Protocol Title:	The Role of HMGB-1 in Chronic Stroke
Principal Investigator:	Bruce T. Volpe, MD
Sub-Investigator:	Richard Libman, MD Adam Stein, MD
Study Coordinator:	Johanna Chang, MS

Introduction

The acute pathophysiology of stroke depends on the innate immune response that begins in a clotted vessel and overwhelms the perivascular and parenchymal affected regional anatomic sector with all of the effector molecules arising from mostly pro-inflammatory cascades. The chronic pathophysiology of stroke is less clear as the innate inflammatory response fades and matures into an adaptive immune response. We want to investigate whether the pro-inflammatory circumstances extend over longer time in patients with stroke by measuring a candidate serum cytokine – HMGB-1 – that is secreted long after an initial insult, causes organ damage, and can be inhibited. Building on the work of the Tracey lab here at Feinstein on sepsis [1], and working in the Diamond lab we have developed new information about the effect of HMGB-1 on the brain. **We hypothesize that persistent HMGB-1 serum levels in patients with stroke retard functional recovery.** A first step in the test of this hypothesis is to gather serum data in patients with stroke longitudinally. Because we will have collected timed serum samples and stored them in the bio-repository at Feinstein, the hypothesis will be enriched by reexamining the serum samples for other known cytokines, other danger associated pattern molecules (DAMP), and growth factors that may also contribute to a prolonged inflammatory state for some stroke victims. If it turns out that in some patients the inflammatory condition wanes soon after the acute stroke then it will be important to determine the functional outcome compared to those in whom the inflammatory condition persists. While not in the scope of this particular protocol, we have long experience using modern interactive robotic techniques to measure and also to enhance motor function in patients recovering from stroke [2, 3], as our long term goal is to influence future stroke-rehabilitation practice.

Specific Aims

As a first step in achieving a long-term goal to improve stroke recovery, we will determine when and how long the circulating HMGB-1 levels are elevated in patients with stroke. We will also compare the cytokine levels of individuals after stroke with those of health, age-matched controls. We will:

1. Measure the levels of HMGB-1 in patients with acute stroke. We currently have the tools available to measure the levels of HMGB-1 in humans by ELISA (IBL-International, Toronto; ST51011).

2. Measure the level of cytokines, DAMPs and growth factors downstream from HMGB-1 secretion. If we find that levels of HMGB-1 are elevated after stroke, we will assay the levels longitudinally over a 3 month period. We will return to the bio-repository to assay samples for IL-1, IL-6, IL-8, IL-10, TNF and BDNF, all molecules known to be stimulated by HMGB-1 secretion [4, 5], and for which there are well developed ELISA protocols and further there are well developed assays for the Meso-Scale Diagnostics (MSD: Gaithersberg,MD) in the Diamond lab. The interleukins and TNF are well known cytokines involved in pro-inflammatory response. BDNF is a growth factor involved in repair [6, 7]. As HMGB-1 levels are negligible in normal healthy volunteers we do not expect that patients with acute stroke will have low levels. While there is some support to use a published lower level of 20 ng/ml as a cutoff for further cytokine and growth factor analysis [5], it seems prudent under the circumstances of the current lack of knowledge about the longitudinal characterization of cytokines and growth factors after stroke to

analyze the blood samples over time for HMGB-1 and for the cytokines and growth factors mentioned.

3. Compare the level of cytokines individuals with acute stroke with those of age-matched, healthy controls. In order to establish baseline cytokine levels, we will collect serum samples from healthy, age-matched controls and assay them for HMGB-1, IL-1, IL-6, IL-8< IL-10, TNF, and BDNF. We predict that cytokine levels will be negligible in healthy subjects and their samples will serve as a control for individuals post-stroke.

Background and Significance

Stroke, cerebrovascular accident, is the leading cause of brain injury and the leading cause of permanent disability. Over 800,000 people per year are afflicted with stroke in the US, and despite effective prevention campaigns like smoking cessation and blood pressure control, and medical treatments that have decreased the mortality from acute stroke, well over 5 million survivors now have permanent disability at a cost of billions spent for chronic care [8]. Post stroke treatment depends on supportive care and the imperfect spontaneous recovery of function abetted by compassionate rehabilitative efforts. Recent pre-clinical work [7, 9, 10] has provided a rationale that demonstrates that neuroplastic changes that underlie recovery might be improved by increased intensity of motor activity during therapy. Our group has been on the forefront of this development [11-14]. We have developed and used interactive robotic devices to deliver adaptive motor training that progressively increases training intensity, provides just-enough challenge to keep the patient focused and interested, and, importantly, has demonstrated that patients undergoing these robotic treatment protocols have significantly improved motor function [2, 3].

Ischemia and reperfusion triggers innate immune response first in the compromised vessel, in the perivascular and the brain parenchymal space. Early innate immune events follow clotting, platelet-leucocyte aggregation, complement activation [15, 16], increased arachidonic acid metabolites and reactive oxygen species [17, 18], and cytokine release, especially IL-1 [19-21]. Up-regulation and release of selectins, integrins [22, 23], proteases (like matrix metalloproteinases) [24, 25], and loss of NO (nitric oxide) [26] provide platforms for leucocyte adhesion, rupture of the blood-brain barrier, and matrix proteolysis. Mast cell degranulation releases histamine and additional proteases increasing signals for inflammatory cell migration to blocked and leaky vessels. The parenchyma of the brain fares no better; cytokines and chemokines [27-30] disrupt the microglia-neuron communication and TNF, depending on its source [31-34], provokes continued pro- but also insufficient anti-inflammatory signals and ATP is released, extracellular glutamate rises and cells die. Left out of this brief highlight summary of the early response of the immune system to stroke is the complete list of activated and secreted cytokines; including IL-6, IL-10, IL-12, IL-17, IL-18, IL-20, IL-23 [35-41], growth factors [42-44], and enzymes [26, 45, 46]. Persistent cell death initiates a new phase of the immune physiology the adaptive response. Altering these complex cascades will be a challenge. Furthermore, any intervention will require a certain immediacy and timeliness that currently exceeds standard and even premium health care delivery systems. More likely effective interventions will come from elucidation of the ongoing inflammatory response.

Interferon and TGF sensitize and prime T cells and antigen presenting cells (dendritic cells), and danger associated pattern molecules (DAMP) like HMGB-1 which in turn activate toll like receptors (TLR). Recent work on the humoral immune response has focused on the increased

production of antigen presenting cells (APCs), lymphocyte infiltration and the expression of co-stimulatory molecules like CD80 [47]. Some experimental pre-clinical evidence suggests that T- and B- cell deficient mice have reduced lesion size after exposure to experimental stroke [48], and progress in delineating mechanism has ruled against antigen recognition, or T-cell receptor co-stimulation, or thrombus formation [49]. But the mechanism for stroke exacerbation caused by humoral and adaptive immune response is not clear. Nor is it clear that the adaptive response is only to exacerbate the ischemia, as experiments with regulatory T cells and IL-17 for example, demonstrate an anti-inflammatory response [50, 51]. Lurking in the literature though, is the persistent rise of HMGB-1 levels and pre-clinical experiments show the rise continues for weeks to months. We believe this persistent pro-inflammatory signal represented by increased serum HMGB-1 needs investigation.

Progress Report/Preliminary Studies

To that end, it is important to note that the medicine group at Northwell, under the guidance of the Tracey group at Feinstein, has assayed the HMGB-1 levels in serum samples collected from some patients with brain and cardiac ischemia [52, 53]. Their data show that collection of acute blood samples in the Northwell is possible and, importantly for the purpose of this protocol that the some stroke and cardiac patients have elevated HMGB-1 serum levels hours after the ictus (**Fig 1A &B**). The variable serum levels of HMGB-1 are due to different assays, currently there is a standard ELISA kit for HMGB-1 analysis in the serum (IBL-International, Toronto; ST51011). Of note, the levels of HMGB in serum from age-matched healthy controls were typically less than 20ng/ml of HMGB-1 [5]. Importantly the level of HMGB-1 increased significantly with patients who had their blood sampled after longer times from the onset of their stroke (**Fig 1C**). The progression and persistence of HMGB-1 is not known in the clinical literature, although Tracey's lab has demonstrated that HMGB-1 levels persist in sepsis experiments for weeks (see **Fig 2.**).

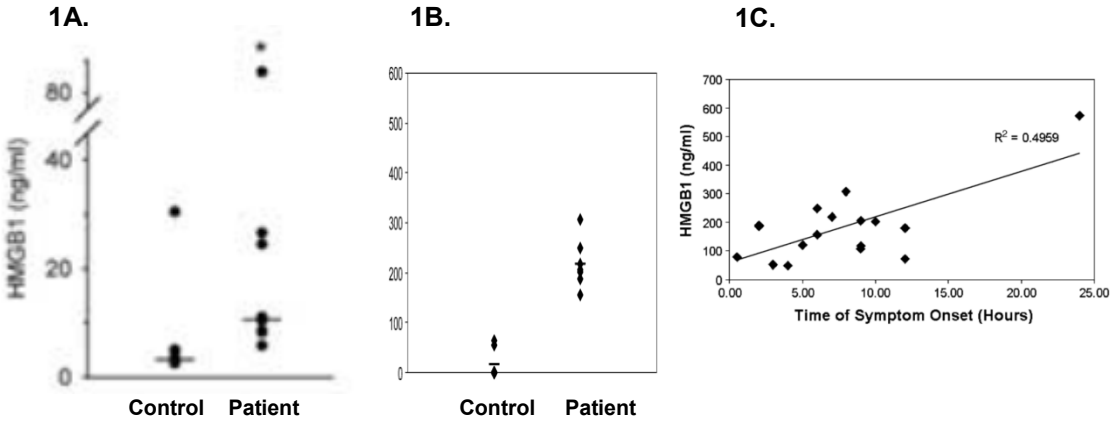


Fig 1A. Stroke patients (N=8) had significantly higher HMGB1 serum concentrations than controls. Patients were included into the study within 24 h after onset of symptoms.
Fig 1B. Stroke patients (N=7) had significantly higher HMGB1 serum concentrations than controls.
Fig 1C. HMGB serum levels rise as a function of symptom onset. Serum from patients with myocardial infarction (N=9) and cerebral ischemia (N=7) obtained on arrival in the emergency department and correlated with the historical onset of symptoms (R^2= 0.49, P<0.01).

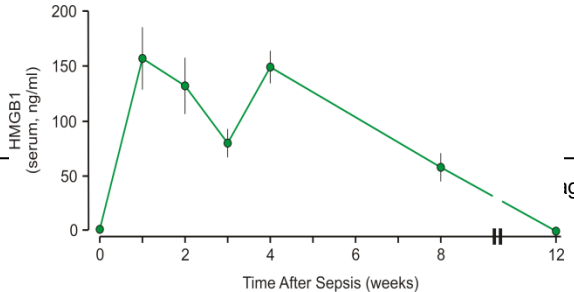


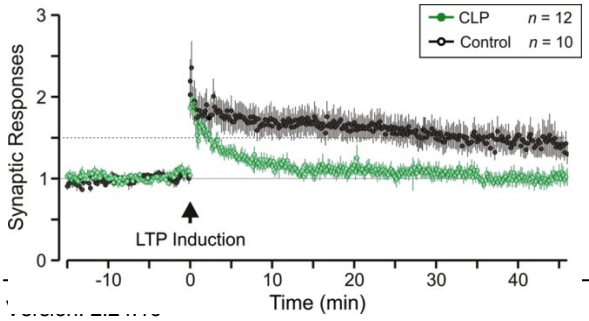
Fig 2. Animals exposed to severe sepsis (standard cecal ligation and puncture; N=50) demonstrate persistent elevation of HMGB-1 serum measurements.

In an animal model we have been exploring the effect of severe sepsis using a standard stress of cecal ligation and puncture (CLP) on the brain. HMGB1 levels do not rise immediately following CLP but an elevation is sustained for several weeks, making it an ideal therapeutic target. These preliminary results support a rationale for the proposed protocol.

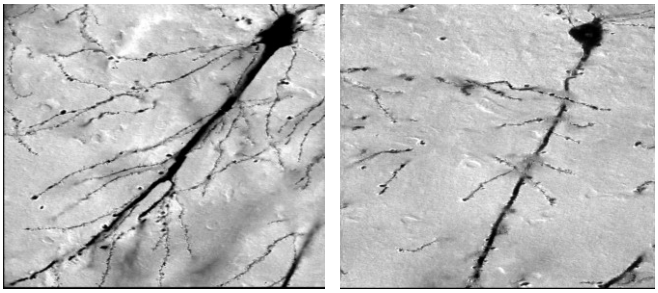
Sepsis is a common clinical problem with a 30% acute mortality, but the survivors do not return to work and sustain loss of both cognitive function and immunocompetence [54-56]. To understand the persistent cognitive impairment in sepsis survivors, we subjected mice to sepsis induced through cecal puncture and ligation (CLP). At 4 weeks following CLP sepsis, mice displayed a loss of long term potentiation (LTP) in hippocampal slice preparations; a measure that can be taken as a loss of synaptic plasticity (**Fig3A**). Also on histologic analysis these animals demonstrated a loss of dendritic arborization and spine density in CA1 neurons of the hippocampus (**Fig 3B**). To ask whether these deficits were a consequence of systemic immune activation, we measured the performance of mice treated with severe sepsis and sham exposed mice on a clock maze. The latency of a mouse's escape is generally thought to depend on memory, and is further thought to be mediated in part by hippocampal neuronal function. **Fig 4A** depicts the performance of mice exposed to severe sepsis and demonstrating memory impairment. In another experiment (**Fig4B**), we treated mice exposed to CLP sepsis for one week with antibody to the inflammatory cytokine HMGB1, beginning one week following CLP. Antibody-treated mice displayed partial improvement in cognition, demonstrating that prolonged systemic inflammation contributes to the post-sepsis cognitive decline and suggesting a therapeutic strategy for neuroprotection in sepsis survivors.

We know that overwhelming sepsis and stroke are different conditions and impose a number of different stresses on the patient/experimental animal. However, the two conditions share some molecular mediators; especially HMGB-1. Also both stroke and sepsis cause immediate rise in TNF and IL-1, sentinels in the innate immune system. High levels of these cytokines persist for hours to days in mice, but return to baseline by 3 days. This first wave of cytokines is followed by a later but more sustained elevation of HMGB1. Increased levels of HMGB1 persist for weeks with a return to baseline levels in mice at around eight weeks following the initial insult. This information is not entirely clear in animal stroke models or in patients with stroke, a void that this protocol intends to correct.

3A.



3B. SHAM **SEPSIS**



4 WEEKS AFTER EXPOSURE TO SEPSIS OR SHAM

Fig 3A. Hippocampal neuron circuits in a slice preparation from animals exposed to severe sepsis (CLP) procedure fail to generate and maintain LTP. Hippocampal neuron circuits from control (sham) animals generate normal LTP. **Fig 3B.** Using Golgi staining techniques in which random but entire neurons are filled with peroxidase positive material, animals exposed to severe sepsis (CLP) lose dendritic arborization and, on higher magnification, demonstrate significantly decreased spine density compared to the hippocampus of sham (control) treated animals (1.11 ± 0.29 spines/μ vs. 0.54 ± 0.21 spines/μ; P<0.001).

4A. **4B.**

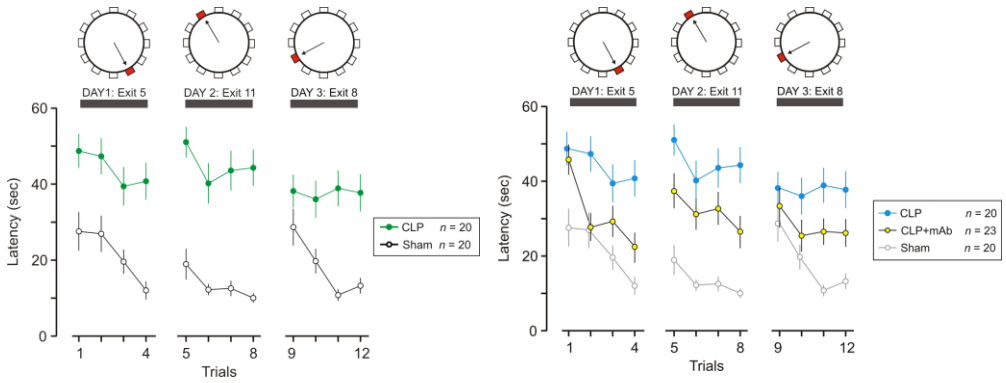


Fig 4A. Animals exposed to severe sepsis (CLP) demonstrated impaired learning in a clock maze. **Fig 4B.** This impairment was altered by treating the animals with a neutralizing anti-HMGB-1 antibody.

Research Design and Methods

We will obtain blood samples from 100 consecutive patients with ischemic or hemorrhagic stroke admitted to the Northwell stroke service. As we are interested in assay of HMGB-1 from the time from stroke, the admission sample timing with respect to stroke will be noted and we will obtain subsequent blood samples at 7, 14, 30 and 90 days after the time that the stroke occurred. So if the patient comes in 24- or 48-hours after the onset of symptoms, we will collect the admission sample and collect the 7-day sample 6 or 5 days after admission respectively. Given the challenges of collecting samples within a 24 hour time period, the collection window will be extended such that day 7 and day 14 samples will be collected plus or minus one day of the target (e.g. between day 6-8 or day 13-15, respectively) and day 30 and day 90 samples will be collected plus or minus 7 days of the target (e.g. between day 23-37 and day 83-97, respectively). Each of the 5 samples will be 4 cc each (green top tube). We will also obtain a one-time DNA sample, via the collection of a 10cc lavender tube (EDTA) at the time of the first sample collection. If this is not possible we will collect this sample at the time of a subsequent research blood sample collection. The total volume of blood drawn for each study subject will be no more than 30 cc over the duration of the study. Additionally, we will collect a one-time blood sample from up to 30 healthy, age-matched controls. Control subjects will be NSLIJHS employees, members of the local community recruited with the Northshore Recruitment Registry and GAP Registry recruitment tools, and enrolled subjects' family members recruited by the Research Coordinator and Principal Investigator. Each control sample will be 4cc total (green top tube)

The blood will be centrifuged and prepared for HMGB-1 analysis by NS-LIJ Biorepository staff. If we miss sample collection across the 7, 14, 30, and 90 day time points the available collected samples from a subject will be analyzed. We will measure HMGB-1 and if the levels are elevated (>20ng/ml) we will return to the banked samples in the bio-repository to repository to assay samples for IL-1, IL-6, IL-8, IL-10, TNF and BDNF, all molecules known to be stimulated by HMGB-1 secretion [4, 5].

The following data will be collected for each subject with stroke:

- Age (at time of stroke)
- Gender
- Stroke risk factors (history of hypertension, smoking, estrogen supplements)
- Concurrent infections (at time of stroke)
- Stroke type (ischemic or hemorrhagic)
- Stroke location
- Stroke severity (NIH Stroke Scale)
- Stroke outcome (Rankin scale) – measured at admission & discharge
- Contact information
- Medical record number

Inclusion criteria:

- All patients admitted to the stroke service at Northwell will be eligible for inclusion in this pilot study.
- Patients ≥18-years of age

Exclusion criteria:

- Patients <18-years of age

The following data will be collected for each control subject:

- Age (at time of stroke)
- Gender
- Stroke risk factors (history of hypertension, smoking, estrogen supplements)
- Concurrent infections (at time of stroke)
- Contact information

Inclusion criteria:

- Patients ≥18-years of age
- No report of stroke risk factors (hypertension, smoking, estrogen supplements) or concurrent infections during screening at time of participation.

Exclusion criteria:

- Patients <18-years of age
- Report of stroke risk factors or concurrent infections during screening at time of participation.

Because of the collaborative efforts of the Department of Neurology and the Physical Medicine and Rehabilitation groups we will be able to track the patients from the acute care facility to the rehabilitation units. We know that the combined admissions to the stroke units at the two facilities are nearly 900 patients per annum. We also know that the patients often stay within the greater Northwell Health System and that over 700 patients with stroke are admitted to the rehabilitation units.

Blood sampling for acute stroke subjects will take place on the subjects' in-patient units during their stay in Northwell Health System. We expect, as the average length of stay is 10 days, to collect an admission and day 7 sample. Many patients with stroke are transferred to the rehabilitation service and we expect to collect blood samples for day 14 and 30 on those services. The average length of stay for a patient with stroke on an in-patient rehabilitation unit is 30 days. We are set up in the CRC to collect the 90 day sample and the control subjects' samples. We will ask patients to return to the CRC for this sample collection.

Banking of samples for future use:
The Tissue Donation Program (TDP) collects and stores biological samples and associated clinical data as a resource for investigators in the Northwell Health System. Through the development and implementation of tissue specific sub-protocols, these materials may be available to the larger biomedical community. A portion of each blood sample collected for this study will be stored by the TDP and made available for future research according to TDP established procedures.

Plasma will be stored from each blood sample collected. DNA will be isolated and stored once on each patient with stroke at the time of the first blood collection. If for some reason this is not possible, a DNA sample will be isolated from the next available blood sample.

These samples stored by the TDP can be shared by other investigators who submit protocol for use of stored samples to the TDP's Committee for Participant Protection (COPP). Once approved by the COPP, stored samples and associated clinical data can be distributed to the investigator in a completely de-identified fashion. Investigators receiving samples will not have access to the link between a bar coded label and subject identity.

Statistical Considerations and Data Analysis

Specific Aims:

1. Measure the levels of HMGB-1 in patients with stroke. Because the level of HMGB-1 in healthy control subjects is negligible and because survivors of sepsis had levels of HMGB-1 < 20 ng/ml, we believe it is reasonable to assume that levels above 20ng/ml are not normal [5]. Also we are collecting longitudinal samples and we expect that if the pro-inflammatory condition clears in these patients with stroke the HMGB-1 level will fall below 20 ng/ml if not lower.

2. Measure the level of downstream pro-inflammatory molecules and growth factor. We will measure IL-1, IL-6, IL-8, IL-10, TNF and BDNF, all molecules known to be stimulated by HMGB-1 secretion. If we find that HMGB-1 is elevated we expect to find elevations in the other

molecules. If HMGB-1 is not elevated it will be important to measure the serum level of the other cytokines to insure that some other pathophysiology of stroke and stroke recovery is not also involved in activating cytokines. The serum growth factor BDNF level may change in an alternate direction.

Endpoint Variables:

1. HMGB-1
2. Downstream cytokines activated by HMGB-1 including: TNF;IL-1; IL-6; IL-8; IL-10; and growth factor, BDNF.

3. Compare the level of cytokines individuals with acute stroke with those of age-matched, healthy controls. In order to establish baseline cytokine levels, we will collect serum samples from healthy, age-matched controls and assay them for HMGB-1, IL-1, IL-6, IL-8< IL-10, TNF, and BDNF. We predict that cytokine levels will be negligible in healthy subjects and their samples will serve as a control for individuals post-stroke.

Statistical Methods.

This will be a prospective pilot study of patients diagnosed with acute stroke. We will obtain serial blood samples (admission, 7, 14, 30, 90 d after stroke) to examine whether the blood levels of HMGB-1, and TNF,IL-1, IL-6, IL-8, IL-10, and BDNF are elevated, and if they are, determine their temporal characteristics.

Recruitment of patients with stroke will take place among those who admitted to North-Shore and LIJ hospital stroke service.

Specific Statistical Aims. In this pilot we will gather information from patients in the acute phase of stroke and the early recovery phase of stroke. We aim to measure over a three month period the HMGB-1 level, and the downstream pro-inflammatory cytokines such as TNF, IL-1, IL-6, IL-8 and IL-10, and also the growth factor BDNF. We plan to evaluate these serum levels in 100 patients. This data will permit a formal estimate of the number of patients required to determine correlation among the cytokines and growth factor levels, and also correlation with stroke severity and possible disability outcome.

Outcome Variables. The following variables will be collected from the study group. Blood will be collected on admission, 7 14, 30 and 90 days after stroke. We will also collect stroke type – ischemic or hemorrhagic; stroke severity as measured by the NIH stroke scale; stroke outcome as measured by the Ranking scale.

Statistical Analysis. The statistical analysis for this pilot study will be primarily descriptive in nature. The main statistical analysis will employ repeated measures analysis to describe the patterns of change in HMGB-1 and the cytokine values over time. Other techniques will be explored if the data warrant it; such as examining pairs of outcome variables (HMGB-1 and TNF, HMGB-1 and IL-1, or HMGB-1 and stroke severity; as pioneered at Feinstein by Dr. O Bloom and as in [57]).

Potential secondary analysis includes stratification by stroke severity.

SAMPLE SIZE CONSIDERATIONS
The proposed sample size is 100 consecutive patients with stroke and 30 healthy, age-matched controls. This sample size is a sample size of convenience based on feasibility and availability of resources and is not based on any formal power calculations.

Sample Size Justification
This is a pilot study, the purpose of which is to determine if HMGB-1 is elevated in patients after stroke. There is scant longitudinal clinical information about HMGB-1 in patients with stroke, and the current information about some of the other cytokines is that they are gone within hours- days. Recent hospital data from the stroke service indicate admission of 600 patients with stroke per year at Northwell and 300 per year at LIJ. The rehabilitation service admits around 700 patients with stroke per year. We should be able to gain complete information (admission, 7, 14, 30 and 90 days after stroke) on 100 patients within two years.

Protection of Human Subjects

RISKS TO SUBJECTS:
Human Subjects Involvement & Characteristics: We anticipate enrolling 100 human. Inclusion and exclusion criteria are stated above in the Research Design and Methods section.

Sources of Materials: Sources of research material on patients with stroke will be obtained specifically for this study from existing health records and we will obtain blood specimens.

Potential Risk: Minimal risks of blood withdrawal include pain from the needle being inserted through the skin into the vein, bruising, clot formation under the skin, lightheadedness, possible fainting and rarely infection.

One additional risk concerns the risk to confidentiality incurred with any collection of medical data.

ADEQUACY OF PROTECTION AGAINST RISKS:
Recruitment and Informed Consent: Patients with acute stroke will be initially identified either by clinicians in the Northshore-LIJ Health System or by the Research Informatics Department at Feinstein who will extract candidacy information through Sunrise Clinical Manager Software. Dr. Ron Kanner, Chair of Neurology is aware of these studies and we will require the active participation of the Stroke team led by Dr. Richard Libman and the participation of his colleague Dr. Jeffrey Katz. Also, Dr. Adam Stein, Chair of Physical Medicine and Rehabilitation is aware of these studies and will direct us to the participating residents. Additional Northwell Health System physicians who have appropriate patient populations will be made aware of the research study protocol and procedures, and given an overview of the study through contacts with the study personnel. David Ballard, Director of Research Informatics at the Feinstein Institute of Medical Research is also aware of these studies and will develop software with his team to extract candidacy information from Sunrise Clinical Manager, including patient name/age, stroke type/location, admission date/admission status, medical record number, and contact information. This information will be provided to the study coordinator to identify potential study participants efficiently within the acute time window. The study coordinator will then contact treating physicians with the names of potential patients before contacting the patients for

recruitment. The patient will then be contacted by either the study coordinator (with permission of the treating physician) or the treating physician directly. If the patient expresses interest in participation, consenting study personnel or the physician will obtain informed consent (if the physician is listed as an investigator on this study). Alternatively, the physician may either, 1) provide the patient with the study coordinator's contact information or 2) provide the patient's contact information to study personnel with the patient's permission, which will be documented in the medical record.

If the patient is unable to provide consent and a family member is not present, the study coordinator (with permission of the treating physician) will phone the patient's next-of-kin in the following order of contact: patient's spouse, patient's child, patient's parent, patient's extended family (e.g. aunt/uncle, niece/nephew). During the phone call, the coordinator will discuss the study and arrange a time to meet and sign the consent form if the family member voices interest in the patient's participation.

After a discussion about the study with a potential subject or a potential subject's legally authorized representative (LAR), interested parties will be given a copy of the consent form by one of the study investigators. The investigator will review and explain the consent form. All information about the study will be provided. Ample time will be given for individuals to ask questions regarding participation and to have questions answered prior to signing the consent form. If so desired, those interested will be given a copy of the consent form so that they may have the opportunity to discuss participation further with family and/or advisors. Only those investigators listed in the study protocol will obtain informed consent. If an individual chooses to enroll, the consent form will be signed before participation begins. Once an individual joins the study and informed consent is obtained, the subject will receive a signed copy of the consent form. The subject may withdraw from the study at any time without repercussions to subsequent care.

If the patient is awake, alert, and oriented to person, place, and time, and demonstrates appropriate cognitive and communicative abilities as determined by the treating physician, the patient will be deemed to have the appropriate capacity to consent.

If it is determined that a patient is unable to consent for him/herself, due to a lack of capacity or lack of comprehension, consent will be sought from the patient's LAR. Assent from the adult patient will be obtained as appropriate. If such a subject regains his/her ability to make healthcare decisions, he/she will be given the opportunity to provide consent. This consent will be documented using the Addendum to Consent by Research Proxy for Continuing Participation in a Research Study form.

If the patient provides the consent delegate with assent to participate in the research but, due to a physical disability, is unable to sign the consent form, two witnesses will sign the document affirming their presence during the consent process and the patient's physical disability as reason for an absent signature.

Healthy, age-matched control subjects will be recruited from NSLIJHS employees, enrolled subjects' family members, and members of the local community identified by the Northshore Recruitment Registry and GAP Registry recruitment tools. Recruitment will be done with direct contact or via telephone and will not be done by anyone who has, or can anticipate having, a supervisory role over the employee.

A study investigator will obtain informed consent from interested persons. After a discussion about the study with a potential subject, interested persons will be given a copy of the consent form by one of the study investigators. The investigator will review and explain the consent form to the person. All information about the study will be provided. Ample time will be given for persons to ask questions regarding participation and to have questions answered prior to signing the consent form. If so desired, those interested will be given a copy of the consent form so that they may have the opportunity to discuss participation further with family and/or advisors. Only those investigators listed in the study protocol will obtain informed consent. If a person chooses to enroll, the consent form will be signed before participation begins. Once an individual joins the study and informed consent is obtained, the subject will receive a signed copy of the consent form. The subject may withdraw from the study at any time without repercussions to subsequent care.

Protection Against Risk: Experienced and certified personnel, using standard precautions to avoid unnecessary complications related to the procedure, will perform phlebotomy. Whenever possible, blood draw will occur concurrently with blood draws being performed for routine patient care.

To protect subjects' confidentiality, each subject will be assigned a number, and all samples and data will be stored with the subject number only and not the subject's name. Data will be stored on a password-protected computer. Subject charts with medical history and assigned subject numbers will be kept in locked file cabinets in the CRC. Access to charts will be granted only to study investigators and CRC staff. Charts will be kept confidential and will not be shared with any third parties without permission from the subject.

POTENTIAL BENEFIT TO THE SUBJECTS AND OTHERS*:*

The subjects enrolled in this study will not benefit directly due to their participation. However, the information obtained from this study may benefit patients suffering from spinal cord injury, in the future. The risks to subjects are reasonable in relation to the anticipated benefits to others.

SCIENTIFIC VALUE:

The results of this study may help to improve stroke recovery.

Inclusion of Women and Minorities

Subjects will be selected without regard to gender, race, or ethnicity. Recruitment flyers will be posted around the Northwell Health System, as many uninjured adults visit or accompany friends and family members to the outpatient and inpatient facilities. Given the size and location of the health system, we believe that we will reach a multiethnic/multiracial audience of both genders. If we find that members of minority groups are not responding to the flyers, we will post additional flyers in neighborhoods with larger proportions of those minorities.

Inclusion of Children

Children will be excluded from this study because the incidence of such patients is low enough at NSLIJ that we cannot expect to recruit them.

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