurocritical care specialists encounter in their daily practice, as well as important research questions in the field of neurocritical care.

Neuroimaging plays a critical role in the identification of potentially salvageable regions of the brain at risk for secondary injury. For example, a common problem encountered in the

neurointensive care unit (neuroICU) is weighing the risk and benefits of recanalization interventions in acute ischemic stroke patients. Imaging techniques can help assess the extent of irreversibly injured tissue and tissue at risk for secondary injury.

The most prevalent imaging modalities used in the neuroICU are: (1) computed tomography (CT) including CT-angiography (CTA), CT-perfusion (CTP), and xenon CT, (2) magnetic resonance imaging (MRI) including MR angiography (MRA), MR-Perfusion, MR spectroscopy and functional MRI, and (3) positron emission tomography (PET) and single photon emission CT (SPECT). Brain imaging is routinely used by neurocritical care specialists in the management of acute/evolving ischemic stroke, subarachnoid hemorrhage, traumatic brain injury, and coma, among others. Brain imaging also plays an increasingly important role in the assessment of the extent of brain injury, the potential for recovery, and prognostication of long-term outcome.

Measurements that can be obtained by brain imaging and which may be useful in neurocritical care management include: (1) brain anatomy and tissue injury (CT, MRI T1, T2, FLAIR and susceptibility images), (2) vascular tree anatomy (CTA and MRA), (3) extent of recently irreversibly damaged tissue (DWI MRI), (4) extent of brain tissue at risk (CT/MR-perfusion measurements and perfusion-diffusion mismatch), (5) regional cerebral blood flow and oxygen extraction fraction (PET), (7) regional vascular reactivity and cerebrovascular reserve (CTP, SPECT, and fMRI), (8) cerebral metabolic rate of oxygen utilization (PET), (9) blood oxygen level dependent (fMRI), or other (PET/SPECT) metabolic measures of cortical activity, (10) degree of metabolic stress and neuronal injury (MR spectroscopy), and (11) pharmacologic/molecular imaging (PET/SPECT). Measurements of these parameters should ideally be quantifiable, accurate and reproducible, in order for them to be used as surrogate markers for outcome measures of therapeutic interventions in neuroICUs.

The use of neuroimaging in the neuroICU is ubiquitous and growing continuously as more advanced techniques and applications become available. Important research questions that may be pursued utilizing neuroimaging are: (1) assess risks and benefits of acute ischemic stroke interventions stratifying the risk for stroke extension, stroke recurrence, hemorrhage and malignant edema formation, (2) evaluate for the presence and effectiveness of collateral circulation, (3) assess intracerebral hemodynamic parameters to help predict the risk of vasospasm in subarachnoid hemorrhage, (4) improve prognostication of neurological outcomes, (5) preoperative risk assessment, (6) evaluate potential residual cognitive capabilities of subjects “trapped” in comatose or vegetative states (notably, fMRI has been used to re-establish communication with such patients in select cases [25–28]), (6) guide clinical management in traumatic brain injury, (7) study how measurements of cerebrovascular anatomy and physiology, including measures of vascular reactivity [29, 30], impact patient management and outcomes.

Brief Account of Neuroimaging Techniques

Ultra fast helical CT scanning provides high definition cerebral angiograms, and easily quantifiable CT-source image, and CT-perfusion parameters. CT scanners are widely available and CT imaging can be performed rapidly making CT particularly suitable for evaluation of acute neurological problems. CT clearly has its niche in the neuroICU; however, it typically provides less information on tissue status than MRI, and has the disadvantage of radiation exposure, which has recently raised concerns particularly in the pediatric population [31, 32].

In the last decade, MRI techniques have become the standard of care and are employed more and more frequently in the neurocritical care setting. High field (3T) magnets with multiple

phase array acquisition systems are becoming more and more available and have improved signal-to-noise ratio and spatial resolution. Recently introduced algorithmic approaches allow for quantification of brain tissue at risk in the setting of vascular compromise [33, 34]. Functional MRI is being used in conjunction with CO₂, or acetazolamide challenges to measure regional cerebral vascular reactivity [35], a surrogate measure of the capacity of the tissue to maintain its oxygenation. The signal-to-noise ratio and resolution of specialized MRI methods such as magnetic resonance spectroscopy (MRS) and measurement of regional oxygen extraction fraction also have improved [36–38]. MR imaging can be challenging in the neuroICU because of the duration of image acquisition with its associated sensitivity to motion artifact, and the interference of metallic objects (pacemakers, aortic-balloon pumps, and defibrillators) that prevent MRI acquisition; however, MR imaging protocols are becoming faster and MRI access for neuroICU patients in tertiary referral centers is increasing.

PET/SPECT will remain for the foreseeable future, the province of specialized centers given the need for radioactive isotopes. However, these technologies are invaluable research tools, because their sensitivity for molecular/ metabolic imaging exceeds that of MRI techniques by ~ 6 orders of magnitude. Advanced design of radioactive probes that target specific molecular ligands has already started to revolutionize the field of molecular diagnostics based on PET neuroimaging. PET measurements of the oxygen extraction fraction provided the basis for the Carotid Occlusion Surgery Study (COSS). PET/MRI combination scanners are now available for animal research [39]. Human PET/MRI scanners will gradually become more available, allowing simultaneous molecular-pharmacological and high-resolution anatomical-vascular neuroimaging.

Neuroimaging Endpoints

All too frequently, stroke trials have failed because of inclusion of study populations with non-uniform disease etiology, and because of endpoints prone to a high degree of variability. Neuroimaging can help identify patient populations with uniform disease etiology, and provide objective and reliable measurements (“neuroimaging biomarkers”) for surrogate, quantifiable, endpoints. Choosing neuroimaging endpoints guided by physiological principles will increase study power, and may provide valuable information on underlying pathophysiology. Ideal neuroimaging trials should be designed prospectively and provide both clinically relevant and physiologically meaningful information.

Examples of surrogate neuroimaging endpoints that might be useful include: (1) volume/ location of damaged tissue, (2) volume of hypoperfused tissue and quantification of the conferred risk, (3) fraction of the territory at risk that was eventually saved, (4) quantification of regional vascular reactivity, (5) regional CMRO₂ measurements, (6) oxygen extraction fraction, and (7) molecular imaging endpoints.

It is also important to note the need to define and validate specific “neuroimaging phenotypes,” defined loosely as a set of neuroimaging measurements, that correlate with a particular clinical phenotype or pathological entity. Neuroimaging phenotypes can serve as a valuable starting point for defining appropriate patient populations for the investigation of particular disease mechanisms. Proteomics and genomics research based on well characterized, quantifiable, neuroimaging phenotypes holds particular promise for the future.

How Can We Use Neuroimaging Techniques to Assess Recovery/Improvement After Neurocritical Care Illness?

Neuroimaging techniques provide visualization of the evolution of vascular status and neural recovery over time. Applying standard multi-modality MRI sequences at defined time points in the course of recovery, allows for the assessment of tissue viability, vascular recanalization, and tissue reperfusion [40]. Algorithms for accurate prediction of tissue outcome based on imaging data should be optimized to allow the use of imaging data as surrogate biomarkers for the assessment of the success of an intervention. The development of multicenter collaborations and centralized repositories of imaging data will facilitate measuring the impact of advanced imaging on neurocritical care outcomes.

Assessing as to what patterns of cortical reorganization correlate with (or maybe promote) recovery is a complex but important question. Under normal circumstances, neural activity generates a proportional, over-compensatory, vascular response which can be measured by functional MRI (BOLD signal). By following the pattern of the response elicited by a standardized stimulation paradigm (such as, finger tapping for example), we can derive information about the patterns of reorganization that occur during recovery. Regional vascular reactivity maps following a CO₂ challenge can help avoid the potential pitfall of interpreting BOLD signal changes of vascular origin as reflecting neural plasticity [29].

Translational Aspects. Animal Models. Imaging for Basic Research

Animal models are pertinent for testing specific hypotheses. Both rodent and primate models for common neurocritical care conditions promise to be extremely useful. For example, macaque fMRI has been developed as an experimental model for measuring cortical patterns of recovery following brain injury [41, 42], and can be used to test a number of interventions designed to promote recovery, such as cortical stimulation [43, 44]. Pluta et al. [45], recently showed that sodium nitrite infusions prevent vasospasm in a primate model of subarachnoid hemorrhage, raising the hope that such treatment will benefit human populations.

Although here we focus on neuroimaging modalities that can be applied to humans, one other emerging experimental research tool deserves specific mention. Laser scanning two-photon microscopy methods [46] have unparalleled power for monitoring the structure and function of neural cortical circuits, as well as the dynamics of the supplying vasculature, in vivo [47]. Recently, the methodology for inducing and studying specific models of microvascular injury using two-photon microscopy has been developed [48–50], providing a model for microvascular stroke, hemorrhage or endothelial leakage, and allowing the study of therapeutic manipulations on cortical micro-vascular dynamics. Two-photon methods are also ideal for studying local cortical circuit repair, as they can monitor both the structure and function of neuronal units in ~300–500 μ m fields of view. Channel rhodopsin and other emerging optogenetic methods that can be used to manipulate cortical circuit activity hold promise for elucidating underlying neuronal circuit recovery mechanisms.

Paving the Road for establishing Multi-Center Neuroimaging Trials

Overall, the amount of information that can be obtained by a well organized neuroimaging effort is staggering. In order to be successful we will need to: (1) develop a neurocritical care neuroimaging research roadmap, and (2) apply standardized neuroimaging protocols across centers.

The coordination of multi-center MRI and fMRI trials has already been performed by other groups. The Biomedical Informatics Research Network Functional and Structural Neuroimaging Calibration Study (fBIRN) consortium, for example, has organized a network of multiple sites and multiple experimental platforms, and has proposed a set of guidelines

for the management of large scale multi-center neuroimaging trials (http://www-calit2.nbirn.net/research/function/fbirn_publications.shtm). Common challenges include: (1) data standardization to be able to combine results across sites, (2) efficient data sharing and management plan, (3) coordination and communication across sites, (4) prevention of site bias in patient selection, and (5) prevention of site bias in experimental neuroimage acquisition parameter design and execution. fBIRN has proposed specific recommendations which include: (1) develop a training check list of requirements for the sites to adhere to, (2) implement a multi-center pilot to test the study design, (3) designate *one* experienced scientist to travel among sites and ensure that the experimental parameters, design, and execution align, (4) have *one* person or group analyze the data obtained on the pilot and prove consistency, (5) develop an automated quality assurance scheme to run for each data set, which will ensure, that during the trial, imaging quality remains consistent, and (6) implement an automatic pre-processing analysis pipeline including motion correction, smoothing, slice timing correction, and B0 field inhomogeneity correction. Networks such as fBIRN may function as a template for the future design of multi-center neuroimaging trials in neurocritical care populations.

In conclusion, a carefully selected neuroimaging research agenda will play a central role in future neurocritical care research. In order to make advancements in this field we should establish a committee to design a neurocritical care imaging agenda that should include the following:

- i. Identify and prioritize neurocritical care research questions that can be answered by neuroimaging trials.
- ii. Decide on the optimal imaging modalities for each question.
- iii. Identify neurocritical care centers for participation in a neuroimaging research network (different centers might be ideal for different questions).
- iv. Decide on a few priority questions to focus our efforts on. The ideal criteria would be that the neuroimaging information gathered is applicable to more than one clinical process or study. Efforts should be coordinated with those from other committees in the neurocritical care research network.
- v. Consider alignment with existing neuroimaging consortia and networks as applicable.
- vi. Design a plan to organize the technical and administrative aspects of coordinating neuroimaging data collection for multi-center trials.
- vii. Support efforts to use imaging modalities for basic and translational research pertinent to neurocritical care.

Genomics, Proteomics, and Metabolomics Research in Neurocritical Care (Presenter: Kinga Szigeti)

Where Is the Field of Genomics, Proteomics, and Metabolomics Generally?

Technological and scientific advances in molecular biology and bioinformatics have made it possible to perform large scale analyses of gene and protein expression in specific disease phenotypes. Extracting patterns of gene, protein, or metabolite expression associated with specific phenotypes have the potential to yield: (1) specific markers of particular disease states associated with the studied phenotype, and (2) novel molecular and pathophysiological understanding of disease etiology that identify new targets for therapy.

To derive the most benefit from “omics” analysis, the following requirements must be met: (1) the patient population should be appropriately and accurately phenotyped resulting in large numbers of highly phenotyped patients for study; (2) normative data should be obtained in a variety of normal population samples to set the statistical range of expected expression patterns; (3) appropriate bioinformatics techniques should be applied to assess significance; (4) technological methods should be selected to maximize sensitivity and minimize experimental noise; (5) establishment of research networks with standardized approaches and centralized databases that allow free access for approved research projects; and (6) animal and cell culture experiments should be performed in parallel with human studies to allow detailed investigation and confirmation of generated hypotheses.

The complex issues involved in neurocritical care disease states, demand careful patient selection and thoughtful formulation of questions to be addressed by “omics” research. Examples of research questions that may provide fertile ground for analysis include: determining unique signature differences between hemorrhagic and ischemic strokes; determining genetic factors that predispose to intracranial aneurysms, arterial dissections of the head and neck, and formation of malignant cerebral edema; identifying response patterns to pharmacological interventions to differentiate between inflammatory and infectious febrile states; characterizing biomarker patterns that identify transient ischemic attacks; and determining genetic or proteomic patterns that correlate with neural plasticity and the propensity for recovery.

What can We Learn from Existing “Omics” Repositories?

Although current repositories exist, it would be most efficient to tailor “omics” data collection to the specific needs of the neurocritical care population. Omics research is powerful, but faces several challenges that include: standardization of the collection and analysis of “omics” data, access to high quality “platform” technologies and advances in bioinformatics, identification of valid molecular markers, and limited overlap of gene expression signatures between patients and animal models. One particular issue that arises in identifying and correlating specific patterns of expression with disease phenotypes, is the contrasting demands of sensitivity versus specificity. A layered approach, in which we separate the questions we want to address into (1) hypothesis forming (requiring high sensitivity) and (2) hypothesis testing (requiring high specificity), is likely to yield the best results.

Lessons from existing “omics” efforts provide important information about effective trial design [51, 52]. In particular, we can learn about establishing standards for collection, presentation, and analysis of genomic, proteomic, and metabolomic data, akin to the established MIAME (minimum information about a microarray experiment) format for genomic data [53]. Practical lessons about methodology can be assimilated from large collaborative programs, such as, the “Inflammation and Host Response to Injury, Large-Scale Collaborative Research Program”. The Human Proteome Organization (HUPO) and the Proteomics Standards Initiative (PSI) set the standard for data representation in proteomics, to facilitate collaborative data exchange (www.hupo.org). Proteomics study and data interpretation requires collaborative efforts from mass spectrometry scientists, bioinformatics experts, biochemists, protein chemists, translational researchers, and clinicians. This collaboration ensures the design of experiments that acquire high quality data to understand the biological and clinical relevance of “omics” measurements. Proteomics profiling can take a “candidate” or “discovery” approach. Targeted (candidate) approach measures a specific subset of known proteins, whereas, discovery approach identifies all proteins that change expression in response to a stimulus. While targeted approach is faster and more sensitive, it may discount important and yet unknown protein pathway interactions. In contrast, the discovery approach can explore unknown pathways,

but is more time/effort intensive, statistically demanding, and may not detect low abundance proteins. The development of new technological approaches has started to close the gap between targeted and discovery proteomics making more in-depth clinical proteomic investigation feasible.

How Can We Create an Integrative Method of Analysis that Focuses on Important Functional Interaction Maps of Molecules and Processes that Play a Role in Cell Organism and Function?

Major efforts, both on a local and international level have been devoted towards these goals. Functional interaction requires in-depth translational research from the bench to bedside. For now, we can help identify pathways that are important, and perform pilot “omics” trials targeting specific known pathways, keeping an open mind about interactions or pathways that are not known. There is a need to develop system level approaches to analyze the functional interaction maps derived from “omics” analysis. The development of organized and maintained pathways analysis tools (www.ingenuity.com) can help delineate the complex relationships that exist between the expression of different proteins or genes, and will likely be critical for analyzing “omics” data, forming the basis for subsequent hypothesis testing.

Do We Need Long-Lasting Interactions With Quantitative Scientists?

Omics research is a large scale unbiased approach, and to be successful, requires, “omics” neurocritical care consortia to be organized efficiently. Close collaboration between centers is needed to develop large homogeneously characterized sample data sets. Establishment of central databases and consortiums will be essential. There is a need for bioinformatics expertise, as well as for close collaborations between scientists and clinicians. A tri-partite structure in which three teams coexist and interact closely on a regular basis may be one way to proceed. For example, there could be: (1) a clinical team that understands the clinical questions and determines appropriate phenotypes for study, (2) a bioinformatics team that is critical in specifying the study design and analysis approaches, and (3) a translational scientist/molecular biology team that synthesizes the molecular and pathophysiological information to extract information about basic cellular and molecular pathways, and processes to achieve maximal translational benefit. Establishing animal and cell culture models for diseases appropriate for “omics” research will be a critical function of the translational team.

Why Is It Important to Carry Out Pharmacogenomics Studies?

It is important because so much of neurocritical care is devoted to intervention, and pharmacogenomic investigations may offer a rapid route to improving patient care. Pharmacogenomic studies can be added to Genomic Wide Association Studies (GWAS) by ascertaining drugs administered, and responder status, or can be designed independently for specific therapeutic interventions.

How Will a Research Network Impact Genomics and Proteomics Studies in Neurocritical Care?

A network approach is critical for genomics and proteomics as it will allow for large sample sizes of the phenotypes interrogated. Single centers likely will not be able to ascertain adequate sample sizes for these studies.

How Do We Create and Adopt Standard Operating Procedures (SOPs)?

Sample Access—Extracted DNA, RNA, and protein can be redistributed to investigators. Initially access may be limited to network members. As samples are limited, decisions for

sample redistribution may need to be the responsibility of a scientific committee responsible for the evaluation of study proposals.

Sample Processing and Storage—Many genomic and proteomic assays are confounded by batch effects, thus centralized sample handling is fundamental. Centers for storage, and DNA protein and RNA extraction should be identified, and SOPs must be used for local sample handling and shipment. Samples should be collected and labeled through SOPs and processed at a central location. Barcode labels containing a unique identifier, a human-legible identifier, a sample number, and the sample type (whole blood, buffy coat, serum or plasma) are recommended. Storage conditions should be part of the SOP and need to be uniform. Software packages (e.g. Freezerwork) are available for sample tracking.

Phenotyping—Especially in a “noisy” neurocritical care setting, where multiple interventions are carried out in an individual patient, meticulous clinical phenotyping is crucial. For example, intravenous albumin, blood products, and contrast dye may affect proteomic and metabolomic analyses. Phenotyping needs to be predefined by consensus criteria. Misclassification is a major confounder and decreases power by introducing noise to the data. Phenotypic data that can be measured quantitatively (e.g. size of aneurysm on imaging study) are the least likely to result in misclassification.

Data Sharing—NIH has established guidelines for data sharing for GWAS and requires adherence for NIH funded studies. Data sharing has already fundamentally accelerated the yield of GWAS and consortia are emerging as standard across the “omics” fields. In most instances, data sharing requests are evaluated by a scientific committee and granted according to scientific merit. Data repositories are strongly encouraged for any publication. Repositories for proteomics already exist from EMBL (PRIDE database), NCBI (GenBank), and other sources.

Genetic Dissection of Complex Traits in Neurocritical Care

With the advent of technology, allowing detection of markers at the whole genome level, and the progress in cataloging genetic variants to serve as marker maps, a new era of GWAS has developed. A large wave of GWAS applied to the common diseases and quantitative traits have been published and cataloged for public access in dbGAP, and at the National Cancer Institute (NCI)-National Human Genome Research Institute (NHGRI)’s catalog of published GWAS. These studies consistently demonstrate that, common variants explain only a fraction of heritability and any given variant has a moderate effect size at best. New approaches focusing on the missing heritability have been proposed, with current attention focused on the identification of rarer high potency variants via high throughput sequencing, as well as on the exploration of gene-environment interactions.

Large sample sizes are the key factor for the conduct of well-powered GWAS, a major challenge for the disease entities encountered in neurocritical care. Prioritization of diseases for genetic association studies has been based on the estimated heritability of the disease; however, information on heritability for most neurocritical care conditions is sparse. Furthermore, high heritability is no guarantee of a successful GWAS, while there are many conditions of relatively low heritability for which genetic risk factors have been found. It is also crucial to collect detailed information on non-genetic exposures, since, for diseases such as subarachnoid hemorrhage, in which heritability is estimated to be high [54], environmental factors including smoking, alcohol intake, and hypertension appear to play a potent, if not more potent, effect on risk, as any genetic risk factors. Sample sizes in the thousands are required for GWAS, and to detect a geneXenvironment interaction of similar effect, a sample size of ~ 4 times more is required [55].

For case–control studies, the selection of controls needs careful evaluation as well, taking into account, accuracy of phenotyping and the prevalence of the disease in question. Data from the Welcome Trust Case Control Consortium suggests that population based controls are a reasonable approach, if the sample size is adequate. As historical control genotype data is available for large number of controls, one potential approach is to use these historical controls, provided that the genotyping is validated on a subset of these controls by the assay used for genotyping the cases [56].

Replication of the association detected in the discovery cohort is of utmost importance and requires an independent cohort. The goal of replication is to identify the association signals that are truly reproducible. When designing GWAS, efforts should be made to follow the recommendations of the NCI-NHGRI Working Group on Replication in Association studies [57].

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Appendix

The First Neurocritical Care Research Conference Investigators:

Organizing Committee

Chair: Suarez JI, MD, Baylor College of Medicine, Houston, TX; Calvillo E, RN, Baylor College of Medicine, Houston, TX; Geocadin R, MD, Johns Hopkins University, Baltimore, MD; Hall C, MD, UT Southwestern University; Le Roux PD, MD, University of Pennsylvania, Philadelphia, PA; Livesay S, MS, RN, ACNP, St Luke's Episcopal Hospital, Houston, TX; Mayer SA, MD, Columbia University, New York, NY; Vespa P, MD, UCLA, Los Angeles, CA; Wijman CAC, MD, PhD, Stanford University, Palo Alto, CA; Zaidat OO, MD, MS, Medical College of Wisconsin, Milwaukee, WI.

Speakers

Bleck TP, RUSH School of Medicine, Chicago, IL; Chang C, MD, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI; Cooper DJ, MD, Alfred Hospital and Monash University, Melbourne, Australia; Guntupalli KK, MD, Baylor College of Medicine, Houston, TX; Daily J, PhD, Case Western Reserve University, Cleveland, OH; Diringer MA, MD, Washington University School of Medicine, St Louis, MO; Robertson CS, MD, Baylor College of Medicine, Houston, TX; Hanley DF, MD, Johns Hopkins University, Baltimore, MD; Hemphill C III, MD, PhD, University of California in San Francisco, San Francisco, CA; Koroshetz W, MD, Deputy Director, NINDS, Bethesda, MD; Mirski MA, MD, PhD, Johns Hopkins University, Baltimore, MD; Palesch Y, PhD, Medical University of South Carolina; Qureshi AI, MD, University of Minnesota, Minneapolis, MN; Silbergleit R, MD, University of Michigan, Ann Arbor, MI; Smirnakis SM, MD, PhD, Baylor College of Medicine, Houston, TX; Szigeti K, MD, PhD, Baylor College of Medicine, Houston, TX.

Attendees

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