Systems neuroimmunology: a review of multiomics methodologies to characterize neuroimmunological interactions in spinal and cranial diseases

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Neuroimmunology plays a critical role in our understanding of the pathophysiological processes that underlie a variety of diseases treated by neurosurgeons, including degenerative disc disease (DDD), glioblastoma (GBM), aneurysmal subarachnoid hemorrhage (aSAH), and others. Compared with traditional methods in neuroimmunology, which study one pathway or gene at a time, emerging multiomics methodologies allow for holistic interrogation of multiple immune-signaling pathways to test hypotheses and the effects of therapeutics at a systems level. In this review, the authors summarize key concepts for gathering and analyzing multiomics data so that neurosurgeons can contribute to the emerging field of systems neuroimmunology. Additionally, they describe 3 use cases, based on original research published by their group and others, that utilize transcriptomic, metabolomic, and proteomic analyses to study immune-signaling pathways in DDD, aSAH, and GBM. Through these use cases, techniques for performing machine learning and network-based analyses to generate new clinical insights from multiomics data are shared. The authors hope that neurosurgeons might use this review as a summary of common tools and principles in systems immunology to better engage in creating the immunotherapies of tomorrow.

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THE cellular microenvironment of the nervous system is composed of a dynamic milieu of vascular, immune, and neural cell types that interact with one another in a way that produces complex physiological responses.¹ Neuroimmune interactions, in particular, play a critical role in both normal physiology and the pathophysiological processes that underlie cranial and spinal neurosurgical disease. In fact, the immune system has been found to have a key role in a variety of conditions treated by spine, tumor, and cerebrovascular neurosurgeons including 1) the immune-mediated progression of degenerative disc disease (DDD),² 2) the susceptibility of brain tumors like glioblastoma (GBM)^{3–6} to novel immunotherapies, and 3) the response of brain tissue to extravasated blood products during aneurysmal subarachnoid hemorrhage (aSAH).⁷

Systems neuroimmunology is an emerging framework for interrogating neuroimmunological interactions at a systems level, which can be used to study the immune processes that drive neurosurgical disease.^{8–10} Systems neuroimmunology leverages multiomics data from genomic, proteomic, metabolomic, and other sources to arrive at a systems-level view of which genes, proteins, and metabolites of the immune system are active during healthy versus diseased states. The evolution of systems neuroimmunology into its modern form stems from advancements in molecular technologies, such as expression profiling, microchip array technology, immunological assay methods, and large-scale epitope screening.⁹ Neurosurgeons are particularly well poised to engage in systems neuroimmunology research, as their role is essential in gathering the tissue samples that generate multiomics data.

ABBREVIATIONS AF = annulus fibrosus; aSAH = aneurysmal subarachnoid hemorrhage; CCN1 = cellular communication network factor 1; DDD = degenerative disc disease; DGE = differential gene expression; GBM = glioblastoma; HSV = herpes simplex virus; ICH = intracerebral hemorrhage; IL = interleukin; IVD = intervertebral disc; MALDI-TOF = matrix-assisted laser desorption/ionization time of flight; NP = nucleus pulposus; OV = oncolytic virus; PPI = protein-protein interaction; scRNA-seq = single-cell RNA sequencing; TNF = tumor necrosis factor.

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FIG. 1. A summary of multiomics approaches used to characterize neuroimmunological interactions in DDD and GBM. **A:** Proteomics can be used to identify susceptibilities of GBM to immunotherapy based on PPI network analysis. **B:** Metabolomics can identify metabolites that modulate immune interactions after aSAH. **C:** Transcriptomics can identify differentially expressed genes in immune-signaling pathways that drive DDD progression. LC/MS = liquid chromatography/mass spectrometry; RNA-seq = RNA sequencing. Created with BioRender.com.

In this article, we hope to provide neurosurgeons with a practical guide for how they might use systems neuroimmunology in their practice to advance the collective understanding of neuroimmunological interactions in neurosurgical disease. We have selected 3 use cases that feature original research from our laboratories and others that apply systems neuroimmunology to the study of immunological interactions in spinal and cranial neurosurgical disease. These use cases each respectively demonstrate how multiomics data, including transcriptomic, metabolomic, and proteomic data, may be used to generate insights into how the immune system drives the progression of a broad variety of neurosurgical diseases including DDD, aSAH, and GBM (Fig. 1).

Multiomics Technologies

Systems neuroimmunology has successfully matured as a discipline due to technological advances in gathering multiomics data in a high-throughput fashion, as well as robust analysis pipelines for identifying alterations in immune signaling. In this section, we present some general principles related to generating, analyzing, and interpreting multiomics data so that neurosurgeons can better engage in this line of research.

Generating Multiomics Data

Systems neuroimmunology as applied to the study of neurosurgical pathologies relies on robust multiomics data sets generated by carefully curating tissue samples from healthy and diseased subjects. Neurosurgeons are essential to the process of collecting multiomics data for systems neuroimmunology research because they are directly involved in procedures that result in the excision of diseased tissue. For example, as described in use case 1, a standard lumbar discectomy and fusion can result in the excision of inflamed disc tissue, which can be used for systems neuroimmunology research. Several enabling technologies such as microarrays, RNA sequencing, and mass spectrometry then allow for multiomics data to be gathered from direct tissue biopsies, CSF, or blood (Table 1).

Generating Genomic and Transcriptomic Data

Genomic and transcriptomic data can be gathered from high-throughput technologies that quickly survey global gene expression patterns. Through microarray technology, global gene expression in tissue samples can be measured in parallel by thousands of genes and quantified to deduce signaling pathways and regulatory networks.¹¹ The emergence of single-cell RNA sequencing (scRNA-seq) tech-

TABLE 1. A summary of multiomics studies including the data source, enabling technologies, and insights created for neuroimmunology

Authors & Year	-Omic Approach	Data Source	Enabling Technologies	Application in Neuroimmunology
Costantino et al., 2017 ³⁷	Genomics	Genes	DNA microarray	Genome-wide association study in 906 pts, including 486 pts w/ SpA; revealed association of SpA w/ <i>MAPK14</i>
Bydon et al., 2020 ²	Transcriptomics	RNA transcripts	RNA sequencing	Transcriptomics data from IVD samples showed immune-signaling pathways were upregulated in DDD
Ma et al., 2021 ⁴	Proteomics	Proteins	MALDI-TOF	A computational analysis of PPIs in GBM cell lines identified opportunities to strategically improve immunotherapy design
Valentín-Guillama et al., 2018 ⁵	Metabolomics	Small molecules & metabolites	Mass spectrometry, HPLC	Metabolomic data from gp120-treated glioma cells showed that HIV-1 can promote proliferation & activation of glycolysis, resulting in increased protein synthesis

gp120 = envelope glycoprotein 120; HPLC = high-performance liquid chromatography; pts = patients; SpA = spondyloarthritis.

nologies allows transcriptomic data to be gathered from individual cells.^{12,13} RNA sequencing requires single-cell suspensions to be obtained, which is typically achieved with high yield through mechanical disaggregation and enzymatic dissociation. Several technologies, such as flow cytometry, can be used to then isolate individual cells. After isolation, cells are lysed to extract RNA, which is subsequently amplified by polymerase chain reaction.¹² It is important to assess the quality of the extracted RNA by measuring its integrity number. Given that RNA molecules are susceptible to degradation, the RNA integrity number is an important measure that can be used for quality control.¹⁴ Before sequencing can be performed, the RNA must be converted into double-stranded complementary DNA, which can be achieved through poly-T oligo-attached magnetic beads. Sequencing can then be conducted, with reads mapped to a reference transcriptome or genome, and quantified to gene counts per gene or transcript.¹⁵ An analysis of differential gene expression (DGE) enables quantification through computational statistical models to distinguish overlapping transcripts between samples. In use case 1, we discuss a study that identified molecular regulators associated with DDD in an effort to establish promising candidates for therapeutic targets.^{12,16}

Generating Proteomic and Metabolomic Data

Mass spectrometry is the foundational technology for generating proteomic data and allows for characterization of proteins through analyzing their mass-to-charge ratio.¹⁷ Studying proteins in this fashion is a formidable task due to the fact that genes can generate multiple proteins through sequence polymorphisms, alternative splicing, and posttranscriptional modifications. These challenges have been overcome with several technological advances.¹⁷ For example, matrix-assisted laser desorption/ionization time of flight (MALDI-TOF), allows for the visualization of proteins in addition to their various proteoforms.¹⁸ With this technology, a matrix is used to coat the tissue under study, which aids in desorption and ionization of endogenous biomolecules during laser irradiation. Then, individual mass spectra are collected to generate signal intensity maps and ion images across the sample area. As thousands of these ion images are created through a single MALDI-TOF experiment, researchers can use top-down

protein identification methods to gain molecular contexts of the tissue sample under study.¹⁸ In use case 2, we discuss how mass spectrometry is used to generate metabolomic data from tumor cell lines.

Analyzing Multiomics Data

Broadly, algorithms for analyzing multiomics data can be subdivided into clustering and network-based approaches.¹⁹

Clustering Analysis

Clustering algorithms are a type of unsupervised machine learning algorithm that can infer groups of similar samples based on a distance metric by using a weighted sum of expression values in multiomics data sets derived from different samples.²⁰ Samples that have a distance apart from each other less than a given threshold are considered similar and are clustered into the same group. Common clustering algorithms used in neuroimmunology research include t-distributed stochastic neighbor embedding (t-SNE) and uniform manifold approximation and projection (UMAP).²¹ In Fig. 2A, we demonstrate an example of the hierarchical clustering that was employed in use case 1 to assess tissue-to-tissue and patient-to-patient variation in musculoskeletal tissue samples from patients with DDD.

Network-Based Analysis

Network-based approaches for analyzing multiomics data sets seek to identify dependent relationships and interactions between genes, RNA transcripts, and proteins. Such algorithms use metrics such as pairwise correlation, mutual information, or weighted coexpression to assess genes that are closely dependent on one another and to create a network.²² Some common network-based algorithms include NetDecoder (<https://netdecoder.hms.harvard.edu>), to derive context specificity, and the visualization packages Cytoscape (<https://cytoscape.org>) and NetworkX (<https://networkx.org>).^{21–23} In Fig. 2B, we show how a network-based algorithm was used to infer the immune-signaling pathways that were active in patients with DDD from use case 1.

For neurosurgeons just getting started with using such analytical approaches, there are several public multiomics

FIG. 2. Examples of different approaches employed to analyze multiomics data. **A:** Clustering methods can be used to assess patient-to-patient and tissue-to-tissue variation in musculoskeletal tissue samples from patients with DDD. **B:** Network methods can be used to assess immune-signaling pathways that are active in patients with DDD. **C:** Overrepresentation analyses can be used to detect signatures of resistance to OV therapy in GBM. AGE-RAGE = advanced glycation end products–receptor for advanced glycation end products; KEGG = Kyoto Encyclopedia of Genes and Genomes; NOD = nucleotide-binding oligomerization domain. Panels A and B reprinted from *Gene*, 750, Bydon M, Moinuddin FM, Yolcu YU, Wahood W, Alvi MA, Goyal A, Elminawy M, Galeano-Garcés C, Dudakovic A, Nassr A, Larson AN, van Wijnen AJ, Lumbar intervertebral disc mRNA sequencing identifies the regulatory pathway in patients with disc herniation and spondylolisthesis, 144634, Copyright 2020, with permission from Elsevier.

data resources that may be applied to neuroimmunology (Table 2). We advise, as a best practice, that different omics data sources should be integrated, which can serve to strengthen associations and reduce noise; in particular, similarity network fusion (SNF) is a commonly used method for integrating different omics data sources.²³

Generating New Insights From Multiomics Data

Clinical insight generation is a critical component of multiomics pipelines in which neurosurgeons can actively participate. Often, the networks derived from the network-based analyses described above can yield insights in and of themselves. For example, in use case 3, we have described how performing a network analysis on GBM mul-

tiomics data elucidated mechanisms of resistance to oncolytic viral therapies. Additionally, we have described how the same network analysis allowed us to postulate that the measles virus was a more effective oncolytic viral vector compared with herpes simplex virus (HSV) for GBM with cellular communication network factor 1 (CCN1) resistance signatures (Fig. 2C).

Additionally, once an interaction network has been derived from multiomics data, novel clinical insights can be gathered by performing *in silico* simulations of network behavior. Network simulator tools like BioNSi (<https://bionsi.wixsite.com/bionsi>) can estimate the behaviors of genes and proteins in a network once certain interactions are deleted or added.²⁴

TABLE 2. Resources for public multiomics data sets

Authors & Year	Data Resource	Multiomics Type	Description
Edgar et al., 2002 ³⁸	GEO	Genomics	A public, curated repository of microarray experiment data
Darmanis et al., 2017 ³⁹	GBMseq	Transcriptomics	A public repository of scRNA-seq on 3,589 cells from GBM samples in a cohort of 4 pts
Xie et al., 2015 ⁴⁰	HGCC	Transcriptomics	An open resource, useful to both basic & translational GBM research, containing a biobank of 48 GBM cell lines & an associated database containing high-resolution molecular data
Schmidt et al., 2018 ⁴¹	ProteomicsDB	Proteomics	A public, curated repository of proteomic data sets from a variety of host organisms
Joshi et al., 2019 ⁴²	TcellSubC	Proteomics	A public proteomic data set of 6,572 proteins in the human CD4+ T-cell proteome
Sud et al., 2016 ⁴³	Metabolomics Workbench	Metabolomics	A public, international repository for metabolomic data

GEO = Gene Expression Omnibus; HGCC = Human Glioblastoma Cell Culture; TcellSubC = T-Cell Subcellular Atlas.

Systems Neuroimmunology and DDD

Immunology Review in DDD

DDD is a degenerative process affecting the mechanical integrity of intervertebral discs (IVDs) in the spine, which are composed of an inner nucleus pulposus (NP), outer annulus fibrosus (AF), and the bordering cartilaginous endplate.²⁵ DDD is thought to be an age-related phenomenon that includes inflammation and cell apoptosis leading to decreased proteoglycan synthesis with resultant disc dehydration and loss of disc height. Multiomics can facilitate translational research to identify key genetic networks with sufficiently high levels of expression to make practical targets for genetic molecular therapy directed at ameliorating DDD.

Several studies in the neuroimmunology literature have elucidated the immune pathways that drive the progression of DDD. Initially, cells from the NP and AF secrete pro-inflammatory molecules that recruit immune cells to the IVD.²⁶ Cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, and IL-17 increase in concentration in the IVD, resulting in an inflammatory microenvironment.²⁶ Such an environment reduces the structural integrity of the NP and AF, leading to herniation and annular tears. The degenerated IVDs subsequently amplify the inflammatory cascade through further production of TNF- α , IL-1, IL-6, IL-17, interferon (IFN)- γ , and inflammatory mediators such as nitric oxide and prostaglandin E2.^{27,28}

It is imperative that neurosurgeons seeking therapeutic targets for DDD have a thorough understanding of the immunopathology for spine degeneration. In our first use case, we present work from our group that uses transcriptomic analysis to gain insight into DDD pathogenesis.

Use Case 1: Transcriptomics to Characterize Inflammatory Pathways in DDD

Our group published a study that aimed to identify immune-signaling pathways distinct to DDD progression.² DDD may lead to spinal stenosis, disc herniation, and/or degenerative spondylolisthesis, which may manifest in the patient as radiculopathy or myelopathy. To further enhance the understanding of disc herniation and degenerative spondylolisthesis pathogenesis, the transcriptome data of IVD samples from lumbar-degenerated discs were

analyzed. Disc tissues were harvested intraoperatively and subsequently underwent RNA sequencing.²

A total of 33 adult patients who underwent lumbar discectomy to treat DDD were included in our study. During lumbar discectomy, NP samples from 33 adult patients were carefully dissected in the operating room by the primary surgeon. The harvested samples were then frozen in liquid nitrogen and stored until ready for processing. Of the 33 samples, only 10 samples met our threshold RNA integrity number necessary for next-generation sequencing.

The NP tissue biopsies were subsequently ground into powder and homogenized, then quantified with a spectrophotometer. Next-generation RNA sequencing was then performed to assess DGE. Computational analysis compared NP RNA sequencing with bone, cartilage, growth plate, and muscle tissue.²

When we compared transcriptomic data from NP samples with other musculoskeletal tissues in patients with DDD, we were able to identify several immune-signaling pathways that were upregulated. When comparing the NP samples with other musculoskeletal tissues, 702 genes demonstrating significant upregulation and 3734 genes with significant downregulation were found. To better understand the unique regulatory pathway for disc herniation and degenerative spondylolisthesis, the DGE profile revealed 1514 upregulated and 281 downregulated mRNA molecules. Furthermore, STRING analysis (<https://string-db.org>) demonstrated differentially expressed genes for each gene ontology term from different types of networks, with distinct cellular functions for each biomedical condition. Specifically, we found several differentially expressed genes in chemotactic signaling (*CXCL10*, *CXCL11*, *IL1RL2*, and *IL-6*) and matrix-degrading pathways (*MMP16*, *ADAMTSL1*, *ADAMTSL5*, *ADAMTSL8*, *ADAMTSL12*, and *ADAMTSL15*). Neurosurgeons can leverage these identified gene regulatory networks, or conduct similar transcriptomic analysis, to strategically engineer novel pharmacological approaches for the treatment of lumbar DDD and other degenerative diseases.

Systems Neuroimmunology and aSAH

Immunology Review in aSAH

Extravasated blood products that are released after intracerebral hemorrhage (ICH) or aSAH are known trig-

gers of inflammation in the brain.^{29,30} While primary injury, which occurs within hours after ICH, is related to the mechanical distortion of brain tissue due to hematoma expansion, secondary injury, which occurs on a much longer time scale after the initial insult, is often mediated by immune signaling. Red blood cell lysis occurs approximately 24 hours after an initial ICH and has been demonstrated to activate resident microglia and astrocytes. These neuroglia in turn release cytokines such as TNF- α and IL-1 β , which recruit circulating immune cells, including neutrophils and macrophages.²⁹ In aSAH, the pathway toward neutrophil recruitment is similar, and cytokines like IL-6, IL-1 α , IL-1 β , IL-8, and TNF- α have been implicated as contributors to cerebral vasospasm.³⁰

High-throughput methods from multiomics are enabling researchers to interrogate changes in the CSF of patients with aSAH and ICH. Here, we summarize recent work from Koch et al.⁷ that used metabolomics and machine learning to study biomarkers in the CSF of patients with aSAH.

Use Case 2: Metabolomics to Evaluate Outcomes After aSAH

In their study, Koch et al. used metabolomic data derived from the CSF samples of 81 patients with aSAH to identify key metabolites in an immune-signaling pathway that were predictive of poor outcomes after aSAH.⁷ CSF samples were obtained from unclamped external ventricular drains at 3 different time points (0–5 days, 6–10 days, and 11–15 days) after admission. CSF samples were additionally obtained in a control cohort of 16 patients with nonruptured cerebral aneurysms through either a lumbar drain or lumbar puncture.

Several steps were taken to prepare CSF samples for metabolomic analysis. Samples were centrifuged to pellet out cellular material, and the resulting supernatant was aliquoted and treated with acetonitrile/methanol (3:1) with citrulline-d8, in order to prepare the samples for processing through a high-performance liquid chromatography system. Mass spectrometry was then used to quantitatively measure the concentrations of a total of 138 metabolites.

To process the metabolomic data, the authors used machine learning models to identify which metabolites were most strongly associated with modified Rankin Scale scores at discharge and at 90 days after discharge. They found that the concentrations of key metabolites that alter the nitric oxide signaling pathway, including symmetric dimethylarginine, dimethylguanidine valeric acid, and ornithine, were associated with a poor modified Rankin Scale score at discharge and at 90 days.

The methodology of Koch et al. provides an example of how multiomics can elucidate how key metabolites that regulate immune-signaling pathways can also shape outcomes after neurosurgical disease. Their findings provide key biomarkers for predicting outcomes and point to potential drug targets for modulating the immune response after aSAH. Neurosurgeons may use a similar multiomics workflow as that of Koch et al. to study immune-signaling pathways that are upregulated in the CSF samples of patients with epilepsy, spinal cord injury, and other neurosurgical diseases.

Systems Neuroimmunology and GBM

Immunology Review in GBM

GBM is an aggressive brain tumor characterized by significant cellular and molecular heterogeneity, as well as complex interactions with the host immune system.³¹ Recent work characterizing the GBM microenvironment has led to the discovery of functionally distinct immune cells in tumors, including tumor-infiltrating dendritic cells, regulatory T cells, cytotoxic T cells, and tumor-associated macrophages, each of which uniquely shape the response to immunotherapies.³² For example, regulatory T cells recruited by GBM cells can promote tumorigenesis by suppressing the cytotoxic effect of tumor-infiltrating dendritic cells and cytotoxic T cells.³³ Given how complex these interactions between immune cells and GBM cells are, there has been an increasing appreciation of the need for systems-based approaches to develop new immunotherapies for GBM.³⁴

Herein, we present use case 3, our second use case based on original work that uses systems neuroimmunology to better understand how the immune system interacts with GBM, in which we aimed to determine potential therapeutic targets for HSV-1–derived oncolytic viruses (OVs) to treat GBM. This use case underscores how proteomic data analysis can generate insights about GBM neuroimmunology.

Use Case 3: Protein-Protein Interaction Analysis to Identify Tumor Susceptibility to Immunotherapies

Immunotherapies use the host immune system to target GBM cells and are broadly divided into 2 categories: passive and active.³⁵ Passive immunotherapies, such as monoclonal antibodies, require continuous administration over time as they produce short-lived, yet specific, immune responses. On the other hand, active immunotherapies, including OVs, are a direct stimulation of an immune response with long-term effects. OV immunotherapy for cancer dates back to the 1990s, when HSV-1 was engineered to selectively replicate and target tumor cells. Since then, active immunotherapies have made significant strides and are the focal point of many clinical trials.³⁵

Our team previously published a study³ that aimed to further understand HSV-1 OV resistance by investigating the role of CCN1 in the GBM intracellular state. Found in most GBM microenvironments, CCN1 expression has been shown to predict resistance to OV. This work builds on a study by Haseley et al.,³⁶ which found that CCN1 binds and activates cell-surface integrin $\alpha 6 \beta 1$, promoting an antiviral and protumor state. GBM with high concentrations of CCN1 (CCN1^{high}) has demonstrated worse rates of overall and progression-free survival.³

To further understand the downstream effect of CCN1, transcriptomic data of LN229 GBM cell lines were mapped to known protein-protein interactions (PPIs) in the iRefIndex (<https://irefindex.vib.be>) database. These PPIs were synthesized into networks and analyzed in NetDecoder to compare CCN1^{high} with CCN1^{low} GBM phenotypes in order to elucidate critical differences between the two states. High-impact genes, network routers, key targets, and CCN1-specific edges were identified through informa-

tion flow analysis. We further compared these cell states through network and motif modeling, overrepresentation analysis, and assessment of gene dependencies.³

After studying differential edge flows, we identified 39 nodes and 12 binary edges that may potentially determine susceptibility of CCN1^{high} GBM to OV. Furthermore, CCN1^{high} states were shown to exploit *IDH1* and *TP53* and increase dependency on *RPL6*, *HUWE1*, and *COPS5*. Our findings were reproduced in 65 other GBM cell lines and 174 clinical sample data sets from patients with GBM for validation.³

Our study identified novel pathways, proteins, and interactions critical to the CCN1^{high} GBM phenotype. Conducting a generalized network model and systems-level analysis allowed us to identify several innate immune pathways in GBM that CCN1 leverages to disrupt HSV-1 OV immunotherapy. Additionally, we found opportunities to strategically improve the HSV-1 OV design. For example, because measles virus is not present in the resistance signatures that we identified through overexpression analysis (Fig. 2C), we postulated that measles virus may be a better vector than HSV-1 for GBM treatment.³ In a similar vein, other neurosurgeons can use this approach to design more effective immunotherapies for GBMs and other CNS malignancies.

Conclusions

Recent evidence has demonstrated that neuroimmune-mediated processes have an essential role in both the normal physiology of the nervous system and the pathophysiological processes that drive neurosurgical disease. The study of the multiome, which integrates genomic, transcriptomic, proteomic, and other omics data sources, is driving new discoveries in neuroimmunology. Compared with traditional approaches in neuroimmunology, multiomics methods allow for holistic interrogation of multiple immune-signaling pathways at a systems level. In this review, we described 3 use cases in which multiomics approaches were used to investigate the neuroimmunology of different conditions treated by neurosurgeons, including DDD, GBM, and aSAH. We hope that the methods outlined here will catalyze further research using systems immunology and empower neurosurgeons to generate future discoveries in neuroimmunology.

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Disclosures

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Author Contributions

Conception and design: Zamanian, Bhandarkar, Monie. Acquisition of data: Zamanian, Bhandarkar, Monie. Analysis and interpretation of data: Zamanian, Bhandarkar, Monie, Moinuddin, Vile. Drafting the article: Zamanian, Bhandarkar, Monie, Moinuddin, Vile, Quiñones-Hinojosa. Critically revising the article: Zamanian, Bhandarkar, Monie, Moinuddin, Vile, Quiñones-Hinojosa. Reviewed submitted version of manuscript: Zamanian, Bhandarkar, Monie. Approved the final version of the manuscript on behalf of all authors: Bydon. Statistical analysis: Monie. Study supervision: Bydon.

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