Modulation of Inflammatory Cytokine Responses to Neurosurgery and Traumatic Injury via Sympathoadrenal Activation

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

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Abstract

Traumatic brain injury (TBI) is a major cause of death and disability worldwide. TBI elicits sympathetic nervous system (SNS) activation with massive catecholamines secretion and modulation of neuroimmune networks. Catecholamines incites cerebral and systemic immune cells to synthesize and secrete multiple pro- and anti-inflammatory cytokines. Our study aims to evaluate temporal changes in circulating pro- and anti-inflammatory cytokines Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-10 in association with plasma epinephrine (E) and norepinephrine (NE) levels, in traumatic brain injury (TBI) and elective neurosurgical patients. This also examines possible interrelationships between these mediators and neurological outcomes as evaluated by extended Glasgow Outcome Scale (GOSE) score. From the data gathered within this study, it was determined that high circulating catecholamine levels affect the pro- and anti-inflammatory cytokine balance, and patients with higher peak cytokine levels do sustain worsened neurologic outcome.
Acknowledgments

I would like to thank Dr. Sandro Rizoli (Supervisor) and Dr. Shawn Rhind (PAC committee member) for their continuous support and guidance. Without their mentorship and crucial feedback, this scientific work would not have been possible. Their endless efforts made this project a success. An additional special thanks to Dr. Shawn Rhind for providing me with the equipment and necessary training for molecular characteristics of this study. This was vital for me to be able to complete this project. I would also like to thank Dr. Ori Rotstien (PAC committee member) and Dr. Andrew Baker (previous PAC committee member) for their insights and thought-provoking questions that guided me to publish this work.

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List of Contributions

Dr. Sandro Rizolli and Dr. Shawn Rhind were the chief contributors throughout the length of this study. Their contributions included providing research equipment, designing the trial, providing revision of statistical data analysis, providing insightful oral as well as written feedback on key concepts within the study, and writing the proposal application that got this project the funding that it required.

Mrs. Sandy Trpcic, Dr. Adic Perez, and Mrs. Yangme Li became involved in the initial phase of this project through the collection and handling of patient blood samples.
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<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>Adm</td>
<td>Admission</td>
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<tr>
<td>AIS</td>
<td>Abbreviated Injury Score</td>
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<tr>
<td>ATP</td>
<td>Adenosine tri phosphate</td>
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<tr>
<td>BB</td>
<td>Beta-blocker</td>
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<tr>
<td>BBB</td>
<td>Blood-brain-barrier</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium</td>
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<tr>
<td>CARS</td>
<td>Compensatory anti-inflammatory response syndrome</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>Conc.</td>
<td>Concentration</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>DAMPs</td>
<td>Damage associated molecular patterns</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>E</td>
<td>Epinephrine</td>
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<td>EIA</td>
<td>Enzyme immunoassay</td>
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<td>EIR</td>
<td>Excitatory inhibitory ratio</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>GC</td>
<td>Glycocorticoids</td>
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<td>GCS</td>
<td>Glasgow Coma Scale score</td>
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<tr>
<td>GOSE</td>
<td>Glasgow Coma Outcome Scale Score - Extended</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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x
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
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<td>ICP</td>
<td>Intracranial Pressure</td>
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<td>ICU</td>
<td>Intensive care unit</td>
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<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin-10</td>
</tr>
<tr>
<td>K+</td>
<td>Potassium</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<td>MAP</td>
<td>Mean arterial pressure</td>
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<tr>
<td>MODS</td>
<td>Multi-organ dysfunction syndrome</td>
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<tr>
<td>Na+</td>
<td>Sodium</td>
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<tr>
<td>NE</td>
<td>Norepinephrine</td>
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<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cell</td>
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<tr>
<td>PSH</td>
<td>Paroxysmal Sympathetic Hyperactivity</td>
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<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SAH</td>
<td>Subarachnoid hemorrhage</td>
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<tr>
<td>SAS</td>
<td>Sympathoadrenal system</td>
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<tr>
<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
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<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
</tr>
<tr>
<td>SPM</td>
<td>Spectrophotometric</td>
</tr>
<tr>
<td>STBI</td>
<td>Severe Traumatic Brain Injury</td>
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<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
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TNFR - TNF receptors
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Chapter 1
Review of Literature

1.1 Introduction

Traumatic brain injury (TBI) is the most significant injury contributing to major health related issues and socioeconomic problems amongst most countries. As such, TBI is a major public health problem, responsible for thousands of deaths every year in Canada. After TBI, surviving patients suffer from long-term physical disabilities, cognitive, motor or speech-language deficits. Previously, it was a major cause of mortality and morbidity in young individuals that live in high-income countries, largely due to a rise in traffic collisions. However, now we also see an increase in traffic related injuries as more vehicles are on the road in middle-income and low-income countries[1–4] .

Immediately after TBI, a complex stress response is initiated, which is characterized by profound alterations in neuroendocrine and immune functions, designed to re-establish homeostasis. Activation of the hypothalamic-pituitary-adrenal (HPA) axis, and the sympathetic nervous system (SNS) with secretion of catecholamines and glucocorticoids, along with intricate neuroimmune interactions, are recognized as central pathways in the pathogenesis of post-traumatic complications [5–7] .

After TBI, spontaneous activation of the SNS occurs with massive release of Epinephrine (E) and Norepinephrine (NE); these can alter the production of multiple inflammatory mediators in peripheral blood immune cells and in various organs. Growing evidence indicates that catecholamines play an important role in the regulation of host immune and inflammatory
responses. Experimental studies demonstrated that catecholamines modulate monocyte–
macrophage functional activities and alter their local production of cytokines, thereby initiating
and amplifying the inflammatory response to trauma. Hence, it is likely that the posttraumatic
hyper-adrenergic state following TBI, with its pro-inflammatory effects is harmful to the
already injured brain [8, 9].

Both primary and secondary insults also activate the release of multiple cellular mediators,
including inflammatory cytokines, chemokines, glutamate, reactive oxygen/nitrogen species and
complement. TBI can induce neuronal cells to synthesize and secrete inflammatory cytokines,
such as peptides of interleukin (IL) family and tumor necrosis factor (TNF)-α. The synthesis
and release of chemokines and adhesion molecules, in turn act to mobilize immune cells and
activate glial cells in a parallel and synergistic fashion. Microglia and macrophages possess
multiple toll-like receptors through which they can detect a wide array of host-derived damage
associated molecular patterns (DAMPs) within the CNS, responding rapidly to tissue damage.
The inflammatory response elicited by trauma is a key component of TBI. Trauma causes not
only neuroinflammation within the brain, but also leads to a systemic inflammatory response
(SIRS) [10, 11].

After injury, breakdown of blood-brain-barrier (BBB) facilitates the infiltration of peripheral
blood leukocytes (i.e., neutrophils and monocytes) into the brain parenchyma and activation of
resident glial cells (e.g., astrocytes and microglia). Activated microglia have been implicated as
the primary mediators of progressive neurodegeneration after brain trauma, due to their release
of inflammatory and cytotoxic agents. These cytotoxic factors further disrupt the BBB,
upregulate expression of cell-surface adhesion molecules, chemokine receptors, thereby sustaining cellular recruitment and activation. This perpetuates the inflammatory response in the injured brain and ongoing cellular dysfunction [12–16].

Our study aims to measure circulating catecholamine levels (E and NE) and pro/anti-inflammatory cytokines in patients sustaining moderate-to-severe TBI or undergoing a controlled neurosurgical insult and examines the interrelationships between changes in these SNS hormones, inflammatory cytokines, and clinical indicators of injury severity and neurological outcome. This is achieved by correlating circulating levels of inflammatory mediators with plasma catecholamine concentrations [15].

1.1.1 Epidemiology of TBI

Each year, more than 8 million patients are treated for head injuries in Canadian and US emergency departments, constituting 6.7% of the 120 million total emergency department visits. The mortality percentage can reach 40% within the individuals having severe TBI, and neurologic morbidity among the survivors is also high. World Health Organization’s World Report on Road Traffic Injury Prevention (2004) estimated that road traffic accidents would be within the top three leading causes of the worldwide burden of disease, ahead of HIV and tuberculosis, by 2020. Injuries due to falls and violence are among the other causes of TBI. Incidences of violence related TBI are higher in USA because of firearms users. Furthermore, blast injuries of the brain are now recognized as a specific class of TBI [17–26].
1.1.2 Economic burden

The economic burden of the hospital care for TBI patients is also huge. Intracranial Injury and fractured skull were ranked as the second (with $55.5 million) and sixth (with $16 million) most expensive diagnoses among all injury patients hospitalized in Ontario between April 1, 1999 and March 31, 2000. In U.S. and Australia, the cost was estimated $56 billion and AU$8.6 billion annually [27–31].
1.2 Background

1.2.1 Classification of Traumatic Brain Injury

TBI is comprised of a two-stage injury process: primary and secondary injury. The primary brain injury occurs due to the direct mechanical impact/trauma to the brain cells. Secondary injury occurs as a blend of intracranial physical and biochemical alterations in the brain and systemic extracranial insult [32, 33].

1.2.1.1 Primary brain injury

Primary brain injury is a direct insult to the intracranial structures, including damage to brain parenchyma, and injury to neurons. Intracranial hemorrhage and vasogenic edema can occur due to direct vascular damage. Laceration is the most severe form of primary brain injury. Compression of the brain and severe neurologic dysfunction can occur due to axial hematomas within the brain parenchyma and extra axial hematomas in the subarachnoid, subdural and epidural spaces. Primary brain injury is further classified as (1) focal brain injury or (2) diffuse brain [33, 34].

1.2.1.1.1 Focal injury

The brain is floating and supported by the fluid in the skull. The brain tissue is soft and can be stretched or compressed easily. Impact to the head often results in skull fracture, scalp laceration, contusions, intracranial hematomas and development of focal TBI. The impact also causes successive transfer of energy to the cerebral tissues, resulting in nerve cell depolarization. This produces leads to uncontrolled excessive release of excitatory neurotransmitters resulting in pathological events called excitotoxicity. Additionally,
contusions, which are focal surface injuries occurring due to damage to small blood vessels, can be seen anywhere in the brain; the frontal and temporal areas, however, are more vulnerable to injury. Contusions are common features of TBI and their presence confirm that a head injury has occurred. However, these may be absent in diffuse brain injuries [35, 36].

1.2.1.1.2 Diffuse axonal injury (DAI)

In TBI, DAI mostly occur due to acceleration and deceleration of skull that results in collision of brain inside of the skull, inflicting bruising to the brain. In addition, the head, which can move freely on impact, can produce loss of consciousness as compared to immobilized one that results in local deformations of skull and brain. The brain is comprised of tiny and delicate cells called neurons. The axon, which is a part of neuron, is responsible for communication between neurons. When the brain moves, the axons stretch and tear, eliciting injury to the neuron and may result in potential death of neurons instantly or over the course of a few days. This develops across the whole brain that is why it is referred to as diffuse axonal injury. It was observed that the structural changes in axonal and white matter were correlated with cognitive deficits and behavioral changes, even 6 months after mild TBI [35, 37, 38].

1.2.1.2 Secondary brain injury

Secondary brain injury occurs due to vigorous interaction between inflammatory, ischemic and cytotoxic processes. It can take place immediately, or delayed for days after trauma. The first stage of cerebral injury is comprised of direct tissue damage, impaired regulation of cerebral blood flow (CBF) and metabolism. These ischemic stimuli trigger accumulation of lactic acid due to anaerobic glycolysis, increased membrane permeability, and sequential edema formation.
Because of inadequate aerobic metabolism, cellular energy state is not maintained due to which depletion of adenosine tri phosphate (ATP) stores and failure of energy-dependent membrane ion pumps takes place. Energy failure causes an imbalance of ion homeostasis and dysregulated influx of sodium (Na\(^+\)) and calcium (Ca\(^{2+}\)) into neurons. In second stage, sequential terminal membrane depolarization with enormous release of excitatory neurotransmitters (glutamate, aspartate) occurs. The normal re-uptake of glutamate by astrocytes through an ATP-dependent Na\(^+\) cotransport system, declines or stops due to destruction and energy depletion of neighboring astrocytes, consequently increasing the levels of glutamate. Human microdialysis studies validate that elevated extracellular glutamate levels are linked with worse outcome [32, 34, 36].

1.2.1.2.1 Ions imbalance pathogenesis in TBI

The massive extracellular glutamate concentration initiates enormous influx of Ca\(^{2+}\)and Na\(^+\) into the neurons and glial cells. Intracellular increase of Ca\(^{2+}\) activates several intracellular enzymes inflicting severe damage within the cell and eventually cell death. The presence of high extracellular glutamate levels causes membrane channels to open, which in turn leads to Na\(^+\) influx, membrane depolarization, secondary influx of chloride Cl\(^-\) and water resulting in excitotoxic swelling [36, 39].

After TBI, potassium (K\(^+\)) ions are released into the extracellular spaces. Such K\(^+\) influx seems to be related to widespread depolarization, which may block normal neural activity, consequently spreading of depression in cerebral cortex. Severe upsurges in K\(^+\) hinders the membrane transport systems, metabolisms, and synaptic functions. Energy homeostasis may
also be disturbed due to elevated extracellular $K^+$ levels. $K^+$ induces glial cells to uptake oxygen and denies injured neurons of their oxygen demand leading to anoxic neuronal damage that may occur in brain after the injury [16, 40–44].

Magnesium ion ($Mg^{2+}$) is vital for cellular processes like glycolysis, respiration, oxidative phosphorylation, biosynthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein, and helps sustain the levels of $Na^+$ and $K^+$. Substantially Low $Mg^{2+}$ after TBI may disrupt glucose utilization, energy metabolism, oxidative phosphorylation and biosynthetic pathways, leading to neurodegeneration of the area. As $Mg^{2+}$ controls movement and surge of intracellular $Ca^{2+}$, any change in $Mg^{2+}$ concentrations in the brain may lead to $Ca^{2+}$ facilitated neurotoxicity after TBI [16, 45].

1.2.1.2.2 Reactive oxygen species (ROS) and TBI

The kinin system, excitotoxicity, activation of the innate immune system results in neutrophil recruitment, mitochondrial alterations and microglial activation leading to generation of ROS. ROS can easily damage cell membranes containing high levels of polyunsaturated fats and cholesterol. Since brain tissue is rich in lipid, it is predominantly sensitive to oxidative injury. ROS trigger downstream pathways and produces oxidative damage, alterations in tight junctions, and play a vital role in facilitating BBB permeability changes due to TBI. The inflammatory molecules and cells pass through permeable BBB in and out of the injured brain, initiating cascade of responses in the brain and other organs. The most important events contributing toward the pathology of TBI are reactive astrogliosis, microglial activation, infiltration of immune cells in the CNS, neurodegeneration and post-traumatic neurological
deficits. Pro-inflammatory cytokines and ROS, together, can damage neuronal cell leading to cell death. These inflammatory mediators activate arachidonic acid, coagulation cascades, and induce nitric oxide (NO) production, leading to secondary brain injury [11, 46].

1.2.1.2.3 Effect of nitric oxide in TBI

NO as a free radical, reacts with oxygen radicals to form peroxynitrite, resulting in lipid peroxidation, cell membrane lysis and DNA fragmentation. NO, facilitate ROS and glutamate to produce tissue damage. NO produces massive vasodilation leading to the loss of brain pressure auto regulation [36, 47, 48].

1.2.1.2.4 Role of edema in TBI

Edema has a significant role in secondary brain injury and in serious cases, pressure and swelling produced within the stiff hollow cranial dome, consequently lead to herniation of structures in the brainstem and death. Several pathological conditions, triggered by brain injury, can induce cerebral edema. There are two types of edema: vasogenic and cytotoxic. One form of vasogenic edema takes place at the tight junctions of endothelial cells that bound the movement of macromolecules across the BBB. Another type of vasogenic edema is linked to the available arachidonic acid that produces slight change in vasomotor system, and enhances endothelial cell permeability for small and large tracers leading to edema. In cytotoxic edema, BBB may not be involved, and instead, all of the cellular elements of the brain are involved. For example, the cytotoxic edema, which occurs during hypoxic conditions due to failure of the ATP dependent Na\(^+\) K\(^+\) pump. Na\(^+\) and water rapidly accumulates within cells due to osmotic pressure. The elevated levels of extracellular excitatory amino acid neurotransmitters such as glutamate and
glycine can induce acute swelling in dendrites and cell bodies. Direct mechanical traumatic insult can also precipitate cytotoxic edema and distortion of the neuronal membrane. This leads to astrocytic swelling as the astrocytes attempt to maintain cellular homeostasis [49].

1.2.1.2.5 Intracranial pressure (ICP) potentially deadly sequel to TBI

ICP is a known and potentially fatal consequence of TBI. According to existing studies, the skull is a cranial vault that contains brain parenchyma, arterial and venous blood vessels and CSF. In normal physiological conditions, these components exist in a state of balanced dynamic equilibrium. This state of equilibrium is known as intracranial compliance. Intracranial compliance decreases as ICP upsurges. When ICP upsurge crosses the limits of the compensatory mechanisms, cerebral perfusion is hampered leading to cerebral ischemic response, or Cushing reflex. Elevated ICP, results in reduced CBF, further leading to rise in Carbon dioxide (CO₂) levels, which are detected locally at the vasomotor center. The vasomotor center triggers SNS response resulting in elevated mean arterial pressure (MAP) ultimately rise in cerebral perfusion pressure (CPP) [33, 50].

1.2.1.2.6 Role of catecholamine in TBI

Disproportionate activation of the sympathetic system occurs after TBI, which triggers adrenal medulla and superior cervical ganglia to release of catecholamines. Injury can also activate adrenal medulla and superior cervical ganglia, completely independent of central activity. Catecholamines circulating centrally, released from the ganglia or peripherally from the adrenal medulla, enter back to cerebral cortex resulting in sympathetic storms. The release of both central and peripheral catecholamines (E and NE) produce clinical complications like
tachycardia, hypertension, diaphoresis, tachypnea, and mydriasis. The spillover of catecholamines from neurologic system leads to a threefold increase in plasma levels of NE. This condition may persist for 10 days and it is stated, that NE levels can take up to 6 months to normalize. The possibility that dead/dying neural cells could release catecholamines centrally after TBI cannot be ruled out [15, 27, 51].

1.2.1.2.7 Role of cytokines in TBI
1.2.1.2.7.1 Pro-inflammatory cytokines
Cytokines are proteins that are important for the defense and repair of tissue after trauma. They are secreted by cells that are responsible for the communication between cells, in both paracrine and endocrine fashion. Various types of pro-inflammatory cytokines expressions increase after head trauma, among those, IL-β and TNF-α are well studied cytokines, which play a vital role in inflammatory responses following TBI, and a number of studies examining focal injury have known their involvement in secondary neural injury. IL-1β activates astrocytes and microglia to produce TNF-α, and IL-6 and can induce growth factors like nerve growth factor within the central nervous system (CNS). Primarily immunoreactivity of TNF-α is detected in microglia, perivascular astrocytes, and oligodendroglia. In an experimental animal model, 3 to 6 hours after head trauma, an increase in TNF-α expression is evident in the hippocampus and related cortices. In humans, production of TNF-α and IL-1β, takes place at very early stage of the traumatic impact. TNF-α act as vital mediators to produce inflammatory response. It increases the adhesion and activation neutrophils, upregulates toxicity of eosinophils, exerts a chemotactic effect on monocytes and prompts the release of other immunoregulators. Cytokines like IL-1, IL-6, and TNFα can act as endogenous pyrogens, eliciting fever in
response to endotoxin. After TBI or stroke, their elevated levels could thus produce increases in body temperature, by acting on the brain directly and indirectly. The resulting hyperthermia is believed to aggravate the biochemical cascades involved in the secondary brain injury [52–58].

1.2.1.2.7.2 Anti-inflammatory cytokines

IL-10 is very well studied anti-inflammatory cytokine. It acts like immunosuppressive or immunostimulatory agent. In humans, activated monocytes, macrophages and mast cells have the capacity to produce IL-10. IL-10 could inhibit Th2-induced inflammation and differentiation of Th1 cells, thereby preventing the release of IFN-α and IL-2. IL-10 limits monocyte and macrophage function as it can inhibit major histocompatibility complex (MHC) class II expression on the surfaces of antigen-presenting cells and prevents superoxide and NO release from inflammatory cells. It is capable of limiting the effects of pro-inflammatory cytokines and chemokines (TNF-, IL-1, IL-6, IL-8, MIP-1), secreted during allergic reactions. It also prevents the release of chemoattractants such as RANTES and IL-8. IL-10 increases the release of anti-inflammatory mediators, like IL-1 receptor antagonist and soluble TNF-α receptors [59–61].

1.2.2 SNS activation after TBI

1.2.2.1 TBI and the catecholamine surge

After TBI, an extreme stimulation of the sympathetic system takes place, which leads to massive releases catecholemines with clinical manifestations. In 1929, Penfield discovered the first case of what we now call sympathetic storm, where he described a 41-year old patient with sympathetic hyperactivity. Under normal physiological conditions, the sympathetic reaction to a
trauma is aimed at reverting the adverse effects of the injury. In TBI, however, this process can easily shift from being a preserving one to a damaging one. This sort of response usually occurs after a variety of severe cerebral insults, where it has been characterized by paroxysmal autonomic and motor over activity. Most literature refers to this resulting condition as dysautonomia. It is important to note, however, that the adrenal medulla and the superior cervical ganglia, are potential sources of catecholamines. Rosner et al conducted a study and observed a 500-fold increase in plasma E levels as well as 100-fold increase in plasma NE levels for patients with severe head injury. This was precisely what Beckman, lams et al had established before. The result was that both E and NE plasma levels were elevated as a key reaction to energy transferred to the brain corresponding to injury severity. Previous studies observed hyperadrenergic state in patients with severe TBI, as well as nontraumatic subarachnoid hemorrhage. This state can be found anywhere between the benign SIRS to the paroxysmal sympathetic storms (PSS) and are most noticeable in the first week of injury [15, 62–72].

Following tables shows similar studies conducted for evaluating Catecholamines and Cytokines in TBI Patients

**Table 1. Studies Evaluating Catecholamines in TBI Patients**

<table>
<thead>
<tr>
<th>Author(s), Year</th>
<th>Patients (n / GCS)</th>
<th>Catecholamine Evaluated</th>
<th>Sample Time(s)</th>
<th>Biofluid / Assay Method</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaatte et al., 1975</td>
<td>45 / NA</td>
<td>E, NE</td>
<td>Adm</td>
<td>Plasma / SPM</td>
<td>Plasma E, NE concentrations were at the highest (0.95 ± 0.28 pg/l) and (1.08 ± 0.12 pg/l), respectively on admission</td>
</tr>
<tr>
<td>Hörttag et al.,</td>
<td>15/NA</td>
<td>E, NE</td>
<td>NA</td>
<td>Plasma/RIA</td>
<td>Plasma concentrations of NE and in some cases also E begin</td>
</tr>
<tr>
<td>Year</td>
<td>Authors</td>
<td>Study Group</td>
<td>Methodology</td>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>------</td>
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<td>-------------</td>
<td>-----------------</td>
<td>------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>1980</td>
<td>Nayak et al.</td>
<td>65/NA</td>
<td>E, NE, Adm</td>
<td>Plasma/Spectrophotofluorimetric</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The mean plasma NE 6.68 ng/ml (SD ± 1.14) and E 1.01 ng/ml (SD ±0.66) were observed. The levels were significantly elevated in all types of head injury when compared to normal.</td>
<td></td>
</tr>
<tr>
<td>1981</td>
<td>Clifton et al.</td>
<td>48/10 &lt;10</td>
<td>NE</td>
<td>Plasma/RIA</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Patients with head Trauma had an elevated plasma NE below CGS 10.</td>
<td></td>
</tr>
<tr>
<td>1984</td>
<td>Ikeda et al.</td>
<td>60/4-15</td>
<td>NE</td>
<td>Plasma/HPLC</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The good outcome consisted of patients with good recovery or moderate disability (mean plasma NE 0.69±0.08 ng/ml). The poor outcome consisted of patients with severe disability and persistent vegetative state (mean plasma NE 1.01±0.17 ng/ml). The mean plasma NE levels in &quot;poor&quot; and &quot;dead&quot; group were significantly higher than that in &quot;good&quot; group (p&lt;0.001).</td>
<td></td>
</tr>
<tr>
<td>1987</td>
<td>Woolf et al.</td>
<td>21/4 ≤4</td>
<td>E, NE, NA</td>
<td>Plasma/RIA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>28/11 ≤11</td>
<td></td>
<td>Patients with GCS scores of 3 or 4, E and NE were elevated four- to fivefold (plasma conc. E 400.2 ± 108.1 and NE 1502.6 ± 265.2), compared to patients with GCS scores of &gt;11 (plasma conc. E 101.8 ± 13.6 and NE 413.3 ± 47.1), and were correlated with patient outcome 1 week after injury.</td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>Woolf et al.</td>
<td>24/NA</td>
<td>E, NE, NA</td>
<td>Plasma/RIA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E levels elevated three- to sevenfold NE Values were significantly elevated in TBI patients with E conc. 1.26±0.32 pmol/mg and NE 5.80±1.40 pmol/mg when compared with Normal Subjects (E 0.23±0.04 and NE 1.79±0.15pmol/mg). NE and E may also correlate with the severity of brain injury.</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>Woolf et al.</td>
<td>124/3-15</td>
<td>E, NE, Adm</td>
<td>Plasma/RIA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Higher NE concentrations (4.88±0.44 nmol/L) always and epinephrine levels usually were associated with worsening indexes of injury severity.</td>
<td></td>
</tr>
</tbody>
</table>
Brain-injured patients with GCS values of 3 or 4 on admission and NE concentration was above 6.088 nmol/L were significantly more likely to die or remain neurologically unchanged.

<table>
<thead>
<tr>
<th>Study</th>
<th>GCS</th>
<th>Sample Size</th>
<th>Sample Type</th>
<th>Method</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson et al., 1993</td>
<td>38/3-13</td>
<td>E, NE</td>
<td>Adm</td>
<td>Plasma, Serum/HPLC</td>
<td>Both NE and dopamine levels increased with time since injury. During the first 48 hours, the total GCS scores for majority of patients were &lt;8.</td>
</tr>
<tr>
<td>Mautes et al., 2001</td>
<td>29/ &lt; 8</td>
<td>NE</td>
<td>Adm</td>
<td>Plasma, CSF/HPLC</td>
<td>Elevated plasma NE levels (&gt; 275 pg/ml) were observed in 50% of all samples. There was no correlation between GCS and the levels of NE in plasma or CSF either in samples. Four (14%) out of 29 patients died (Plasma NE 3600±7600 ng/l, GCS ≤8) during the observation period.</td>
</tr>
<tr>
<td>Johansson et al., 2011</td>
<td>75/3-15</td>
<td>E, NE</td>
<td>Adm</td>
<td>Plasma, Serum/RRIA</td>
<td>In the present study, non-survivors had higher Catecholamines levels compared with survivors and adrenaline was an independent predictor of mortality in accordance with previous findings.</td>
</tr>
<tr>
<td>Patel et al., 2012</td>
<td>40/ ≤ 8</td>
<td>E, NE</td>
<td>Adm</td>
<td>Plasma, Urine/HPLC</td>
<td>Direct correlation exists between severe TBI and catecholamine surge. Immediately after TBI, plasma epinephrine and norepinephrine (base line 168±416 pg/ml) levels increase several-fold, and remained elevated in those who have persistent coma or are moribund. Those with initial catecholamine levels that are only mildly elevated have been found to improve to a GCS &gt; 11 at 1 week.</td>
</tr>
</tbody>
</table>

**Abbreviations:** Adm, admission; n, sample size; E, Epinephrine; NE, norepinephrine; SPM, spectrophotometric; RIA, radioimmunoassay; EIA, enzyme immunoassay; HPLC, high performance liquid chromatography; Spectrophotofluorimetric.
<table>
<thead>
<tr>
<th>Author(s), Year</th>
<th>Patients (n / GCS)</th>
<th>Cytokine(s)</th>
<th>Sample Time(s)</th>
<th>Biofluid / Assay Method</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hensler et al/ 2000</td>
<td>32 / ≤8</td>
<td>IL-10</td>
<td>Adm</td>
<td>Plasma / ELISA</td>
<td>IL-10 levels in severely injured patients were increased (0-75 pg/ml) within 3h of trauma as compared to healthy controls and dropped to control values within 12 h.</td>
</tr>
<tr>
<td>Maier, B et al/ 2007</td>
<td>352 / ≤8</td>
<td>IL-6, IL-8, IL-10</td>
<td>Adm</td>
<td>Plasma / ELISA</td>
<td>IL-10 values immediately increased with peak values on the day of admission, rapidly declining to baseline levels during the subsequent 48 h.</td>
</tr>
<tr>
<td>Helmy et al 2012</td>
<td>12 / ≤8</td>
<td>IL-1β, TNF-α</td>
<td>Every 12h</td>
<td>Plasma / Milliplex</td>
<td>Current literature on TBI and Cytokines focuses on a relatively small group of mediators. The two cytokines most commonly implicated in a pro-inflammatory role are IL1β and TNF. They have synergistic actions in cell culture models and have concomitant production</td>
</tr>
<tr>
<td>Schneider Soares et al/2012</td>
<td>93 / ≤ 8</td>
<td>IL-10</td>
<td>Adm</td>
<td>Serum / ELISA</td>
<td>Serum IL-10, anti-inflammatory cytokine estimation showed a statistically significant correlation with TBI severity determined by GCS. This is supported by the subset of patients with severe TBI (GCS ≤ 8), non-survivors were found to display higher conc. of serum IL-10 (120 pg/ml) than survivors (70 pg/ml) first time point</td>
</tr>
<tr>
<td>Liao et al 2013</td>
<td>28 / 3-15</td>
<td>TNF-α</td>
<td>6, 12, 24, 48, 8, 72 h</td>
<td>Plasma / EIA</td>
<td>Uninjured subjects (n =6) had low conc. of TNF-α (29.46±2.1 pg/ml). ↑ Of TNF-α conc. in TBI patients at 6,12,24,48 and 72 hours was 1.9, 2.7, 3.0, 3.5, 4.1,-fold respectively to uninjured controls. ↑ in the conc. of STBI subjects were</td>
</tr>
</tbody>
</table>
significantly greater than those from MTBI subjects throughout the entire study period (6 h to 2 w after injury).

IL-1β levels were measured in available CSF and serum samples. Mean daily and mean weekly CSF IL-1β levels for the TBI groups were higher than controls. Our results show high serum IL-1β concentrations, relative to CSF, in our cohort with IL-1β levels that are suggestive of a blood-to-brain gradient for IL-1β.

### Abbreviations:

- Adm, admission; n, sample size; E, Epinephrine; NE, norepinephrine; SPM, spectrophotometric; RIA, radioimmunoassay; EIA, enzyme immunoassay; Milliplex Human High Sensitivity 9-plex; Milliplex, Multi-Analyte Profiling Human Cytokine/Chemokine 42 analyte premixed kit; Flow Cytometry; Concentration; ↑, Increase; STBI, Severe Traumatic Brain Injury; MTBI, Moderate Traumatic Brain Injury; ELISA, Enzyme-linked immunosorbent assay

#### 1.2.2.2 SNS mediated role of epinephrine and norepinephrine

Mainly, SNS mediates its adrenergic effects by NE and partially by E through nine different adrenoceptors, (α1A, α1B, α1C, α2A, α2B, α2C, β1, β2, β3). Human and rodent peripheral blood mononuclear cells (PBMCs), macrophages, neutrophils and lymphocytes express various categories of adrenergic receptors. Under physiologic conditions, when there is low sympathetic activity, nerve terminals only release NE, whereas in the event of high activity, both NE and neuropeptide Y (NPY) are released. When E and high doses of NE, bind to β2 adrenoceptors, they activate adenylate cyclase, leading to elevated levels of intracellular second messengers such as cAMP. Catecholamines interact with ligands of adrenergic receptors to activate and/or inhibit cAMP, Ca2+, diacylglycerol and inositol triphosphate. Consequently, endogenously secreted catecholamines activate cells responsible for producing catecholamines as well as neighboring cells in an autocrine/paracrine manner. Catecholamines are able to activate cellular
adrenergic receptors, trigger intracellular second messengers, and finally control the cell functions [73–75].

1.2.2.3 Mechanism of sympathetic hyperactivity

Sympathetic hyperactivity is also hypothesized as an upsurge in activity of the SNS, produced by loss of balance between the sympathetic and parasympathetic nervous systems. The specific mechanism of dysfunction includes loss of cortical control, dysregulation of autonomic balance, and/or there may be disturbance of relay mechanisms. The irregular stimulation or functional disconnection of the systems, critical for autonomic control is the most common mechanism for producing paroxysmal sympathetic hyperactivity (PSH) or sympathetic hyperactivity. The focus of preliminary reports regarding PSH are on increased activity of the diencephalon and its influences on direct activation or loss of inhibition. In TBI patients, levels of E and NE seem to be several fold higher than in controls. It is established that a correlation exists between the increase in catecholamine levels, TBI severity, and clinical outcome [76–84].

According to disconnection theories, the loss of higher-level control over one or more excitatory center(s), is termed as dysautonomia. Conventional disconnection theories propose that regulation of the upper brain stem and diencephalic regions by cerebral cortex, (paroxysms which are driven by central excitatory foci) is lost as a result of injured pathways from the cerebral cortex to the midbrain. Excitatory inhibitory ratio (EIR) model, another disconnection theory, states, that the excitatory spinal cord processes, are released from their inhibitory effects when damage to the brain stem and diencephalic centers occur. Essentially, spinal cord modulates the afferent stimuli from the periphery, as well as the efferent centrally originating
signals. Normally, the spinal cord modulates the EIR as brain activity and brain stem output increases, thus protecting the end organ. In TBI patients, however, the excess brain activity overpowers EIR of brain stem and subsequently overpowers the neighboring spinal EIR as well. Consequently, the spinal cord is not able to inhibit or balance out the highly elevated levels of catecholamines, hence leaving the peripheral organs unprotected. Consequently, dysautonomia of multiple end organs, such as smooth muscle rigidity or tachycardia, develops. An overlap condition occurs when both sympathetic over activity (hyperthermia, tachycardia, hypertension, tachypnea, and sweating) and motor over activity (rigidity, spasticity, and dystonia) are exhibited at the same time. Typically, the parasympathetic nervous system reduces the effects of augmented activity of the SNS and returns the body to homeostasis. In sympathetic storm, this feedback is not produced and the individual suffers from unrestrained state of stress [15, 76].

1.2.2.4 Potential adverse effects of untreated sympathetic hyperactivity
Catecholamines surge trigger a stress response that has significant involvement in morbidity and mortality after head injuries. These centrally and peripherally produced catecholamines exhibit certain clinical conditions. The common signs observed in moderate to severe brain injury patients are agitation and restlessness accompanied with fever, posturing, tachycardia, hypertension, and diaphoresis, cardiac arrhythmia, noncardiogenic pulmonary edema, and immunosuppression [72, 76, 85].

In 1902, Cushing defined the phenomenon of severe rise in intracranial pressure inducing an elevation of systemic blood pressure. This association between injury of the central nervous system and impairment of the cardiovascular system is now well recognized. A disorder with
hypertension and elevated catecholamine production, mimicking phaeochromocytoma, has been studied in patients with brain tumors, following stroke and during surgery for intracranial aneurysm. Cushing’s response, though induced by a catecholamine surge, is a response to increased ICP and reduction of cerebral perfusion [72, 86, 87].

TBI patients, admitted in an intensive care unit, are sedated in order to lessen stress on the brain. As mentioned above, failure to lower the stress response in brain can cause sympathetic storming which occurs in 15% to 33% of patients with severe TBI who are comatose (Glasgow Coma Scale (GCS) < 8). A consequence of sympathetic storms is substantial rise in cerebral blood volume, which leads to elevated ICP. Primarily, NE preserves integrity of the blood-brain barrier however if sustained levels remain for too long, the blood-brain barrier becomes permeable, thus producing cerebral edema, aggravate cerebral ischemia and necrosis. Sympathetic hyperactivity also induces vasoconstriction leading to cerebral tissue ischemia. Circulating catecholamines can induce substantial fluid shifts producing an overload of the pulmonary system resulting in neurogenic pulmonary edema [15, 76, 88–90].

TBI induces a surge in metabolic demands, which in case of sympathetic storm events produces intense catabolism. Patients affected by sympathetic hyperactivity undergo an increase in energy expenditure by up to 75%. This hypermetabolic state complicates patient’s outcome even further, produces hyperglycemia, hyperthermia diaphoresis, hypernatremia, renal insufficiency, thickening of pulmonary secretions, risk of muscle wasting, and weight loss. Proper diagnosis of fever and preservation of normothermia are vital for patients with traumatic brain injury, otherwise presence of an infection can also produce hyperthermia [15, 76].
After a severe head injury, the extents of apnea and catecholamine higher levels are directly proportional to the amount of energy transferred to the brain stem. Hence, most patients with severe head injury at the accident site, depending on the resumption of breathing, sustained apnea-induced hypoxic brain and cardiac injury amplified by raised levels of stress catecholamines, modulates morbidity and mortality in survivors. Stress linked immense sympathetic discharge and head injury-induced apnea occurs with the onset of severe TBI and has significant influence on subsequent outcome. The pooled effects produced by hypoxia, hypercarbia, acidosis and blood pressure surge along with the effect of catecholamine on tissues can lead to synergistic injury effect in the host. Continued sympathetic activation can induce heart damage, which begins as pivotal myocytolysis related to constant level of catecholamines. This can cause damage to myocardium and, if severe, academically death [72, 91].

1.2.3 Effects of TBI on circulating catecholamines (E, NE) and neurological outcome

Among severe TBI patients, some undergo sudden periodic increased stress response, or storming. After injury, within the first 24 hours, storming may occur. The sympathetic storm is likely linked to poor neurological function triggered by injury, and is most commonly seen in TBI populations [91–93].

1.2.3.1 TBI modulation of circulating catecholamines

After TBI, physiological and neurobehavioral disability is due to the pathologic alterations in the brain's endogenous neurochemical systems, can be regarded as possible mediators causing damage. Changes such as the over-stimulation or inhibition of neurotransmitter discharge, alterations in pre- or postsynaptic receptor affinity, neurotransmitter transport or signal
transduction mechanisms may produce harmful effects on brain blood flow and metabolism or exert direct toxic effects on neurons or glial cells. Among the neurotransmitter systems implicated in the pathogenesis of posttraumatic CNS dysfunction are the catecholamines. Molecules of plasma catecholamines produced in response to brain injury infiltrate the physical and enzymatic barriers between blood and brain. Catecholamines help to initiate recirculation, due to their effect on secondary transient ischemia and on the development of neuronal necrosis. It is also anticipated that NE is involved in both the recovery of function and maintenance of that recovery following both sensorimotor cortical removal and cortical bruising after brain injury in rats [5, 72, 94, 95].

1.2.3.2 Catecholamine levels and outcome in TBI patients

In 1987, researchers studied plasma levels of E, NE, and DOP in 33 patients with head trauma. It was revealed that the plasma concentration of these catecholamines was inversely proportional to the level of consciousness and GCS score of the patients. Patients with increased levels of E, NE and DOP had lower GCS scores. Patients with very low CGS score had plasma catecholamine levels of 4 to 5 times more than normal individuals. In head trauma patients, it was proposed, that assessment of serum E and NE might be a beneficial way for early evaluation of prognosis. With severe brain trauma (GCS 3 to 4), the level of plasma NE, E, and DA is increased and this increase correlates with the level of coma; these responses gradually decreased as the GCSs improved. E and NE levels remained elevated in those who had persistent coma or are moribund. Patients with mildly elevated catecholamine levels were found to improve to a GCS > 11 at 1-week time-point. Data from various studies on TBI patients advocates that SNS stimulation may induce poor outcome [5, 96–102].
Autonomic responses to cerebral injury, whether traumatic or nontraumatic, are well known in about 25 to 30% of patients with severe TBI. It is stated that the level of sympathetic activation seemed to correlate with the severity of brain injury and intracranial hypertension. Increased circulating catecholamine levels may cause such electrocardiographic abnormalities as arrhythmias and ST-T wave changes, and can induce myocardial infarction and necrosis seen after head injury. Upsurge in TBI severity also correlates with reduced heart rate variability (HRV) which is regarded as dysfunction of autonomic nervous system (ANS). Clinically, these vague irregular events, using process of differential diagnosis, are termed ‘sympathetic storms’ or ‘autonomic storms’, frequently manifest with “aggression” or “agitation”. It is observed; continued sympathetic upsurge is linked with increased length of stay (LOS) in intensive care unit (ICU), lower cognitive ability and higher cognitive fatigue [97, 103–105].

In the acute phase of cerebral trauma, clinical assessment of the extent of brain damage and prediction of recovery remains difficult. Unquestionably, an important role in the improvement of the patient is played by the above-mentioned changes in the activity of the sympathoadrenal system (SAS) and the hypothalamopituitaryadrenocortical system (HPAS). Recent research revealed that the elevated levels of hormones of SAS and HPAS correlate with the severity of neurological damage and recovery. The excessive volume of catecholamines often results in extra cranial manifestations, like systolic arterial blood pressure, which is directly correlates with the level of circulating catecholemines. Outcomes of TBI patients can be improved by limiting secondary injury. After normalization of neurologic function, the autonomic dysfunction syndrome and subsequent catecholamine excess recedes [5, 62, 85, 106–108].
1.2.3.3 Norepinephrine levels as outcome markers in TBI

In patients with TBI, abnormal levels of NE are observed. These abnormal levels are inversely proportional to the patient’s GCS; patients who remain comatose constantly and have higher NE levels (up to 7 times higher than normal) with elevated blood pressure, heart rate and body temperature. While those who have normalized GCS after injury also have normalized NE levels. NE levels are considered as effective outcome markers [96, 97, 100, 109].

1.2.3.4 Sympathetic surge and parasympathetic system activation

After acute trauma, an instant sympathetic outburst provides the necessary quick response to compensate the effects of the injury. Normally the elevated sympathetic state is balanced by inhibitory effect of parasympathetic system and the normal physiological status of system is attained. In sympathetic storm or sympathetic hyperactivity, there is an upsurge of the sympathetic response with a reduced parasympathetic response or miscommunication between the two systems. Usually, in the normal physiological state, a person has little neurological activity with negligible attentiveness and vigilance. Due to stimulation and sympathetic storming, activation of reflexive motor responses can change apparently passive status of an individual into a state of confusion [91].

Some severe TBI patients undergo sudden periodic increased stress response, or storming. Within the first 24 hours after injury, storming may occur, possibly linked with poor neurological function triggered by injury. This is most common in the TBI population. The apparent signs of this outburst are anxiety, mental uneasiness resulting from brain injury, unconsciousness due to impaired function of the reticular activating system and hypertension.
(HTN) which would result from impaired function of catecholamine systems in the hypothalamus or the vasomotor center. Other signs include hyperthermia that - in turn - encompasses catecholaminergic mechanisms of thermoregulation, motor dysfunction which could implicate extrapyramidal dopamine systems and finally, increasingly ischemic conditions through stimulation of α-adrenergic receptors in the cerebral vasculature, leading to vasoconstriction. Elevated catecholamines have the potential to cause extra-cranial manifestations such as arrhythmias, pulmonary edema, and immunosuppression. Suppressing this catecholamine surge in non-cardiac surgery, burn and neurosurgical patients, beta-adrenergic blockers have emerged as effective and safe treatment for such patients. Due to this established adrenergic blocking effect, a number of authors used beta-blockers for the same purpose in TBI patients and found it effective [91, 110–112].

1.2.3.5 Activation of sympathoadrenomedullary axis in TBI

Studies have largely recognized that head injury, along with other traumas, causes response involving the whole organism. TBI can lead to structural damage to the central nervous system, and also stimulates the sympathoadrenomedullary axis, which triggers a cascade of deadly consequences, with subsequent worsening of neurological deterioration and severe metabolic or systemic disorders. TBI and multiple stimuli can stimulate adrenergic and noradrenergic neurons in the brain, which eventually affects the peripheral SNS. This results in increased plasma concentrations of catecholamines, adrenocorticotropic hormone (ACTH) and glucocorticoids (GCs). As a response to the stress, the released catecholamines may reduce the extent of ischemic brain damage [97, 113].
Catecholamine levels are one of the suggested indicators in trauma patient’s prognoses. Fear, along with trauma, incites SNS. Conversely, pain is a potent activator of sympathoadrenal axis, which elevates sympathetic tone and the release of catecholamines. Moreover, injury, bleeding and hypovolemia can activate SNS. Increased catecholamine concentration can induce arteriolar constriction and consequently reduce oxygen and metabolic substrates transport to the tissues, resulting in catabolic metabolic state, which is seen in severe trauma patients [98].

1.2.4 Effects of TBI on central and peripheral inflammatory responses through dysregulation of pro- and anti-inflammatory Cytokine balance

1.2.4.1 Pathological responses after TBI

TBI is a multifaceted pathology comprising of several pathophysiological mechanisms of leading to neural cell injury, such as breakdown of BBB, cerebral edema, excitotoxicity, mitochondrial dysfunction and altered cerebrovascular reactivity. The inflammation signifies a common pathological reaction to any neurological disorder including brain trauma. A number of studies have established the role of neuroinflammation induced by TBI, comprising of local infiltration of circulating immune cells, local cytokine production and astrocytic stimulation and propagation [114–116].

1.2.4.2 Expression of inflammatory mediators in the CNS and their role

Cerebral inflammation, though initially thought to be primarily harmful for injured brain, but now is documented, that it can have both beneficial and injurious roles. It is beneficial when the inflammation is present for a defined period. If the inflammation sustains indefinitely, it can
cause various neuropathologies. Pro-inflammatory Cytokines, chemokines, and cell adhesion molecules are the main mediators of this inflammatory response [115, 117, 118].

Recent studies show that cytokines play an important role in propagating the inflammatory response, in addition to promoting neurotoxicity via excitotoxicity and oxidative injury. Moreover, there is growing evidence that cytokines are neuroprotective through angiogenic, neurotrophic, and other mechanisms known to reduce CNS damage [119–121].

In healthy CNS tissue, most inflammatory mediators are comparatively not active and have low or undetectable levels. Conversely, in case of a tissue injury or infection, they can immediately produce various actions. Moreover, studies have shown accumulation of blood borne immune cells contained in the parenchyma in both injured patients and animal models of brain trauma. These activated cells discharge mediators including free radicals, prostaglandins, complement factors and pro-inflammatory cytokines, which consequently induce the expression of chemokines and cell adhesion molecules and mobilize immune and glial cells to the injured tissue. Additionally, neurons, microglia, astrocytes and oligodendrocytes may produce inflammatory mediators and cytokine receptors, which are expressed persistently throughout the CNS although at low levels. As time passes, the successive productions of anti-inflammatory mediators suppress both cellular and humoral stimulation [10, 122].

1.2.4.3 Integrity BBB after TBI

In the acute post-traumatic period, pathological modification of the brain tissue, like impairment of BBB, occurs due to which the circulating neutrophils, lymphocytes and monocytes penetrate the injured tissue. This directly affects the neuronal survival and death. When integrity of the
BBB is compromised due to some pathological disorders, cytokines may also cross the BBB via active transport or through leaky sites of endothelium. Hence, inflammatory mediators, either secreted within the brain, or originating from the periphery, can affect the central nervous system (CNS). BBB disruption after TBI facilitates neuro-inflammation following injury [10, 119, 123].

1.2.4.4 Neuroinflammatory response in the injured brain

Inflammation is a complex process that comprises of several injury signals, cellular reactions and changes in the microenvironment. The role of Inflammation in the central nervous system may be structure or area specific. Its function is to safeguard tissues and organs locally from attacking pathogens across epithelial barriers. The wound becomes filled with blood-derived material comprising of monocytes, which develops in to a plug. These monocytes migrate from the blood into the injured neural tissue where they are converted into macrophages. In the adjacent neural tissue, reactive gliosis starts, which surrounds along the boundaries of the wound by the proliferation and migration of glial cells [13, 118].

After injury, some inflammatory mediators are locally released to control the cellular alterations that occur. The incursion of inflammatory cells to the site of injury may be controlled by the concentration and location of inflammatory factors expressed in the injured CNS. It has been studied that soon after the injury, CNS may generate an initial intrinsic inflammatory response by increasing the levels of IL-1 and TNF-α in and around the injury site before leukocyte migration [12, 124, 125].
1.2.4.5 Role of microglia and astrocytes in brain neuroinflammatory response

Microglia, which are mononuclear phagocytes and resident cells in the CNS, are recognized as the likely sensors of brain injury and producers of cytokines. They are stimulated and proliferated after injury and look like blood macrophages in their capability to secrete factors, scavenge, engulf and clear cellular remains in and around the wound site. After brain trauma, and in other pathophysiological conditions, microglia and blood-derived macrophages are activated and are accumulated swiftly at the site of injury. Activated microglia, synthesize cytokines and trophic factors that may elicit twofold effects on adjacent cells. Microglia release IL-1, which is immunomodulatory, trigger astrogliosis in vivo, and neovascularization at trauma sites [126–128].

The resident macrophages, microglia and astrocytes in CNS can synthesize TNF-α, which are pro-inflammatory during the acute phase of CNS inflammatory response and immunosuppressive during the chronic phase. The mechanisms that regulate the expression of different cytokines are usually related, for instance, TNF-α induces IL-1 and IL-6, whereas IL-1 can stimulate expression of both IL-6 and TNF-α. Hence, after an injury to the brain tissue, the initial upregulation of cytokines results in infiltration of other inflammatory mediators to the area of injury and secondary cytokine signaling is initiated [12].

The elevated levels of activated astrocytes may be useful for injured neurons as they induce neurotrophic effects, control neurotransmitter concentration, repair the extracellular matrix, regulate the blood-CNS interface and transport processes. Astrocytes also give trophic support to injured cells by releasing neurotrophins and pleiotrophins, and quarantining the site of injury.
This isolation is accomplished through transformation of normal basal lamina to ectopic basal lamina for safeguarding neighboring tissue. Astrogliosis occurs when peak levels of IL-1 are produced, comprising of cellular hypertrophy and hyperplasia, signifying that the secretory action of mononuclear phagocytes affect the astrocytes [12, 127, 129].

1.2.4.6 Cytokines and chemokines produced after TBI

Cytokines are polypeptides in nature may mediate intracellular communication and play a key role in tissue homeostasis of the matured organism, in addition to their contribution in immune processes. Cytokines secreted by neurons are mainly involved in cellular communication, while cytokines from glial cells promote neuronal growth, survival and repair. Cytokines are the biggest group of mediators. A number of cytokines, like IL-1β, and TNF-α, are vastly studied cytokines, involved in the inflammatory response [115, 130, 131].

**IL-1β** is a key contributor in the inflammatory response, following trauma, through associated IL-1 receptors (type 1). It is pro-inflammatory, secreted by macrophages and monocytes. IL-1β takes part in the activation, control of the acute phase response, and induces the production of IL-6. It is also responsible for elevating the expression of leukocyte adhesion molecule. In addition, IL-1β is responsible for mediating the damage to the BBB. Conversely, IL-1β may contribute to safe guard the CNS by inducing protective factors for neurons and glial cells [12].

**TNF-α** is considered as a pro-inflammatory and pro-coagulative cytokine and is involved in the development of SIRS and disseminated intravascular coagulopathy. The patients affected by septic complications after trauma have higher levels of TNF-α. TNF-α along with IL-1β mediates the BBB injury. Furthermore, it also activates apoptosis of neurons. TNF-α is
produced by resident macrophages, astrocytes and microglia in the CNS, after traumatic brain injury. Hemorrhage stimulates the production of TNF-α rather than the tissue damage. In the pathogenesis of TBI, TNF-α may produce both destructive and protective effects via different TNF receptors (TNFR). Moreover, TNF-α triggers apoptosis of neurons by stimulating TNFR1 to elicit comprehensive brain damage. Alternatively, TNF-α promotes growth and proliferation of neurons and oligodendrocytes via TNFR2 [12, 115].

**IL-10** is a pleiotropic cytokine. Macrophages, monocytes, B-cells, keratinocytes and Th2-cells can synthesize IL-10. It is involved in compensatory anti-inflammatory response syndrome (CARS), has an inhibitory effect on cytotoxic T-cells, macrophages, NK cells, and it is immunosuppressive. The benefits of IL-10 have also been established in a number of inflammatory or trauma animal models. Administration of IL-10 in animals suffering from experimentally induced TBI inhibited the production of pro-inflammatory cytokines such as TNF and IL-1. In addition, it also observed that IL-10 down regulates the activation of glial cells and improve the damage outcome [132].

**Chemokines** are regarded as key mediators; they are highly basic in nature and have affinity for acidic extracellular components, attracting inflammatory cells from circulation into the injured site [12].

Chemokines also called chemotactic cytokines, consist of a small group of inflammatory mediators that control leukocyte activation and migration and play a vital role in embryogenesis of the nervous system, homeostasis, and host defense. In the nervous system, glia and neurons release chemokines. These cells also express a range of chemokine receptors [115].
1.2.4.7 Systemic inflammatory response following acute traumatic brain Injury

After acute TBI, the concentration of inflammatory mediators increases in the blood circulation. This upsurge of inflammatory mediators leads to systemic inflammatory response, and when it is out of control, it results in complications of hyper-inflammation, subsequently immunosuppression, multi-organ dysfunction syndrome (MODS) and in some cases even death. The systemic reaction to trauma is divided into the early and delayed phase. The immediately occurring phase includes the cardiovascular response; this is helpful to revive from the hemodynamic changes due to hemorrhage. The delayed phase instead comprises of immunological and metabolic alterations. These signs may appear in hours or could take up to several days. It is the delayed phase, which induces lifelong effects and plays a significant role in secondary effects of acute traumatic brain injury [12].

1.2.4.8 Neuroimmune regulation of systemic inflammation

The CNS and systemic immune system communicate with each other via the neuroimmune system, which coordinates the overall pro-inflammatory and anti-inflammatory immune responses by the CARS. IL-1β, IL-6 and TNF-α, present in the circulation, are the major cytokines targeted in CARS to inhibit SIRS [12, 132].

Neuroimmune system consists of two efferent branches, the HPA-axis, responsible for the secretion of GCs, and the SNS, which forms an anatomical relationship between the CNS and systemic lymphoid organs, thus resulting in elevated levels of IL-10. These efferent pathways are anti-inflammatory, therefore providing a mechanism to control the severity of systemic inflammatory response [12].
A bidirectional relationship exists between the endocrine and immune system via the HPA-axis. The CNS cytokines, including IL-1, IL-6 and TNF-α, strong activator of the HPA-axis, and it can result in worsened systemic inflammatory surroundings. Alternatively, GCs may act in an inhibitory or facilitative manner regarding inflammation, depending upon the various parameters such as concentration, compartment and time elapsed following the stimulation of the stress response. Furthermore, when elevated, cortisol gives a negative feedback that inhibits excessive peripheral inflammation, however under certain circumstances; cortisol may also assist peripheral and CNS pro-inflammatory cytokine generation [119, 133, 134].

The release of corticotropin releasing factor (CRF) induces the release of adrenocorticotropic hormone (ACTH), also called corticotropin from adenohypophysis into the systemic circulation. Consequently, this activates the adrenal cortex, thus raising immunosuppressive GCs levels. Subsequently, the increase levels of GCs may inhibit the production of pro-inflammatory cytokines and elevate the levels of TGF-β and IL-10, which are anti-inflammatory in their response. GCs likewise lowers the level of major histocompatibility complex class II (MHC II) expression on antigen presenting cells (APCs), therefore diminishing the ability to exhibit foreign antigens and to activate the immune response. GCs also increase the production of acute phase proteins (APPs), which have anti-inflammatory response [12].

CRF also activates the SNS, which is considered as the second effector limb, subsequently elevating the secretion of catecholamines from the spleen, pancreas, lungs and diaphragm into the circulation. Moreover, catecholamines elevate the levels of IL-10 and reduce the release of
TNF-α, by acting directly on the circulating monocyte activity, thus manage to avoid the hyper-inflammation [12, 132].

In 1992, researchers discovered that the brain produces IL-1β under pathological conditions. Other studies have established that, due to the breakdown of BBB, peripherally synthesized cytokines enter the brain through the blood stream or cerebrospinal fluid (CSF) and secrete into the brain parenchyma, consequently linking the brain to the immune system. Furthermore, leukocytes migration is induced by chemokines, due to their chemotactic action. The role of chemokines in signaling the CNS has been well established and reported in various studies in the 1990s [11, 135].

CARS prevent the development of SIRS. The locally developed pro-inflammatory cytokines released in the brain parenchyma in acute TBI, may diffuse to the preoptic area (POA) and trigger CARS prematurely, thus inducing the compensatory events for hyperinflammation in an under stimulated systemic condition leading to immunosuppression. An increase in ICP due to trauma may cause activation of SNS stimulating both efferent pathways of CARS. Additionally, SIRS causes hyper-inflammation, whereas CARS leads to immunosuppression. The body typically experiences both SIRS and CARS syndromes in alternating phases unless equilibrium is reached [12, 17, 132].

1.2.4.8.1 Systemic changes following acute TBI and SIRS
SIRS is regarded as the response of the whole body inflammation. Normally, the inflammation is locally confined; therefore, development of systemic inflammation indicates an ineffective immune response. SIRS is viewed as preliminary hyper-inflammatory reaction to trauma,
triggering an increase in levels of inflammatory mediators in circulation. Moreover, the spillover of cytokines from the local injury site into the circulation plays a vital role in development of SIRS. The huge increase in levels of such inflammatory mediators in systemic circulation could be harmful to the organs, thus increasing the risk of MODS. In addition to intracerebral pro-inflammatory action, SIRS elicits post-traumatic cerebral damage and systemic complications as stated by the secondary injury concept. Furthermore, the interaction between activated neutrophils and vascular endothelium leading to neutrophil adhesion and transendothelial migration play a critical role in the pathophysiology of SIRS [12].

1.2.4.8.2 Multiorgan dysfunction syndrome (MODS)
MODS is a high mortality complication of trauma and systemic inflammation. It may occur due to infectious or non-infectious reasons. Moreover, MODS typically may occur due to the changes in the functions of systemic inflammatory and immunological processes. The abnormally elevated level of the inflammatory mediators may be the core reason of cellular dysfunction and organ failure.

MODS has been explained in the “two-hit” theory, described by its pathogenesis. The “first-hit”, which is asymptomatic, refers to the primary insult that activates and prepares the immune system to produce a response. On the other hand, the “second-hit” refers to another injury that may be mild, but releases higher concentration of inflammatory mediators, as a result of activated immune system, ultimately causing hyper-inflammation and extensive tissue damage. The plasma elastase levels have been established to correlate with the increased tissue damage.
Other studies have suggested that immunosuppressed state leads to MODS, rather than hyper-inflammation therefore the actual root cause for MODS is not clearly understood [12, 136].

1.2.5 Interrelationships between catecholamines (E/ NE) and cytokine responses

1.2.5.1 Endogenous catecholamines as modulators of immune and inflammatory cells response

According to substantial data available, besides being vital neurotransmitters and hormones, catecholamines are crucial immunomodulators during health and disease. Only in the mid-1990s, it was learnt that lymphocyte-derived catecholamines modulate lymphocyte functions in an autocrine and paracrine manner, offering these cells a useful way to perform their actions with precision and crosstalk with nearby cells. The HPA axis and the autonomic nervous system, comprising of the adrenergic SNS, the vagus-mediated parasympathetic nervous system, and the enteric nervous system, are main systems involved in this crosstalk [138–141]. Moreover, these cells express adrenergic and cholinergic functions consequently coexist in the nervous system and in the immune system. These mediators act as a universal language of a neuro-endocrine-immune modulating network, which allows the nervous, endocrine, and immune system to control their functional responses positively or negatively, and thus permit the body to adjust swiftly to different alterations of internal and external situations.

Catecholamines can modify the functions of human PBMCs, via direct communication using sympathetic nerve fibers that supply lymphoid organs. The adrenergic receptors expressed on, human PBMCs, assist these interactions [73, 140, 141].
1.2.5.2 Occurrence of cytokines in the brain

CNS resident cells may express cytokines under inactive physiological conditions, but cytokines can also be produce during injury and development. In the brain, however, infiltrating macrophages under pathological conditions, may express cytokines. In the CNS, the cellular expression of cytokines is strictly controlled physiologically. Conversely, in some pathological conditions, the expression of many cytokine genes become structurally and temporally modified. The origin of various cytokines affecting CNS directly might be from peripheral immune cells or neuronal cells within the CNS [9, 142].

1.2.5.2.1 Peripheral immune cells

Peripheral immune cells are the key source of cytokines. The peripherally produced cytokines by the immune cells are able to cross the blood–brain barrier, even in normal, healthy conditions.

1.2.5.2.2 Neuronal cells within the CNS

Even in the normal healthy state, CNS neuronal cells are capable of producing cytokines. Within the brain, neurons and glial cells produced cytokines, which may contribute in the complex autonomic, neuroendocrine, metabolic, and behavioral responses to brain injuries, triggered by trauma, ischemia, infection, or inflammation. The brain has CNS–cytokine complex, which is made up of neurons and glial cells. The brain neurons and glial cells not only produce cytokines and express cytokine receptors, but also increase cytokine signals, which sequentially can have deep effects on neurotransmitter, corticotropin-releasing hormone (CRH) function and also on behavior. There is growing consideration that many cytokines also play a
vital role in the central nervous and endocrine systems. The monitoring pathways that regulate
the immune system consist of mediators from the brain, most markedly catecholamines (CA),
and several hormones, like corticosteroids, produced by the endocrine system [9, 143–145].

1.2.5.2.3 Peripherally secreted cytokines and chemokines control after TBI

After TBI, the inflammatory cells are mobilized due to activation of signaling pathways.
Secretion of multiple inflammatory mediators like cytokines, chemokines and DAMPs are
elevated. DAMPs consecutively restimulate the inflammatory mediators and worsen the
damage. Cytokines has a key role in generating body’s response to TBI [11].

1.2.5.3 The pro-inflammatory and anti-inflammatory cytokines balance

The balance between the pro-inflammatory and anti-inflammatory cytokines is closely linked to
neurotoxic and neuroprotective mechanisms. Neurodegeneration takes place when pro-
inflammatory cytokines, like IL-1 or TNF−α are produced, irrespective of their origins whether
CNS or systemic. In CNS, anti-inflammatory cytokines preserve homeostasis, which inhibit
inflammatory responses, thus safeguarding the cell viability. Pro-inflammatory and anti-
inflammatory cytokines indirectly suppress the synthesis of each other, providing a further
regulation of the balance between neurodegenerative and neuroprotective effects. Due to this,
the cytokines help to select populations of mature cell types by increasing survival of some and
removing others through apoptosis [9, 131, 146, 147].

Acknowledging that the probability that pro-inflammatory cytokines are not neurotoxic, it is
likely that they modify neuronal survival, by intracellular receptor-receptor interactions and by
cell-cell interactions. After brain injury, primarily microglia, produces IL-1 and TNF-α, but in
the CNS, cytokines may also be produce by astrocytes, oligodendrocytes, and other cells, which may interact with each other. This is reinforced by another perception of neurodegeneration that suggests the presence of a diverse level of regulation between pro- and anti-inflammatory cytokines in the brain. This perception is based on the principle that within the cell, cross talk between heterologous receptors on a single cell can either stimulate or impede receptor activity. TNF- receptors, which represent a death signal, interacts with insulin-like growth factor 1 (IGF1) receptors, which are receptors for survival signal, may antagonize survival signal, and consequently inhibit IGF1-facilitated neuro protection [9, 148].

1.2.5.4 Role of catecholamines and cytokines receptors in the inflammatory response

The immune organs are innervated by the sympathetic nerve fibers and, when SNS is activated, signaling molecules discharge in the surrounding area of immune cells. Hence, sympathetic neurotransmitters, together in the CNS and in the periphery modulate cytokine balance. Various immune cells, express neurotransmitter receptors that are sensitive to monoamines, and activation of these receptors, modulate the production of cytokines and other immune or inflammatory mediators like chemokines and free radicals. As soon as the neurotransmitters interact with the target cells, they occupy their proper receptors and start transducing signals, triggering the cytokine production of the cell. To produce their effects, the catecholamine, E and NE bind to transmembrane spanning G-protein combine with cell surface receptors called adrenoceptors. Sufficient data is available regarding the immunomodulatory effect of catecholamines in which cAMP plays one of the key roles. Consequently, occupation of neurotransmitter receptors that activate or inhibit adenylate-cyclase also effect the cytokine
profile of the system. With the activation of β2-adrenergic receptor, adenylyl cyclase is stimulated through stimulatory G proteins (Gs) and subsequent elevation of intracellular cAMP levels. Several immune cells express both α2- and β2-adrenoceptors on the surface. α-2 adrenoceptors on macrophages, when occupied, induce suppression of the intracellular cAMP level. Since it is established that cAMP suppresses the inflammatory immune response, sympathetic control of the innate immune response is supposed to be essentially immunosuppressive [9, 138, 149–152].

NE and the adrenergic drugs could affect the immune response directly, through adrenergic receptors expressed on macrophages and likewise on other immunologically capable cells. The immune response is also affected indirectly through alteration of locally available NE levels induced by the action of release-regulating presynaptic α-2-adrenoceptors (α-2-AR) situated on sympathetic nerve terminals. Stimulation of the presynaptic α-2-adrenoceptors results in a negative effect on NE discharge, leading to reduced extracellular NE levels. Mostly direct effect of NE is possible through several immune cells, which express the β-2 adrenoceptors. The secreted catecholamines induce their action in an autocrine or paracrine feedback manner. The catecholamines from adrenal medulla directly stimulate and modify intracellular functions of immune and inflammatory cells. Hyperactivity of the SNS over the time, in conjunction with end-organ damage and dysfunction, lead the researchers to the use of β-blockers and adrenergic neuron blocking drugs in TBI [9, 15, 73].
1.2.5.5 Catecholamine–cytokine balance

The modulation of cytokine balance by catecholamines is controlled by the period when effective concentration of these monoamines is maintained. Catecholamines maintain their volume by keeping a balance between their vesicular release in extracellular area, their reuptake by the monoamine transporter and their breakdown by MAO system. Inflammatory cytokines induced intense stimulatory effects on the HPA axis hormones, CRH, as well as effects on neurotransmitter metabolism. Mostly these effects are facilitated by the cross-talk of cytokines and their receptors within the HPA axis tissues that expedite the integration of cytokine signals [9, 153, 154].

Numerous external and/or internal stimuli, like bacterial endotoxin, lipopolysaccharide (LPS), viral infections, prostaglandins, cytokines, are capable of triggering the immune system via macrophages, causing production of elevated quantities of pro-inflammatory cytokines, such as interleukin (IL)-1, IL-12, and TNF-α. Recent studies have also provided sufficient data indicating the association concerning stress or other conditions that effect catecholamine levels and cytokine production. According to numerous studies, in spite of suppressing certain immune processes, pro-inflammatory activation in the periphery is associated with the SNS stimulation. This peripheral pro-inflammatory activation may, in turn, affect inflammatory processes in the CNS. A brief interaction of E with mononuclear cells in vitro, inhibited endotoxin- induced production of TNF-α, producing anti-inflammatory effect, whereas pre-exposure for 24 hours lead to an increased TNF-α production and consequently induce pro-inflammatory effect. E reduced TNF-α and elevated IL-10 production during endotoxemia in healthy volunteers, when infusion was started 3 hours prior to injection of endotoxin. It was also
observed that E triggered a dose-dependent reduction in LPS-induced IL-1β production, which was probably facilitated through adrenergic β receptors [9, 149, 155–157].

Regarding the inflammatory response, the role of catecholamines is not defined. It has been established that E can activate proteases, thereby initiating discharge of kinins and indirectly increasing capillary permeability. Moreover, histamine and bradykinin, were known to release E, and thus cause an increase in the local concentration of catecholamines at the site of injury. On the other hand, it has been proposed that E might be a normal anti-inflammatory agent causing a reduction in capillary permeability [158].

Anti-inflammatory cytokines can produce an anti-inflammatory effect, either like IL-10, which produces its own anti-inflammatory effect, or by the receptors like soluble TNF receptors II, which blocks the pro-inflammatory stimuli by binding to their cell-surface receptors. It is acknowledged that IL-10 impedes LPS-induced IL-1β production. Hence, it is possible that E inhibits LPS mediated IL-1β production in whole blood at least partly by increasing the release of IL-10. It is seen that E does not inhibit IL-1β production in the presence of anti-IL-10. Therefore, we can conclude that inhibiting effect of E on IL-1β production depends on the synchronized enhancing effect of E on IL-10 production. It has been established that the inhibition of IL-1β production by E also depends on the synchronized inhibiting effect of E on TNF production. The production of IL-1β during gram-negative bacteremia in vivo is partly dependent on TNF production, and Inhibition of TNF production by E might consequently contribute to E- induced inhibition of IL-1β production. Anti-TNF inhibition of LPS-induced IL-1β production indicates that TNF is partly responsible for LPS-induced IL-1β production in
whole blood. It is shown that E inhibits the production of potent pro-inflammatory cytokine IL-1β, providing more support for the perception that E may act to reduce disproportionate pro-inflammatory effects of cytokines during the early phases of systemic infection [9, 138, 149, 157].

1.2.5.6 Bidirectional communication between cytokines and neurotransmitters

CNS and the immune system can communicate with each other bi-directionally. The brain affects the immune system through the HPA axis (GCS) and by the sympathetic (E and NE) and parasympathetic (acetylcholine (ACh)) pathways of the autonomic nervous system (ANS). The ANS is divided into the sympathetic (noradrenergic) and parasympathetic (cholinergic) nervous system, which usually produce opposing effects on various functions. The internal and external stimuli disturb physiological stability of these systems, resulting in stimulation of the HPA-axis and the SNS. SNS postganglionic fibers are mostly noradrenergic, and the effects on target organs depend upon the type of receptor expressed on those cells. The ANS releases its neurotransmitters from varicosities in a paracrine manner into their surroundings where the distribution of neurotransmitters may be across several micrometers. This type of association is signified as “nonsynaptic”, and targets a larger cell population than synaptic connections. In a highly stimulated state such as sepsis, with this nonsynaptic transmission, there is a risk of transmitters like NE, may spill over into the circulation and produce undesired effects in distant organs [74, 159, 160].

The physiological parallel to the adrenergic system, is cholinergic system, which is a vital part of human macrophage and lymphocyte regulation. Human macrophages regulate their cytokine
release through interactions of ACh with nicotinic acetylcholine receptors expressed on cell membranes. Activation of the α7 sub-unit of the nicotinic acetylcholine receptors on macrophages strongly inhibit the production of TNF-α IL1β, and other cytokines. Human peripheral blood lymphocytes also have known to express a number of cholinergic mediators including Ach [73].

The severity of brain injury is linked to an upregulated sympathetic activity and a downregulated parasympathetic tone. The established autonomic imbalance reduces HRV and apparently the probability of myocardial ischemic injury due to amplified metabolic demand. Acute myocardial infarction patients when treated with propranolol, restores the parasympathetic tone and reduces sympathetic activity [83].

1.2.5.7 Muscarinic control of inflammatory response

Activation of the vagus nerve considerably downregulates the synthesis of IL-1, IL-6, IL-8 and TNF, but does not affect the release of the anti-inflammatory cytokines IL-10 and transforming growth factor (TGF). As such, this essentially acts as a centrally regulated inhibitor of the immune and inflammatory reaction to a variety of infectious and traumatic insults. Even though the vagal control of the inflammatory response in under central control, the exact mechanism is not completely understood. The muscarinic receptors (M1-subtype) may play a vital role as they are widely distributed within the brain [15].
1.2.6 Potential value of beta blocker therapy on TBI outcome

1.2.6.1 TBI and non-neurologic organ dysfunction

A number of TBI cases that result in death are triggered by non-neurologic organ dysfunction. In a study, it was observed that 89% of TBI patients had non-neurologic dysfunction, out of which 18% suffered cardiovascular failure while 23% had respiratory failure. Most authors believe that after TBI, hyper-adrenergic state is the main cause to extracranial injuries. Brain and cerebral vasculature express adrenergic receptors. Efforts are being made to explore the effect of stress, on CBF and energy metabolism, which has lead the researchers to conclude that β-adrenergic receptors play key role in the stress that effects on cerebral blood and energy metabolism. They strongly suggest that catecholemines, systemic from adrenal medulla, central from locus ceruleus and sympathetic from the superior cervical ganglia are the main sources, which may activate cerebral β-adrenergic receptors. As a result, there is a need to search for treatments, efficacious enough, in reducing the effects of sympathetic hyperactivity, which could help improve survival chances for patients with TBI. Nowadays, the authors of several retrospective studies are studying whether β-blockers could be beneficial for patients with TBI.

It is observed that use of β-blockers, in high-risk patients, undertaking non-cardiac surgery, minimize in hospital deaths. Not all beta blockers can cross BBB, hence survival benefit of beta blockers, to the TBI patient, are systemically or centrally mediated is currently unknown [83, 161–164].

1.2.6.2 Use of beta blockers after TBI

β-blockers illicit their neuroprotective effects through reducing CBF, less consumption glucose and oxygen, and thus reducing cerebral metabolism. β-blockers can slow down the
catecholamine induced catabolic state in burn victims. In a study, 11 patients with severe head injury were given anti-hypertensive therapy such as beta 1- antagonist, metoprolol, and alpha 2-agonist, clonidine. They were also administered dihydroergotamine as a potential precapillary vasoconstrictor. It was found that nine patients survived with excellent neurological outcome. It was debated that maintaining the CBF to normal levels, leads to retain normovolemia and presents improved efficiency to reduce the interstitial tissues volume. Studies conducted in the 1970s observed potential link of β- blockers use and improved mortality in those patients suffering from hyperadrenergic state after intracranial pathology like organ dysfunction and death. A decline in metabolic requirement of between 5% and 18% was observed when patients with severe head injury were administered propranolol. This may be due to the effects of β-blockers on heart rate, because 10% of resting energy may be consumed due to tachycardia. In two randomized, controlled trials conducted on the patients-with severe TBI, it was observed, that patients treated with propanol had a decreased intensity and duration of hyperadrenergic state, improved neurological recovery and less complication regarding the cardiac and respiratory systems. Blocking with β- blockers locally, may leads to reduction in severity of intracerebral posttraumatic catecholamine induced vasospasm, limiting the chances for development local ischemia. Propranolol has been mainly used in early studies because of its more lipophilic nature than most of the beta–blockers, due to this property, propranolol can readily cross the blood brain barrier, hence providing better central as well as peripheral action and propranolol has also shown to reduce hyperthermic response to brain injury. In another study, 420 patients with severe TBI (head AIS> 3) were examined to evaluate the effectiveness of βB, of these 420 patients, 174 received βB for two or more consecutive days. Even though
these patients were significantly older, more severely injured and had a much lower chance of survival, yet they had a significantly lower mortality rate (5.1% vs 10.8%). In recent times, two reviewing studies of human patients with TBI were published. In the first study, out of 4,117 trauma patients admitted in three years, 303 received β-blockers. Patients treated β-blockers were 30% less likely to die (p 0.0001). In the subset of head-injured patients with head injuries and GCS of 13 or less, it was found that β-blockade provided significant chances of survival [15, 71, 80, 83, 109, 110].
Chapter 2
Research aims and hypotheses

Injury severity is known to cause an exponential rise in catecholamines levels leading to an intense sympathetic discharge. This dramatic posttraumatic surge of catecholamine is believed to be associated with poor patient outcome, and maybe used as prognostic biomarkers. This assertion is supported by previous studies that have found high catecholamine levels were linked with injury severity (GCS) and neurologic outcome (GOSE).

Cerebral and systemic immuno-inflammatory alterations are a common element of many molecular and cellular abnormalities seen after TBI. Evidence indicates that catecholamines play a prominent role in regulation of host immune and inflammatory responses. Experimental studies demonstrated that catecholamines modulate monocyte–macrophage functional activities and alter their local production of E, NE, and cytokines, thereby initiating and amplifying the inflammatory response to trauma. High catecholamines levels can induce immune cell extravasation, cellular adhesion molecule expression and inflammatory mediator releases. All of which signifies that the posttraumatic sympathetic surge and hyperadrenergic state following TBI are harmful to the injured brain with bad outcome. Recent studies, however, have indicated that patients taking β-blockers, which attenuate catecholamines release, have a good outcome and these finding strengthens the hypothesis that catecholamines contribute to the pathogenesis of TBI. In order to understand the potentially harmful effects of catecholamines in TBI, our study will correlate their levels to pro- and anti-inflammatory mediators (TNF-α, IL-1β, and IL-10).
Thus, the aims of the study were as follows:

#1: Compare E, NE and pro/anti-inflammatory cytokine concentrations in TBI patients with those found in neurosurgical patients and healthy controls.

#2: Examine the potential inter-relationship between catecholamine and cytokines in association with clinical outcomes between TBI and Neurosurgical patient groups.

As a result of the above aims, the following hypothesis were formed:

#1: Catecholamine and Cytokine concentrations observed in TBI patients will be highly elevated in comparison to those found in neurosurgical patient groups and healthy controls. Catecholamine and Cytokine levels in neurosurgical patient group will also be elevated in relation to the levels within healthy patient group.

#2: High circulating levels of catecholamines are associated with dysfunctional pro/anti-inflammatory cytokine release and poor neurologic outcome in TBI and neurosurgical patients.
3.1 Study design

Our study focused on determining alterations in pro- and anti-inflammatory cytokine responses in relation to changes in SNS activation, after either TBI or neurosurgery. To accomplish this, we studied a cohort of 181 patients sustaining isolated moderate-to-severe TBI in a prospective blinded manner. In addition, we examined the inflammatory profiles in a group of 17 elective neurosurgery patients. We assessed circulating plasma catecholamines (E, NE), pro-inflammatory (IL-1β, TNFα) and anti-inflammatory (IL-10) cytokines over a 24-hours period. We also correlated the plasma levels of catecholamines with the severity of trauma using the extended GOSE score at discharge, adjusted for the age and initial GCS score. Three Level 1 trauma centers, Sunnybrook Health Sciences Center, St. Michael’s Hospital in Toronto Canada and Los Angeles County + University of Southern California (USC) and Medical Center (Los Angeles, CA), participated in this study by collecting the blood samples of the admitted TBI and neurosurgical patients.

3.2 Study participants

The participants (n=248) consisted of isolated TBI patients (n=181) (severe TBI n=143; moderate TBI n=38), neurosurgical patients (n=17) and healthy control group (n=50). They were aged between 16 and 96 years with a mean age of 47. Only patients sustaining isolated, severe brain trauma with a GCS score of 3-4, or patients with moderate brain trauma (GCS > 9) and a non-head Abbreviated Injury Score (AIS) < 2 were considered for this study. Patients
who had suffered head trauma for more than 3 hours before admission, aged less than 16 years, pregnant, or showing an absence of vital signs, were not included in this study. Upon admission to Sunnybrook Health Sciences Center, St. Michael's Hospital and LA County Hospital, informed consent was obtained for the qualifying patients. For the patients who were unable to provide informed consent, it was obtained from the substitute decision maker. Moreover, if there was no substitute decision maker available, then informed consent was deferred in accordance with the Tri-Council Policy Agreement for Research in Emergency Health Situations (Article 3.8). The delayed consent was obtained from the next-of-kin and subsequently from the patient’s themselves if they recovered sufficiently.

3.3 Blood sample collection and handling

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**Figure 1-A** Illustration of blood drawing schedule
Venous blood samples for catecholamines (E, NE) and cytokines (IL-1β, TNF-α, and IL-10) were drawn from patients at admission to emergency department, 6-, 12-, and 24-hours post-injury. A 10-mL K$_2$EDTA (Vacutainers, Becton Dickinson) was used to draw the blood samples from the patients and centrifuged at 1600x g for 15 minutes at 4°C. The separated plasma was divided into two aliquots (VWR 1.5 ml Micro-Centrifuge Tubes) of 1.5 mL each and were stored at -70°C. These aliquots were transported to participating Defense Research and Development Canada Laboratory in Toronto. Upon receipt, one of the EDTA plasma sample (1.5 mL) was thawed, centrifuged, separated into 150 µL aliquots and frozen at -70°C. Thus, all the aliquoted samples had the same freeze-thaw history at the time of analysis. The second EDTA plasma sample was assessed E and NE quantitation.

3.3.1 Protocol of circulating catecholamines analyses

Estimation of plasma (pg/L) E and NE concentrations was performed in duplicate, using a direct competitive enzyme immunoassay method according to the manufacturer’s instructions (Bi-CAT EIA, Alpco Diagnostics, Salem, NH). Extraction of plasma E and NE was performed, by using a cis-diol-specific affinity gel, acylated, and then derivatized enzymatically into N-acylmetanephrine and N-acylnormetanephrine, respectively. Antibody bound to the solid phase catecholamines were detected by an anti-rabbit IgG-peroxidase conjugate using tetramethylbenzidine as a substrate. This colorimetric reaction was terminated by the addition of 0.25 M sulphuric acid and the absorbance measured at 450 nanometres (nm) and 630 nm using a multi-detection micro plate reader (PerkinElmer VICTOR 3, Waltham, MA). Quantitative analysis of unknown samples was performed by comparing their absorbance with a
reference curve prepared with known standard concentrations included in the kit. Detected antibody was inversely proportional to catecholamine concentrations of the sample.

### 3.3.2 Protocol of circulating pro and anti-inflammatory cytokines analyses

Table 3 Reagent Specifications

<table>
<thead>
<tr>
<th>Product</th>
<th>Specification</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MULTI-SPOT® 96-well 10-Spot Human TH1/TH2 10-Plex Plate</td>
<td>N05010A-1</td>
<td>2-8°C</td>
</tr>
<tr>
<td>SULFO-TAG Detection Antibody Blend</td>
<td>50X</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Human TH1/TH2 10-Plex Calibrator Blend</td>
<td>1 μg/ml of each</td>
<td>≤ 70°C</td>
</tr>
<tr>
<td>Diluent 2</td>
<td>R51BB-4 (8 ml) R51BB-3 (40 ml)</td>
<td>≤ 10°C</td>
</tr>
<tr>
<td>Diluent 3</td>
<td>R51BA-4 (5 ml) R51BA-5 (25 ml)</td>
<td>≤ 10°C</td>
</tr>
<tr>
<td>Read Buffer T</td>
<td>4X R92TC-3 (50 ml) R92TC-2 (200 ml)</td>
<td>Room Temperature</td>
</tr>
</tbody>
</table>

List of products and their storage temperatures.

In this assay, all samples were analysed in duplicates, using multiplexed high density plate immunoassay format (10-Plex Kit: IL-10, TNFα, and IL-1β). The standards were prepared with Diluent 2. Diluent 2 was added in wells of MULTI-ARRAY, or MULTISPOT plate and incubated for 30 minutes on VWR Microtiter plate shaker at 300-1000 rpm at room temperature.

In addition, 25 μL of each standard, control and sample was added to two adjacent wells and the plate was incubated on plate shaker for 2 hours at 300-1000 rpm at room temperature. Upon completion of incubation, the plates were washed three times on BioTek ELx50 Microplate Strip Washer using PBS+0.05%Tween 20. Twenty-five μL of Detection Antibodies were added in each well and the plate was sealed and incubated on plate shaker for 2 hours at room temperature (300-1000 rpm). The plates were then again washed three times as before 150 μL
of Read Buffer was added in each well of the plate and the plate was read using the SECTOR Imager 6000 reader. The electrochemiluminescence signal was measured by photodetectors and analysis was performed using Discovery Workbench 3.0 software.

3.3.2.1 Specifications of plate and reagents used
TH1/TH2 human 10-plex kits were used to analyze IL-1β, TNFα, and IL-10. Multi-spot 96 well 10 spot human TH1/TH2 10 Plex Plates (N05010A-1) were used in the assay. Each well had 10 carbon electrodes, which were pre-coated with one of the 10 anti-cytokine antibodies of interest.

3.3.2.2 Reagent preparation
3.3.2.2.1 Calibrator
The Calibrator stock was thawed on ice and the reagents were allowed to reach the room temperature. Diluent 2 and Diluent 3 were separated into required sized aliquots.

3.3.2.2.2 Prepare calibrator and control solutions
The cytokines calibrators had an initial concentration of 1 μg/mL and were diluted using diluent-2. The calibration curve was prepared according to the instruction listed below. It generated an 8-point standard curve from 2500 pg/mL to 0.61 pg/mL, and would be calibrated to accommodate the range of blood samples. The measurements were repeated twice for each concentration. Moreover, 25μL of calibrator was added to first two columns of Multi-Array or Multispot plate. In addition, 4-fold serial dilution steps were prepared for the assay whereas only Diluent 2 was utilized for the 8th point.
Stock calibrator was diluted by taking 10 μL of the Human TH1/TH2 10-Plex Calibrator Blend (Ultra-Sensitive) and added 990 μL of Diluent 2 to make diluted calibrator stock. 50 μL of diluted calibrator stock was then added to 150 μL of Diluent 2 in order to prepare the highest calibration point STD-01. The 4-fold serial dilutions were repeated six additional times in order to prepare seven calibrators. The 8th calibrator is only Diluent 2 (zero calibrator).

The initial concentration for the Detection Antibody stock was 50X. In order to dilute it to 1X, which is the required concentration, 60 μL aliquot of Detection Antibody stock was added to 2.94 mL of Diluent-3.

3.3.3 Determination of circulating cytokine concentrations

![Diagram of electrochemiluminescence process]

**Figure 1-B** Process of electrochemiluminescence
This assay employed a sandwich immunoassay format in which the captured antibodies were coated in a single spot or in a patterned array on the bottom of the wells in the plate. A 96-well 10 spot human TH1/TH2 10 Plex Plates (N05010A-1) was used to estimate cytokines (IL-1β, TNF-α, and IL-10) in a multiplex cytokines assay. Each well had 10 carbon electrodes, which were pre-coated with one of the 10 anti-cytokine antibodies of interest. During one or more incubation periods, the plasma EDTA samples, a solution consisting of anti-cytokine antibody labeled (labeled detection antibody) with an electrochemiluminescent (ECL) compound, SULFO-TAG label were added. The added cytokine binds with the antibody immobilized on the surface of working electrode. With the addition of the labeled detection antibody, the bound cytokine completes the sandwich. At this point MSD read buffer was added to provide the suitable chemical environment for electrochemiluminescence. Upon completion of the preparation of the plate, it was inserted into Sector Imager 6000 for analysis. Sector Imager 6000 applied an electrical current through the electrodes bounded inside the plate wells to emit light. The mechanism for generation of ECL from ruthenium tris(bipyridine) complexes at an oxidizing electrode and the presence of tripropylamine read buffer enhanced the electrochemical signal. The luminescent signals were captured via charged-couple device camera and the intensity of the emitted light was measured by photodetectors. Furthermore, analysis was performed using Discovery Workbench 3.0 software. The method provided a quantitative measure.
3.3.4 Advantages meso scale discovery technology over elisa and bead-based multiplex assay

Rather than using a traditional singleplex enzyme-linked immunosorbent assay (Elisa), our study employed multiplex Meso Scale Discovery Technology (MSD). This was done because MSD support analysis of multiple inflammatory molecules at the same time, requires smaller sample volume, and has high throughput multiplex analysis all of which mean it is efficient and effective in terms of time and cost. The MSD technology of multiplex assays is also reproducible and reliable regarding detection of different proteins across a wide dynamic range of concentrations, and also provides higher frequency quantifiable endogenous cytokines when compared with other multiplex assays [165–169].

MSD multiplex assay has been shown to be better in most situations when compared with Cytometric Bead Array (CBA). This was documented in a study, which illustrated that when cytokines were measured using MSD and CBA, had similar sensitivities on standard curves, MSD experienced better recovery of exogenous recombinant cytokines added to serum and better quantification of endogenous cytokines in serum. In serum, MDS was consistently able to quantify levels of IL-12p70, TNF-α, and IL-10, which were undetectable by CBA assays [170].

3.3.5 Data entry and storage

Measurements taken within the study were entered into a central Microsoft excel sheet. Measurements for catecholamine and cytokine concentrations were taken at the admission, 6, 12 and 24-hour time points. In addition to this, the gender, age, GOSE score, AIS head, and ISS were also recorded. Excel formulas were used to calculate averages, standard deviation and
standard error of mean. The following tables presented below show the counts of number of

time points collected.

Table 4 Time points collected

<table>
<thead>
<tr>
<th></th>
<th>Admission</th>
<th>6 hours</th>
<th>12 hours</th>
<th>24 hours</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>180</td>
<td>167</td>
<td>160</td>
<td>154</td>
<td>661</td>
</tr>
<tr>
<td>NE</td>
<td>180</td>
<td>167</td>
<td>160</td>
<td>154</td>
<td>661</td>
</tr>
<tr>
<td>IL-1β</td>
<td>104</td>
<td>103</td>
<td>111</td>
<td>102</td>
<td>420</td>
</tr>
<tr>
<td>TNF-α</td>
<td>183</td>
<td>173</td>
<td>169</td>
<td>158</td>
<td>683</td>
</tr>
<tr>
<td>IL-10</td>
<td>183</td>
<td>170</td>
<td>165</td>
<td>156</td>
<td>674</td>
</tr>
<tr>
<td>Total</td>
<td>830</td>
<td>780</td>
<td>765</td>
<td>724</td>
<td>3099</td>
</tr>
</tbody>
</table>

Number of measurements of that were collected at each time point for each Catecholamine and Cytokine.
Abbreviations: E, Epinephrine; NE, Norepinephrine; IL, interleukin; TNF, tumor necrosis factor.

3.3.6 Statistical analysis

Results are expressed as means ± standard error of the mean (SEM) unless otherwise stated.

Statistical analyses were performed using a StatView 5.0 (SAS Institute) microcomputer
software package, with statistical significance set at $P < 0.05$. Possible interactions were
analyzed using repeated measures analysis of variance (ANOVA); a conservative correction to
sphericity, Greenhouse-Geisser correction, was applied to all repeated ANOVA. When
significant $F$ ratios were observed, differences among control and patient groups at each time
point were examined using Bonferroni’s post hoc tests.
Chapter 4
Results

4.1 Participant characteristics

A total of 248 subjects were enrolled during the study period. Participants were categorized into three main groups, comprising isolated TBI patients, elective neurosurgical patients and healthy controls. TBI patients (n=181) were subdivided into patients sustaining either severe TBI (n=143) or moderate TBI (n=38), as defined by their GCS scores. Neurosurgical group (n=17) and healthy volunteers (n=50) served as control groups for comparison with the TBI cohorts. Healthy volunteers were recruited by local advertisement. Tables 4 and 5 summarize the demographic and clinical characteristics of the TBI patients and neurosurgical patients / healthy controls, respectively. TBI and neurosurgical patients were admitted to Sunnybrook Health Sciences Centre, St. Michael’s hospital and LA County Hospital after severe head injury or for elective neurosurgery. Enrolled patients were predominantly males (n=194, 78%) with a mean age of 44 ± 19. Mean ISS values within severe and moderate TBI patients were 26 ± 0.94 and 18 ± 1.79 respectively. Mean AIS values for the same groups were found to be 4 ± 0.09 and 3 ± 0.20 respectively. Average GCS values in severe and moderate TBI patient groups were 5 and 11, respectively while the corresponding value in Neurosurgical patients was 10. All TBI patients enrolled in this study had an elapsed time between trauma and admission to the emergency department of less than 3 hours, were at least 16 years of age, were not pregnant and showed no absence of vital signs prior to admission.
Table 5. Demographic and clinical outcomes of TBI patients stratified by TBI severity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pooled TBI Patients (n=181)</th>
<th>Moderate (n=38)</th>
<th>Severe (n=143)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47.14 ± 21.0</td>
<td>51.16 ± 20.5</td>
<td>46.08 ± 21.0</td>
</tr>
<tr>
<td>Gender (% Male)</td>
<td>137 (75.7%)</td>
<td>27 (71.0%)</td>
<td>110 (76.9%)</td>
</tr>
<tr>
<td>ISS</td>
<td>23.64 ± 11.55</td>
<td>18.02 ± 11.05</td>
<td>25.12 ± 11.80</td>
</tr>
<tr>
<td>AIS Head</td>
<td>4.02 ± 1.11</td>
<td>3.45 ± 1.20</td>
<td>4.17 ± 1.01</td>
</tr>
<tr>
<td>GCS</td>
<td>5.96 ± 3.09</td>
<td>10.79 ± 1.38</td>
<td>4.67 ± 1.93</td>
</tr>
</tbody>
</table>

Demographic data for patients with severe and moderate TBI that were admitted to Sunnybrook and St. Michael’s hospital. All values are expressed as Mean ± Standard Deviation. Abbreviations: ISS, Injury Severity Score; AIS, Abbreviated Injury Score; GCS, Glasgow Coma Scale; GOSE, Glasgow Outcome Scale Extended.

Table 6. Demographic and clinical variables of control groups

<table>
<thead>
<tr>
<th></th>
<th>Neurosurgical (n=17)</th>
<th>Healthy (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>51.17 ± 16.28</td>
<td>30.32 ± 7.65</td>
</tr>
<tr>
<td>Sex (% Male)</td>
<td>58.8%</td>
<td>92.0%</td>
</tr>
<tr>
<td>GCS</td>
<td>10.07 ± 1.31</td>
<td>NA</td>
</tr>
</tbody>
</table>

Demographic data for patients undergoing elective neurosurgery and healthy controls. All values are expressed as Mean ± Standard Deviation. Abbreviations: GCS, Glasgow Coma Scale

4.2 Catecholamine levels in TBI Patients, Neurosurgical, and Healthy Controls

Plasma concentration of E and NE in TBI and neurosurgical patients relative to healthy control subjects are summarized in Figure 2-A and 2-B respectively. E and NE levels in TBI patients were found to be higher than those observed in healthy controls and neurosurgical patients at all time-points ($p < 0.05$ at admission, 6, 12, and 24 hours versus healthy controls and $p < 0.05$ at admission, 6, and 12 hours versus neurosurgical controls for E and $p < 0.05$ at admission, 6, 12, and 24 hours versus healthy and neurosurgical controls for NE). E concentrations were elevated in TBI patients at the time of admission (877 pg/ml), approximately 13- and 8-fold higher than
healthy and neurosurgical patients respectively, but can be seen to decrease over the next 24 hours.

![Graph](image1)

**Figure 2-A** Epinephrine concentration (pg/ml) in all TBI \((n=181)\) and neurosurgical \((n=17)\) patients vs. healthy controls \((n=50)\). Values are expressed as Mean ± SEM. \(p\) values calculated using ANOVA.

![Graph](image2)

**Figure 2-B** Norepinephrine concentration (pg/ml) in all TBI \((n=181)\) and neurosurgical \((n=17)\) patients vs. healthy controls \((n=50)\). Values are expressed as Mean ± SEM. \(p\) values calculated using ANOVA.
NE concentrations were also elevated within TBI patients at admission (4547 pg/ml) with levels 8-fold and 7-fold greater than those in healthy and neurosurgical patients, respectively. E and NE concentrations in neurosurgical patients were observed to be higher at all time points than the corresponding values in healthy patients ($p < 0.05$ at admission, 6, 12, and 24 hours versus healthy controls for E and $p < 0.05$ at admission, 6, 12, and 24 hours versus healthy controls for NE).

### 4.3 Cytokine levels in TBI, neurosurgical, and healthy controls

![IL-1β Levels in TBI, Neurosurgical, and Controls](image)

**Figure 3-A** Interleukin [IL-1β] concentration (pg/ml) in TBI patients ($n=181$) vs. Neurosurgical patients ($n=17$) vs. healthy controls ($n=50$). Values are expressed as Mean ± SEM. $p$ values calculated using ANOVA.

Compared to healthy subjects, TBI patients displayed higher IL-1β levels at all time points ($p < 0.05$ at admission, 6, 12, and 24 hours versus healthy controls) and also remained higher at 6, 12 and 24 hour time points when compared to neurosurgical controls ($3A p < 0.05$ at 6, 12 and 24 hours versus neurosurgical controls). IL-1β levels in TBI patients were lowest at admission and peaked at 24 hours (0.57 pg/ml) (>4-fold increase versus healthy controls and over 2-fold...
increase versus. Neurosurgical patients at 24 hours). The reverse was found in the neurosurgical patients, where an overall decrease in IL-1β levels was observed at 24 hours, with peak levels occurring at the 6 h time point and the nadir at 24 hours ($p < 0.05$ at admission and 6 hours versus. healthy controls).

The TNF-α levels were highest in TBI patients compared to both neurosurgical patients and healthy controls all time points (3-B, $p < 0.05$ at admission, 6, 12, 24 hours versus healthy controls and $p < 0.05$ at admission, 6, 12, 24 hours versus neurosurgical controls). The observed concentrations in neurosurgical patients were also higher at all time points relative to healthy controls ($p < 0.05$ admission, 6, 12, and 24 hours versus healthy controls). TNF-α concentration within TBI patients was observed to be the lowest at admission and the peak value at 24 hours (5.69 pg/ml). Neurosurgical patients showed an overall decrease until 12 hours, followed by an
increase at the 24-hour time point ($p < 0.05$ at admission, 6, 12, and 24 hours versus healthy controls).

**Figure 3-C** IL-10 concentration (pg/ml) in TBI patients ($n=181$) vs. neurosurgical patients ($n=17$) vs. healthy controls ($n=50$). Values are expressed as Mean ± SEM. $p$ values calculated using ANOVA.

Plasma IL-10 level were found to be elevated at all time points in TBI patients when compared to healthy controls and neurosurgical patients (Figure 3-C). TBI patients exhibited the highest IL-10 values at admission (49.5 pg/ml) (21-fold above healthy controls; 3-fold above neurosurgical patients, $p < 0.05$ at all time points versus healthy controls and $p < 0.05$ at admission versus neurosurgical) with the lowest levels observed at 24 hours. By contrast, the concentration of IL-10 in neurosurgical patients did not fluctuate over the 24-hour sample period, but remained higher in at all time points compared to healthy controls ($p < 0.05$ at admission, 6, and 12 hours versus healthy controls).
4.4 Catecholamine levels in severe, moderate TBI and healthy controls

**Figure 4-A** Epinephrine [E] concentration (pg/ml) in Severe (n=143) and Moderate (n=38) TBI patients vs. healthy controls (n=50). Values are expressed as Mean ± SEM. p values calculated using ANOVA.

**Figure 4-B** Norepinephrine [NE] concentration (pg/ml) in Severe (n=143) and Moderate (n=38) TBI patients vs. healthy controls (n=50). Values are expressed as Mean ± SEM. p values calculated using ANOVA.
Plasma E concentrations were higher in severe TBI patients at all time points when compared to moderate TBI and healthy controls (4-A; $p < 0.05$ at admission, 6, 12, and 24 hours versus healthy controls and $p < 0.05$ at admission, 6, and 12 hours versus moderate TBI patients). In moderate TBI patients, these values were higher than those observed in healthy controls on admission, 6 and 12-hour time points ($p < 0.05$ at admission and 6 hours versus healthy controls). E concentrations in moderate and severe TBI patients were maximal on admission, (1043 pg/ml) (15-fold higher in severe vs. healthy and 236.8 pg/ml in moderate; > 3-fold higher for moderate versus healthy followed by a decrease over the next 24 hours.

NE plasma concentration in severe TBI patients was found to be higher than that observed in both moderate TBI patients and healthy controls, at all time points (4-B, $p < 0.05$ admission, 6, 12, and 24 hours versus healthy controls and moderate TBI patients). The levels of NE in moderate TBI patients also remained higher at all time points when compared to healthy controls ($p < 0.05$ at admission and 6 hours versus healthy controls for E and $p < 0.05$ at admission, 6, 12, and 24 hours versus healthy controls for NE). In moderate TBI patient group, an overall decrease was observed over 24 hours, with the lowest concentration observed at 24 hours post-injury.

### 4.5 Cytokine levels in severe, moderate TBI and healthy controls

Levels of IL-1β in severe TBI patients were higher at all time points compared to healthy controls (5-A, $p < 0.05$ at 6, 12, and 24 hours versus healthy controls) and were elevated relative to values observed in moderate TBI patients over the sample period ($p < 0.05$ at 6, 12, and 24 hours vs. moderate). IL-1β increased over a period of 24 hours in severe TBI patients, whereas
levels did not fluctuate significantly in moderate TBI patients. The highest concentration of IL-1β was observed at 24-hour post-injury for both severe and moderate TBI patients (0.62 pg/ml in moderate and 1.04 pg/ml in severe; > 3-fold higher for severe vs. healthy).

Levels of TNF-α in severe TBI patients were higher than those in healthy controls at all time points, while also being higher than the levels in moderate TBI patients at 6, 12, and 24 hours (5-B, \( p < 0.05 \) at admission, 6, 12, and 24 hours versus healthy controls and \( p < 0.05 \) at 6, 12, and 24 hours versus moderate). Similarly, TNF-α levels were also higher in moderate TBI patients compared to healthy controls (\( p < 0.05 \) admission, 6, and 24 hours versus healthy controls).

**Figure 5-A** Plasma Interleukin (IL)-1β concentrations (pg/ml) in Severe (\( n=143 \)) and Moderate (\( n=38 \)) TBI patients vs. healthy controls (\( n=50 \)). Values are expressed as Mean ± SEM. \( p \) values calculated using ANOVA.
**Figure 5-B** Plasma Tumor Necrosis factor (TNF)-α concentrations (pg/ml) in Severe (n=143) and Moderate (n=38) TBI patients vs. healthy controls (n=50). Values are expressed as Mean ± SEM. p values calculated using ANOVA.

**Figure 5-C.** Plasma IL-10 concentrations (pg/ml) in Severe (n=143) and Moderate (n=38) TBI patients vs. healthy controls (n=50). Values are expressed as Mean ± SEM. p values calculated using ANOVA.
TNF-α levels increased over the 24-hour post-injury period in both moderate and severe TBI patients with the maximum concentration observed at 24 hours (7.28 pg/ml in severe and 6.31 pg/ml in moderate).

Figure 5-C displays IL-10 levels in severe TBI patients which were higher at all time points when compared to healthy ($p < 0.05$ admission, 6, 12, and 24 hour versus healthy controls) and moderate TBI groups ($p < 0.05$ at admission and 6 hours versus moderate TBI patients). IL-10 levels in moderate TBI patients were also found to be higher at all time points when compared to healthy controls ($p < 0.05$ at admission, 6, 12, and 24 hours versus healthy controls). The graphs illustrate that IL-10 levels peaked in both severe and moderate TBI patients on admission (52 pg/ml in severe and 39 pg/ml in moderate; >21-fold higher for severe vs. healthy, 15-fold higher in moderate vs. healthy). Both severe and moderate TBI patients experienced an overall decrease in IL-10 concentrations over the 24-hour observation period.

### 4.6 Relationships between catecholamine and cytokine levels in TBI patients

#### Table 7 Correlations between circulating catecholamines and cytokines

<table>
<thead>
<tr>
<th>Variable</th>
<th>E</th>
<th>NE</th>
<th>IL-1β</th>
<th>TNF-α</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>0.71*</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.21*</td>
<td>0.26*</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.05</td>
<td>0.15*</td>
<td>0.43*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>0.45*</td>
<td>0.35*</td>
<td>0.21*</td>
<td>0.53*</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: E, Epinephrine; NE, Norepinephrine; IL, interleukin; TNF, tumor necrosis factor. Statistical significance $p < 0.05$ via Pearson’s r.

Linear regression analysis was performed to examine potential relationships between the plasma concentrations of the measured catecholamines (E, NE) and inflammatory cytokines (IL-1β,
TNF-α, IL-10 levels in all TBI patients. The data indicate significant positive associations between these variables (Table 7). A significant positive correlation was observed between peak plasma E levels and IL-1β ($r=0.21$, $p=0.02$) and IL-10 ($r=0.45$, $p=0.01$), but not with TNF-α. Similarly, peak NE levels were highly correlated ($p < 0.01$) with all three cytokines. Overall, the strongest cytokine relationships to both E and NE were observed with IL-10. Additionally, peak E and NE values were strongly correlated to each other ($r=0.71$, $p=0.001$), as were IL-1β, IL-10 and TNF-α ($p=0.05$).

Peak values were used as the difference between patient groups, which were maximal at that time point. The peak time for E, NE and IL-10 were on admission whereas the peak time point for IL-1β and TNF-α was 24 hours. The peak values of Catecholamine in table 8 and for cytokines are summarized in Table 9.

### Table 8. Peak Concentration (pg/ml) for Catecholamine E, NE

<table>
<thead>
<tr>
<th>Catecholamine</th>
<th>Severe</th>
<th>Moderate</th>
<th>Neurosurgical</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>1043.07(119.93)</td>
<td>236.81(46.93)</td>
<td>106.86(19.10)</td>
<td>65.28(5.39)</td>
</tr>
<tr>
<td>NE</td>
<td>5314.12(645.71)</td>
<td>1592.25(273.45)</td>
<td>619.71(144.93)</td>
<td>568.52(31.61)</td>
</tr>
</tbody>
</table>

*Peak concentration for E and NE in all patient groups. E and NE peak values occurred at admission time.*

*Abbreviations: E, Epinephrine; NE, Norepinephrine;*

### Table 9. Peak Cytokine Concentration (pg/ml) for IL-10, IL-1β, and TNF-α

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Severe</th>
<th>Moderate</th>
<th>Neurosurgical</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>52.37(7.84)</td>
<td>39.14(13.12)</td>
<td>10.43(2.27)</td>
<td>2.33(0.28)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.04(2.15)</td>
<td>0.63(0.33)</td>
<td>0.37(0.19)</td>
<td>0.24(0.07)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>7.29(7.84)</td>
<td>6.31(3.78)</td>
<td>5.12(1.88)</td>
<td>3.95(0.18)</td>
</tr>
</tbody>
</table>

*Peak concentration for IL-10, IL-1β, and TNF-α in all patient groups. IL-10 peak values occurred at admission time while IL-1β and TNF-α values occurred at 24 hours.*

*Abbreviations: IL, interleukin; TNF, tumor necrosis factor.*
The TBI patients exhibiting higher E levels on admission were significantly correlated with high levels of IL-1β and TNF-α at 24 hours; with IL-1β being statistically significant. Higher levels of E were also directly related to significantly increased IL-10 levels on admission.

Moreover, TBI patients exhibiting higher NE levels on admission displayed significantly higher levels of IL-1β and TNF-α at 24 hours post injury. Similarly, IL-10 was also found to be higher on admission among those TBI patients displaying elevated NE at admission.

4.7 Relationship between Catecholamine and Cytokine vs GOSE Outcome

![E Values According To Dichotomized GOSE](image)

**Figure 6-A.** Peak E concentrations (pg/ml) in all TBI patients stratified according to the Extended Glasgow Outcome Scale (GOS-E) score. Poor (GOS-E 1-4) and Good (GOS-E 5-8) outcomes. Peak values for E were on admission p values calculated using ANOVA. Values are expressed as Mean ± SEM. *p < 0.05 vs. patients with good outcome.
**Figure 6-B.** Peak NE concentrations (pg/ml) in all TBI patients stratified according to the Extended Glasgow Outcome Scale (GOS-E) score. Peak values for NE were on admission. Values are expressed as Mean ± SEM. *p < 0.05 vs. patients with good outcome.

**Figure 7-A.** Peak IL-1β concentrations (pg/ml) in all TBI patients stratified according to the Extended Glasgow Outcome Scale (GOS-E) score. Peak values for IL-1β occurred at 24 hours. Values are expressed as Mean ± SEM. *p < 0.05 vs. patients with good outcome.
Figure 7-B. Peak TNF-α concentrations (pg/ml) in all TBI patients stratified according to the Extended Glasgow Outcome Scale (GOS-E) score. Peak values for TNF-α occurred at 24 hours. Values are expressed as Mean ± SEM. *p < 0.05 vs. patients with good outcome.

Figure 7-C. Peak IL-10 concentrations (pg/ml) in all TBI patients stratified according to the Extended Glasgow Outcome Scale (GOS-E) score. Peak values for TNF-α occurred at admission. Values are expressed as Mean ± SEM. *p < 0.05 vs. patients with good outcome.
For figures 6(A-B) and 7(A-C), patients with GOS-E of lower moderate disability, upper moderate disability, lower good recovery, and upper good recovery (GOS-E 5-8) were classified as “Good” while patients who died, were in a vegetative state, or suffered lower severe disability, or upper severe disability (GOS-E 1-4) were classified as “Poor”.

Figure 6-A and 6-B illustrate the relationship that was observed between GOSE outcome and concentration of E and NE in TBI patients. The results show that patients with a bad GOSE outcome were found to have higher concentrations of E and NE in plasma when compared to those patients with a good GOSE outcome. The difference between patients with good GOSE outcome and bad GOSE outcome were found to be statistically significant for E and NE ($p < 0.05$).

Figure 7 (A–C) illustrates the relationship that observed between GOSE outcome and the concentration of IL-1β, TNF-α and IL-10 in TBI patients. The peak values used for these graphs occurred at 24 hours for IL-1β as well as TNF-α, while it occurred at the time of admission for IL-10. From the graphs, it was observed that TBI patients had higher peak concentrations of these cytokines if they were classified as having a bad GOSE outcome.
5.1 Summary
This study had the following main objectives: (i) evaluate E, NE, proinflammatory (IL-1β and TNF-α) and anti-inflammatory cytokine (IL-10) levels in TBI patients; and (ii) examine the effect of Injury severity and outcome on catecholamine and cytokine plasma levels in TBI patients, and association of those levels with patient’ outcome.

The main findings of our study were the following: (i) E, NE and IL-10 levels peaked at admission, while IL-1β and TNF-α levels peaked at 24 hours, and; (ii) patients with severe TBI had higher plasma catecholamine and cytokine values at all time points compared with moderate TBI, neurosurgical, and healthy controls. Moderate TBI patients were found to have higher plasma catecholamine and cytokine levels than healthy controls, while neurosurgical patients also had higher plasma catecholamine and cytokine values than healthy controls.

The following factors constitute the novelty of this study: (i) our study comprised one of the largest sample sizes of isolated TBI patients (n=181) to date, which measured both catecholamine and cytokine, responses. A study involving this number of isolated TBI patients had not been conducted previously; (ii) our study also looked at plasma catecholamine and cytokine levels in neurosurgical patients (n=17) since neurosurgical patients also undergo a controlled traumatic insult.
5.2 TBI and sympathetic nervous system activation

After TBI, a rapid and robust sympathetic surge may serve as a protective mechanism to minimize the effects of the injury resulting from the primary insult. This causes individuals afflicted with severe TBI to experience a spontaneous periodic stress response, or sympathetic storming. The reduced neurological functions due to sympathetic storming may include pupillary dilation, tachycardia and hyperthermia as well as alterations in metabolic functions which results in increased release of glucose, basal metabolic rate, energy requirements and impaired CBF. This ischemic environment triggers anaerobic glycolysis resulting in increased lactic acid concentration and membrane permeability, leading to edema formation. The sympathetic storm can lead to dysfunctions that range from loss of cortical control over autonomic function, loss of physiological regulatory mechanism, disinhibitory control of sympathetic outflow, and disruption/dysregulation of autonomic nervous system [34, 76, 91].

In our study, E and NE levels were elevated immediately after TBI and stayed raised primarily in those patients who were in critical condition or had a persistent coma. Initially this surge of E and NE levels seems to confer some protection by augmenting perfusion of vital areas of the brain. It has also been studied that, cerebral ischemia produced after TBI, can triggered both the sympathetic and vagal systems probably by releasing inhibitory control, with the principal effect enforced by the vagal system [104, 108].

5.3 E and NE responses following TBI

According to our study, E and NE levels were highly elevated at admission time point after which they decreased over the next 24 hours. The lowest observed values for E and NE
occurred at 24 hours. The findings of our study, pertaining to behavior of E and NE levels, were consistent with other studies. Similar studies found that plasma E content was highest at admission but had dropped by the 6-hour mark after which it continued to decline further. They also reported a several-fold increase in E and NE concentration during the first few days [86, 97, 171].

This noticeable plasma upsurge of E and NE after TBI, can be attributed to their release from the adrenal medulla that may occur as a result of hypovolemia due to profuse blood loss and can also be due to NE being released from sympathetic nerve endings triggered by tissue hypoxia and acidosis [107].

The reasons pertaining to the decrease of E and NE could be that sympathetic over activity has the ability to diminish spontaneously. With the reintegration of the injured brain, stimulation of the presynaptic α2-adrenoceptors which downregulate NE release, leading to decreased extracellular NE levels. The physiological activities of catecholamines can also be decreased by cellular reuptake through norepinephrine transporters, followed by their intracellular inactivation by monoamine oxidase (MAO) [9, 172, 173].

In our study, NE concentration was found to be approximately 5 times higher than E concentration following TBI. This is because plasma E is released from adrenal medulla and sympathetic ganglions, whereas NE is released from sympathetic ganglions, adrenal medulla and from sympathetic nerve endings. The plasma levels of NE depend upon the release and subsequent clearance from circulation, any alteration in the clearance may change plasma concentrations of NE. Another study observed plasma E and NE concentration in patients with
fluctuating levels during the erratic phases of recovery after TBI. This release from the neurologic system resulted in a threefold increase in plasma levels of NE, which persisted for at least 10 days and take up to 6 months for normalization. Since NE is generally accepted as an index of sympathetic nervous activity, any increase in its release after TBI might be a protective mechanism while the decline might hamper recovery of functions [15, 174, 175].

5.4 Cytokines (IL-1β and TNF-α) cause brain and systemic inflammation

Neuroinflammatory events following TBI are known to produce adverse clinical outcomes and secondary brain damage due to the release of several neurotoxic substances as well as post-traumatic inflammation, which stimulate neurorestorative mechanisms. The main event in the progression of acute inflammation within CNS is infiltration by neutrophils (PMN), followed by monocytes/macrophages with the activation of resident microglia, astrocytes and neurons. Microglia, in particular, have an innate inflammatory capacity which helps to produce proinflammatory cytokines, ROS and reactive nitrogen species (RNS). RNS leads to triggering of vascular dilatation, fluid extravasation and the ensuing edema, which is mediated by the ion channels on these glial cells. During TBI, cytokines are released within a few minutes of tissue injury. Initially, only the pro-inflammatory mediators are released, however, they are able to induce the synthesis of anti-inflammatory cytokines, which helps in restoring the normal homeostatic state [35].

During TBI, the BBB becomes permeable, which allows proinflammatory mediator such as IL-1, IL-6, and TNF-α into systemic circulation. The initial release of TNF-α, which is known to cause central/systemic inflammation, is intended to protect site of injury and fight foreign
bodies, however, when release is dysregulated, it can have a destructive effect. Greatly elevated TNF-α level can cause damage to end organs as well as increase risk of MODS. When the inflammation cannot be contained locally, it leads to SIRS [12].

5.5 IL-1β, TNF-α and IL-10 behavior following TBI

Our study found that IL-1β and TNF-α levels were lowest at admission, but increased over the next 24 hours. The highest observed values for IL-1β and TNF-α occurred at 24 hours. IL-10 values were highly elevated at admission after which they decreased over the next 24 hours with the lowest observed value occurring at 24-hour time point. The findings of our study concerning behavior of IL-1β, TNF-α and IL-10 levels were consistent with what was observed in other studies [114, 117, 142, 176].

Immediately after TBI, injured CNS induces an acute upsurge of pro-inflammatory cytokines IL-1β and TNF-α. The injured brain tissue has the tendency to increase extracellular ATP levels, which facilitates CNS microglia activation as well as synthesis and release of IL-1β. Other studies also confirmed microglia activation, increased IL-1β levels, and cell death, which may occur, even up to one year after TBI. This makes IL-1β a useful marker of chronic inflammation. In the experimental models of head injury, TNF-α was upregulated within the brain after few hours of the initial injury. This was also observed in a different study on closed head injury patients that increased TNF-α level were detected in human cerebrospinal fluid and serum. IL-1β and TNF-α can act synergistically in order to further increase injury [12, 19, 117, 177, 191].
The findings of our study exhibit that at admission time, the levels of IL-1β and TNF-α were lower than the corresponding values at 6, 12, and 24-hour time points. This was observed in all patient groups. At admission time point, the values of E and NE were at their highest and both play a role in limiting the production and release of IL-1β and TNF-α. In previous studies it was documented that E and NE can inhibit the production of TNF-α by stimulating β adrenergic receptors. It was also established, that elevated levels of E and NE, via cAMP, increase the levels of IL-1 receptor (IL1ra) and soluble TNF receptors (sTNFRS), which are endogenous competitive antagonist to IL-1β and TNF-α respectively, hence explaining the low levels of IL-1β and TNF-α at admission time point. It was also seen that cyclic AMP analogs such as adenylyl cyclase (AC) activators as well as phosphodiesterase (PDE) inhibitors can increase production of IL-10 and slow down microglia activation, thus suppressing proinflammatory cytokine production. Finally, other studies have found that as plasma levels of E and NE rise accordingly, the IL-10 levels are also increased and plasma concentrations of IL-1β and TNF-α decrease. The data collected in this study also showcases this behavior. These opposing behavior that IL-1β and TNF-α show against the IL-10 can be explained by the balance that is attained due to an inhibitory relationship between the proinflammatory and anti-inflammatory groups of cytokines which can indirectly suppress production of each other protecting cell viability as well as maintaining homeostasis. The decrease in E and NE also influences the decrease in cAMP, which in turn decrease levels of receptors, IL1ra and sTNFRS, hence allowing IL-1β and TNF-α to start increasing [9, 117, 156, 177–181].

In our study, it was found that IL-10 peaked at admission. This could be because immediately after TBI, resident microglia, infiltrating monocytes and macrophages rapidly secrete IL-10,
mediated by SNS discharge [168]. The elevated plasma concentrations of anti-inflammatory IL-10 prevent detrimental effects, produced by overwhelming increased levels of pro-inflammatory cytokines [188]. There is clinical evidence that “sympathetic storming”, surgery and psychological stress may also play a role in increasing IL-10 levels. Finally, E, NE and cAMP are able to increase the synthesis of the anti-inflammatory and immunosuppressive cytokine IL-10, which also has the effect of downregulating monocytic pro-inflammatory functions [165, 182–184].

Our findings indicated that IL-10 value at the 24-hour time point is much lower than all earlier values. This may be due to early deactivation of monocytes thorough IL-10 itself, as it can down-regulate its own synthesis in phagocytic cells. The proinflammatory and anti-inflammatory cytokines inhibit synthesis of each other, thereby, creating a balance between neurodegenerative and neuroprotective effects. Finally, studies have shown that TNF-α can inhibit NE which leads to a decrease in cAMP and, hence, a decrease in IL-10 production [9, 184, 185].

In our study, it was observed that patients with severe TBI had higher IL-1β and TNF-α levels at all time points compared to moderate, neurosurgical patients and healthy controls. Similar studies have also been able to validate these findings. It was also observed that IL-10 levels were also more elevated in patients with severe TBI than in all other patient groups. In earlier studies, it was established that patients with severe injuries showed elevated IL-10 levels when compared to patients with moderate trauma. The same study stated that IL-10 levels in all patient groups were more elevated than the healthy control group. Thus, the findings of our
study in relation to IL-1β, TNF-α, and IL-10 levels in severe TBI patients compared to moderate TBI patients are consistent with previous studies [186–188].

Table 10 IL-1β peak value comparison with other studies

<table>
<thead>
<tr>
<th>IL-1β</th>
<th>No. Of Patients</th>
<th>Peak Value</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our Study</td>
<td>181</td>
<td>0.95 pg/ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Toshiyaki et al. 2004</td>
<td>23</td>
<td>0.5 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Tadahiko et al. 2005</td>
<td>35</td>
<td>1.5 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Alptekin et al. 2006</td>
<td>16</td>
<td>52.8 pg/ml</td>
<td>Serum</td>
</tr>
</tbody>
</table>

Peak values for IL-1β as found in other similar studies
Abbreviations: IL, interleukin;

Table 11 TNF-α peak value comparison with other studies

<table>
<thead>
<tr>
<th>TNF-α</th>
<th>No. Of Patients</th>
<th>Peak Value</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our Study</td>
<td>181</td>
<td>6.31 pg/ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Goodman et al. 1990</td>
<td>21</td>
<td>7500 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Stuart et al. 1994</td>
<td>50</td>
<td>12.19 pg/ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Morganti-Kossman et al. 1997</td>
<td>36</td>
<td>157 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Esther et al. 1999</td>
<td>28</td>
<td>6.3 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Tadahiko et al. 2005</td>
<td>35</td>
<td>5.1 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Deborah et al. 2011</td>
<td>25</td>
<td>96.9 pg/ml</td>
<td>Serum</td>
</tr>
</tbody>
</table>

Peak values for TNF-α as found in other similar studies
Abbreviations: TNF, tumor necrosis factor.

Table 12 IL-10 peak value comparison with other studies

<table>
<thead>
<tr>
<th>IL-10</th>
<th>No. Of Patients</th>
<th>Peak Value</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our Study</td>
<td>181</td>
<td>49.95 pg/ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Morganti-Kossman et al. 1997</td>
<td>28</td>
<td>98.5 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Esther et al. 1999</td>
<td>28</td>
<td>98.5 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Toshiyaki et al. 2004</td>
<td>23</td>
<td>12 pg/ml</td>
<td>Serum</td>
</tr>
<tr>
<td>Bernd Maier et al. 2007</td>
<td>378</td>
<td>170 pg/ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>C.Kirchoff et al. 2008</td>
<td>33</td>
<td>90 pg/ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Deborah et al. 2011</td>
<td>25</td>
<td>1136 pg/ml</td>
<td>Serum</td>
</tr>
</tbody>
</table>

Peak values for IL-10 as found in other similar studies
Abbreviations: IL, interleukin
5.6 Role of catecholamines and cytokines in neurosurgical patients

In neurosurgical patients, the increase in E and NE levels at admission can be attributed to the operative stress, different methods of anesthesia as well as other therapeutic interventions, such as tracheal suction that can stimulate the SNS. It is known that such procedures are able to cause surgical trauma leading to a sudden increase in physiological and immunological responses that are required to maintain homeostasis and survival. In such cases, the magnitude of damaged tissue can be used to determine the traumatic response, which can be correlated with the severity of surgical stress [107].

The response of the human body to different harmful stimuli is characterized as a physiological ‘stress response’. Any insult that alters the human tissues can affect the fine balance of homeostasis. Patients who undergo trauma or elective surgery react to surgical stress by triggering specific mechanisms, which include afferent neural stimuli and inflammatory mediator release. Injuries to CNS activate local neuroimmune cells and the influx of circulating peripheral leukocytes, initiated by an endogenous up-regulation of cell adhesion molecules, chemokines and cytokines. Cytokines comprise of inflammatory (IL-1β, TNF-α) and anti-inflammatory (IL-10) mediators, which are promptly secreted after CNS injuries and are crucial for the initiation, proliferation, and cessation of the inflammatory response and elicit changes in hemodynamic, endocrine and immune responses. These cytokines regulate the function of the activated cells in order to maintain the total body homeostasis. Mediated by the HPA axis and SNS, there is a delicate balance between pro- and anti-inflammatory cytokines after injury or surgery. Neurosurgical processes and TBI related poor responses may produce infections.
associated with immunosuppression. Beyond the stress of surgery, additional variable factors, such as blood transfusions, pain, and hyperglycemia result in perioperative immunomodulation. However, the catecholamine levels observed in the current study during surgical stress was relatively small in comparison to the E and NE in TBI patients [176, 189, 190].

5.7 Relationship between plasma catecholamine concentration and patient outcome

According to our study, patients with TBI were classified into moderate or severe TBI patient groups based on their GOSE score. A GOSE score of 1–4, is considered as poor outcome while GOSE score of 5–8 was reflected as good outcome. In our study, it was noted that patients with poor GOSE outcome had higher E and NE levels at admission. According to one study, (Woolf et al.) levels of E and NE were significantly higher in patients who died or remained persistently vegetative than in those with better outcomes. It was further stated that the patients with the two worst prognosis after TBI had higher E and NE levels than those with better outcomes while patients with severe disability had greater NE levels when compared to those with good recovery. Another study stated that E and especially NE levels were a good indicator of injury severity, but only if the injury happened within the brain [96, 106, 180, 191–193].

According to certain studies, catecholamine levels were negatively related to GCS score. Patients with low GCS score (3 to 4) generally had E and NE levels, which were increased by several folds, compared to healthy patients. The values in patients with moderate GCS score (8-10) showed intermediate levels. The increasing levels of circulating biogenic amines, which act as indicators of brain injury severity, are also able to elicit autonomic disturbances that occur after head injury [97, 107, 191, 194].
5.8 Relationship between plasma cytokine concentration and patient outcome

In general, we observed that within our study, lower patient outcomes were associated with higher IL-1β and TNF-α values at 24 hours as well as higher IL-10 levels at admission. Previous studies and reports validate this observation as they found that, depending on the amount and the situation, increased synthesis of pro-inflammatory cytokines are associated with poor outcomes in TBI patients, and the plasma concentration observed in these patients were far higher than those in healthy patients. Hence, it would be safe to say that based on our findings, as well as evidence from the previous studies, a negative correlation exists between GOSE score and IL-1β/TNF-α and IL-10 levels at 24 hours and at admission time points respectively [178].

5.9 Correlation between catecholamines and cytokine responses

Our findings showed that peak values of E and NE at admission corresponded with peak levels of IL-1β and TNF-α at 24 hours, as well as peak levels of IL-10 at admission. This observation can be validated by a previous study, which found that TNF-α was influenced by E and NE. The production of TNF-α was affected in a dose-dependent manner by different catecholamine concentrations, and further went on to state that this finding was statistically significant. One of the relationships between IL-1β/TNF-α and NE can be described by the fact that lower NE concentrations can activate α2 receptors which can further stimulate TNF-α and IL-1β production in hepatic Kupffer cells. At high NE concentrations, the same pro-inflammatory cytokines are inhibited by β2-adrenergic receptors. Both E and NE are also known to induce IL-10 release from monocytes as this can be proven by the fact that patients with sympathetic storm have higher IL-10 levels. Furthermore, IL-10 has an effect on both TNF-α and IL-1β while
both IL-1β and TNF-α also have an effect on IL-10 thorough activating processes that indirectly inhibits the production of each other and this provides a fine tuning balance between the neurodegenerative and neuroprotective effects [15, 152, 179, 181, 195].

5.10 Study limitations
Although the study was carefully planned and executed to the best of abilities, there were still some limiting factors that could have affected the result of the study. One of the main limitations of the study was the inadequate availability of plasma samples at all time-points; this shortage of plasma for each sample time meant that a lesser number of analyses were possible for the specific cytokines, including IL-1β and TNF-α as compared to the number of measurements per time point for IL-10, E, and NE. Consequently, potential differences between patient groups were not found be to statistically significant for IL-1β and TNF-α. Furthermore, since the final measurement for this study was taken at 24 hours where the peak concentrations were also observed, it is possible that the plasma concentrations might have continued to increase beyond the 24 hours, therefore making this a limitation of the study. And, despite using state of the art electrochemiluminuensence multiplex analyses, the measurements were taken from peripheral blood therefore, we are not certain of precise cellular origin of inflammatory markers. Neurosurgical patients used for this study were useful for comparison to more severe trauma patients; however, we cannot fully exclude differences in the inflammatory or stress response that may be attributable to concurrent stressors and other comorbidities. Finally, achieving the most accurate measurements would require drawing the samples from the CSF or intrathecally, however since this was not accessible; therefore, the samples had to be taken from the peripheral blood.
5.11 Future studies

After TBI, the catecholamines surge exacerbates the harmful effect of the secondary injury thorough vasoconstriction, reducing cerebral perfusion and oxygenation, ischemia, and subsequent development of extracranial organ dysfunction. This makes it important to find treatments that are effective in reducing sympathetic hyperactivity and thus increasing chances of survival. We now know that brain and cerebral vasculature have adrenergic receptors. This leads researchers to believe that activation of cerebral β-adrenergic receptors was due to catecholamine release. However, there is increasing evidence to suggest that adrenergic receptor blocking agents, such as β-blockers can provide a survival benefit in severe TBI patients, by reducing the effect of E and NE on β2 adrenergic receptors and consequently down regulating the hyperadrenergic state observed after TBI. So far, a great number of studies have been conducted to determine whether β-blockers could be beneficial for patients afflicted with TBI. A blockade could potentially result in the attenuation of intracerebral posttraumatic catecholamine induced vasospasm, which reduces the likelihood of local ischemia and protects end organs which are prone to being damaged as a result of brain-injury induced catecholamine surge.

Various studies have also debated that idea that maintaining the CBF to normal levels leads to retain normovolemia and presents improved efficiency to reduce the intestinal tissues volume. The neuroprotective effects of β-blockers can be facilitated through reduced CBF, less glucose, and oxygen consumption, which in turn reduces metabolism. It has been shown that β-blockers can slow down catecholamine induced catabolic state in burn victims. It is further believed that anti-hypertensive therapy aimed at decreasing cerebral perfusion can be used to control brain
edema and secondary brain injury. Furthermore, other studies and clinical trials have explored the use of β-blockers in TBI patients, and in each case found patients who were administered β-blockers had better neurologic outcome and higher chances of survival. However, since not all β-blockers can cross BBB, it is unknown to what extent their survival benefit can apply to TBI patients. Different clinical circumstances have been able to show that the blockade can be cardioprotective, which is very important in controlling the damage caused by TBI as both rhythm disturbances and myocardial necrosis are known to occur following TBI.

There is increasing evidence to suggest that adrenergic receptor blocking agents, such as β-blockers, can provide a survival benefit in severe TBI patients by reducing the effect of E and NE on β2 adrenergic receptors and consequently down-regulate the hyperadrenergic state observed after TBI. In elderly patients, cardiovascular comorbidities are associated with increasing age and when combined with a hyperadrenergic state, the outcomes of these patients can be severely worsened. Exposing such patients to β-blocker therapy at the time of their ICU admission results in a noticeable decrease in mortality [15, 83, 100, 109, 161–164, 196].

Traumatic injury to the central nervous system initiates inflammatory processes, such as the synthesis of proinflammatory mediators that contribute detrimental effects to secondary tissue damage. Studies based on animal models have shown that neutrophil accumulation in the brain starts occurring at 4 hours and peak by 24-72 hours. Therefore, it is reasonable to believe, that the administration of anti-inflammatory cytokines such as IL-10 could be neuroprotective and could potentially decrease pro-inflammatory response to TBI. Several in vivo models of tissue injury have shown IL-10 to be protective. Furthermore, studies conducted on rats found those
who were treated with IL-10 before ischemia-reperfusion had significant lung neutrophil infiltration and tissue injury. A study was conducted which found that treating rats 30 minutes before and 1 hour after injury with IL-10 improved neurobehavioral outcome. [197, 198]

Interleukin-1 (IL-1) together with TNF-α are known to contribute towards the development of sepsis-induced multiorgan dysfunction syndrome (MODS). However, in 1981, the antagonist receptor for IL-1 (IL-1Ra) was purified and was found to be a key factor in regulating systemic inflammatory responses. IL-1Ra was tried in several rodent models, which were affected with head trauma and the results showed IL-1Ra to have a neuroprotective effect. Studies have also found that to date, no significant safety issue has been reported concerning IL-1Ra (Anakinra) and have also found that it offered greater survival chances to those populations it was used on. [199, 200]

5.12 Conclusion
The main purpose of this study was to investigate the putative relationship between posttraumatic SNS overactivity and dysregulation of the central and peripheral inflammatory response to injury, as evidenced by alterations in key circulating pro- versus anti-inflammatory cytokines. In order to accomplish this, the study was focused on answering two main questions: i) Can high-circulating catecholamines directly and indirectly increase dysfunctional inflammatory cytokine release, and are they associated with poor neurologic outcome in both TBI and neurosurgical patients, and; ii) Do those TBI patients who develop persistent neurological sequelae, demonstrate sustained inflammatory cytokine alterations relative to patients with good outcome. From the data gathered within this study, we can determine that
high-circulating catecholamine levels affect pro- and anti-inflammatory cytokine balance and patients with higher peak cytokine levels do sustain worsened neurologic outcome. Further studies on limiting the damage caused by initial catecholamine release as part of secondary injury need to be carried out as there is evidence that patient outcome and mortality can improve if the effects of the catecholamine can be inhibited.
References


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Appendices

1 Appendix 1 - Glasgow Coma Scale

Eyes open

- Spontaneously: 4
- To Speech: 3
- To Pain: 2
- Never: 1

Best motor response

- Obeys Commands: 6
- Localizes Pain: 5
- Flexion withdrawal: 4
- Decorticate flexion: 3
- Decorticate extension: 2
- No response: 1

Best Verbal Response

- Orientated: 5
- Confused: 4
- Inappropriate words: 3
- Incomprehensible Sounds: 2
- Silent: 1
Appendix 2 - The Glasgow Outcome Scale Definition of Terms

1 - Dead

2 - Vegetative State

*Unable to interact with environment; unresponsive*

Patients who show no evidence of meaningful responsiveness. Patients who obey even simple commands, or who utter any words, are assigned to the better category of severe disability. Vegetative patients breathe spontaneously, have periods of spontaneous eye-opening when they may follow moving objects with their eyes, show reflex responses in their limbs (to postural or painful stimuli), and they may swallow food placed in their mouths. This non-sentient state must be distinguished from other conditions of wakeful, reduced responsiveness--such as the locked-in syndrome, akinetic mutism and total global aphasia.

3 - Severe disability

*Able to follow commands/ unable to live independently*

This indicates that a patient is conscious but needs the assistance of another person for some activities of daily living every day. This may range from continuous total dependency (for feeding and washing) to the need for assistance with only one activity--such as dressing, getting out of bed or moving about the house, or going outside to a shop. Often dependency is due to a combination of physical and mental disability--because when physical disability is severe after head injury there is almost always considerable mental deficit. The patient cannot be left overnight because they would be unable to plan their meals or to deal with callers, or any
domestic crisis which might arise. The severely disabled are described by the phrase "conscious but dependent."

4 - Moderate disability

*Able to live independently; unable to return to work or school*

These patients may be summarized as "independent but disabled," but it is perhaps the least easily described category of survivor. Such a patient is able to look after himself at home, to get out and about to the shops and to travel by public transport. However, some previous activities, either at work or in social life, are now no longer possible by reason of either physical or mental deficit. Some patients in this category are able to return to certain kinds of work, even to their own job, if this happens not to involve a high level of performance in the area of their major deficit.

5 - Good recovery

*Able to return to work or school*

This indicates the capacity to resume normal occupational and social activities, although there may be minor physical or mental deficits. However, for various reasons, the patient may not have resumed all his previous activities, and in particular may not be working.
3 Appendix 3 - Limitations

**Known confounders for outcome**

We will adjust to age, admission GCS, features of head CT scan (Marshall). We expect to have at least 50 events (poor outcome GOS score) among the 200 patients, thus at least 10 events per variable, which will allow us to perform the logistic regression analysis.

**Patients who die before 24 hrs**

The correlation between catecholamine levels and inflammatory mediators, neuro-injury biomarkers, cardiac troponin and coagulation in severe TBI patients will be investigated using the Spearman correlation at the different time points. Considering the hypothesis that the catecholamine levels are triggers of the inflammatory response, we will investigate the correlation between catecholamine levels at baseline and inflammatory markers at 24 hours by using last observation carry forward for those who die before 24 hours.