January 2017

Concussion Biomarker Discovery For Prognosis In Adolescent Athletes (compete) Pilot Study

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A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by

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2017

Abstract

Concussion management in the ED is inconsistent due to a lack of available empirical testing. This project aims to assess the feasibility of a large multi-centered study for concussion biomarker discovery in the ED.

This prospective cohort pilot and feasibility study was conducted in the adult and pediatric EDs of an urban, academic, Level 1 trauma center. Twelve patients with concussions and twelve age- and gender-matched control patients presenting within 6 hours of an injury sustained during recreational activity were enrolled. ED blood specimens were banked for future proteomic analysis. Clinical outcomes were collected via online survey. Patient recruitment strategies were refined in three phases: (1) identification and notification by clinical staff, (2) email notification automatically-generated by the electronic health record (EHR), and (3) patient financial incentives provided at enrollment and upon completion of the symptom diary.

After Phase 1, the patient identification rate improved from 0% to 22% ($p<0.001$) with EHR-generated notifications. In Phase 3, the enrollment rate improved from 38% to 100% ($p=0.01$) with financial incentives.

This pilot and feasibility project is the first step to toward the goal of identifying new diagnostic and prognostic biomarkers as well as novel targets for therapy. EHR-based paging and financial incentives for participation increased subject identification and enrollment rate to inform and optimize the enrollment and recruitment strategies for the eventual larger study.

Acknowledgements

I would like to express my deep gratitude to Dr. Edward Melnick, my research mentor, whose guidance and encouragement made this project possible. I would also like to extend warm thanks to Dr. Vincent Pieribone, whose ideas and lab space were vital for day-to-day operations. I am also grateful for the contributions of Dr. Madeline McCarthy and Dr. Kirsten Bechtel, who provided valuable feedback and comments on the writing associated with this research.

This project was supported by the Gershon/Yale One-Year Fellowship.

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Introduction

What is mild traumatic brain injury?

Mild traumatic brain injury (mild TBI or mTBI) is an alteration in brain function caused by either direct head trauma or impulse forces transmitted to the head. The injury typically affects memory and orientation and may involve loss of consciousness (LOC). It can also be associated with symptoms such as headache, difficulty concentrating, irritability, and insomnia.(1) The terms *mTBI* and *concussion* are often used interchangeably in US sports-related literature, but some consider mTBI to be a more severe injury than concussion. *Concussion* is a historical term used to refer to brain "shaking" and resultant clinical symptoms not necessarily associated with pathologic injury.(2) For the purpose of this study, mTBI and concussion will be used as synonymous terms. The most widely accepted sports-related definition of concussion in both clinical and research settings worldwide is from the 2012 Zurich Consensus Statement on Concussion in Sport(2,3):

> Concussion is a brain injury and is defined as a complex pathophysiological process affecting the brain, induced by biomechanical forces.
>
> Several common features that incorporate clinical, pathologic and biomechanical injury constructs that may be utilized in defining the nature of a concussive head injury include
>
> 1. Concussion may be caused either by a direct blow to the head, face, neck or elsewhere on the body with an 'impulsive' force transmitted to the head.

2. Concussion typically results in the rapid onset of short-lived impairment of neurological function that resolves spontaneously. However, in some cases, symptoms and signs may evolve over a number of minutes to hours.

3. Concussion may result in neuropathological changes, but the acute clinical symptoms largely reflect a functional disturbance rather than a structural injury, and as such, no abnormality is seen on standard structural neuroimaging studies.

4. Concussion results in a graded set of clinical symptoms that may or may not involve LOC. Resolution of the clinical and cognitive symptoms typically follows a sequential course. However, it is important to note that in some cases, symptoms may be prolonged.

The Centers for Disease Control and Prevention (CDC) has developed a similar conceptual definition for mild TBI(4):

Occurrence of injury to the head, resulting from blunt trauma or acceleration or deceleration forces, with one or more of the following conditions attributable to the head injury during the surveillance period:

- Any period of observed or self-reported transient confusion, disorientation, or impaired consciousness
- Any period of observed or self-reported dysfunction of memory (amnesia) around the time of injury
- Observed signs of other neurologic or neuropsychological dysfunction
- Any period of observed or self-reported LOC lasting 30 minutes or less.

The CDC definition of concussion is widely used by physicians in the United States and is the primary definition for this study. Per the CDC guidelines, symptoms associated with concussions can be physical (e.g. headache), cognitive (e.g. fogginess), emotional (e.g. irritability), or sleep-related (e.g. insomnia) (Table 1).

Epidemiology

Sports-related concussions have traditionally received little attention because sports culture has provided implicit acceptability of the condition. Players are celebrated for "highlight reel" hits and are praised for displays of "toughness" when injured players remain in the game. In a culture centered on playing through pain, concerns for player safety have been obscured by the desire to win at all costs.(5) A recent large class action lawsuit between retired football players and the National Football League has brought the issue to the forefront and has led to the reevaluation of long-standing sporting ideals.(5)

Concussions account for approximately 75% of TBIs each year, a number that has risen due to increased awareness and reporting of concussions in youth sports.(6,7) Approximately 1.4 to 3.8 million TBIs, including concussions, occur each year.(6,8,9) This estimate includes those for which no medical care is sought. In youth football, concussions account for 4-9.6% of all injuries with increasing concussion rates at higher levels of competition.(5) An estimated 182,000 young football players experience at least one concussion each year.(5) These numbers are probably underestimated because many concussions go unrecognized.(9) Although an estimated 5.3 million Americans currently live with TBI-related sequelae, MTBI is considered a "silent

epidemic" because external observers may not recognize many of its acute and lasting functional alterations (e.g. memory impairment).(6,10)

Post-concussive syndrome

Post-concussive syndrome (PCS) is a term referring to long-term concussion symptoms, including headache, fatigue, dizziness, irritability, and insomnia, that do not remit in the immediate period following head trauma. There are two major definitions of PCS, and they differ in definition of concussion, timing, and duration of symptoms. In it's definition of PCS, the International Classification of Diseases, 10th revision (ICD-10) requires that the inciting concussion include LOC and that symptoms appear within four weeks.(11) The Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V) makes no specific mention of onset timing and does not specify the occurrence of LOC, but states that symptoms should last at least three months.(12)

In adults, concussive symptoms usually resolve within 7-10 days.(3) In children, estimates of symptom resolution vary widely, but symptoms typically resolve within two weeks. Approximately 25-35% of concussion patients are symptomatic at one month and less than 5% are symptomatic at one year.(3) Studies seeking to reveal risk factors predictive of longer duration of symptoms suggest that psychosocial factors play a significant role in younger patients. Psychological traits, insufficient support systems and maladaptive coping strategies have all been found to affect PCS duration.(13) Hou et al found that negative mTBI perceptions, perceived stress, anxiety, depression, and all-or-nothing behavior were associated with the presence of PCS at 3 months and 6 months post-injury.(14)

Emotional and neurobehavioral dysregulation can also develop after mTBI as a consequence of damage to the orbital prefrontal cortex. These changes in emotion and cognition may increase the risk for suicidal behavior, and concussions have been associated with increased rates of suicide.(15),(16) Moreover, Hoge et al linked mTBI with LOC to increased rates of post-traumatic stress disorder (PTSD) and depression in combat veterans.(17)

Lasting manifestations of concussions

In the days following a concussion, the brain is in a hypermetabolic state, predisposing it to repeated injury. If concussions recur, they can become increasingly debilitating. A study of high school athletes by Collins et al demonstrated that those with three or more prior concussions have increased rates of LOC, anterograde amnesia, and confusion with subsequent concussions.(18) A recent study also found that recent or repeated concussions predict longer PCS duration.(19) In rare cases, this second head injury can lead to rapid onset diffuse cerebral edema. This phenomenon, known as second impact syndrome, is often fatal and can cause severe lasting neurologic disability in those who survive it.(3,20) Even in cases where a second concussion does not occur in the immediate post-concussive period, repetitive head trauma has been linked to neurodegenerative disease.

Nearly a century ago, pathologist Harrison S. Martland observed chronic neuropsychiatric and motor dysfunction in former boxers. These patients displayed a clinical picture including personality change, emotional lability, memory impairment and dementia, as well as pyramidal and extrapyramidal dysfunction and cerebellar

impairment. Later studies suggested that repetitive TBI from boxing might be the cause of this progressive neuropathology, and the term 'dementia pugilistica' was coined.(21) Observations of similar neuropsychological findings in participants of other contact sports, including football, hockey, and wrestling, indicated that this disease was not limited to boxing. The term 'chronic traumatic encephalopathy' (CTE) was created to characterize the symptoms of 'dementia pugilistica' and reflect the realization that head injuries from all forms of contact sports can cause this condition. Studies have suggested that CTE might have a dose-risk association, with increased TBI exposure leading to increased risk of impairment.(21) The neuropathologic basis of CTE is thought to stem from a number of mechanisms. Cerebral atrophy, tau abnormalities (neurofibrillary tangles), aβ amyloid plaques, and neuroinflammation all possibly play a role. However, the relatively small number of observations in the literature have not been able to formally characterize the clinical and pathological manifestations of the disease.(21) CTE has recently been linked to a series of homicides involving NFL players and has been shown to correlate with increased levels of depression, PTSD, and suicide in military personnel as well.(21,22) These findings have brought attention to how critical it is to it increase our understanding of TBI-related neurodegeneration.

Current guidelines on return to play

To mitigate the well-documented consequences of recurrent concussions, graded return to play protocols have been employed in recent years to prevent athletes from returning to the field before their concussions have resolved. The physical rest recommended before return to play includes restrictions on sports participation,

recreational activity, and leisure activities such as bike-riding, skateboarding, and rollerblading.(3) Some clinicians have advocated “cocoon therapy,” which restricts patients to a darkened room for several days before gradual return to activity; but this stricter protocol has shown no additional benefit. Return to play typically does not occur for at least a week after the injury, and generally, no athlete is allowed to return to play on the same day as his/her concussion.(23) One accepted graded return to play program is the protocol established in the Zurich 2012 Consensus Statement on Concussion in Sport.(2) This protocol advises no activity initially, followed by aerobic activity, sports-specific non-contact drills, full-contact practice, and finally a full return to play (Table 2). Full return to play is considered when athletes have returned to baseline function and are symptom-free at each step of the graduated protocol. Sport-specific return to play protocols have also recently been developed for football, basketball, baseball, softball, soccer, wrestling, cheerleading, lacrosse, and ice hockey.(3)

Computerized neurocognitive tests are being increasingly utilized to diagnose and track athletes’ recovery from mTBI. These tests measure reaction time and ability to process information. The most common of these tests is the imPACT test. This test is first administered to the athlete in the preseason to establish the athlete’s neurocognitive baseline and is re-assessed after a head injury. The post-injury results are compared to the baseline to determine if a concussion has occurred. These computerized tests are prone to false negatives as athletes may deliberately underperform on the baseline evaluation to avoid being removed from play following a head injury.(24)

Pathophysiology

Trauma to the brain triggers structural changes and subcellular disruptions within the axon. One of the first changes is microperforation of the axolemma (cell membrane surrounding an axon) and increased membrane permeability. This phenomenon has been observed as the influx of the normally excluded protein, horseradish peroxidase, into axonal cells after head injury. Extracellular calcium can enter through the disrupted axolemma, leading to caplain activation. Caplain is a calcium-dependent protease that causes cytoskeletal changes within the axon that disrupts anterograde and retrograde transport. This disruption can lead to swelling of contiguous axons and eventual axotomy (cutting of an axon). Disruption of transport may also be mediated by microfilament compaction secondary to dephosphorylation.(6)

Although mTBI was originally thought to be an exclusively acute injury, there is evidence showing that mild TBI can have chronic effects through excitotoxicity (neuronal damage caused by overactivation of glutamate receptors), oxidative damage, mitochondrial damage, blood-brain barrier breakdown, and the activation of inflammatory elements.(25,26)

Following TBI, NMDA and AMPA receptors can be over-activated by glutamate. Calcium is released within activated neurons, leading to enzymatic hyperactivity and DNA fragmentation.(27,28) Additionally, TBI can lead to increased levels of reactive oxygen species and decreased levels of antioxidants. Mitochondrial damage results from a combination of both mechanisms.(29,30)

Blood brain barrier (BBB) breakdown is another important mechanism of lasting injury following TBI. The BBB consists of endothelial cells closely associated with astrocytes and glial cells. It normally keeps the brain's environment free of blood-borne factors and immune cells.(31) Following TBI, there can be an upregulation of matrix metallopeptidase 9, which digests tight junctions and disrupts proper BBB function. When the BBB breaks down, leukocytes can enter and increase the osmotic forces on the brain.(32) This leads to edema, which can progress further to ischemia, cell damage, and cell death.(33)

Neuroinflammation is perhaps the longest-lasting mechanism of brain damage following TBI, lasting for up to 17 years.(34) The inflammatory response after the initial injury defends the site from invading pathogens and repairs the damaged cells. Complement activation and cytokine release from neutrophils, monocytes, and lymphocytes lead to stimulation of inflammatory cells and microglia.(25,35) Microglia separate the injured tissue from healthy tissue, but when microglial activation is excessive it leads to exaggerated proinflammatory cytokine release and breakdown of the BBB.(25,36) Astrocytes also aid in the survival of neurons and reduce glutamate excitotoxicity. However, by isolating and encapsulating axons, they can interfere with regeneration if their levels are too high.(36,37)

Though there are no FDA-approved pharmaceutical interventions for mTBI, potential therapies for more severe TBI have targeted some of the aforementioned injury mechanisms. Progesterone, a neurosteroid synthesized in the central nervous system, is thought to exert neuroprotective properties by decreasing cerebral vasogenic edema,

protecting the BBB, and modulating the inflammatory cascade to improve neuronal survival.(38) Progesterone's therapeutic role is controversial, however, as studies have shown differing results with regard to mortality and unfavorable outcomes following TBI.(38,39) Additional investigations have been conducted on neurosteroids other than progesterone.(39) GABAergic neurosteroids like allopregnanolone (a progesterone metabolite) hold some promise due to their ability to mitigate post-injury excitotocicity.(39) Further investigation of these neurosteroids in TBI is warranted as questions about ideal dosing and the confounding effects of injury heterogeneity still exist.(39)

Knowledge gap and variation in concussion aftercare

Concussions are often diagnosed in accordance with institutional or local guidelines. This and the lack of a standardized approach add subjectivity and variation to concussion diagnosis. Using the Zurich Consensus definition of concussion, a recent study at a level I trauma center found that concussion was mentioned as a final diagnosis in only 31% of cases. Concussion-specific instructions were given in only 62% of ED cases and were much more likely to be given if there was LOC. Activity restrictions were given in only 34% of cases and were more likely for sports-related injury.(40) Given the risks associated with a premature return to play, comprehensive identification of concussion patients is imperative. Standardization relies heavily on the accurate and consistent diagnosis of the condition itself, which in turn is inhibited by the current lack of an empiric approach to diagnosis.

One of the likely sources of variation in concussion care is the fact that concussions are diagnosed clinically. Diagnosis relies more on subjective assessment rather than objective data. Symptoms are reported by athletes and families and may be skewed by a desire for social acceptance;(5) i.e., players can also under-report their symptoms to return to play sooner. While the clinical symptoms of acute concussion can be identified during the evaluation of a forthright patient, the potential chronic symptoms of concussion might not appear until years after the head injury.(5) When these delayed symptoms present, they are difficult to link to the inciting event.(5)

What is a biomarker?

Biomarkers are genes, proteins, metabolites, or other quantifiable physical indicators that lend diagnostic, prognostic, or predictive knowledge to the understanding of biology or disease.(41) Biomarkers have been identified for many disease states including troponins for acute coronary syndromes, LDL for hypercholesterolemia, and p53 for cancer.

The biomarker hypothesis of brain injury suggests that trauma to the brain causes release of neuronal and/or glial proteins into biofluids (e.g. blood, CSF, urine) and the levels of these biomarkers is correlated with the severity or presence of injury. Assessment of mTBI biomarkers can be structured around three fundamental points. First, the biomarker should be specific to the brain and appear rapidly after injury. Secondly, testing for the marker should be sensitive, reliable, reproducible and cost-effective. Finally, the biomarker should be diagnostically or prognostically useful or predictive of response to therapy.(42)

Much of the diagnostic challenge with concussions lies in the subjectivity of self-reported symptoms and the variability of the guidelines with which it is diagnosed. The combination of a biomarker with neurological assessment could overcome both problems by lending itself to an empirical test for the diagnosis or prognosis of concussions.

Most promising concussion biomarkers and why they are inadequate

A large number of studies have sought to identify a useful biomarker for mTBI. S100b, a calcium-binding protein found in high concentrations in Schwann cells and astroglial cells, once showed significant promise. S100b is a rapid marker for detection of mTBI and its testing is relatively inexpensive.(43) However, the marker is limited in its sensitivity. Ingebrigtsen et al found that s100b is associated with the development of symptoms in only 67% of patients with negative CT after injury.(42,44) S100b has also demonstrated limited specificity due to peripheral sources of the protein. The protein is detected in increased concentrations in multiple clinical settings, including minor head injury, hypoxic brain injury, intracranial hemorrhage, skull fracture, brain contusion, diffuse axonal injury, and stroke. Furthermore, s100b is not a reliable prognostic index because even very elevated values are compatible with complete neurological recovery.(43,45)

Glial fibrillary acidic protein (GFAP) is a monomeric filament protein that is the major component of the astrocyte cytoskeleton. It is found exclusively in the central nervous system, making it a specific marker of central nervous system pathology. Recent studies have examined GFAP as a marker of brain injury. In one study of 108 adults patients

with mild and moderate brain injury, the accuracy for CT-positive injury was 79%, with a sensitivity of 97%, specificity of 18% using a cut-off level of 0.035 ng/mL.(42,46) A similar study in a pediatric cohort yielded comparable results.(47) Metting et al(48) reported that GFAP had a negative predictive value of 82% for MRI findings 3 months after injury, showing that low levels of GFAP can predict normal MRI results. In a comparison study, GFAP was found to be a more sensitive marker for mTBI than s100b overall. However, the majority of the patients with mTBI in the study had no detectable GFAP, possibly indicating limited specificity and diagnostic utility in this group.(42,48)

Neuron-specific enolase is a glycolyic pathway enzyme located in the neuronal cytoplasm. It was initially found to be expressed in neurons, but was later identified in erythrocytes, thrombocytes, oligodendrocytes, and neuroendocrine cells. Though the marker is a good indicator of mortality and poor outcome following TBI, it lacks specificity. Elevated serum neuron-specific enolase is a marker of hemolysis, small cell lung cancer, neuroendocrine bladder tumor, neuroblastoma, and ischemic stroke.(42)

Tau is a microtubule-associated axonal protein. Various studies suggest that its release into the biological fluids reflects the degree of axonal degeneration. However, multiple groups have provided evidence that tau has limited diagnostic value for mTBI. Additionally, studies by Bazarian et al and Ma et al both suggest tau does not have prognostic value in the identification of postconcussive syndrome either.(42) Conversely, a recent study by Shahim et al showed that t-tau was elevated in a cohort of 28 concussed professional hockey players. T-tau levels at 1 hour were also predictive of

the duration of PCS symptoms.(49) This most recent data is promising and warrants replication in a larger cohort to confirm its diagnostic and prognostic utility.

Calpain-derived αII-spectrin N-terminal fragment (SNTF) is an N-terminal fragment of alpha spectrin shown to have increased accumulation in damaged axons. It is undetectable in undamaged axons, but is generated following stretch injury secondary to intra-axonal calcium overload and spectrin proteolysis.(42) Siman et al showed that an elevation in the SNTF was associated with changes on diffusion tensor imaging and was correlated strongly with cognitive impairment that lasted at least three months.(50) Another recent study quantifying SNTF levels in concussed hockey players found that SNTF levels were elevated at several time points from one hour to six days following injury. SNTF had particular diagnostic accuracy for players with post-concussive symptoms lasting at least six days. At the one hour to six day time points, protein levels were elevated 2.5-fold in players with symptoms lasting six or more days. However, SNTF levels were unchanged in players with more rapid recoveries.(51) Though promising, SNTF might lack the sensitivity necessary for diagnosing mild concussions.

The role of imaging in concussion management

Uncomplicated concussion involves functional and microstructural damage not visible on CT scan.(52) As a result, CT imaging has no role in the diagnosis of concussion. Nevertheless, CT is important in identifying structural and potentially life-threatening diagnoses such as intracranial hemorrhage.(53) The Canadian CT Head Rule (CCHR) is a validated clinical decision rule developed to determine when CT scans are warranted in mTBI.(53) Additionally, there is growing evidence that handheld quantitative

electroencephalogram (QEEG) can gauge small abnormalities in brain activity associated with CT-positive mTBI.(54) Despite the availability of more cost-effective alternatives, CT scans are still overused in the ED.(55)

Though intracranial lesions secondary to mTBI can be missed by CT scans, quantitative MR methods, including diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy imaging (H-MRSI) have shown the ability to detect injuries missed by CT and other conventional techniques. DTI has been able to uncover micro-structural alteration in axons in mTBI, but it is limited by the fact that mTBI often leaves the axon structurally intact but dysfunctional.(52) H-MRSI can be used to study cell status, measuring such parameters as membrane turnover, neuronal integrity, cellular energy/density, and astroglial proliferation. These parameters are measured via the N-acetylaspartate, creatine, choline, and myoinositol markers. A recent study of 26 mTBI subjects showed that these patients had an isolated decrease in white matter N-acetylaspartate, suggesting a diffuse axonal dysfuction.(56) Given that N-acetlyaspertate is nearly 100% specific to neurons(57) and 90% of the patients in the study had an unremarkable clinical MRI, H-MRSI could be both a sensitive and specific marker for mTBI.(52) A subsequent study also showed that H-MRSI changes were also sensitive to PCS in patients with normal neuroimaging, suggesting that H-MRSI might also have some prognostic value.(52)

Current proteomic technologies and their limitations

Proteomics, the study of the entire complement of proteins produced by an organism or cellular system, has great potential for use in the discovery of biomarkers of

human health and disease. The human proteome contains an estimated 20,000 proteins, which has made accurate quantification of biomarkers a big challenge.(58) Up until recently, large scale proteomic profiling has been performed using mass spectrometry and antibody–based methods. Though mass spectrometry has shown great potential for proteomic quantification, it is limited by a lack of sensitivity, specificity, reproducibility, and throughput. Antibody-based methods such as enzyme-linked immune-sorbent assays (ELISA) are more sensitive than mass spectrometry. However, these technologies require larger sample sizes as they cannot be multiplexed above a few simultaneous measurements because secondary antibodies can cross-react with surface immobilized proteins including, but not limited to, primary antibodies. This feature of antibody-based methods limits their specificity.(58)

Aptamer-based proteomic technology

Aptamers are a class of single-stranded oligonucleotides with the ability to bind to protein targets with high affinity and specificity. While their uses in therapeutics and catalysis have been around for over twenty years, their use in proteomics is first appeared in the literature in 2010. Aptamers can be used to measure proteins in complex matrices such as plasma through a process that transforms protein concentrations into corresponding aptamer concentrations. These aptamer concentrations are quantified on a DNA microarray (a collection of microscopic DNA spots attached to a solid surface).(58)

This technology is capable of simultaneously measuring thousands of proteins, and aptamers' multiplexing capability allows for biomarker discovery unencumbered by an

incomplete knowledge of biology. While traditional methods require researchers to know which marker they plan to measure, aptamer-based methods can act as a proteomic "search engine" for thousands of low-abundance proteins in body fluids. Additionally, aptamer-based proteomic technology has the advantage of measuring proteins from small sample sizes (as low as 15 microliters) and with high throughput.(58)

Previous applications of aptamer technology

Early applications of aptamer-based technologies (SOMAscan, SomaLogic, Boulder, CO) in Duchenne muscular dystrophy (DMD), Alzheimer's disease, tuberculosis, coronary heart disease, and pre-term birth, have yielded promising results. Duchenne muscular dystrophy is a severe myopathy caused by a lack of dystrophin protein production.(59) Unfortunately, dystrophin, the most obvious DMD biomarker, is measurable only though invasive muscle biopsy. Aptamer-based proteomic technology has identified 26 serum DMD biomarkers. These novel biomarkers are involved in muscle degeneration, inflammation, and fibrosis pathways.(59,60) Several of the biomarkers also appear to be responsive to dystrophin regeneration.(61) In the field of Alzheimer's research, aptamer-based proteomic technology has found several complement proteins associated with rapid cognitive decline(62) as well as two modifiable markers of cognitive aging.(63) In a Ugandan tuberculosis study, aptamer-based proteomic technology discovered markers associated with both tuberculosis treatment(64) and successful treatment response (defined by 8-week culture status).(65) Additionally, aptamer-based proteomic technology helped develop a 9-

protein biomarker panel found to be superior to the Framingham model in predicting cardiovascular events in patients with stable coronary heart disease.(66) Recently, aptamer-based proteomic technology was also used to confirm the involvement of coagulation and immune-related pathways in pre-term birth.(67)

Machine learning and its utility in proteomic analysis

Many scientific disciplines aim to model the relationship between inputs and outputs using mathematical models. Creating these models becomes challenging when they involve complex real-world phenomena. Machine learning mitigates this challenge by constructing algorithms that can learn and make predictions from data.(68) Machine learning methods have been used to develop speech recognition, self-driving cars, and effective web searching in the past decade alone.(69) Recent advances in processing capacity and digital data gathering have facilitated the application of machine learning to a wide array of fields, including medical diagnosis.

Machine leaning in the biologic realm can be conducted in either a supervised or unsupervised fashion. "Supervised" machine learning involves the training of a model based on samples with known class labels associated with them. "Unsupervised" classification, also known as clustering, simply groups samples with similar attribute profiles.(68,70) In biology, researchers frequently want to know if a sample is associated with a disease state. In the creation of a model that can accomplish this task, samples can be labeled as "diseased" or "not diseased." Supervised machine learning classification uses these labels to determine variables predictive of classification. A brief description of the five most common supervised classification machine learning models

(Naïve Bayesian classifiers, rule-based learners, decision trees, support vector machines, and artificial neural networks, Figures (1-3) is provided below.(70)

Naïve bayesian classifier model

Naïve Bayesian classifiers are statistical methods based on Bayes' theorem. Bayes' theorem states that the probability of a given outcome can be predicted by the conditions surrounding the outcome.(70) The conditional probability is given by:

$$P(A|B) = \frac{P(B|A)P(A)}{P(B)}$$

Where A represents the outcome, B the predictor, $P(B|A)$ the conditional probability of B given A (i.e. the likelihood), P(A) the probability of A (i.e. the prior), and the P(B) the probability of the predictor (i.e. the evidence). This technique is efficient and trains on a relatively small number of outcomes, but it is sometimes limited by its assumption that attributes are independent of each other.

Random forest model

Rule-based learners generate sets of rules that are readable by humans (e.g. If attribute A<2 OR attribute B>4, THEN Class=1) and explain the process by which a sample is sorted into a particular class. Decision trees structure the knowledge used to discriminate samples in a tree-like structure. Samples are classified by following the tree along its relevant branches. Branching is carried out in a rule-based fashion with set cutoff values. A random forest builds on the concept of decision trees. It builds multiple trees from a data set. Each tree only has access to a random subset of attributes from the samples. Each tree predicts a class, and the class chosen by the majority wins out (Figure 1).

Support vector machine model

Support vector machines predict sample classes based on the concept of the linear separability of classes (Figure 2). Support vector machine are defined by the criteria they use to create the optimal linear classifier (the classifier with the maximum separation between classes), their identification of the minimum number of training instances (or "support vectors") needed to define this optimal classifier, and their use of kernels (pattern recognition algorithms) to transform the original set of variables into a higher order non-linear space in which the linear separability happens.

Artificial neural networks

Artificial neural networks are analogous to the connections and function of nerves in the nervous system (Figure 3). In this model, the "neurons" are computational elements (usually linear classifiers) that are connected in a variety of ways and become activated or deactivated based on the signal they receive. Though each linear classifier used in the neural network is simple, the use of multiple neurons and layers allow this method to be applied to complex problems.(70)

Feature selection in biomarker discovery

In proteomic analysis, any of the aforementioned machine learning algorithms can be used to classify biological samples. For proteomic biomarker discovery, machine learning is taken one step further - the researcher is not only given the ability to classify samples, but also to determine which elements within a sample are most important in reaching its final classification. Machine learning "feature selection" methods allow for

the selection of the most significant attributes within a sample and the removal of redundant or irrelevant attributes.(70)

Rationale for assessing feasibility through piloting

A pilot study is a small investigation that tests research methods before their implementation in a larger study. The purpose of a pilot is to evaluate the feasibility of the study's approach. Prior to conducting a large-scale investigation, a researcher may choose to examine the recruitment, retention, assessment, randomization, and implementation methods of the study to make sure they are adequate.(71)

It is important to note that a pilot study's purpose is not to test a statistical hypothesis. Instead, it is used to gauge the probability of success of the larger study given a set of procedures or evaluate a feasibility question (e.g. determine the consent proportion). Since a pilot study does not test a hypothesis, inferential statistical tests are not conducted. Additionally, sample sizes in pilot studies are not determined by power calculations, but rather patient flow and budget restrictions.(71) Though there are limitations to the role and interpretation of pilot studies, they are a necessary first step in a study that uses novel methods or interventions.

In this concussion biomarker discovery project, the purpose of a pilot study is twofold. First, piloting was used to determine the typical concussion patient flow in the ED setting to help predict recruitment and enrollment rates. This information will be used to guide RA staffing decisions to maximize the yield for enrollment. By assessing the reasons that patients were excluded or refused participation in the pilot, inclusion/exclusion criteria and recruitment methods can be optimized for the larger

study to achieve sample size goals and desired statistical power. Secondly, with a pilot study, novel biomarkers can be identified from the collected samples so that in the eventual larger study, less expensive traditional methods could be employed to assess the utility of those biomarkers.

Statement of purpose

Specific hypotheses of the thesis

The COMPETE protocol for concussion biomarker discovery in the emergency department (ED) is feasible for successful subject recruitment and sample processing methods.

Long-term hypothesis

Proteins not normally present in significant quantities in the blood will appear and/or become elevated following concussion in adolescent athletes but not following isolated extremity injury in age- and gender-matched controls. Serum levels of these proteins will correlate with concussion severity and time to resolution of concussive symptoms.

Specific aims of the thesis

Assess the feasibility of a study protocol for concussion biomarker discovery in the ED. The long-term objective of this project is to Identify a biomarker or set of biomarkers linked to concussions.

Methods

Study design

Setting

Twenty-four athletes between the ages of 13 to 25 years were recruited from the Yale-New Haven Hospital Pediatric and Adult ED (urban, academic Level I trauma center with over 200,000 patient visits per year between the York Street and Chapel Street campuses).

Assembling the research team

A research team was assembled including an emergency physician with research expertise in mTBI and recruitment strategies; a neurophysiologist with expertise in biomarker discovery; a pediatric emergency physician with an expertise in injury; and a medical student who functioned as a research assistant (RA), responsible for patient enrollment and data collection.

Training

Prior to, and during the recruitment of patients, the RA was trained in research-related clinical procedures, including peripheral venous phlebotomy, urine collection, and urine pregnancy testing. The RA practiced procedures through formal medical school training as well as supplementary training by hospital nursing staff. Whenever possible, the RA carried out these procedures on study recruits.

The RA completed training in the use of OnCore (OnCore, Forte Research Systems, Inc., Madison, WI), an electronic database linked to the Yale-New Haven Hospital billing system, and used this database to register study participants.

Registration in this system ensured that study procedures, including blood/urine collection and sample processing, were charged to a research account and not to the subjects.

Subjects/eligibility criteria

Twelve concussion patients, ages 13-25 years, presenting within 6 hours of a mTBI sustained during recreational activity were enrolled prospectively. Minor head injury was defined as a blunt injury associated with LOC, amnesia, or disorientation and a Glasgow Coma Scale (GCS) score of 13–15. Since we sought patients with mTBI, there was no requirement for subjects to undergo CT imaging. However, if a subject did receive CT imaging, the CT had to be negative for structural brain injury (e.g., epidural hematoma). Patients who experienced a seizure following their injury, had a known bleeding disorder or coagulopathy, had an acute focal neurological deficit, or were pregnant were excluded. To ensure no pregnant patients were enrolled, all female subjects underwent urine pregnancy testing (often as part of standard care in the ED).

Twelve age- and gender-matched control patients presenting with isolated extremity injury sustained during recreational activity were also enrolled. A successful age match was defined as presentation within 366 calendar days. Isolated extremity injury was defined as a fracture, sprain, strain, contusion, abrasion, or laceration to an extremity in the absence of both blunt head injury and rapid deceleration injury that could result in a concussion.

Script development

The patient recruitment script was developed to standardize the method with which patients were recruited (see Appendix). In the script, the RA introduces him/herself to the subject, explains the study, details the patient's role in the study, introduces incentives, and explains the patient's right to refuse enrollment or withdraw from the study at any time. The script was drafted by the RA and reviewed by the research team. It was then revised based on how well it supported recruitment.

Clinical database

A Filemaker (Go 14, Filemaker, Inc., Santa Clara, CA) data collection database on a HIPAA-compliant handheld tablet was used for clinical data collection. The database included inclusion/exclusion criteria checklists, subject and parent consent forms (signed electronically), and subject questionnaires. Filemaker data was entered on a tablet computer and securely stored on a HIPAA-compliant cloud and intermittently copied to a spreadsheet accessible only to the RA and principal investigator.

Development of feasibility study

While conducting our pilot study, we collected feasibility data in the following areas of focus – "practicality", "adaptation", and "limited efficacy" – as outlined by Bowen et al.(72) "Practicality" explored the extent to which our protocol could be carried out in the setting of limited resources, time, and commitment from ED staff.(72) "Adaptation" was reflected in our focus on updating our protocol to optimize recruitment potential.(72) "Limited efficacy" was demonstrated by the testing of our protocol in a limited fashion. As in other "limited efficacy" studies, we used convenience samples,

with intermediate rather than final outcomes, shorter follow-up periods, and our study had limited statistical power.(72)

Participant identification, screening, and recruitment

In phase 1 (first 35 days) of recruitment, the RA posted informational posters in the ED and attended morning nursing staff meetings. Nurses, medical residents, and attending physicians notified the RA of potentially eligible patients via telephone page. In phase 2 (36 days), eligible patients were identified via page through the electronic health record (EHR). The EHR automatically generated a page to the RA whenever a patient aged 13-25 years old with chief complaint of head injury presented to the ED. In the final phase of recruitment (167 days), patients were identified via EHR and two $25 incentives in the form of gift cards were added to increase enrollment and completion of follow-up symptom diaries.

When patients were identified, the RA reviewed the patient's chart for the purposes of obtaining information about the injury, including time of the injury, mechanism, location and site of the injury, symptoms, and any relevant past medical history. With this information, the RA determined if patient history warranted in-person screening (i.e. the patient could not be excluded based on this initial information). If RA was unavailable at the time, he later reviewed the chart to determine if an eligible patient was missed. Potentially eligible patients were screened in person. Prior to consenting patients, the RA verified eligibility of the patient using an electronic checklist on a handheld tablet device. Subjects that were 18 years of age or older underwent

informed consent. Subjects younger than 18 years of age underwent informed assent, and their parent or guardian also underwent informed consent.

Prior to recruitment of controls, the RA met with triage nursing staff to create a list of chief complaints capable of encompassing most of the extremity injuries in the YNHH ED (Table 3). These chief complaints were added to the existing head injury paging system during the final recruitment phase.

Data collection

After the consenting process, demographic information including race, ethnicity, insurance type, and education level was collected. Patients also provided detailed clinical information that served as the acute post-injury data point (Figure 4). The data collection inventory used in the ED and follow-up was based on the U.S. Military Neurobehavioral Symptom Checklist.(73) Clinical data collected included:

- Site of injury
- LOC
- Amnesia
- Confusion
- Dazed state
- Headache
- Nausea
- Vomiting
- Dizziness
- Loss of balance
- Coordination
- Mood change
- Vision change
- Light sensitivity
- Hearing difficulty
- Sensitivity to noise
- Body numbness or tingling
- Changes in taste and/or smell
- Concentration
- Forgetfulness
- Difficulty making decisions
- Slowed thinking
- Fatigue
- Date/time of symptom resolution (if applicable
- Laceration/abrasion/ecchymosis at injury site

Specimen collection and processing

The RA collected 3 mL blood and 10 mL urine specimens. If the subject was already scheduled to undergo phlebotomy as part of his/her ED evaluation, an additional tube of blood was drawn for the study and collected in a heparinized, lavender EDTA tube. If the subject was not scheduled for phlebotomy as part of their ED evaluation, blood was collected by venous blood draw for the study's purposes only. When patient venipuncture was difficult to perform, the RA elicited assistance from nursing and/or technical staff. Mid-stream urine specimens were collected in sterile urine collection cups. Samples were coded at the time of collection.

Blood and urine specimens were sent to the Yale-New Haven Hospital laboratory after collection. There, blood tubes were sealed and centrifuged (at room temperature 2200 x g for 15 minutes). Serum was drawn off, aliquoted, and frozen at -20ºC. Urine samples were frozen (at -20ºC). Within the next 24 hours, the RA transported the specimens on dry ice to an outside laboratory. Here, the samples were frozen and stored at -80ºC. For proteomic analysis (pending at this time), all samples will be express mailed on blue ice to Somalogic.

Lab communications

In this study, direct communication with lab staff was essential to daily operations. Prior to beginning the study, protocols were reviewed and approved by lab administrators and billing was arranged through OnCore. After collection, study samples were sent to the hospital chemistry lab through a tube system along with a requisition form. This form labeled the samples with a Human Investigations

Committee number assigned to the study and included the details for specimen processing and handling. In the early stages of the study, the RA called lab technicians directly to confirm their understanding of the research protocol. After samples were frozen overnight, the RA contacted lab staff in a designated, outside research lab to set up a transport time.

Outcome measures

The primary outcome measure for the eventual machine learning analysis will be the presence or absence of clinical head injury. Proteins will be pursued as biomarkers if their concentration in the blood or urine of the concussion subjects is greater than in controls by two standard deviations. Approximately 1300 proteins will be assessed. The secondary outcome measure will be time to full recovery of concussion symptoms.

Follow-up survey

Follow-up was conducted through electronic surveys emailed to patients using the Qualtrics Survey Tool (Qualtrics, LLC, Provo, UT, Figure 5). Patients provided contact information in the ED and the RA contacted subjects and/or parents at regular intervals by phone call or text message to remind them to complete surveys on follow-up days. A $25 gift card incentive was sent to patients upon completion of follow up. Follow-up surveys included the same clinical questions as the initial inventory, but also inquired about how symptoms affected patients' daily lives and how others perceived the symptoms.

Consideration for timing of follow-up

Patient follow-up for head injury patients was conducted at 24-72 hours after injury, one week after injury, and then one, three, and six months after the injury to assess for symptoms after the ED visit. Head injury patients were followed until they were symptom free for two consecutive checkpoints (or for six months if symptoms persisted for that long). For control patients, follow-up was conducted at 24-72 hours after injury and one week after injury to ensure that no head injury symptoms were present in these patients.

Although concussions typically resolve within two weeks in pediatric populations, and even sooner in adult populations,(3) symptoms can last up to a year in a small subset of patients.(3) To capture the most salient data points, follow-ups were frontloaded and continued for many months.

Recruitment goal

This was a pilot study, so power calculations were not used to determine the sample size or recruitment goal. The sample size was instead based on the batching logistics of proteomic analysis. To eliminate the "batch effect," i.e. systematic error introduced when microarray data is processed in multiple batches, the whole study was processed in a single batch.(74) The batch size for the assay was 24, and this number was divided into twelve experimental subjects and twelve controls. The choice of one batch of data over multiple batches was also influenced by budget limitations. Findings from the proteomic analysis will inform power calculation for the larger study.

Feasibility

The feasibility of the study methods was determined by the ability of the research team to optimize recruitment within the study period. Feasibility findings from this pilot study will inform staffing needs for the subsequent, larger and adequately powered study. As the frequency of sports-related concussion is dependent on the scheduling of recreational activities, clinical data including date and time of arrival in the ED was collected to determine when best to staff for and recruit future patients.

Data and safety monitoring

The Human Investigation Committee of Yale University reviewed and approved the study (protocol #1503015422). After a patient was deemed eligible for the study, if the subject was not a minor, the patient was approached to obtain informed consent in the patient's room within the ED. If the subject was a minor, informed assent from the patient and parental permission were solicited. Minor patients did not require re-consent after reaching the age of majority during the time of enrollment.

This was a minimal risk study, which targeted populations wherein impaired decision-making capacity was unlikely (minor head injury only). During the consent process (and throughout the trial), research staff educated parents and patients about concussions and how clinical trials work. Potential risks were enumerated and described without overstating benefits.

Protected health information including name, address, telephone number, e-mail address, medical record number, birth date, admission date, and discharge date was collected. These data were stored on secured servers and HIPAA-compliant electronic

devices. After the study, all identifiable information will be removed from the stored data.

Student responsibilities

The medical student's responsibilities in this study included study design as well as those described above for the RA. The student assisted in the development of the questionnaires that were administered to study subjects and also tested the Filemaker application used to administer the survey. He developed the standardized script used to approach potential subjects in the ED. He was solely responsible for recruiting and consenting patients in the ED. Blood drawing responsibilities were split between the medical student and nurses in the ED. The medical student collected patient urine. He labeled all of the samples, filled out lab requisition paperwork, and transported all of the samples to the YNHH laboratory. Here, lab staff processed the samples. The student then transported all of these samples to a second laboratory for storage. After storing the samples, the student followed up with patients via phone and email for up to six months. He was also responsible for tracking that all follow-ups were completed on time. Once the follow-up process was complete, he administered all patient incentives (at time of enrollment and after follow-up) in person or by mail. He logged all of the automated recruitment pages he received and analyzed these data. The student was also deeply involved in the process of selecting an appropriate machine-learning model for the proteomic data.

Results

Subjects

We reached our goal of recruiting twelve concussion patients in the study. Additionally, we enrolled twelve age- and gender- matched controls with isolated extremity injuries sustained during recreational activity not involving the head. Demographic, mechanism/timing of injury, and past medical history characteristics are described in Table 4. Ages and genders of patients were closely matched, at an average of about 16 years old and 2/12 (17%) female in both the head injury and control groups. Most patients were white (non-Hispanic). Socioeconomically, patients were almost evenly split between private insurance and Medicaid. Football injuries were the most common cause of potentially eligible head injury pages at 9/39 (23%), while basketball was the most common cause of potentially eligible control pages at 8/27 (30%) (Table 4).

Subject flow

Out of the 24 total subjects in the study, sixteen were recruited using automated paging (Figure 6). The vast majority of pages, 483/549 (88%), were for ineligible subjects. These patients had a chief complaint of "head injury", but did not meet inclusion/exclusion criteria (e.g. no LOC/confusion/amnesia, non-recreational injury, or injury occurring more than six hours before ED presentation). Most ineligible patients had several reasons for exclusion, making a detailed breakdown of these patients became very cumbersome for a single RA to complete. Consequently, that analysis was foregone in this thesis. There were five eligible potential subjects who were approached in the ED, but refused enrollment. Fear of needles was the most common reason for

enrollment refusal (n=2), followed by general disinterest (n=2) and distress from a vasovagal episode (n=1).

Recruitment optimization

In Phase 1 of recruitment, ED staff informed the RA of potentially eligible patients via telephone. In Phase 1, ED staff did not identify any eligible patients; fourteen potentially eligible patients were identified via EHR query. For this query, the EHR was retrospectively searched for all patients aged 13-25 who presented to the YNHH ED with a chief complaint of head injury during Phase 1. Of these patients, the ones who met inclusion/exclusion criteria were deemed potentially eligible. In Phases 2 and 3, the EHR identified 183 potentially eligible head injury patients (previous figure of 549 total pages includes pages for controls) of which 39 were eligible (Table 5). Patient identification rate improved from 0/14 (0.00±0.00) to 39/183 (0.21±0.06) ($p<0.001$) with EHR-generated notification. In Phase 2 (pre-incentive), 3/8 patients approached in the ED were enrolled; in Phase 3 (post-incentive) 8/8 eligible patients were enrolled (Table 5). Enrollment rate improved from 0.38±0.43 to 1±0.00 ($p=0.01$) with financial incentives. Non-enrolled patients did not differ significantly from enrolled patients in mean age (16.80 vs. 16.23, $p=.64$) or percentage of female patients (20% vs. 17%, $p=.88$). Enrollment rate was used instead of recruitment rate (i.e. patients recruited/month) because recruitment was dependent on RA availability.

When were subjects recruited?

Enrollment began August 7th, 2015. By April 14th 2016, all twelve head injury patients had been enrolled. Automated paging was then narrowed to find age/sex matches for

unmatched head injury patients. During the initial paging period, the RA received 37/66 (56%) of pages for eligible patients on weekends - Friday, Saturday, or Sunday - and 44/66 (66%) of potentially eligible pages were received at night - 6pm to 5am (Table 6). In total, 53/66 (80%) of pages for potentially eligible patients were received on weekends and/or nights. Of the nine eligible football-related concussion pages, eight (89%) occurred during the months of September and October, with the lone exception occurring on November 1st.

Proteomic samples

Blood and urine samples were collected from all 24 of the enrolled patients and stored as described earlier in this document. All of these samples were usable for future proteomic analysis.

Concussive symptom data

Among the twelve concussion subjects, three reported LOC, six reported post-concussive amnesia, seven reported feeling confused, and ten reported feeling dazed. All of these patients also reported other concussive symptoms, including headache, difficulty concentrating, and confusion (Table 7). Based on the U.S. Military Neurobehavioral Checklist, all twelve subjects remained symptomatic at 24-72 hours, three were symptom-free at one week, eight were symptom-free at one month, nine were symptom-free at three months, and ten were symptom-free at six months with one lost to follow-up (Table 7).

Discussion

Interpretation

In this prospective cohort pilot and feasibility study of twelve adolescent athletes with concussion and age- and gender-matched controls, we found that identification and enrollment of patients was significantly improved by the implementation of automated EHR paging and additionally through patient incentives provided at the time of enrollment and upon completion of follow-up surveys.

Generalizability

The protocol demonstrated in this pilot study shows potential for use in future concussion biomarker discovery projects. Automated pages are practical because they do not rely on busy ED staff to identify patients and cost-effective because medical students can respond to evening pages while fulfilling daytime commitments. Indeed, given the demanding nature of the ED environment and the typically off-hours presentation of head injury patients, automated pages and financial incentives could be the preferred recruitment strategy for smaller concussion biomarker discovery projects. To optimize recruitment, larger, funded studies should plan and budget to preferentially staff RAs on nights and weekends.

Evidence of feasibility

We conducted our feasibility study in accordance to the principles outlined by Bowen et al.(72) With “practicality”, we examined the performance of our recruitment strategy in the time- and resource-strained setting of a busy ED. In phase one of recruitment, it quickly became apparent that ED staff was too busy to report potentially

eligible patients. A clear majority of our eligible patients also presented on nights and weekends, proving "camping" in the ED for long blocks of time as an inefficient and unreasonable recruitment strategy. Automated EHR paging significantly improved patient identification and alerted the RA about eligible patients during all hours. However, many eligible patients still lacked motivation to participate after this first adjustment. With a focus on "adaptation", we added gift card incentives to the study. This small amount of compensation incentivized eligible patients to enroll in the study – even those with an aversion to needlesticks –and complete the follow-up protocol. Our study demonstrated "efficacy" because we reached our recruitment goal within the one-year goal we defined for ourselves. The efficacy of the strategy is limited mainly by the fact that the cohort of 24 patients was a convenience sample. At times, the RA was available to screen every page he received, but at other times, the RA was not around the hospital and could not screen patients.

Limitations

Concussion patients and controls in this study were young, healthy, and age- and gender-matched, likely creating an environment that minimized baseline proteomic variability. Though our age- and gender-matched controls were adequate for use in a small pilot study, there may still be proteomic variability between individuals that could not be accounted for with this matching method. The ideal control group would consist of pre-season samples from the same athletes, but this would require banking specimens from a large population of athletes in anticipation of a potential head injury. This alternative approach will be employed if there is too much variability in the pilot

proteomic data. Though this protocol has the potential to identify acute (<6 hours) biomarkers, it will not track the trend of biomarker levels over time.

One of the principal limitations of this study was its sample size. As a pilot study, sample sizes were determined by practical and financial limitations rather than power calculations. Furthermore, there was no binary hypothesis to test, making power calculations even less appropriate. It is difficult to infer that the biomarkers identified in this pilot study are in fact associated with mTBI. However, with candidate biomarkers identified, the performance of the biomarker panel can be tested in a larger study. That said, the proteomic data will be large and important biomarkers have been identified with a small sample sizes, as low as in the 30's in existing aptamer studies.(65)

This study was limited by its use of a single RA. The RA missed far more eligible patients than he was available to recruit. A larger study would benefit from a team of RA's and more ED coverage. Another recruitment-related limitation of this study was the large amount of head injury pages required to find eligible patients. To improve the yield of the pages, nursing triage notes would have to be coded in addition to chief complaints.

Another limitation of the study is the self-reported follow-up surveys used to determine the duration of concussion symptoms. These surveys are subject to recall bias. One could also argue that the inclusion of a post-follow-up incentive could encourage subjects to under-report symptoms. This problem could be circumvented with the use of formal neuropsychiatric follow-up visits, though such intensive follow-up would have required resources beyond the scope of this pilot project.

Conclusions

EHR-based paging and financial incentives for participation increased subject identification and enrollment rates to inform and optimize the enrollment and recruitment strategies for the eventual larger study. Our next step will be developing an extensive profile of changes in the blood proteome following concussion.

Tables and Figures

Table 1: CDC - Concussion signs and symptoms

Signs	Symptoms	
• Appears dazed or stunned • Is confused about events • Answers questions slowly • Repeats questions • Can't recall events prior to the hit, bump, or fall • Can't recall events after the hit, bump, or fall • Loses consciousness (even briefly) • Shows behavior or personality changes	**Thinking/Remembering:** • Difficulty thinking clearly • Difficulty concentrating or remembering • Feeling more slowed down • Feeling sluggish, hazy, foggy, or groggy **Emotional:** • Irritable • Sad • More emotional than usual • Nervous	**Physical:** • Headache or "pressure" in head • Nausea or vomiting • Balance problems or dizziness • Fatigue or feeling tired • Blurry or double vision • Sensitivity to light or noise • Numbness or tingling • Does not "feel right" **Sleep:** • Drowsy • Sleeps less than usual • Sleeps more than usual • Has trouble falling asleep

Source: Centers for Disease Control and Prevention, Heads Up: Concussion in High School Sports. A Fact Sheet for Parents.(75)

Table 2: Graduated return to play protocol

Stage	Functional Exercise	Objective
No activity	Symptom-limited physical and cognitive rest	Recovery
Light aerobic exercise	Walking, swimming or stationary cycling keeping intensity <70% maximum permitted heart rate	Increase heart rate
Sport-specific exercise	Skating drills in ice hockey, running drills in soccer. No head impact activities	Add movement
Non-contact training drills	Progression to more complex training drills, for example, passing drills in football and ice hockey. May start progressive resistance training	Exercise, co-ordination and cognitive load
Full contact practice	Following medical clearance participate in normal training activities	Restore confidence and assess functional skills by coaching staff
Return to play	Normal game play	

Source: Consensus Statement on Concussion in Sport 4th International Conference on Concussion in Sport.(2) Adapted from Silvia Bressan and Franz E. Babi *Diagnosis and Management of Pediatric Concussion.(3)*

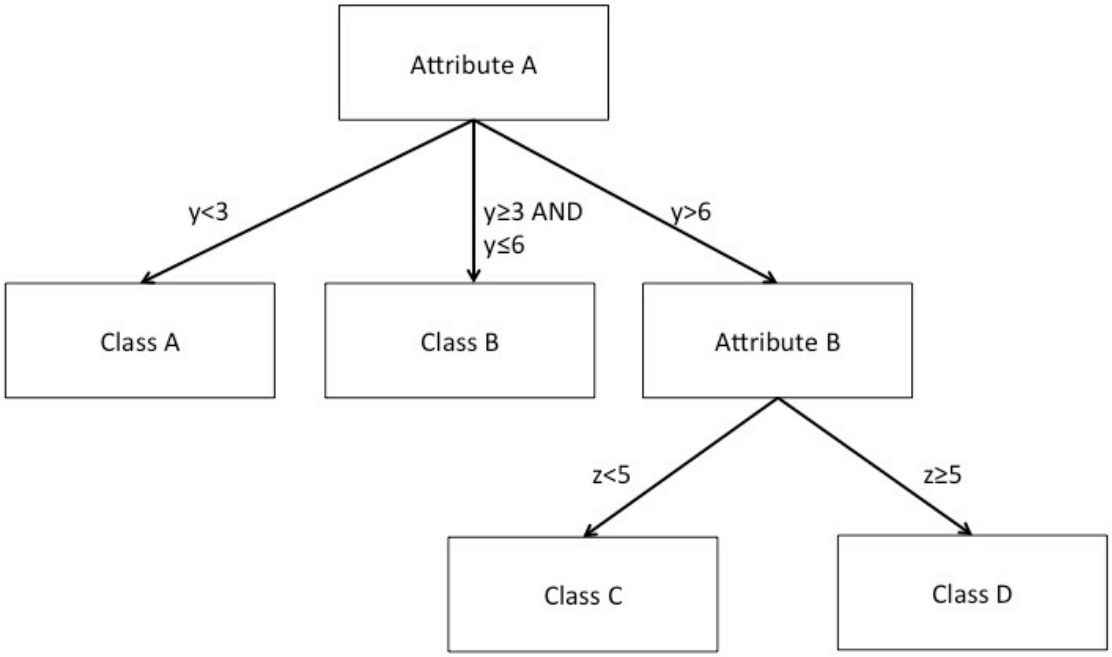

Figure 1: A graphical representation of a decision tree. This example sorts into four classes using two attributes.

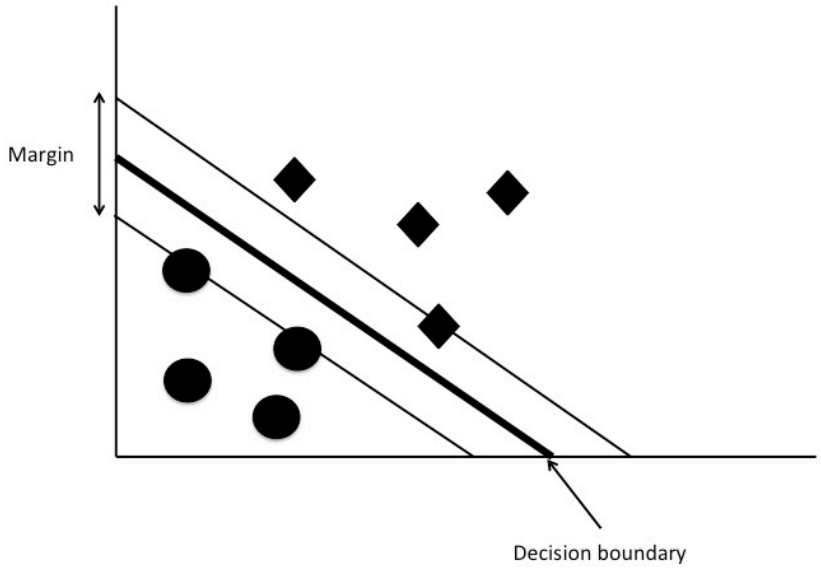

Figure 2: A graphical representation of a support vector machine linearly separating two classes.

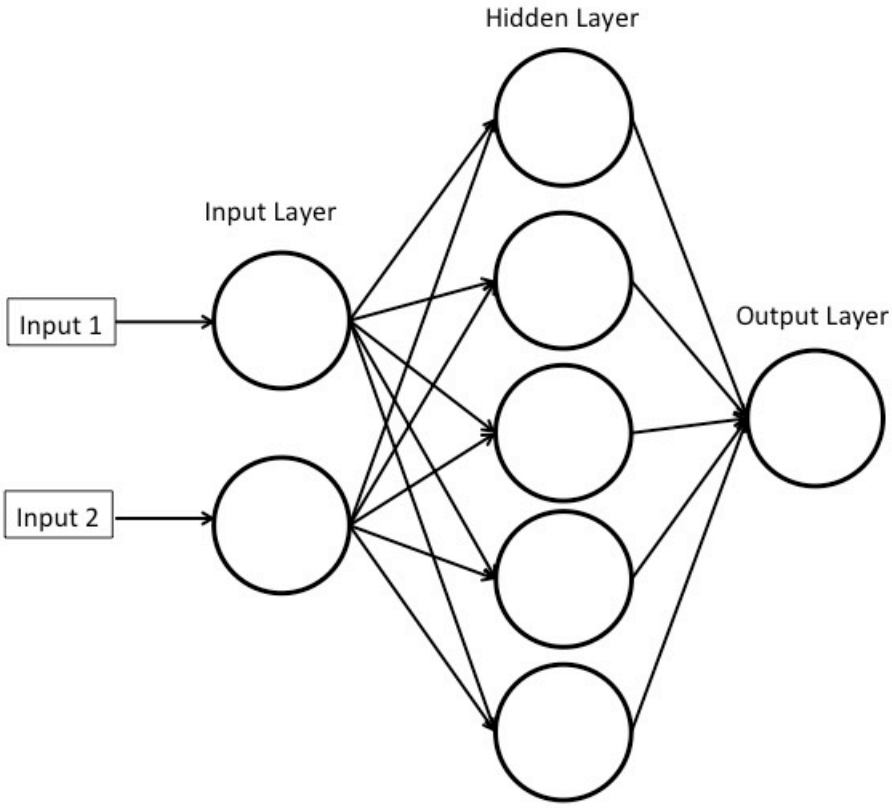

Figure 3: A graphical representation of artificial neural networks and the layers involved in the generation of a model.

Table 3: Chief complaints included in the EHR query for control subjects

• ankle injury • ankle pain • ankle swelling • arm injury • arm pain • arm swelling	• clavicle injury • clavicle pain • clavicle swelling • elbow injury • elbow pain	• hand injury • hand pain • hand swelling • hip injury • hip pain • hip swelling	• shoulder injury • shoulder pain • shoulder swelling • thumb injury

• wrist injury • wrist pain • wrist swelling • leg injury • leg pain • leg swelling	• elbow swelling • finger injury • finger pain • finger swelling • foot injury • foot pain • foot swelling	• joint injury • joint pain • joint swelling • knee injury • knee pain • knee swelling	• thumb pain • thumb swelling • toe injury • toe pain • toe swelling • wrist injury • wrist pain • wrist swelling • injury

Figure 4: Sample patient data entry screen on tablet

Please rate the following symptoms with regard to how much they have disturbed you since your injury using the following scale:

0 = None – Rarely if ever present; not a problem at all

1 = Mild – Occasionally present, but does not disrupt my activities; I can usually continue what I'm doing; doesn't really concern me.

2 = Moderate – Often present, occasionally disrupts my activities; I can usually continue what I am doing with some effort; I feel somewhat concerned

3 = Severe - Frequently present and disrupts activities; I can only do things that are fairly simple or take little effort; I feel like I need help.

4 = Very severe - Almost always present and I have been unable to perform at work, school or home due to this problem; I probably cannot function without help

	None	Mild	Moderate	Severe	Very Severe
	0	1	2	3	4
Feeling dizzy	○	○	○	○	○
Balance problems, loss of balance	○	○	○	○	○
Poor coordination, clumsy	○	○	○	○	○
Headaches	○	○	○	○	○
Nausea	○	○	○	○	○
Vision problems, blurring, trouble seeing	○	○	○	○	○
Sensitivity to light	○	○	○	○	○

Figure 5. Screen capture from online follow-up survey with the U.S. Military Neurobehavioral Symptom Checklist

Table 4: Baseline characteristics of recruits

Characteristic	Head Injury (n=12)	Control (n=12)	P-value[A]
Age (yr), mean±SD	16.23±1.65	16.46±1.76	0.74
Male (%)	10 (83)	10 (83)	.99
Race			
White (%)	9 (75)	10 (83)	0.85
Black (%)	3 (25)	4 (33)	0.67
Other	0 (0)	0 (0)	0.99
Ethnicity			
Hispanic (%)	2 (17)	2 (17)	0.99
Non-Hispanic (%)	10 (83)	10 (83)	0.99
Insurance Type			
Private/HMO (%)	7 (58)	6 (50)	0.70
Medicaid (%)	5 (42)	6 (50)	0.70
Other/Self-pay (%)	0 (0)	0 (0)	0.99
Inciting Recreational Activity			
Football (%)	2 (17)	0 (0)	0.15
Basketball (%)	2 (17)	3 (25)	0.63
Wrestling (%)	0 (0)	3 (25)	0.08
Recreational vehicle (%)	3 (25)	2 (17)	0.63

Soccer (%)	1 (8)	0 (0)	0.34
Floor Hockey (%)	1 (8)	0 (0)	0.34
Volleyball (%)	0 (0)	1 (8)	0.34
Lacrosse (%)	1 (8)	0 (0)	0.34
Other (%)	2 (17)	3 (25)	0.63
Chronic conditions			
Prior Concussion(s) (%)	3 (25)	3 (25)	0.99
Asthma (%)	4 (33)	3 (25)	0.67
Migraines (%)	1 (8)	1 (8)	0.99
ADHD (%)	0 (0)	2 (17)	0.17
Extremity Fracture (%)	2 (17)	2 (17)	0.99

[A] P-value calculated with two-sided t-test (homoscedasicity/heteroscedascity determined with F-test)

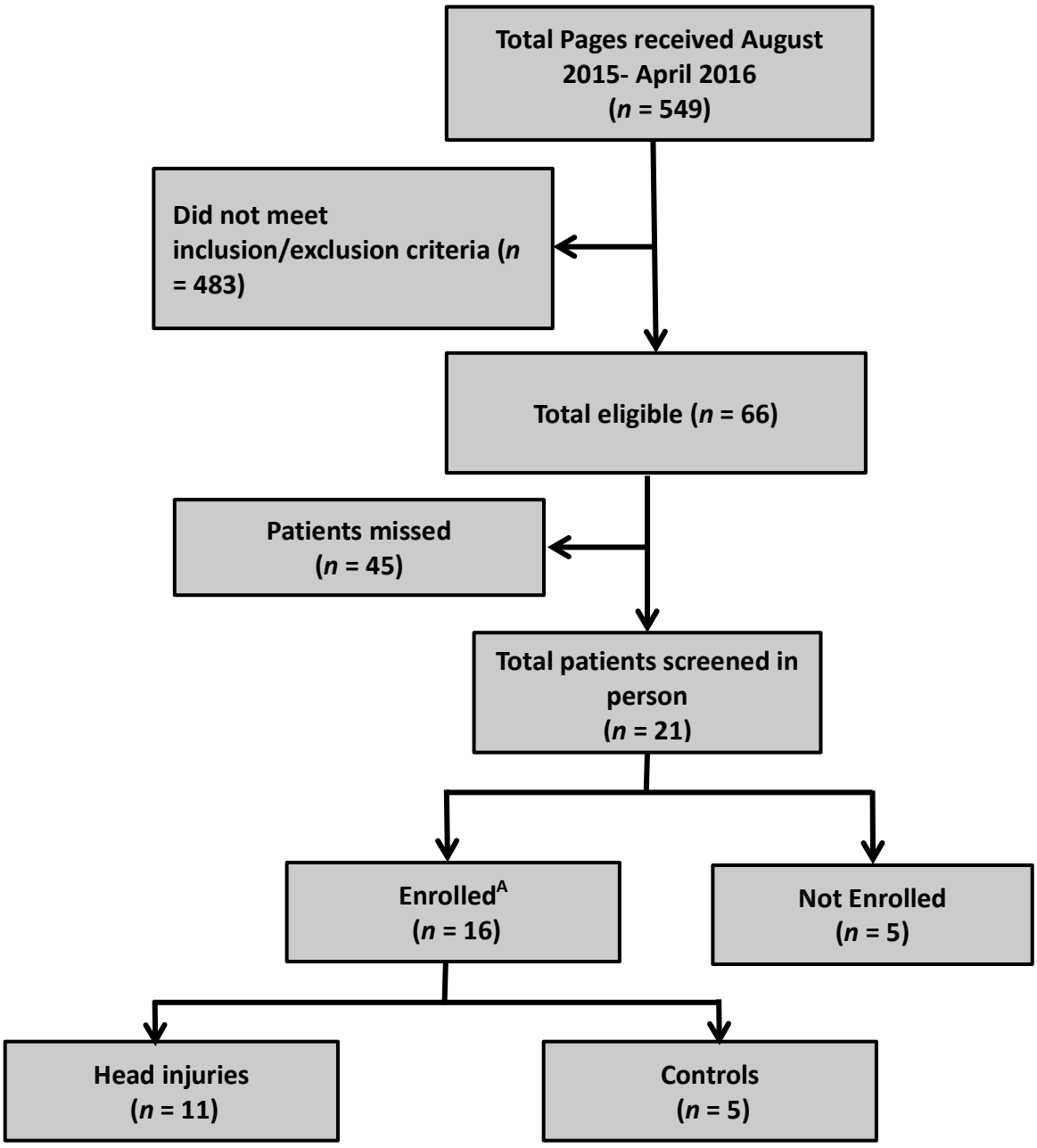

Figure 6: Patient recruitment and enrollment flowchart

[A] 1 head injury subject and 7 controls were enrolled without the EHR paging system

Table 5: Head injury recruitment by phase

	Phase 1[A] (35 days)	Phase 2 (36 days)	Phase 3 (167 days)	Total
Identified[B]	0	66	117	183
Eligible[C]	0	17	22	39
Recruited[D]	0	8	8	16
Enrolled	0	3	8	11

[A] Phase 1 – Notification by ER staff; Phase 2 – Automated EHR paging; Phase 3 – Automated EHR paging with added incentives for subjects

[B] "Identified" patients were those with a chief complaint of "head injury" for whom the RA received an EHR page.

[C] "Eligible" patients met inclusion/exclusion criteria after patient interview or EHR review (if RA was unavailable and patient was missed). Fourteen such patients in Phase 1 were identified retrospectively.

[D] "Recruited" patients were approached by the RA and asked to participate in the study

Table 6: Night and weekend head injury pages

Time of page	Head Injury (n=39)	Control (n=27)	p-value[A]
6am – 5pm (%)	9 (23)	13 (48)	.03
5pm – 6am(%)	30 (77)	14 (52)	.03
Friday, Saturday, Sunday	21 (54)	16 (59)	.67
Monday - Thursday	18 (46)	11 (41)	.67

[A] P-value calculated with two-sided t-test (homoscedasicity/heteroscedascity determined with F-test)

Table 7: Clinical outcomes from online survey (all data are % followed by standard deviation, n=12 for all calculations)

Symptom	<6 hrs	24-72 hours	1 week [A]	1 month	3 months	6 months
Feeling Dizzy	83.3 (10.8)	91.7 (8.0)	41.7 (14.2)	16.7 (10.8)	16.7 (10.8)	0.0 (0.0)
Balance Problems	50.0 (14.4)	50.0 (14.4)	33.3 (13.6)	25.0 (12.5)	16.7 (10.8)	0.0 (0.0)
Poor Coordination	58.3 (14.2)	50.0 (14.4)	33.3 (13.6)	25.0 (12.5)	16.7 (10.8)	0.0 (0.0)
Headache	91.7 (8.0)	91.7 (8.0)	83.3 (10.8)	41.7 (14.2)	25.0 (12.5)	0.0 (0.0)
Nausea	25.0 (12.5)	16.7 (10.8)	8.3 (8.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vision Problem	50.0 (14.4)	33.3 (13.6)	25.0 (12.5)	16.7 (10.8)	8.3 (8.0)	0.0 (0.0)
Sensitivity to Light	50.0 (14.4)	58.3 (14.2)	33.3 (13.6)	16.7 (10.8)	16.7 (10.8)	0.0 (0.0)
Hearing Difficulty	8.3 (8.0)	16.7 (10.8)	0.0 (0.0)	0.0 (0.0)	8.3 (8.0)	0.0 (0.0)
Sensitivity to sound	50.0 (14.4)	66.7 (13.6)	50.0 (14.4)	8.3 (8.0)	16.7 (10.8)	0.0 (0.0)

Numbness/ tingling	41.7 (14.2)	25.0 (12.5)	16.7 (10.8)	0.0 (0.0)	8.3 (8.0)	0.0 (0.0)
Change in taste	NA	16.7 (10.8)	0.0 (0.0)	0.0 (0.0)	8.3 (8.0)	0.0 (0.0)
Appetite Change	NA	16.7 (10.8)	0.0 (0.0)	16.7 (10.8)	8.3 (8.0)	0.0 (0.0)
Poor Concentration	58.3 (14.2)	83.3 (10.8)	50.0 (14.4)	16.7 (10.8)	8.3 (8.0)	0.0 (0.0)
Forgetfulness	33.3 (13.6)	66.7 (13.6)	41.7 (14.2)	16.7 (10.8)	16.7 (10.8)	0.0 (0.0)
Difficulty Making Decisions	33.3 (13.6)	58.3 (14.2)	16.7 (10.8)	8.3 (8.0)	8.3 (8.0)	0.0 (0.0)
Slowed Thinking	66.7 (13.6)	58.3 (14.2)	50.0 (14.4)	25.0 (12.5)	16.7 (10.8)	0.0 (0.0)
Fatigue	66.7 (13.6)	58.3 (14.2)	58.3 (14.2)	16.7 (10.8)	16.7 (10.8)	0.0 (0.0)
Sleep Problems	NA	41.7 (14.2)	33.3 (13.6)	16.7 (10.8)	8.3 (8.0)	0.0 (0.0)
Feeling Anxious	NA	41.7 (14.2)	25.0 (12.5)	8.3 (8.0)	8.3 (8.0)	0.0 (0.0)

Feeling Depressed	NA	25.0 (12.5)	33.3 (13.6)	8.3 (8.0)	8.3 (8.0)	0.0 (0.0)
Irritability	NA	58.3 (14.2)	50.0 (14.4)	8.3 (8.0)	16.7 (10.8)	0.0 (0.0)
Poor frustration tolerance	NA	58.3 (14.2)	16.7 (10.8)	8.3 (8.0)	16.7 (10.8)	0.0 (0.0)
Ringing in the ears	NA	16.7 (10.8)	25.0 (12.5)	0.0 (0.0)	8.3 (8.0)	0.0 (0.0)
Symptoms unresolved	100.0 (0.0)	100.0 (0.0)	75.0 (12.5)	33.3 (13.6)	25.0 (12.5)	8.3 (8.0) [B]

[A] One-week follow-up from one patient was filled in with duplicated one-month follow-up data from the same patient (subject missed one-week follow-up but was compliant thereafter)

[B] Two subjects were lost to follow-up after 3-month follow-up. One remained symptomatic at 6 months (confirmed with EHR query). This patient was included in the "symptoms unresolved" calculation, but none of the other calculations. The status of the other subject is unknown.

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Appendix: Recruitment script

1. Overview

Hi, my name is (name), and I am working as a research assistant in the emergency department. I am not a part of you care team today, but I am helping conduct a study on head injuries called the COMPETE study. It is a study involving young athletes like you that have had head injuries. The hope is that the project will lead to the development of a quick blood or urine test that will be able to help us find out which head injuries are more serious than others. This could, for example, allow football coaches to quickly judge if and when it is safe to allow players to return to the field after a head injury. It is an exciting study, and it has the potential to have a huge impact in both sports and the military. Involvement in the study requires a minimal commitment. If you agree to participate in the study, we would collect quick blood and urine samples today, and over the next 6 months, you would fill out a series of very quick surveys about how you're feeling. I would call you 6 times over the next 6 months and have a 5-minute conversation about how you're feeling.

2a (older adolescents/adults).

Participation in this study is completely voluntary. If you choose to not participate, it will in no way affect the care you are receiving. If you choose to enroll in the study but you change your mind, you are free to withdraw at any time. Withdrawing from the study won't affect your care or your relationship with any of the doctors at XXXXXXX Hospital.

Again, what is involved in the study is the collection of blood [If already drawn, say that it will not require an additional stick] and urine from you today, and after that, a symptom journal to document your symptoms, and six five-minute phone calls over the next few months. You would not have to come back to the hospital to follow up or meet with any of the doctors involved with the study.

Female participant (alone):

Pregnancy causes a lot of changes in blood chemistry, so we ask that female participants of childbearing age also take a pregnancy test. The results of the test will be kept confidential, but a positive test would disqualify you from the study. Additionally, if you were to become pregnant during the study, we would have to withdraw you. So, if there is a reasonable chance that you are pregnant now or may become pregnant soon, we would advise that you not participate.

This is study with very minimal risk to you. Some of the rare risks of blood draws include bleeding and infection, but a sterile needle would be used to draw the blood and

pressure would be applied afterward to minimize infection and bleeding. The other small risk would be leakage of personal information. I would like to assure you that we make every effort to keep the information that we collect confidential. We will not share your information with others unless there is a rare situation where the law requires us to share in the information. If the data is published, any information that can identify you will remain confidential.

Do you have any questions?

Okay, here is a consent form for you to look over and sign. It goes over the details of the study. Whenever you are ready, you can sign the last page of the form, and we can get started.

2b (minors).

Your child's participation in this study is completely voluntary. If you choose to not participate, it will in no way affect the care he/she is receiving. If you choose to enroll in the study but change your mind, you are free to withdraw at any time. Withdrawing from the study won't affect your care or your relationship with any of the doctors at XXXXXXX Hospital.

Again, what is involved in the study is the collection of blood [If already drawn, say that it will not require an additional stick] and urine from your child today, a symptom journal to document his/her symptoms with him/her, and six five-minute phone calls over the next few months. I will call you at a time that is good for both you and your child and we will quickly talk about your child's symptoms. Neither your child nor you would be required to come back to the hospital to follow up or meet with any of the doctors involved with the study.

To Female participant (Gauge interest. Then ask parent to leave.):

Pregnancy causes a lot of changes in blood chemistry, so we ask that female participants of childbearing age also take a pregnancy test. The results of the test will only be known to you, but in the event that the test is positive, we would counsel you on seeking appropriate healthcare and the support of adults around you. If you were to become pregnant during the study, we would also have to withdraw you from the study. So, if there is a reasonable chance that you are pregnant now or may become pregnant soon, we would advise that you not participate.

(Allow parent back in room.)

This is study with very minimal risk to your child. Some of the rare risks of blood draws include bleeding and infection, but a sterile needle would be used to draw the blood and pressure would be applied afterward to minimize infection and bleeding. The other small risk would be leakage of personal information. I would like to assure you that we make every effort to keep the information that we collect confidential. We will not share your information with others unless there is a rare situation where the law requires us to share in the information. If the data is published, any information that can identify you will remain confidential.

Do you have any questions?

Okay, here is a consent form for you to look over and sign. We also have a consent form for your child. It goes over the details of the study. Whenever you are ready, you can sign the last page of the form, and we can get started.

3. Closing.

Thank you so much for your time. It was a pleasure meeting you. Although I am not a part of your care team, I can certainly convey any of your concerns to your team to make sure that all of your needs are met. I will be back with your nurse shortly.