CATECHOLAMINES AS INDEPENDENT PREDICTORS OF OUTCOME IN MODERATE AND SEVERE TRAUMATIC BRAIN INJURY (TBI). THE COMA-TBI STUDY

By

Luis Teodoro da Luz

A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Sciences

University of Toronto

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Catecholamines as Independent Predictors of Outcome in Moderate and Severe Traumatic Brain Injury (TBI). The COMA-TBI Study

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Master of Science
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1. Abstract

Introduction: High levels of catecholamines post brain trauma are associated with severity of injury and neurological outcome. We aimed to overcome methodological limitations of previous studies and demonstrate an independent association between circulating catecholamine levels with neurological outcome in patients with isolated brain injury. Methods: Multi site, prospective observational blinded cohort study. After enrollment, patients had catecholamine levels measured on admission and the independent association with a 6-month neurological outcome was assessed using the Glasgow Outcome Scale Extended. Multivariate logistic regression models estimated the adjusted odds ratio for prediction of unfavorable outcome. Results: 181 patients were enrolled and admission catecholamines were measured. High admission levels were independently associated with severity of injury and with unfavorable outcome. Conclusion: We demonstrated the natural history of catecholamine release early post brain injury and an independent association with unfavorable outcome in a dose response fashion. Catecholamines are biomarkers of outcome in moderate to severe traumatic brain injury.
2. Acknowledgments

This thesis is dedicated to Flavio Henrique Duarte de Araujo, my partner of life, for showing me that there is much more to discover and achieve when leaving our comfort zone. Thanks for the constant emotional support, respect, companionship and love.

I also dedicate this thesis to my parents Sebastião Teodoro da Luz (in memoriam) and Inácia Rosa da Luz, both very simple and respectful people. From mom and dad I learned how to be humble and strong at the same time, characteristics I think are important to face difficulties in life.

I would like to express my deep gratitude to my supervisor Dr. Sandro Rizoli for his continuous support, patience, and helpful advice, for opening the door to a wide range of opportunities. His continuous efforts have made this project possible. I would also like to thank Dr. Andrew Baker and Dr. Leodante da Costa for their insightful feedback and strong guidance to ensure my work was moving in the right path. I would also like to extend my thanks to the members of my outstanding thesis committee members Dr. John Marshal, Dr. Sunit Das, and Dr. Eric Ley.

The COMA-TBI study would not have been possible without a large team of experts that was fundamental in all phases of the study. I would like to express my immense gratitude to the strong work of all members. This unique team is represented by Dr. Capone Neto, Dr. Bartolomeu Nascimento, Dr. Gordon Rubenfeld, Dr. Kenji Inaba, Dr. Alex Di Battista, Dr. Jane T-Vranic, Dr Adic Perez, Dr. Mitra Arjang, Shawn Rhind, Sandy Trpcic, Brandon Lejnieks, Arimie Min, Yangmei Li and Monica Wong.
3. Contributions

The idea of the COMA-TBI study initiated around 2010. In 2011 the project was written and applications for funding and Research Ethics Board approval were submitted. Patient enrollment started in late 2011. Dr. Luis Teodoro da Luz initiated his contribution to the study in 2013 when enrollment was finalizing. Dr. da Luz was involved in updating the research project, reviewing, cleaning and critically analyzing clinical data, coordinating finalization of the data with the research assistants and managers from the 3 study sites. Dr. da Luz was involved in the team effort of performing the statistical analysis and fully responsible for the production of the thesis dissertation. Dr da Luz is also an important contributor in writing the main manuscripts using the COMA-TBI study data.

Dr. Sandro Rizoli was the principal investigator of the study, contributing with all aspects of the trial, from generating the research question, to study design, production of study protocol and application for funding. Dr. Rizoli also coordinated the diverse teams from the 3 different centers, and was involved in all phases of the study and reviewed the statistics analyses, results, the thesis dissertation and the COMA-TBI manuscript.

Dr. Capone Neto contributed to the research question, design, study protocol production, consent forms, application for funding, and for REB approval. Dr. Capone Neto also reviewed the statistical approach and results.

Dr. Alex Di Battista and Dr. Shawn Rhind were instrumental in all phases of the trial, from concept to creation to analysis and more recently, in writing and disseminating the results of the trial. Dr Rhind participated in the concept and writing of the project, arranging for funding, managed the conduction of all laboratorial experiments from sample
collection to results. Drs Di Battista and Rhind have a major role in the analysis of the data, particularly that of the laboratorial results, and writing of the main manuscripts using the COMA-TBI data. Dr Di Battista is using part of the COMA-TBI data for his PhD work.

**Dr. Gordon Rubenfeld** and **Dr. Martin Chapman** made important contributions to the initial concept and its writing by critically reviewing it. Their major contribution was to the methodology for the trial. They participated in the applications for funding and Research Ethic Board approval. They also participated on the initial phases of the clinical trial.

**Mrs. Sandy Trpcic** was instrumental in managing the entire project, from conception to finalization. Mrs. Trpcic has coordinated the efforts from the 3 sites and ascertained that all regulations were followed and all aspects of the work were done.

At SHSC: **Dr. Adic Perez** was the research coordinator with the important role of acquiring the data, storing it and making the results available whenever needed.

At SMH: **Dr. Jane Topolovec-Vranic** was the Principal Investigator and was heavily support by **Dr. Andrew Baker**. Dr Baker was instrumental in all phases of the study, from its conception and writing, to final analysis. **Mrs. Yangmei Li** and **Marlene dos Santos** were the research coordinators responsible for all aspects of the trial, including all regulatory steps, from patient enrollment, consenting, data acquisition, and blood sample handling.

At LA County: **Dr. Kenji Inaba** was responsible for coordinating all aspects of the trial. **Mrs. Monica Wong** was the local research coordinator, coordinating enrollment, consenting, data acquisition and blood sample handling.
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<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
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<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<td>ADC</td>
<td>Apparent diffusion coefficient</td>
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<tr>
<td>AIS</td>
<td>Abbreviated injury scale</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BB</td>
<td>Beta blocker</td>
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<td>BBB</td>
<td>Blood brain barrier</td>
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<td>BDNF</td>
<td>Brain derived neurotrophic factor</td>
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<tr>
<td>CaMKII</td>
<td>Calcium/calmodulin dependent protein kinase II</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<td>CARS</td>
<td>Compensatory anti-inflammatory response syndrome</td>
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<td>CBF</td>
<td>Cerebral blood flow</td>
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<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CIDS</td>
<td>Central nervous system injury-induced immunodepression</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
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<td>CPP</td>
<td>Cerebral perfusion pressure</td>
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<td>CRASH</td>
<td>Corticosteroid Randomization after Significant Head Injury</td>
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<td>CREB</td>
<td>cAMP response element binding protein</td>
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<td>CSF</td>
<td>Cerebral spinal fluid</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>DA</td>
<td>Dopamine</td>
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<td>DAI</td>
<td>Diffuse axonal injury</td>
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<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<td>E</td>
<td>Epinephrine</td>
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<td>ED</td>
<td>Emergency department</td>
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<td>EDH</td>
<td>Epidural hematoma</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>EIA</td>
<td>Enzyme immunoassay</td>
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<tr>
<td>ERK</td>
<td>Extracellular signal-regulated kinase</td>
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<td>FOUR</td>
<td>Full Outline of UnResponsiveness</td>
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<tr>
<td>GPCR</td>
<td>G protein coupled receptor</td>
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<td>HDS</td>
<td>Hypertonic saline plus dextran</td>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
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<td>ICAM</td>
<td>Intercellular adhesion molecule</td>
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<td>ICH</td>
<td>Intra cerebral hematoma</td>
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ICP – Intra cerebral pressure
ICU – Intensive care unit
IL – Interleukin
IMPACT – International Mission on Prognosis and Clinical Trial Design in TBI
IP3 – Inositol trisphosphate
ISS – Injury severity score
IVH – Intra ventricular hemorrhage
GCS – Glasgow coma scale
GFAP – Glial fibrillary acidic protein
GOS – Glasgow outcome scale
GOSE – Glasgow outcome scale extended
HSD – Hypertonic saline plus dextran
LA – Los Angeles
LOC – Level of consciousness
L/P – Lactate/Pyruvate ratio
MAO – Monoamino oxydase
MAPK – Mitogen activated protein kinase
MCP – Monocyte chemoattractant protein
MHC – Major histocompatibility complex
NE – Norepinephrine
NFkB – Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF – Nerve growth factor
NPE – Neurogenic pulmonary edema
NPV – Negative predictive value
NSE – Neuron-specific enolase
OR – Odds ratio
PET – Positron emission tomography
PKA – Activating protein kinase A
PKC – Activating protein kinase C
PPAR-γ – Peroxisome proliferator-activated receptor gamma
PPV – Positive predictive value
PRDX – Peroxiredoxin
PSS – Paroxysmal sympathetic storm
PTX3 – Pentraxin 3
RCT – Randomized controlled trial
RNA – Ribonucleic acid
ROC – Receiver operating curve
S100β – S100 beta protein
S100ββ – Monomer of S100 beta protein
SAH – Subarachnoid hemorrhage
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<td>SHSC</td>
<td>Sunnybrook Health Sciences Centre</td>
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<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
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<td>SMH</td>
<td>Saint Michael’s Hospital</td>
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<td>SNS</td>
<td>Sympathetic nervous system</td>
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<tr>
<td>SSEP</td>
<td>Somatosensory evoked potentials</td>
</tr>
<tr>
<td>T3</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroxine</td>
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<tr>
<td>TBI</td>
<td>Traumatic brain injury</td>
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<tr>
<td>TCDB</td>
<td>Traumatic coma data bank</td>
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<tr>
<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>UCH-L1</td>
<td>Ubiquitin C-terminal hydrolase</td>
</tr>
<tr>
<td>USC</td>
<td>University of south California</td>
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<tr>
<td>VCAM</td>
<td>Vascular adhesion molecule</td>
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<td>VMAT-2</td>
<td>Vesicular aminotransporter</td>
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Catecholamines as Independent Predictors of Outcome in Moderate and Severe Traumatic Brain Injury (TBI). The COMA-TBI Study

CHAPTER 1 – Literature Review

1.1. Introduction

Trauma is a major public health problem responsible for over 6 million deaths and three times as many disabled patients across the world. Traumatic brain injury (TBI) plays a significant role in this onus across all age groups [1]. There is clinical evidence that even mild head injuries can negatively impact on physical, cognitive and social performance [2, 3]. Furthermore, the overall mortality in patients with TBI is approximately 10%, but can reach 40% in patients with severe head injury, specifically [4]. According to the most recent CDC estimates (2004-2006) [5], there are 1.7 million new cases of TBI annually, with 52,000 deaths, 275,000 hospitalizations, and 1.4 million people treated in emergency departments (ED), with approximately 1.4 times as many TBIs occurred among males as among females [5]. In Canada, while the total number of hospitalization for head injuries decreased in the last decade, hospital admissions due to severe TBI have increased. The number of admissions to trauma facilities for severe trauma (all causes) grew from 8,784 in 2000-01 to 10,249 in 2003-04, representing a 17% increase. However, the number of admissions to trauma facilities for severe TBI increased by 46% [4].

Only 5% of patients sustaining a head injury present with a low level of consciousness, with a Glasgow Coma Scale (GCS) less than 12, and the majority of deaths occur exactly in these patients. Consequently, EDs care for large number of patients with minor head injuries, but are challenged when managing patients with moderate and severe TBI who carry enormous chances of dying or becoming disabled [6]. Therefore, our study focused on the latter group of TBI patients with moderate and severe injuries.

The ability to predict outcome in brain injury may significantly impact in patient
management, such as in invasive monitoring (ICP monitoring, tissue brain oxygen monitors, microdialysis, etc), in specific therapeutic strategies and in long-term care, especially in patients suffering moderate and severe TBI. Furthermore, outcome prediction after TBI may facilitate research, improve quality of care and assist with goals of care and end-of-life decisions. However, prediction of outcome after TBI is still inconsistent and difficult. While well-known markers have long been used in clinical practice to help predict outcome, there is controversy regarding how they should be utilized and evaluated. Therefore, identification of other reliable biomarkers or predictors of outcome in severe TBI patients is still needed.

Small observational cohort studies have demonstrated that plasma catecholamine levels (norepinephrine – NE and epinephrine – E) measured at hospital admission in patients with brain injury, rise exponentially as a function of severity [7-11]. Furthermore, levels of plasma catecholamines have been correlated with outcome in this population [7-11]. Clinical studies show a significant association between high levels of both E and NE in TBI patients on admission, and clinical indices, such as GCS score, duration of mechanical ventilation, myocardial damage, endocrine abnormalities, length of stay and neurological outcome [7-11]. However these studies have important methodological limitations such as small sample sizes and no adjustment for confounders. Furthermore, the levels of catecholamines on those studies are not measured early post trauma, especially within the first 24 hours, and this approach is important as we believe that the initial levels have the strongest association with outcome. Another limitation is that the studies generally enroll patients with multisystem trauma and not patients with isolated brain injury exclusively. Our study intends to overcome these limitations with a proper power to detect differences, adjusting for confounders, enrolling patients with isolated moderate to severe TBI direct from the scene of the accident, and measuring catecholamines since the early admission to the hospital. The objective is to demonstrate the natural history of catecholamine release early post traumatic brain injury and the association of the high levels with unfavorable outcome in patients with moderate to severe isolated TBI.

This literature review will provide the following: 1) pharmacology of catecholamines, 2) the role of catecholamines in TBI pathophysiology, 3) catecholamines and current TBI therapeutic strategies, 4) current tools used for prediction of outcome in
brain injury and 5) the role of catecholamines in predicting outcome in TBI.

1.2. Catecholamines

1.2.1. History of Catecholamine Research

Collectively, the term catecholamines comprise the endogenous amines nor adrenaline (norepinephrine), adrenaline (epinephrine) and dopamine. Their investigation constitutes a prominent chapter in the history of physiology, biochemistry and pharmacology. Adrenaline was the first hormone isolated from the adrenals and obtained in pure form, even before the word hormone was coined [12]. It was also the first hormone to have its structure and biosynthesis depicted. Acetylcholine, E and NE were the first neurotransmitters to be demonstrated, and had their intercellular biochemical signals found in intracellular vesicles described. In addition, the β-adrenoceptor was the first G protein-coupled receptor gene to be cloned. More focused catecholamine research began with the preparation by George Oliver and Edward Albert Sharpey-Schafer of a pharmacologically active extract from the adrenal glands [12].

1.2.2. Pharmacology

1.2.2.1. Epinephrine and Norepinephrine Synthesis

Catecholamines are derived from Tyrosine. Tyrosine is sequentially 3-hydroxylated and decarboxylated to form dopamine. Dopamine is β-hydroxylated to yield norepinephrine, which is N-methylated in chromaffin tissue to generate epinephrine [13]. The hydroxylation of tyrosine by tyrosine hydroxylase is generally regarded as the rate-limiting step in the biosynthesis of catecholamines [14]. This enzyme is activated following either direct stimulation of sympathetic nerves or via the actions of adrenocorticotropic hormone (ACTH) on the adrenal medulla. The enzyme is a substrate for activated protein kinase A (PKA), activated protein kinase C (PKC), and calcium/calmodulin dependent protein kinase (CAM Kinase). Kinase-catalyzed phosphorylation may be associated with increased hydroxylase activity [14, 15]. This is an important acute mechanism for increasing catecholamine synthesis in response to elevated nerve stimulation. In addition there is a delayed increase in tyrosine hydroxylase gene expression after nerve stimulation.
This increased expression can occur at multiple levels of regulation, including transcription, RNA processing, regulation of RNA stability, translation, and enzyme stability [16]. These mechanisms serve to maintain the content of catecholamines in response to increased transmitter release. Finally, tyrosine hydroxylase is subject to feedback inhibition by catechol compounds, which allosterically modulate enzyme activity.

1.2.2.2. Epinephrine and Norepinephrine Storage

Epinephrine and norepinephrine are stored in vesicles ensuring their regulated release; this storage decreases intraneural metabolism of these transmitters and their leakage outside the cell. The vesicular amine transporter (VMAT-2) appears to be extensively driven by pH and potential gradients created by an ATP-dependent proton translocase. For every molecule of E or NE taken up, two H⁺ are extruded [17]. Monoamine transporters are relatively promiscuous, and beyond E and NE they also transport dopamine and serotonin besides E and NE [18]. These amines are also transported by other neuronal membrane transporters which are present in the adrenal medulla, liver, placenta, stomach, pancreas and kidney [19].

1.2.2.3. Epinephrine and Norepinephrine Release

The full sequence of steps by which the nerve impulse affects the release of NE from sympathetic neurons is not known. The triggering event in the adrenal medulla is the liberation of acetylcholine by the preganglionic fibers and its interaction with nicotinic receptors on chromaffin cells to produce a localized depolarization [20]. A subsequent step is the entrance of calcium into these cells, which results in extrusion by exocytosis of the granular contents, including E. Influx of calcium likewise plays an essential role in release of NE at sympathetic nerve terminals. Calcium-triggered secretion involves interaction of highly conserved molecular scaffolding proteins leading to docking of granules at the plasma membrane and ultimately leading to secretion [20].

1.2.2.4. Termination of Action

The actions of E and NE are terminated by reuptake into the nerve terminal by amine transporters; dilution by diffusion out of the junctional cleft and uptake at extraneuronal sites by other amine transporters and by metabolic transformation. Two
enzymes are important in the initial steps of catecholamine transformation – monoamino oxydase (MAO) and catechol-\(O\)-methyltransferase (COMT). In addition, E and NE are metabolized by sulfotransferases [21]. The termination of actions by a powerful degradative enzymatic pathway, such as that provided by acetylcholinesterase at sites of cholinergic transmission, is absent from the adrenergic nervous system. Thus, the neuronal reuptake process becomes important and this is observed by the inhibitors of this process (e.g., cocaine and imipramine) that potentiate the effects of the neurotransmitter, while inhibitors of MAO and COMT have relatively little effect. However, MAO metabolizes a transmitter that is released within the nerve terminal. COMT, particularly in the liver, plays a major role in the metabolism of endogenous circulating and administered catecholamines.

**1.2.2.5. Receptors and Specific Actions**

On Figure 1 we illustrate the adrenergic receptors signaling pathways. Adrenergic receptors are G protein coupled receptors (GPCRs) and consist of 7 transmembrane domains, with an intracellular catalytic domain that interacts with subunits. When the receptors are bound by E or NE, their effects are initiated by secondary messenger systems. The receptors subtypes consist of two types, \(\alpha\) type (\(\alpha_1\) and \(\alpha_2\)) and \(\beta\) type (\(\beta_1\), \(\beta_2\), \(\beta_3\)), named primarily on the basis of their specific effects following stimulation [22]. Alpha receptors mediate excitation, while beta receptor activation generally results in relaxation (with the exception of the heart) [22]. What is observed is that alpha receptor activation leads to constriction of blood vessels, while beta receptor activation leads to vasodilation. In the heart, beta receptor activation (mainly \(\beta_1\)) results in increased contractility and cardiac output, and in the lungs (mainly \(\beta_2\)), causes bronchodilation.

The most documented effects of adrenergic receptor activation are related to the cardiovascular system, although the receptors are ubiquitously expressed throughout the body. Ahlquist [23] demonstrated that in the brain, the greatest density of \(\beta\) receptors is found in the cerebral cortex and corpus striatum. In these both areas, NE acts on \(\beta\) adrenergic receptors to increase glutamate reuptake, glucose uptake, and glycolysis. The same author showed that systemic sources of NE are the adrenal medulla and the sympathetic nerve tissue. Sympathetic neurons secrete only NE, and not E and NE is produced by noradrenergic neurons in the locus coeruleus in the brain stem. It seems that
severity of brain damage denotes higher levels of NE in the brain. Abercrombie [24] reported that NE levels measured in the microdialysate from the hippocampus remain normal until >50% of the neurons are destroyed. Higher release of NE was achieved when simulation of excitotoxicity was induced, even when <50% of the cells were depleted.

Xiao [25] reported that the β1 receptor couples exclusively with Gs proteins, while the β2 receptor can couple with either Gs or Gi. When coupled with Gs, adenylate cyclase is activated, which catalyzes the synthesis of cyclic adenosine monophosphate (cAMP) and activates protein kinase A (PKA). Increased cAMP levels catalyze ATP formation. PKA phosphorylates the membrane bound L, N, P, Q, and R type calcium channels, leading to increased calcium influx and membrane depolarization. Increasing calcium causes a phenomenon called calcium induced calcium release, in which inositol trisphosphate (IP3) and ryanodine receptors are stimulated to release calcium stored in the endoplasmic reticulum [26, 27]. The voltage regulated calcium channels poses an auto-inhibitory feedback mechanism that regulates calcium influx. In thalamo-cortical relay neurons, adrenergic stimulation prevents normal deactivation of high voltage activated calcium channels, potentiating calcium entry into the cell [28]. Increased cAMP levels have been shown to prevent the activation of microglia and inhibit the expression of the major compatibility complex (MHC) on astrocytes [28]. A study [28] using porcine corneal epithelial cells observed that isoproterenol increased cellular cAMP concentration, and decreased intracellular calcium. Finally, PKA activates the peroxisome proliferator-activated receptor gamma (PPAR-γ) transcription factor, which couples with retinoic acid receptor in the nucleus to initiate transcription. PPAR-γ transcription is largely anti-inflammatory, and PPAR-γ agonists have been shown to improve insulin sensitivity and reduce blood pressure [29]. PPAR-γ is also involved in the transcription of IL-6, and angiogenic factors. Furthermore, as described by several other studies [30-32], adrenergic agonists can also bind to β1 or β2 adrenergic receptors to activate the calcium/calmodulin (CaM) dependent protein kinase II (CaMKII) which is activated by calmodulin when bound to calcium. Activated, CaMKII phosphorylates the L, N, P, Q, and R type receptors, leading to high levels of cytosolic Ca2+ and phosphorylation of the cAMP response element binding protein (CREB). This process induces the transcription of growth factors such as brain derived neurotrophic factor (BDNF).
Protein kinase A is part of a negative feedback loop, and phosphorylates the β receptor, causing the coupling process to switch to a Gi coupled. Activated Gi protein induces the sequestration of adenylyl cyclase, leading to reduced levels of cAMP and inhibition of PKA. The Gi pathway also activates the mitogen activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway, switching to a pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) transcription profile where tumor necrosis factor alpha (TNFα) and interleukin (IL)-1B are produced. Dvoriantchikova [33], in an animal study, demonstrated that inhibition of NFkB lead to improved neuronal survival after ischemic injury. At high enough NE concentration, surface receptor expression is diminished as the receptors are endocytosed for recycling. Pippig [34], in a study with A431 stimulation of β2 receptors, demonstrated rapid decoupling from Gs, triggered by receptor phosphorylation and a delayed sequestration of the receptors to an internal compartment. Upon removal of the agonist, β2 receptors were recycled to the membrane surface, dephosphorylated, and the receptor function was restored.
1.2.3. Catecholamines in Traumatic Brain Injury

1.2.3.1. Effects in the Brain

Severe trauma elicits a complex stress response, characterized by profound alterations in neuro-endocrine and immune function geared towards re-establishing...
homeostasis. Activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system (SNS) via the secretion of glucocorticoids and catecholamines, along with intricate neuro-immune interactions, are recognized as central pathways in the pathogenesis of post-traumatic complications [35, 36]. TBI in particular leads to immediate and profound SNS activation with massive release of both central and peripheral catecholamines [37]. Patients who have sustained head injury, particularly those with elevated ICP, often exhibit hypertension, tachycardia and other signs of increased sympathetic nervous system activity [37, 38].

Levels of E and NE increase several fold in patients with TBI compared with controls [39-43]. The initial surge is followed by a hyperadrenergic state lasting for a variable time period after the initial trauma, what is also demonstrated in patients with non-traumatic SAH [44]. Several studies have noted a correlation between the increase in catecholamine levels, the severity of TBI, and clinical outcome [37, 39, 42, 43]. In a study by Woolf [45] patients who achieved normalization of their GCS score after injury also had concomitant normalization of their NE levels. Furthermore, those that remain comatose have persistently elevated NE levels, up to seven times normal. Plasma NE levels at 48 hours after TBI may also be predictive of GCS, survival, length of stay, and number of days on a ventilator [45].

Whether the initial catecholamine surge is detrimental or beneficial to the patient who has sustained a TBI is currently unknown. The current evidence, however, suggests that an exaggerated adrenergic response may be harmful to patients with isolated moderate to severe TBI [46]. Certainly, from an evolutionary medicine point of view, maintenance of cerebral blood flow and metabolism would seem to afford a survival advantage. Current evidence shows clinically relevant systemic effects of the catecholamine surge following systemic trauma, in TBI and in non-traumatic brain injury. Deleterious effects to the cardiac, pulmonary, endocrine, and immune systems have been described [47-59].

However, the effects of catecholamine surge and hyperadrenergic state on the brain are less clear. Adrenergic receptors have been identified in the brain [60, 61] and cerebral vasculature, but there is little clinical evidence of the effects of the catecholamine surge on the brain itself. Bryan [62], in a review of the effects of stress (but not specifically that of TBI) on cerebral blood flow and energy metabolism, emphasized the role of beta
adrenergic receptors within the brain as a key mediator of the effects of stress on cerebral blood flow and energy metabolism. The author identified that there are 3 main sources of catecholamines that may stimulate the cerebral beta adrenergic receptors: systemic, from the adrenal medulla; central, from the locus coeruleus; and sympathetic, from the superior cervical ganglia.

In the brain, the direct toxicity of catecholamines intrinsic to the CNS was investigated by Rosenberg [63] in an animal model. The author found that E, NE and dopamine were toxic to neurons and glia. Toxicity was evident after exposure to NE, which was monitored by loss of cells from the cultures. It appeared that toxicity was not mediated by adrenergic receptors because, although isoproterenol (but not phenylephrine) was similar in its toxic effect to NE, and atenolol did not block the toxic effect of NE. The author was able to simulate toxicity by a metabolite of the oxidative degradation of catecholamines, hydrogen peroxide and catalase blocked the toxicity of NE. The neurotoxin 6-hydroxydopamine (6-OHDA) was toxic over the same concentration range as NE. The study suggests that endogenous catecholamines may participate in normal and abnormal cell death, and suggest that caution should be taken on the specific 6-OHDA other supposedly selective neurotoxins.

The blood brain barrier (BBB) normally prevents circulating catecholamines from entering the brain [64, 65]. The BBB is damaged following brain injury as demonstrated by Schoultz [66] who found that trauma to the spinal cord damages the BBB possibly leading to the accumulation of catecholamines in the central nervous system (CNS) from the circulation. Such accumulation could affect the local microcirculation and directly affect cellular function in the brain [67]. Over time, sustained levels of NE lead to a leaky barrier and may induce cerebral edema and ischemia [68]. Furthermore, it has been found that sympathetic activation after experimental TBI may adversely influence cerebral perfusion [69]. Other animal studies have shown that catecholamines enhance the inflammatory response in the brain and further increase edema [70-72]. Han [73] and Ueyama [74] reported that sympathetic stimulation is associated with lower levels of cerebral heat-shock protein 72, an antiapoptotic protein, and increased expression of the so-called immediate early genes indicating cellular activation in response to stress [74, 75]. In line with these findings, neuroprotective effects of β-blockers have been suggested based on attenuation of
cerebral metabolic activity, decreased infarct size, and improved functional outcome in animals subjected to cerebral ischemia and TBI [67, 72, 73, 75]. Administration of propranolol to a murine model of blunt head injury led to a 152% improvement in cerebral perfusion and a 24% reduction in cerebral hypoxia [76]. The authors propose therefore that adrenergic-mediated cerebral vasoconstriction is a mechanism contributing to the secondary events after TBI. Liu [72] demonstrated a protective effect of propranolol after blunt trauma, including better neurologic recovery, better grip test scoring, and reduced brain edema. It was postulated that the effects were a result of propranolol on the vasomotor centers in the hypothalamus.

The catecholamine surge is not unique to TBI and has also been observed after other intracranial processes such as subarachnoid hemorrhage (SAH) [57] and in non-cerebral insults such as burn injuries [47, 77]. Many other investigators have found a clear association between intense sympathetic activity in animal and human studies with SAH, and cerebral arterial vasospasm [78-80]. In experimental studies, both cervical sympathetic gangliectomy and lesioning the ascending catecholaminergic pathways from the pons and medulla oblongata prevented vasospasm in animal SAH models [78, 79]. In a human study [80], it was found that higher levels of catecholamines in cerebrospinal fluid (CSF) were associated with ischemic deficits.

It appears that some regions of the mid brain are associated importantly with the hyperadrenergic storm. Hypothalamic dysfunction, for example, is frequently identified in patients with brain injury. Post-mortem studies have reported that 70% of deaths display hypothalamic injury [81]. The dysfunction in the hyperadrenergic state occurs within the autonomic centers in the diencephalon (thalamus or hypothalamus) or their connections to cortical, subcortical, or brain stem loci that mediate autonomic function. Initially, it was suggested that a loss of cortical and subcortical control of basic functions occurs, including blood pressure and temperature regulation [82]. Later, a mechanism involving activation (or loss of inhibition) of central sympathoexcitatory regions such as the paraventricular hypothalamic nucleus, lateral periaqueductal gray substance, lateral parabrachial nucleus, or rostral ventricular medulla was demonstrated [83]. The subsequent release of adrenomedullary catecholamines during the hyperadrenergic episodes may lead to hypertension, tachycardia, and tachypnea [84, 85].
In summary, the physiopathology of the adrenergic storm in the CNS of patients with brain injury is not completely understood. The existing evidence, however, suggests that an exaggerated adrenergic response may be harmful to patients with brain injury, as some studies have shown an association between catecholamine levels, the severity of brain damage, and functional neurological outcome. High levels of circulating catecholamines seem to be associated with more cerebral ischemia, edema and high intracranial pressures, with consequent worse outcomes.

1.2.3.2. Paroxysmal Sympathetic Storm

The overt early sympathetic response after brain injury is responsible for early local cerebral effects, systemic effects and a syndrome that is characterized by later clinical manifestation. One third of all patients with severe TBI die within the first 3 days of injury. Following this period, the underlying causes of death are mostly the result of sepsis and non-neurologic organ damage that is primarily represented by respiratory failure and cardiovascular dysfunction, the late manifestation of the sympathetic surge. Several studies [59, 86, 87] have demonstrated that non-neurologic multiple organ dysfunction is the result of the interaction between the brain injury and an overt adrenergic response. Patients may develop a syndrome of intermittent agitation, diaphoresis, hyperthermia, hypertension, tachycardia, tachypnea, and extensor posturing. Penfield [88] (1929) first described these symptoms in a 41-year-old man in whom the ensuing post-mortem examination revealed a tumor involving the foramen of Monro. Following this, the author collected a series of patients who had suffered TBI and described the manifestation of symptoms very similar to this initial individual. Later, Rossitch [88] detailed cases of individuals suspected of autonomic dysfunction syndrome. The patients presented signs of sympathetic discharge and extensor posturing after severe closed TBI and acute hydrocephalus. Examining the responses to medications, the authors proposed that TBI was somehow inducing alterations in the opiate and dopaminergic pathways. With a better understanding of the pathophysiology of the sympathetic storm and non-neurologic manifestations of TBI, investigators have studied more closely the potential for beta-adrenergic blocking medications. Despite this, adrenergic hyperactivity after TBI, a condition with high morbidity and mortality, remains poorly elucidated and undertreated.
The etiology and pathophysiology of the sympathetic storm is still being defined. It is believed that TBI may in some patients initiate an overt (exaggerated) activation of the SNS with central and peripheral release of catecholamines. The exaggerated activation of the SNS leads to effector organ activation, including the adrenal gland, initiating the release of catecholamines which causes the manifestation of non-neurologic symptoms. Previous studies have reported a loss of inhibition of central sympathoexcitatory regions. As evidence mounts, it is becoming more apparent that there is little evidence to support the storm being caused by a cerebral disconnection [89]. Disconnection theories state that dysautonomia occurs due to loss of superior control over one or more excitatory centers. Conventional disconnection theories suggest that there is a loss of cortical regulation of the upper brain stem and diencephalic regions (which are the central excitatory foci driving the paroxysms) when pathways from the cerebral cortex to the midbrain are injured [90, 91, 92]. The excitatory: inhibitory ratio (EIR) model, another disconnection theory, suggests that damage to the brain stem and diencephalic centers release the excitatory spinal cord processes from their inhibitory effects [93]. In essence, the spinal cord is responsible for modulating both the afferent stimuli from the periphery as well as the efferent centrally originating signals. Sympathetic output activity in the brain is regulated by the spinal cord, which modulates the EIR, thus protecting the end organ. When the brain stem EIR is overwhelmed by sympathetic cerebral hyperactivity, as seen in TBI, then the neighboring spinal EIR is also overwhelmed, losing its protective effect, and is unable to inhibit or balance large catecholamine surges to the peripheral organs [91, 94]. This is manifested by end-organ dysautonomia of multiple end organs. Sympathetic overactivity (hyperthermia, tachycardia, hypertension, tachypnea, and sweating) and motor overactivity (rigidity, spasticity, and dystonias) may manifest simultaneously [93]. Other potential sources of catecholamines namely from the adrenal medulla, and from the superior cervical ganglia, may be activated by injury completely independent of central activity. The catecholamines released from these areas could circulate centrally back to the cortex resulting in more hyperadrenergic symptoms and further brain injury.

Kupferman [95] demonstrated that hyperthermia in the hyperadrenergic state is produced by hypothalamic disturbances. However, it may also be induced by the hypermetabolic state that is seen in conjunction with sustained muscular contractions.
Brain injury increases the local metabolic demands locally in the brain and is superimposed on the extra metabolic demands caused by the post hyperadrenergic state; events notable for profound catabolism. This hypermetabolic state is characterized by an increase in energy expenditure by up to 75% [96, 97], resistance to nutritional support and ensuing weight loss, which worsens the outcome of patients with TBI [98]. Patients with severe burns for example, who have marked elevated levels of circulating catecholamines, have been shown to have an increased metabolic rate, that can be decreased substantially by the administration of beta adrenergic blocking agents [99].

In summary, the overt hyperadrenergic surge derived from TBI causes cerebral and systemic damage, represented mostly by cardiorespiratory failure and infection. This systemic, non-neurologic damage is harmful in patients with brain injury and worsens outcome. The etiology and pathophysiology of the paroxysmal sympathetic storm is still being characterized, however, disconnection theories state that there might be a stimulation or loss of inhibition of the sympathetic output from the CNS.

1.2.3.3. Effects in the Cardiovascular System

As stated before, the hyperadrenergic surge post TBI can cause significant and harmful cardiovascular dysfunction, leading to hypotension and hypoxia and consequently having a negative impact on the outcome of TBI patients. In patients with the early hyperadrenergic storm, the most common ECG changes are sinus tachycardia [100]. Bradycardia, ST segment changes, and fatal ventricular dysrhythmia occur in only 5% of all patients [101]. The ECG changes due to the sympathetic storm tend to be asymptomatic, and normalization of repolarization occurs in association with resolution of the neurologic insult. However, more extensive neurologic injury resulting in a sustained sympathetic discharge may result in permanent ECG changes, including the development of Q waves [102].

Circulating markers of myocardial damage are elevated in a variety of acute neurological diseases, including ischemic and hemorrhagic stroke, SAH, and TBI. Evidence of myocardial damage demonstrated by increased troponin has been reported in 10–34% of patients with acute stroke [103]. Furthermore, cardiac troponin seems to be elevated in patients with high levels of catecholamines. Rhind [104] in a study with use of
hypertonic saline in TBI patients, demonstrated that cardiac troponin concentrations were significantly correlated ($r=0.455; p=0.0002$) with the Injury Severity Score (ISS), and patients with high cardiac troponin (>0.02 ng/mL) levels also had the greatest concentrations of E, NE and DA. In the same study, patients with fatal outcome had significantly higher serum concentrations of all three catecholamines and cardiac troponin compared to survivors.

It has been hypothesized that myocardial damage in acute neurological events is a result of “massive” activation of the sympatho-adrenal axis resulting in myocytolysis with band necrosis instead of acute coronary thrombosis [105, 106]. Furthermore, the deleterious effects on the myocardium may lead to ventricular hypokinesis [107, 108]. In studies utilizing transesophageal echocardiography or scintigraphy [109-111], a 50% reduction in left ventricular function has been demonstrated. Myocardial necrosis is usually found in patients with premortem ECG disturbances and/or elevated cardiac enzymes. Indeed, such necrosis is a common autopsy finding in patients with severe TBI [112, 113].

In summary, the early recognition of the cardiovascular dysfunction due to the hyperadrenergic state post TBI is fundamental to avoid more systemic complications, especially in patients with extensive neurologic injury which may result in permanent myocardial damage.

1.2.3.4. Effects in the Lungs

Neurogenic pulmonary edema (NPE) is a condition also caused by massive release of E/NE following TBI. It is an under-diagnosed complication reported in only 20% of cases of severe TBI [114], in 32% of those with isolated TBI that die at the scene, and in 50% of TBI victims dying within 96 hours post admission [115].

Regarding its pathophysiology, two different mechanisms seem to coexist, triggered by a sudden increase in ICP (and global decrease in brain perfusion) or a localized ischemic insult in suspected trigger zones (vasomotor centers and pulmonary input and output locations) [116]. The importance of pulmonary dysfunction during elevated ICP has been particularly well described in brain death cases. In these patients, high ICP appears to be associated with the initiation and worsening of the massive catecholamine storm. The
hemodynamic mechanism involves catecholamine-induced intense pulmonary vasoconstriction resulting in an increase in pulmonary hydrostatic pressure, followed by an increase in permeability of pulmonary capillaries compounded by inflammatory mechanisms (also catecholamine-dependent) that further increases the pulmonary capillaries permeability [117]. The entire process may be related to the massive and early release of catecholamines after injury. Several mediators have been implicated in the genesis of NPE, the ensuing sympathetic activation and the resulting endothelial injury [118, 119]. The Neuropeptide Y and NE, which are co-located in large dense vesicles in the sympathetic nerve endings, are secreted in the lungs in large quantities in response to a sympathetic storm [119, 120]. They play an important role in the development of NPE by their vasoconstrictive action and by increasing pulmonary vascular permeability [121, 122]. Vascular endothelial pressure-related insults cause the local release of endothelin-1, which also causes vasoconstriction and are suspected to be involved in experimental TBI models. Inflammatory mechanisms were also linked to the initialization or perennialization of NPE [123].

In summary, NPE is a non-neurological manifestation of the sympathetic surge following TBI, and is under-recognized. There appears to be an association between increased ICP and consequent global cerebral hypoperfusion and NPE, and as well as an association with local ischemic insult in possible trigger zones and inflammatory process.

**1.2.3.5. Effects in Inflammation**

The exacerbated catecholamine release that occurs after brain injury triggers the inflammatory response, since cellular activation at the molecular level until the mobilization of the neutrophils in the peripheral circulation [124]. Traumatic brain injury is followed by a systemic inflammatory response syndrome (SIRS), where inflammation causes the disruption or dysfunction of one or more organ systems [124]. For example, in patients with severe TBI, the presence of at least one organ system dysfunction occurred in 89% of subjects [125]. SIRS results from the release of inflammatory mediators that cause early, delayed, and systemic effects of TBI, including subsequent complement deficit and coagulopathy. Once SIRS is triggered by acute inflammation, it can detrimentally self-propagate [124]. Systemic inflammation causes tissue damage leading to further
inflammation and damage, leaving the body in a vicious cycle of hyperinflammation. Therefore, important inflammatory mediators like interleukin (IL)-1 beta, IL-6 and tumour necrosis factor (TNF) alpha, are targeted in compensatory anti-inflammatory response syndrome (CARS), in an attempt to control the development of SIRS. However, the activation of CARS often leads to immunosuppression and subsequent MODS [124].

Catecholamines can activate both alpha and beta adrenergic receptors, although the general preference for E is beta, and for NE is alpha. In general, α-adrenoreceptor stimulation has immunostimulating effects, while stimulation of the β-adrenoreceptor may be immunosuppressive [126, 127]. As consequence, there is a great deal of uncertainty in regards to the pro vs. anti-inflammatory effects of catecholamines on innate immunity in general, and their specific effects in trauma [128].

After brain injury, particularly, the huge catecholamine surge can render the immune system anergic, and lead to an inability to fight sepsis, possibly culminating in organ failure. The exaggerated release of catecholamines can alter the production of multiple inflammatory mediators (they induce interleukin [IL]-10 release from monocytes, for example) in peripheral blood immune cells and in various organs (spleen, pancreas, lungs and the diaphragm). These immunomodulatory effects have increasingly received attention, especially due to the potential for pharmacological intervention. For example, patients with sympathetic storm post TBI have higher levels of IL-10 and severely depressed monocytic HLA-DR expression, 62% of whom develop severe infections [128]. Another example is shown in a study using isolation of splenic macrophages of a polymicrobial sepsis model, in which further adrenergic stimulation inhibits TNF and IL-6 production by the macrophages [129]. Meisel [130], in a very detailed review article published in 2005, stated that infections post brain injury impede neurological recovery and increase morbidity and mortality. The normal balanced relationship between the CNS and the immune system is deranged leading to the CNS injury-induced immunodepression (CIDS) and infection, a secondary immunodeficiency. The reason why CNS injury initiates a reaction with such a maladaptive response is not understood currently. CIDS can be seen as a consequence of a dysregulation in CARS [130]. Alternatively, CIDS may have evolved as a protective response helping to prevent postinjury auto-agression to CNS [131-133]. However, the interpretation of CIDS ‘function’ is complicated by the fact that a
certain degree of autoimmunity may be needed for regeneration of CNS post injury. More specifically, injury of the CNS would evoke a T-cell-mediated autoimmune response that would reduce the degeneration in the CNS induced by injury [134]. The association between CIDS and the protection of autoimmunity is not known.

As discussed before, sympathetic hormonal regulation of cytokine production and release is highly dependent on which type of receptor is being stimulated and its location. It was recently demonstrated that phagocytic cells (i.e., polymorphonuclear neutrophils and monocyte-macrophages) are sources of catecholamines themselves and that both E and NE directly activate macrophages causing enhanced release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) [135-137]. Experimental studies have shown that catecholamines are not only potent inflammatory activators of macrophages but also that increased levels of phagocyte-derived catecholamines are associated with intensification of the acute inflammatory response, including increased plasma leak of albumin, myeloperoxidase content in lungs, levels of pro-inflammatory mediators in bronchoalveolar lavage fluids, and expression of pulmonary intercellular and vascular cellular adhesion molecules (ICAM-1 and VCAM-1, respectively) [138, 139].

Until recently, the brain was considered an immunologically “privileged” site, but evidence to the contrary exists and is rising, particularly following TBI. Transmigration of leukocytes after blood brain barrier disruption results in the activation of immuno-functioning resident cells of the central nervous system, and both infiltrating peripheral immune cells and activated resident cells subsequently engage in the intrathecal production of cytokines. Cytokines can either support neurotoxicity by promoting excitotoxicity and inflammation, or attenuate the damage through neuroprotective and neurotrophic mechanisms, including the induction of cell growth factors. Interleukin-6 (IL-6) would be a typical cytokine exerting a ‘dual role’ in neuroinflammation. Its specific anti-inflammatory properties include the inhibition of TNF, induction of IL-1RA and stimulation of NGF production, decreasing glutamate-mediated toxicity and oxidative stress, while its proinflammatory properties include inducing chemotaxis and up regulation of chemokine production and adhesion molecule expression. This duplicity of function reflects the contradictory findings in patient outcome studies to date: microdialysate and CSF levels of IL-6 have been shown to be associated with a favorable outcome measured by the Glasgow
outcome scale extended (GOSE) (Table 3), whereas serum measurements have demonstrated a relationship with unfavorable outcome [105, 106].

Based on the role of catecholamines on inflammatory response and the concept of TBI being an inflammatory condition of the CNS, growing evidence suggests that the hyperadrenergic state resulting from severe TBI may cause further impairment of the already damaged brain [53, 124]. Additionally, experimental and clinical studies on the neuroprotective effects of β-blockers and alcohol offer indirect evidence to support the hypothesis that high catecholamine levels are not only predictors of outcome, but in fact, may contribute to the pathogenesis of TBI.

Some experimental TBI studies have suggested that β-adrenergic receptor antagonists can improve functional outcome and lessen cerebral edema [140]. Retrospective studies of TBI patients suggest that β-blockers limit mortality [141], although the exact mechanism(s) of this effect is unknown. In vitro studies demonstrated that E pretreatment significantly increased TNF-α production with lipopolysaccharide stimulation and β2-receptor blockade significantly attenuated it. In vivo studies have demonstrated a significant decrease in TNF-α and IL-6 production in patients treated with β-blockers. A retrospective study evaluated the effect of β-blockers on survival in 174 TBI patients compared to 246 TBI patients not using beta-blockers [142]. This study showed that β-blocker exposure was associated with a significant reduction in mortality in patients with severe TBI, in spite of being an older group of patients and having a lower predicted survival.

Experimental studies have also investigated the beneficial effects of alcohol on TBI and have shown that alcohol can be neuro-protective in low to moderate doses (<240mg/dl). The proposed neuro-protective mechanisms involve a reduction in immediate hyperglycolysis, inhibition of the Nmethyl-D-aspartate receptor, which is involved in excitotoxicity, and decreased pro-inflammatory cytokine production. However, these beneficial effects seemed to be lost at higher doses. Indeed, a study by Tien [143] suggests potential neuro protective effects of alcohol (survival benefit) in low to moderate concentrations (<230mg/dL) compared to an alcohol concentration of zero. However, in higher concentrations it seemed to be detrimental by affecting the host homeostatic
compensatory response to shock. A retrospective study [144] evaluated 14,419 patients with isolated moderate or severe TBI who were tested positive for blood alcohol. After logistic regression analysis, ethanol was associated with reduced mortality (adjusted odds ratio [OR], 0.88; 95% confidence interval [CI], 0.80-0.96; \( p = .005 \)) but with higher complications (adjusted OR, 1.24; 95% CI, 1.15-1.33; \( p < .001 \)). Interestingly, studies published in 1990 and 1991 [145, 146] evaluated the effects of alcohol on catecholamine levels in patients with blunt TBI and multisystem injury within 5 hours of injury. They showed that NE levels had a significant direct correlation with GCS and ISS, and that alcohol intoxication significantly lowered the NE response in patients with lower GCS and with a higher ISS. In those studies, the blunting of the catecholamine response was most marked in those severely injured. Considering the pro-inflammatory effect of sympathetic activation after TBI, the anti-inflammatory effect of alcohol and its ability to decrease catecholamine release after TBI, one can speculate that blunting the catecholamine response would be one of the neuro-protective mechanisms involved in alcohol intoxication as well as in its detrimental effect on shock response.

In summary, injury to the brain causes not only a local inflammation, but also SIRS and systemic tissue damage. Subsequently, CARS occurs in attempt to control the development of SIRS providing negative feedback for the production of inflammatory mediators. However, the activation of CARS often leads to immunosuppression and infection which may result in CIDS, MODS and contribute to a high mortality rate. The various inflammatory mediators play a pivotal role in the activation, development and prognosis of SIRS following acute TBI. A fuller understanding of the pathophysiological processes will undoubtedly help in developing strategies for early diagnosis and therapy, contributing to a decrease in the mortality rate of patients with brain injury.

1.3. Catecholamines and Current TBI Therapeutic Strategies

Currently, there is growing interest in studying adrenergic agonists and antagonists for the treatment of brain injury. Several studies in animals and in humans have investigated the administration of different types of \( \beta \)-blockers and alpha agonists/antagonists. Many studies demonstrate that the use of agonist [147-149] and
antagonist [70, 71, 72, 150-152] medications have protective effects and many other studies [153-156] have reported efficacy with both. This apparent paradox may be explained by the fact that the use of agonist medications also downregulates the receptor activity through negative feedback mechanisms. Brain injury also causes upregulation of expression in some receptors, such as β₂ [157]. Current experimental evidence suggests that the modulation of early adrenergic response to brain injury may improve functional outcome and cerebral edema after treatment with β-blockers.

The studies from the Lund group [55, 158-166] have advocated the use of β-blockers in brain injury. The group has developed a management protocol based on volume-targeted therapy principles. The basis of the protocol is the optimization of fluid flow across the blood brain barrier (BBB) to reduce cerebral edema. The Lund group proposed the following measures to achieve this goal: 1) stress reduction with adequate sedation and catecholamine blockade; 2) maintenance of euvoemia through the use of erythrocyte transfusion and maintenance of a normal albumin level; 3) preservation of cerebral perfusion pressure (60–70 mm Hg for adults and 40–55 mm Hg for children and adolescents); 4) avoidance of cerebrospinal drainage; 5) use of early nutrition; and 6) use of mechanical ventilation to promote normal oxygenation and ventilation. In part the protocol emphasizes the use of metoprolol, a selective β1-antagonist, and clonidine, a α2-agonist used to limit the posttraumatic hyperadrenergic stress response. These investigators advocate the use of these agents to limit the formation of cerebral edema. Clonidine mediates systemic vasodilation and inhibits the release of central catecholamine [167] and metoprolol reduces myocardial contractility, lowers cardiac output, and lowers median arterial blood pressure. Combined, these drugs can be used to hypothetically reduce capillary hydrostatic pressure to the point where fluid filtration halts and reabsorption can occur. Although this induced reduction in blood pressure may lower cerebral perfusion pressure [168], the Lund group has used hemodynamic [158] and microdialysis [167] data to suggest that this effect is well tolerated by patients with brain injuries. Clinical studies indicate that the volume-targeted Lund therapy may reduce the mortality rate following TBI. To date, several studies have been performed indicating improved survival in adults [158, 162, 163] and children [166] treated with Lund therapy. Eke [159] reported a reduction of mortality from 47 to 8% (p<0.001) in patients with severe TBI and high ICP.
In another study, Naredi [162] reported 13% mortality rate in the analysis of a standardized therapy focusing on prevention and treatment of vasogenic edema in patients suffering severe TBI. Of the 33 surviving patients, 27 (71%) were noted to have had a good recovery or moderate disability. In another study [163], these same authors reported that only 1 of 31 patients who underwent treatment with Lund therapy died, and 22 experienced a good recovery or only moderate disability. It should be noted that the significance of these results is controversial due to the nonrandomized nature of the studies and the use of historical controls. Despite these criticisms this group appears to have shown that the adrenergic blocking agent clonidine and metoprolol can be applied to a group of patients with TBI without significant adverse effects.

In an animal study, Liu [71] used a mouse model to analyze the effects of propranolol. The author performed a weight drop impact in BALB-C mice, followed by an intraperitoneal injection of 2.5 mg/kg of propranolol. Mice who received propranolol had a reduction in brain water content and scored higher on the string test (an ordinal scoring system for the ability of the mouse to hold on to a string) and grip time test at one hour after injury. In another study [72] performed in mice injured via a 60 minute middle cerebral artery occlusion followed by 24 hour reperfusion, Han compared the specific β2 inhibitor ICI 118,551 and β2 genetic knockout. They reported that β2 KO animals had a 22% reduction in infarct volume measured by cresyl violet or TTC (2,3,5-triphenyltetrazolium chloride) when compared to controls. Furthermore, the lesion size was reduced 25% in mice treated with ICI 118,551. In a study by Kato [69], the reduction of the acute phase protein IL-6 was reported in CSF after SAH. The author investigated the role of adrenergic inhibitors on cytokine levels in CSF after SAH. They found that rats treated with butoxamine or propranolol, which inhibits β2 receptors, had reduced IL-6 concentrations. In contrast, the β1 inhibitor metoprolol was ineffective. In a rat MCAO model, IV treatment with propranolol (β1/β2), carvedilol (β1/β2/a1), esmolol (β1) or landiolol (β1) resulted in improved neurological deficit scores and smaller infarct volumes [146]. Different types of adrenergic agonists or antagonists have been investigated in animal studies. Junker [156] demonstrated that in mixed cultures treated with glutamate, cell death was blocked by the β2 agonist clenbuterol (1uM) and the effect of clenbuterol was reversed by the nonspecific β1/β2 antagonist propranol, β2 antagonist ICI 118,551,
but not β1 antagonist metoprolol. In the animals, clenbuterol was again protective and this could be reversed by propranolol. However, when they combined clenbuterol with metoprolol, they identified a better neuroprotection than clenbuterol alone.

In humans, several retrospective trauma database analyses demonstrated decreased mortality in TBI patients treated with β-blockers. One study [141] in severe TBI patients identified that β-blockers were associated with reduced mortality (adjusted odds ratio: 0.54; 95% CI, 0.33 to 0.91; \( p < 0.01 \)). In the same study [141] β-blocker therapy was associated with a significant survival advantage in TBI patients with elevated cardiac troponins (OR: 0.38; \( p = 0.03 \)). When the type of β-blockers was compared, lower mortality was observed in TBI patients who received propranolol. More recently, systematic review and meta-analysis performed by Alali [169] included one randomized controlled trial (RCT) and eight retrospective cohort studies. The critical appraisal of the included studies demonstrated moderate to severe risk of bias. They concluded that the current body of evidence is suggestive of a benefit of beta blockers following TBI. However, methodologically sound RCTs are indicated to confirm the efficacy of β-blockers in patients with brain injury.

There are several theoretical pros and cons with regard to the preference for either α- or β-adrenoreceptor antagonists, or both, although blockade of both α- and β-adrenoreceptors with drugs would seem a logical step to try and prevent the complications caused by sympathetic overactivation in acute brain injury. For instance, many experts would agree that blocking peripheral β-adrenoreceptors, with the risk of inducing hypotension, may endanger cerebral blood supply, as elevation of blood pressure (which is preferably left untreated) is generally considered a beneficial compensatory reaction to preserve cerebral blood flow [129].

In an international multicentre RCT (n=114), Cruickshank [170] investigated the association between increased sympathetic activity and cardiac morbidity, and the effect of β-1 selective adrenoreceptor blockade with atenolol. The study was performed in patients with TBI, with or without extracranial injury admitted to an ICU. Treatment with atenolol was administered intravenously, 10 mg every 6h for 3 days, followed by 100 mg orally for 4 days, or a matching placebo. Patients had to be hemodinamically stable, and a strict
inclusion and exclusion criteria was adopted. The follow up was possible in 104 patients and baseline characteristics and concomitant drug use was well matched in both groups. The authors found that NE levels were higher in patients than in controls, but there was no treatment effect of atenolol on these levels. They also measured CKMB and CK (with a known cut off for significant myocardial injury) and concluded that the cardiac enzymes were elevated in 30% of controls and in 7.4% ($p<0.05$) in the group treated with atenolol. Supraventricular tachycardias, ST segment and T wave changes occurred less frequently in the atenolol group. No focal necrotic lesions were detected in 4/4 hearts that were examined post mortem in the atenolol group, whereas the post mortem analysis of 2 patients in the placebo group, showed necrotic lesions in both.

Several recent observational studies confirmed a robust association between the use of $\beta$-blockers and improved outcome, presumably by attenuation of the hyperadrenergic state post-TBI. Cotton [142] found in a retrospective cohort (n=420), based on a large trauma registry, that $\beta$-blocker exposure for at least two days after admission in patients with TBI was associated with an adjusted decreased mortality (5.1% vs. 10.8%) when compared to patients that did not use $\beta$-blockers during the course of their illness. In another study by Inaba [171], 1156 patients were included in a retrospective cohort, and 18% received $\beta$-blockers (n=203). Patients with severe extracranial injuries and nonsurvivable head injury were excluded. Stepwise logistic regression was performed to identify independent prognostic factors for mortality. The main findings were that $\beta$-blocker use is inversely associated with mortality (adjusted OR 0.5, 95% CI 0.3-0.9). The three strongest predictors for death were head abbreviated injury severity score (AIS) $\geq$4 (OR 5.0, 95% CI 2.9-8.6), GCS $\leq$8 (OR 4.5, 95% CI 2.9-6.9), and age $\geq$55 (OR 3.7, 95% CI 2.4-5.8). The stratification of patients with these predictors of mortality in a subgroup analysis identified that they benefit the most from $\beta$-blocker exposure. No $\beta$-blocker use was associated with an OR of 2.32 (95% CI 1.02-10.57) for death in these patients. The above mentioned observational findings were corroborated in a large retrospective single center cohort study [172] (N=2601) with blunt TBI patients. There were 506 patients (20%) using $\beta$-blockers with a mean time to first dose of 5 days (0-132 days). $\beta$-blocker use was considered to be present when more than one dose had been administered according to the pharmacists’ registry. Patients using $\beta$-blockers were older, had more serious associated
injuries, slightly lower GCS on admission, longer ICU length of stay, longer hospital stays, and more frequently experienced ventilator associated pneumonia. The unadjusted data showed equal mortality of 15-16% in both the β-blocker treated and non-β-blocker treated patients. However after adjustment for prognostic variables as selected by a regression model β-blockers were significantly associated with survival (OR for death 0.347, 95% CI 0.246-0.490).

Finally, in a 2014 large retrospective cohort [173] (n=1,755), Schroeppe1 studied patients with brain injury admitted during a 48-month period, who received β-blocker, compared with those who did not. The study excluded patients who received pre-injury β-blocker, deaths within 48 hours, and head AIS score <3 or >5. In addition, propranolol was also compared with all other β-blockers. They found that patients who received β-blockers (n=427) were older (49 years vs. 40 years; \( p < 0.0001 \)), had a higher ISS score (30 vs. 24, \( p < 0.001 \)), and had a higher head AIS score (4.2 vs. 4.0, \( p < 0.001 \)). By univariate analysis, β-blocker patients had a higher mortality (13% vs. 6%, \( p < 0.001 \)); after adjusted analysis, no difference was identified (adjusted OR 0.850, 95% CI 0.536-1.348). Seventy-eight patients (18%) received propranolol during the study. Propranolol patients were younger (30 years vs. 53 years; \( p < 0.001 \)) but more severely injured (ISS, 33 vs. 29; \( p = 0.01 \); head AIS, 4.5 vs. 4.2; \( p < 0.001 \)), with longer hospital stays (44 days vs. 26 days, \( p < 0.001 \)). Mortality was less in the propranolol group (3% vs. 15%, \( p = 0.002 \)). Adjusted analysis confirmed the protective effect of propranolol (adjusted OR 0.19, 95% CI 0.043-0.920). The authors concluded that propranolol is the best β-blocker to limit secondary injury and decrease mortality in patients with traumatic brain injury.

Relevant pharmacological properties with regard to penetrance of specific β-blockers into the brain should be taken into account when studying the effects of these agents on adverse cerebral effects of sympathetic activation. Caution is advised however, in extrapolating pharmacokinetic properties in this regard from healthy subjects to patients with acute brain injury because permeability of the blood-brain barrier is probably much higher in the latter.

In summary, the evidence reports a potential benefit in reducing mortality with the use of β-blocker in patients with brain injury and as consequence it may be justifiable to
proceed to phase II studies in humans. However, several important choices have to be made reflecting the uncertainties that still exist on the clinical significance and pathophysiology of sympathetic overstimulation. Questions to be answered are, for example: 1) the type of β-blocker to be tested (selective versus nonselective, penetration of blood-brain barrier or not); 2) the precise selection criteria for study participation based on the presence or absence of risk factors for sympathetically mediated complications; 3) the primary outcome to be chosen (stress cardiomyopathy incidence, functional outcome, mortality); 4) dosing issues; 5) initiation and duration of treatment; 6) fixed dose treatment or titration to, for instance, a physiological variable such as heart rate, or even assessment of individual sympathetic/parasympathetic tone.

### 1.4. Prediction of Outcome after TBI

“No head injury is so serious that it should be neither despaired or nor so trivial that it can be ignored” (Hippocrates). The Hippocratic aphorism expresses the uncertainty that exists about the prediction of outcome after TBI. It still remains difficult to determine with certainty what the future course of events will be in an individual TBI patient [9, 174-176]. Some of the features that have been used to predict outcome after TBI include the patient age, clinical indices of the severity of injury (GCS, AIS), pupillary diameter and light reflex, hypotension and the results of investigation and imaging studies, particularly ICP and computed tomography (CT). The previous established tools and the additional markers of prediction of outcome in TBI are discussed in sequence.

#### 1.4.1. Age

Age is an important prediction tool while evaluating patients with brain injury. It is well established that in patients older than 60 years prognosis is worse with higher incidence of death, vegetative status or severe disability [171].

Several studies [177-182] have demonstrated that younger individuals do better than others. Mortality rate was identified as being remarkably low in children as early as 1973 [180]. Subsequent studies [183-188] showed that more children survived and had better neurologic outcomes than adults. A prospective study [189] (n=372) in TBI patients with a GCS less than 13 or ISS greater than 16 and age above 14 years showed no
prognostic effect of age up to 50 years. After 50 years, age became an independent predictor of mortality. Sixty appears to be a critical age. Patients older than 60, had significant worse outcomes according to the Traumatic Coma Data Bank (TCDB) [190]. In this study, six months after severe head injury, 92% of those older than 60 were dead, in a vegetative state, or severely disabled. Other studies demonstrated mortality greater than 75% in severely TBI patients older than 60 [182, 186, 191].

Aging is associated with increased number of injuries caused by falls and pedestrian accidents [180, 191-193]. In a prospective study of the TCDB, motor-vehicle crashes were the cause of injury in 55% of patients ages 15–25, whereas only about 5% suffered falls. However, in the age range above 55, 45% suffered falls and only about 15% were in motor-vehicle crashes. Interestingly, in this study the mechanism of injury did not appear as an independent predictor of poor outcome. Older patients had worse outcome compared to younger ones, regardless of the cause of injury. In the same study a marked increase in pre-existing co-morbidities was found with increasing age, which was associated with poor outcomes (death and vegetative state) in 86% vs. 50% in younger patients. However, this correlation was not found in younger age groups. In addition, multiple systemic injuries were less likely in the older age group thus emphasizing the role of the severity of brain injury in determining outcome.

In the TCDB study there was an age-related trend towards increasing the size of intracranial hematomas (ICH) with the largest observed in the oldest group. The chances of survival in patients with ICH decrease with advancing age [191, 193-199]. A significant correlation was noted in the TCDB study between a poor outcome and those patients who had intracerebral hematomas (ICH) or extra cerebral hematomas greater than 15ml, SAH, midline shift, compressed cisterns, which all increased with age. The TCDB group conducted a multivariate logistic regression analysis to evaluate the independent effect of age on outcome from severe TBI. Age was an independent predictor after adjusting to all other factors. One explanation for this is that the brain has a decreased capacity for repair as it ages (more vulnerable, fragile, at higher risk). This has some support in that the proportion of survivors with good neurologic recovery (GOSE scores 3-5) all declined with age [200].
In summary, the current literature demonstrates that age is an important predictor of outcome in brain injury. Currently, most studies have many limitations, including lack of analysis of age as a continuous variable, and the analysis of the effect of confounding variables such as pre-existing medical conditions. Furthermore, the biology of the aging brain and its vulnerability to injury warrants further detailed investigation.

1.4.2. Pupillary Diameter and Light Reflex

The pupillary diameter and light reflex are important tools for the initial assessment and during subsequent re-evaluations of patients with TBI. An increased diameter and a fixed pupil are generally consequences of increased ICP. The identification of increased ICP is of fundamental importance as it impacts outcome and the early management of patients with moderate and severe TBI [201].

Increased ICP results in uncal herniation and compresses the third cranial nerve. This causes reduction in the parasympathetic tone to the pupillary constrictor fibers leading to a dilated pupil. Similarly, destruction of the third nerve parasympathetic brainstem pathway also causes a dilated and fixed to light pupil. Therefore, the pupillary light reflex is an indirect measure of herniation and brainstem injury. Usually, a single dilated and fixed pupil suggests herniation, whereas bilateral dilated and fixed pupils are associated with irreversible brainstem injury in a fully resuscitated patient [201]. A dilated and nonreactive pupil may also be caused by direct orbital trauma without brainstem or intracranial third nerve compression, which should not be associated with death or neurologic outcome. The “blown pupil” is important in the context of a decreased level of consciousness (LOC) and must be assessed for outcome with the LOC and presence of intracranial pathology. Many clinical studies have investigated the prognostic value of the pupillary light reflex. Few studies have rigorously measured the size and reaction of the pupil to light [201] but restricted the analysis to dilated vs. non-dilated pupils, without measuring their size or describing their response to light even though it is implied.

On average 65% of patients with severe TBI have normal reactive pupils after resuscitation, 12% have one abnormal pupil, and 28% have bilateral non-reactive pupils. There is a significant association between pupillary reactivity and other early indicators of outcome; GCS score [178], hypotension [202-204], and compression of basal cisterns on
the CT scan [198]. Several studies [178, 205, 206] demonstrated a strong association between bilateral nonreactive pupils and poor outcome. In two well-designed studies [178, 201], bilateral nonreactive pupils had a greater than 70% positive predictive value (PPV) for poor outcome. In a prospective study [177] of 133 patients with severe TBI, bilateral fixed pupils were found in 35% of the patients and 70% of them had poor outcomes. In another larger study [178] (N=305) on prognostic tools, bilateral nonreactive pupils were associated with 90% mortality. In a recent prospective study [207] in South Africa, the authors developed a simple predictive model for severe TBI using clinical variables that included pupillary reflex. A binary logistic regression model was used, which included: oxygen saturation, GCS and pupil reactivity. The model correctly predicted the outcome in 74.4% of the events, with the odds ratio of 4.405 for a good outcome when both pupils were reactive.

The current literature has many limitations. Future work should consider studying the association between pupil size and light reactivity (appropriately measured) to outcome, as well as the duration of pupillary dilation or fixation. A standardized method of measuring pupil size and reactivity to light would decrease inter-observer variability [201]. Definition of size of dilated pupils is lacking, and some have proposed greater than 4 mm [208] or no constrictor response to bright light. Right or left distinction should be made when the pupils are asymmetric.

In summary, lack of pupillary response to light is a good prognostic tool used to predict outcome. However, direct orbital trauma should be excluded as a causative agent, hypotension should be reversed prior to assessment of pupils, and repeat examination after evacuation of intracranial hematomas should be performed. Pupillary size and reactivity still need to be better defined and properly measured.

1.4.3. Hypotension

Hypotension is an important factor associated with secondary brain injury influencing outcome post TBI as consequence. Many studies have investigated the effect of major secondary brain insults such as hypotension, hypoxemia and hypothermia on the outcome after severe TBI. Furthermore, hypotension seems to be the most robust predicting factor.
Several studies [209-212] found a significant relationship between hypotension, hypoxemia and hypothermia with poorer outcome, which often occur prior to hospital admission. Despite being purely observational, these results have resulted in resuscitation strategies to reduce their occurrence and potential deleterious effects [213]. Miller [214, 215], established the importance of these secondary insults as outcome determinants, but did not study their independence with respect to other predictive factors. In a study [216] (n=717) of the influence of secondary brain insults on outcome, hypotension was defined as a single measurement of a systolic blood pressure less than 90 mmHg. The occurrence of one or more episodes of hypotension during the period from injury through resuscitation or during the shorter period of resuscitation only, was associated with a doubling of mortality and a marked increase in morbidity. Hypotension was an independent and statistically significant predictor of outcome compared to other major predictors, including age, hypoxemia, and the other severe non-TBI injuries. When the influence of hypotension on outcome was analyzed separately, the statistical significance of severe non-TBI trauma as a predictor of outcome was eliminated, suggesting that their effect on the outcome of patients with severe TBI is primarily mediated through hypotension [216]. The same TCDB study [216] revealed that the five most powerful early predictors of outcome are age, positive CT scan for intracranial injury, pupillary reactivity, post-resuscitation GCS score, and hypotension. Of all 5 predictors identified, hypotension is the only one amenable to medical intervention, which was demonstrated by a study from Australia [217]. The study reported that early (within 6 hours) and late (within 48 hours) episodes of hypotension are independent predictors of outcome including mortality and neurologic outcome, dichotomized in good/moderate-to-severe deficits or vegetative/death.

The effects of hypotension on outcome are particularly pronounced in the early time period following TBI. The International Mission for Prognosis and Analysis of Clinical trials in TBI (IMPACT) study [213] performed a meta-analysis of 7 RCTs (n=6,629) aimed at establishing an association between hypoxia-hypotension events before or at hospital admission, and outcome. The study conducted a proportional odds modelling that showed worse outcomes (OR=2.7). In another study by Chestnut [218] (n=493), conducted in patients with severe TBI in whom hypotension was eliminated, demonstrated that for patients without hypotension, 17% died or remained in a vegetative state. For those patients
with early hypotension (at admission), 47% died; for late hypotension (in the ICU), 66% died and for both insults then 77% of the patients died. Both early and late hypotensive episodes were significant independent predictors of outcome after controlling for confounders. Pietropaoli [219] demonstrated that severe TBI patients without previous hypotensive episodes, after had been subjected to surgical procedures, where iatrogenic hypotensive episodes occurred, had significantly worse neurologic outcomes than those without. Additionally, outcome was inversely correlated with duration of intraoperative hypotension.

Other studies [220, 221] further demonstrated strong associations between early hypotension and outcome. Vassar [221], in a multicenter RCT examined initial resuscitation with hypertonic saline versus normal saline in hypotensive multisystem trauma patients. The hypertonic saline group had higher blood pressure, decreased overall fluid requirements, and trended towards improvement in survival. A subgroup analysis of patients with severe TBI revealed that the hypertonic saline group had a significant improvement in survival at the time of discharge. In this study, hypotension appeared to eliminate the generally more favorable outcome afforded by younger age. Carrel [222] also addressed the ability of on-site resuscitation in decreasing secondary injuries and improve outcome. Patients whose secondary brain insults were reversed in the field had a 42% decrease in poor outcomes at three-month follow-up. However, the study did not control for confounding factors.

Secondary brain insults are also associated with the appearance of other factors, which in turn are strongly associated with prognosis. In particular, early hypotension appears to exacerbate the subsequent development of intracranial hypertension in terms of frequency and magnitude [223-225], which may impact on outcome. Lobato [223] reported that the high incidence of hypotension and/or hypoxemia at admission (47% of cases) and the severity of the clinical presentation (82% of patients scored 5 points or less on the GCS, 74% had unilateral or bilateral mydriasis, and 80% had an initial ICP above normal) correlated with a very poor final outcome (87% mortality). In this study, elevation of ICP at any stage was associated with a significantly poorer outcome (77%) as compared to patients with normal ICP courses (43%) (p<0.0001).
In summary, hypotension, occurring at any time from injury through resuscitation phase and ICU admission, has been repeatedly shown to be a strong predictor of outcome in TBI. Hypotension is arguably the only prognostic indicator amenable to therapeutic interventions. The recording of a single hypotensive episode doubles mortality and increases morbidity. The estimated reduction in unfavorable outcome that would result from the elimination of hypotensive secondary brain insults is profound.

1.4.4. Glasgow Coma Scale

The GCS was developed by Teasdale and Jennett [226] in 1974 for measuring the level of consciousness and has become the most widely used clinical tool to measure severity of head injury and, despite its limitations, it is also used to predict outcome.

The initial GCS score, however, may not be reliable due to sedation and intubation and may lack precision for predicting good outcome when it is initially low. An European study [227] showed that the motor score could not be tested in 28% of patients on admission and the full GCS score was not testable in 44%. Additionally, a survey conducted in major trauma centers in the United States [7] found a substantial variability of practice when the initial GCS was assigned for patients who had received prehospital treatment, including neuromuscular blockers. Gale [8] found that the mortality rate for those with a true GCS score of 3-5 was 88%, while it was 65% for those with the same GCS score when a verbal score of 1 was used because of endotracheal intubation. Another study [9] (n=100) described the accuracy of outcome predictions in severe TBI by an experienced neurosurgeon. Outcomes were predicted based on the best GCS scores within 24 hours after injury. Correct prognosis was estimated in only 56% of the cases, poor outcomes were overestimated by 32%-52% and good outcomes underestimate by 35%. Matis [10] suggested that the use of GCS as a sole predictor has a limited predictive value in patients with mild to moderate injury. Indeed, the same authors who developed the GCS found that the predictive accuracy of the initial GCS was poor, in particular when compared to the Glasgow Outcome Scale (GOS), another widely used score to predict functional neurological outcome in TBI [11].

Reliability of the initial measurement and its lack of precision for predicting good
outcome when the initial score is low, are the two most important limitations to using the initial GCS for prognostication. Approximately 20% of the patients with the worst initial GCS score will survive and 8%-10% will have a good neurologic recovery (GOS 4-5), if the initial GCS score is reliably obtained and not tainted by prehospital medications or intubation [227]. Despite these concerns, the GCS score has been shown to have a significant correlation with outcome following severe TBI, both as the sum score or as just the motor component [228-232]. In a prospective study [177] a PPV of 77% for a poor outcome (GOS 1-4) was measured for patients with a GCS score of 3-5 and 26% poor predictive value for a GCS score 6-8.

In summary, when considering the use of the initial GCS score for prognosis, the two most important problems are the reliability of the initial measurement and its lack of precision for prediction of a good outcome if the initial GCS score is low. Currently, the issues that need better investigation are the optimal time after injury for determining the initial GCS, when to assess the GCS score for those who have received paralytic or sedative medication and the reliability of the prehospital GCS score acquisition.

1.4.5. Computed Tomography (CT) Findings

The widespread availability and the speed of image processing have established CT as a cornerstone in the evaluation and outcome prediction of patients with TBI. Several features readily identified on head CT scan have major prognostic significance, including: shift of midline structures, encroachment of the basal cisterns, cerebral infarction, SAH, intraventricular hemorrhage (IVH), diffuse injury, and extra-axial hematomas [233-236]. However, CT scans have many limitations, including the lack of resolution to correctly identify or characterize small white matter lesions as in the setting of diffuse axonal injury [237], for example. The CT scan findings and their association with outcome will be analysed in this section.

1.4.5.1. Abnormal CT

The reported incidence of abnormalities on CT scan in patients with severe TBI varies between 68%-94%. A study in patients with severe TBI [238] showed that any abnormality on CT has a PPV of 78% with respect to an unfavorable outcome. However,
the patients in the study already had an incidence just over 70% of an unfavorable outcome which makes the predictive value of initial abnormalities on CT scan limited. The negative predictive value (NPV), that is, the relation between absence of abnormalities and favorable outcome, is of greater importance and significance [238]. The outcome of patients without abnormalities on initial CT examination is better than in the overall population of patients with severe TBI. Other studies [177, 204, 239, 240] have reported favorable outcomes in 76-83% of patients with a normal CT scan on ED admission.

When determining prognostic significance of lesions on the CT scan, the time elapsed between injury and CT examination must be taken into account. Various authors have addressed the issue of changes on CT appearance over time. In a study [241] with 138 patients, 60 (43.4%) developed a new lesion and only 12 (20%) patients had a favorable outcome, while a favorable outcome was seen in 60 (76.9%) of the 78 patients not developing a new lesion. We identified four other studies [242-245] that demonstrated development of later lesions in patients with an initial CT with isolated ICHs. On average, patients with new lesions evolved with a worse outcome. Lobato [240] demonstrated that new lesions may develop on subsequent CT scans on 33% of the patients. More recently, in the literature, a systematic review and meta-analysis [246] reported that repeated CT scan after TBI results in a change in management for only a minority of patients. However, the role of repeating CT scans in head injury is yet not well established.

In summary, the initial CT scan demonstrates, on average, abnormalities in approximately 90% of patients with severe TBI. Prognosis in patients with severe TBI and demonstrable pathology on initial CT scan is less favorable than when CT is normal. In patients with a normal CT on admission, outcome appears to be primarily related to concomitant extracranial injuries. The absence of abnormalities on CT at admission does not preclude the occurrence of raised ICP, and significant new lesions on repeated CT scans may develop in up to 40% of patients.

1.4.5.2. Classification of TBI according to CT findings

Computed tomography plays an important role in the rapid assessment of patients with TBI in that it detects posttraumatic hemorrhagic lesions and allows selection of patients who require emergency neurosurgery [247]. Several classifications using head CT
scan findings have been proposed in the recent decades. According to Gennarelli [248], conventional classification of CT findings in severely head-injured patients, differentiates between focal (extradural hematomas [EDH], subdural hematomas [SDH], ICH and space occupying contusions) and diffuse head injury. Diffuse injuries are defined by the absence of mass lesions, although small contusions without mass effect may be present. In terms of outcome, patients with diffuse injuries were found to have an intermediate prognosis when compared to patients with EDH or SDH. While acute SDH with low GCS scores had a high mortality, diffuse injuries with higher GCS scores showed a low mortality and a high incidence of good recovery. In practice, some confusion exists between this category of patients with diffuse lesions and the more neuropathologically oriented entity of diffuse axonal injury (DAI). DAI is characterized by wide-spread tearing of axons and/or small blood vessels. Radiologic criteria for diagnosis of DAI are small hemorrhagic lesions at the corticomedullary junction, in the corpus callosum, in the midbrain, and in the brain stem, sometimes in conjunction with some intraventricular bleeding. As demonstrated by Adams [249] and Zimmerman [250] DAI can sometimes be superimposed by generalized brain swelling. Another study [251] has expanded the anatomical patterns of the conventional CT classification, outlining eight categories of injury, mainly subdividing patients with focal lesions which permitted a stronger predictive value than the conventional categorization. Outcome was significantly better in EDH without concomitant brain swelling, simple brain contusion, generalized swelling, and in the absence of lesions.

In 1991, Marshall (Table 7) proposed a new classification in which the category of diffuse injury is further expanded, taking into account signs of raised ICP [206]. The Marshall classification is the most frequently used prognostic method that incorporates the anatomical nature of the injury in the determination of outcome after acute TBI. It uses the findings from CT scans on the status of the mesencephalic cisterns, the degree of midline shift and the presence or absence of local lesions to categorize patients into six different groups [206]. The score allows identification of patients at risk of deterioration from high ICP, which offers the possibility of early intervention [206]. Since its introduction in 1991, this classification has been increasingly used for predicting outcome, including overall survival, GOS, elevated ICP and neuropsychological consequences [252]. There is a very strong relationship between the Marshall CT classification, mortality and the frequency of
elevated ICP [252]. The presence of a midline shift of > 5 mm on the initial brain CT scan and a high or mixed density lesion > 25 cm³ in volume have both been correlated with early death [253]. The relative risk of requiring a delayed operation has been shown to be related to the Marshall CT classification of initial CT scans (diffuse injury IV, 30.7%; diffuse injury III, 30.5%; non-evacuated mass, 20.0%; evacuated mass, 20.2%; diffuse injury II, 12.1%; diffuse injury I, 8.6%) [254]. Ono [255] found that all diffuse brain injury I patients recovered well six months post injury. In the diffuse brain injury II group, age, the GCS score and the presence of multiple parenchymal lesions on CT scans were significantly correlated with outcome. For the diffuse brain injury III and IV groups, the only significant prognostic factor was the GCS score. In patients with a mass lesion, the GCS score was also the only significant prognostic factor in the EDH, but the GCS score and the presence of SAH were predictive factors in the acute SDH group. Outcomes were unfavourable in the majority of patients with ICH. With regard to the frequency of elevated ICP, Hiler [256] reported that the Marshall CT classification correlated significantly only with ICP measured within 24 h of admission. In contrast, the 6 month GOS score correlated with the initial CT scan findings. These results all suggest that the Marshall CT scan classification provides accurate predictions regarding the likelihood of a fatal or non-fatal outcome. Mataró [257] reported a relationship between the acute intracranial lesion diagnosis according to the Marshall CT classification and neuropsychological results and ventricular dilatation indices at 6 months post-injury. Although the Marshall CT classification is an adjunct to clinical parameters and is easy to use, it does not take into account all possible prognostic factors visible on CT and clearly has some limitations. An important limitation is that the classification is partially based on arbitrary assessments and is dependent on the accuracy of measured volumes of focal mass lesions. Furthermore, TCDB categories V (mass lesion surgically evacuated) and VI (mass lesion not operated) are, in part, retrospective in nature as they depend on the decision to operate or not [258]. In addition, the Marshall CT classification does not take the presence of tSAH into account. The incidence of CT documented tSAH in patients with severe TBI is 23 – 63% [259]. It is most common in patients with SDH or haemorrhage contusion and is associated with a worse prognosis [259-261]. Adding SAH to any CT classification system may enhance its
predictive power for outcome and may have consequences for treatment and outcome issues, and in clinical trials [259, 260].

Another classification widely used is the head Abbreviated Injury Score (AIS) [262], (Table 6). The Head AIS is used to classify patients with severe head injury. In this injury scoring system, the severity of injury is rated from minor (AIS=1) to unsalvageable (AIS=6).

Based on this rating system, severe head injury is considered to be Head AIS>3 [263-266]. The AIS is the most widely used anatomic injury severity rating system in the world [267]. Walder [268], have compared the predictive value of the Marshall’s Classification to the worst applicable severity code from the AIS. A high correlation was found between AIS and outcome at six months, the Marshall’s Classification and outcome as well as between GCS score and outcome. The PPV for favorable outcome was shown to be greater for the AIS score than for the Marshall’s classification (95% vs. 71%), when considering outcome categories dead or vegetative.

In summary, a strong correlation exists between the different types of intracranial lesions post TBI and outcome. As consequence, the Marshall Classification yields important prognostic information in TBI. The AIS score shows a strong correlation between the worst intracranial AIS severity code of the initial CT in severe head injury and outcome at six months. Future work should address the complicated issue of how optimally to combine CT characteristics for prognostic purposes and how to improve on currently used CT classifications to predict outcome more accurately.

1.4.6. Additional Strategies to Determine Outcome

1.4.6.1. Clinical Assessment

Other recently proposed prognostic models have used the motor sub-score of GCS: the Corticosteroid Randomization after Significant Head Injury trial collaborators (CRASH) trial [269] and the International Mission on Prognosis and Clinical Trial Design in TBI (IMPACT) [270]. The CRASH model (Figure 2) is used as an aid to estimate mortality at 14 days and death/severe disability at 6 months. The predictions are based on the average outcome of adult patients with GCS of 14 or less, within 8 hours of injury and
can only support (not replace) clinical judgment. The estimations are based on alternative sets that consider low and middle, or high income countries. The CRASH model has been extensively validated and a calculator is available online at no cost.

**Figure 2 – Screenshot of web based calculator.**
Available at http://www.crash2.lshtm.ac.uk/

The IMPACT model (Figure 3) predicts 6 month outcome in adult patients with moderate and severe TBI (GCS ≤12) on admission. By entering the characteristics into the calculator, which is available online or by an app, the models will provide an estimate of the expected outcome at 6 months. The model discriminates well, and is particularly suited for purposes of classification and characterization of large cohort of patients and caution should be used when applying the estimated prognosis to individual patients.
Another score, the Full Outline of UnResponsiveness (FOUR) score [271] (Table 1) attempts to address some of the limitations of the GCS score by removing any verbal assessment and integrating brainstem responses and breathing patterns.
Table 1 – Full Outline of UnResponsiveness (FOUR).

<table>
<thead>
<tr>
<th>Score</th>
<th>Eye response</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Eyelids open and tracking, or blinking on command</td>
</tr>
<tr>
<td>3</td>
<td>Eyelids open but not tracking</td>
</tr>
<tr>
<td>2</td>
<td>Eyelids closed but open to loud voice</td>
</tr>
<tr>
<td>1</td>
<td>Eyelids closed but open to pain</td>
</tr>
<tr>
<td>0</td>
<td>Eyelids closed with pain</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score</th>
<th>Motor response</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Makes sign (thumbs-up, fist, other)</td>
</tr>
<tr>
<td>3</td>
<td>Localizing to pain</td>
</tr>
<tr>
<td>2</td>
<td>Flexion response to pain</td>
</tr>
<tr>
<td>1</td>
<td>Extension response to pain</td>
</tr>
<tr>
<td>0</td>
<td>No response to pain</td>
</tr>
<tr>
<td>0</td>
<td>Generalized myoclonus status</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brainstem Score</th>
<th>Pupil reflexes</th>
<th>Corneal reflexes</th>
<th>Cough</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>One pupil wide and fixed</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Absent</td>
<td>Present</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>Present</td>
<td>Absent</td>
<td>NA</td>
</tr>
<tr>
<td>1</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>0</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respiratory Score</th>
<th>Intubation</th>
<th>Breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Not intubated</td>
<td>Regular</td>
</tr>
<tr>
<td>3</td>
<td>Not intubated</td>
<td>Cheyne-Stockes</td>
</tr>
<tr>
<td>2</td>
<td>Not intubated</td>
<td>Irregular</td>
</tr>
<tr>
<td>0</td>
<td>Not intubated</td>
<td>Apnea</td>
</tr>
<tr>
<td>1</td>
<td>Intubated</td>
<td>Above ventilator rate</td>
</tr>
<tr>
<td>0</td>
<td>Intubated</td>
<td>Breathes at ventilator rate</td>
</tr>
</tbody>
</table>

0 – 16 Total score

In a recent prospective study (n=51), the AUC of the FOUR score in predicting poor functional outcome (GOS score 1, 2 and 3) at 6 months was 0.85, equivalent to the AUC of 0.83 observed with the GCS [272]. The FOUR scale allows testing of eye
movements or blinking that facilitates the detection of locked-in state or the transition from vegetative to minimal conscious state. However, the usefulness of a scale that excludes verbal assessment could be debated especially in the TBI population, the majority of who have moderate or mild injury in which a critical distinguishing feature is verbal performance. Furthermore, respiratory patterns can be difficult to interpret in patients undergoing mechanical ventilation. These characteristics suggest that the clinical utility of the FOUR score may be more relevant in the post-acute phase of TBI.

In summary, the three mentioned prediction models are robust in providing an estimate of the expected outcome at 6 months. They discriminate well and attempt to address some of the limitations of the GCS score. However, those models have limitations that still need to be addressed.

1.4.6.2. Neuroimaging

Many different and novel imaging resources have been explored for prognostication of patients with TBI. As mentioned, the most widely used is the CT scan. Abnormalities such as midline shift, encroachment of the basal cisterns, cerebral infarction, SAH, IVH, DAI, and extra-axial hematomas [233-236] are of robust prognostication significance. Computed tomography has been established as fundamental in the evaluation and outcome prediction in TBI. However, CT has significant limitations particularly when characterizing DAI [237]. The use of Xenon CT is a method used to measure quantitatively the cerebral blood flow (CBF) in 22 cortical mantle regions within 6–12h after severe TBI. The method has been studied prospectively in 120 patients [273] and the study was able to differentiate patients with poor outcome (GOS 1–2) from those with good outcome (p<0.001).

Magnetic resonance as a predictor of outcome tool in TBI has been also investigated [274]. MRI sequences such as diffusion weighted imaging and susceptibility weighted imaging are potentially more sensitive to DAI and have been shown to increase the accuracy of outcome prediction [275-277]. Recent studies [278-280] indicate that diffusion tensor imaging (DTI) in MRI is highly sensitive to traumatic white matter damage and is predictive of long-term functional outcome. Galanaud [281] evaluated 105 comatose patients (≥7 days post TBI) with MRI scans including DTI and constructed a composite DTI score. Prediction of unfavorable outcome (GOS 1, 2 and 3 minus) in this
study, demonstrated a sensitivity of 64% and a specificity of 95%. This small study found that the DTI 1-year prediction model (based on white matter tracts) was found to be more accurate than the IMPACT score. The conventional morphological MRI sequences are not able to identify axonal loss (reduced N-acetyl aspartate-to-creatine ratio) and increased myelin turnover (increased choline-to-creatine ratio) in the cerebral tissue, what is permitted with the early proton magnetic resonance spectroscopy [282]. These changes have been linked to worse long-term outcome [279, 282, 283].

Hilario [284] demonstrated that bilateral brainstem injuries, detected on MRI in the first 30 days after severe TBI, were strongly associated with unfavorable outcome (GOSE 1–3; \( p < 0.05 \)) at 6 months, with a sensitivity of 38%, a specificity of 100%, and a PPV of 100%. Posterior brainstem lesions predicted outcome better (OR 6.8, \( p < 0.05 \)) and disability (OR 4.8, \( p < 0.01 \)), with a sensitivity and specificity of 68 and 76%, respectively. In another study [285], MRI was performed in 106 patients with severe TBI within 4 weeks post trauma. The authors looked at the depth of lesions as a potential predictor of outcome at 1 year. Bilateral brain stem injury was strongly associated with poor outcome in severe compared with moderate TBI (\( p < 0.001 \)) with PPV and NPV of 0.86 and 0.88, respectively. Betz [286] retrospectively assessed the capability of prediction of outcome using DTI in 59 patients with severe TBI. Unfavorable prognosis (death or severe disability) at hospital discharge was associated with lower average apparent diffusion coefficient (ADC) values, axial diffusivity in the genu, and lower fractional anisotropy values in the splenium of the corpus callosum. The body of corpus callosum also had lower fractional anisotropy, ADC, and axial diffusivity values in patients with unfavorable outcome.

In summary, there is an ongoing increase of the importance of MRI sequences such as diffusion weighted imaging and susceptibility weighted imaging which may be more sensitive to DAI and increase the accuracy of outcome prediction, however, CT scan remains the most widely used tool.

1.4.6.3. Brain Physiology/Metabolism

A few novel physiologic measurements and neuro metabolites have been identified and appear to have an effect in outcome after TBI. ICP is commonly monitored in patients with severe TBI. Studies [236, 287] demonstrate that constant elevations of ICP to greater
than 20 mmHg or decreases in cerebral perfusion pressure (CPP) to less than 50–60 mmHg can cause cerebral infarction, herniation, and death following TBI. Other studies [256, 288-290] indicate that changes in ICP elevations cannot be predicted in a reliable or timely manner with either clinical assessment or imaging and the efficacy of treating elevated ICP has been challenged [291-293]. Direct measurements of the brain tissue oxygenation indicates that even when ICP and CPP are within normal limits, significant reductions in brain tissue oxygen pressure may occur, which has been associated with worse outcomes [294, 295]. Consistent with these observations, a few studies suggest that clinical algorithms that target normalization of brain tissue oxygen pressure may have a beneficial effect on outcome [296-298].

The use of cerebral microdialysis probes has identified neurometabolites in the brain interstitium that are associated with outcome in TBI [299, 300]. Timofeev [301], in a large cohort of 223 patients with severe TBI, demonstrated low levels of brain extracellular glucose and elevated levels of lactate to pyruvate ratio (L/P) to be independent predictors of mortality at 6 months. In another study [302], microdialytic assessment identified irreversible neuronal loss and subsequent volume loss in the frontal lobes due to elevations in L/P in the acute setting. Additionally, preliminary microdialysis studies in patients with severe TBI suggest that brain extracellular concentrations of amyloid fragments, tau protein, and neurofilament heavy chain protein may also have prognostic significance [303-305].

In summary, measurement of ICP and brain tissue oxygen pressure may have a beneficial effect on outcome. Furthermore, some interstitial neurometabolites measured in the microdialysis fluid of an injured brain, may have an association with outcome. Further research in this field is still warranted.

1.4.6.4. Electrophysiology

More recently, electroencephalography (EEG) has been used for detection of seizure activity since seizure has been associated with outcome in brain injury [300, 301]. The use of continuous EEG suggests that seizure activity and status epilepticus, often clinically undetected, occur in a significant number of patients with severe TBI, and such perturbations have been linked to outcome [306, 307]. In one prospective, observational,
multicenter study, 109 patients who required neurosurgery for TBI (at median of 9.9h after injury) had cortical spreading depolarization monitoring, which participates in the extension of secondary insults after brain injury [308]. Presence of spreading depolarization was associated with an increased risk of unfavorable outcome (GOSE 1–4) and an OR of 7.58, $p=0.0002$). In a study [309] of 94 patients with moderate to severe TBI who underwent continuous EEG monitoring, seizures occurred in 21 patients, and mortality was 100% in the patients with status epilepticus. Vespa [310, 311] reported that seizures following TBI are associated with increased cerebral metabolic rate, cerebral blood flow and volume, and increased ICP in susceptible patients. Interestingly it has been suggested that seizures cause injury that compounds the primary trauma-induced damage. Hippocampal atrophy was more pronounced in TBI patients with seizures than in those without seizures, and atrophy was most prevalent on the hippocampus ipsilateral to the seizure focus [305]. Currently, the guidelines recommend 7 days of seizure prophylaxis following moderate to severe TBI [312].

The use of somatosensory evoked potentials (SSEPs) in predicting outcome of severe TBI was evaluated in a meta-analysis of 44 studies [313]. The bilateral absence of cortical signals on SSEP had a PPV of 98.7% in predicting adverse outcome 2 months to 3 years after head injury. However, the accuracy of prediction is significantly lower when patients have focal lesions, SDH, and recent decompressive surgery [313]. Houlden [314], in a more recent report, demonstrated that bilaterally absent cortical SSEP responses assessed on the third day after severe TBI were correlated with functional outcomes at 1 year. Furthermore, SSEP helped predict performance on tests of information-processing speed, working memory, and attention 1 year after injury.

In summary, the current evidence concerning the effects of seizures on outcome in TBI patients lacks robustness. Seizure activity may increase cerebral blood flow and consequently ICP. Additional research is needed to determine if early and intensive suppression of seizure activity or periodic discharges may improve outcome after TBI. Additionally, SSEPs on the third day post injury may correlate with outcomes at 1 year.

1.4.6.5. Serum Biomarkers
The prospect of using peripheral blood-based markers synergistically with current clinical diagnostic and prognostic assessments of TBI is favorable for a variety of reasons: (1) it is more clinically accepted compared to other invasive procedures; (2) it is cost effective; (3) it may quickly and accurately provide specific information about the underlying pathophysiology of TBI, which clinicians can then use in the diagnosis and formulation of treatment strategies [315]. Many different biomarkers identified in patients with brain injury, such as S100 beta (S100B) protein, neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), pentraxin 3 (PTX3), interleukin-2 (IL-2), interleukin-6 (IL-6), Ubiquitin C-terminal hydrolase (UCH-L1), brain-derived neurotrophic factor (BDNF), monocyte chemoattractant protein (MCP)-1, intercellular adhesion molecule (ICAM)-5, and peroxiredoxin (PRDX)-6, have been demonstrated more recently and are associated with severity of brain injury and neurological outcome [110, 316-328].

Serum levels of S100B protein and NSE, markers of glial and neuronal damage, respectively, have been extensively studied in patients with hypoxic-ischemic encephalopathy. In some studies, these markers correlated with findings on neuroimaging but were not independently predictive of specific outcomes [316-318]. Other investigations have indicated that S100B and NSE have value not only in determining the severity of TBI but also in classifying their outcomes [319-326, 329]. In a study by Di Battista [329], where peripheral blood was collected from 85 patients with isolated moderate and severe TBI, unfavorable neurological outcome was associated with elevations in S100B, GFAP, and MCP-1. Mortality was related to differences in six of the seven markers analyzed. Combined admission concentrations of S100B, GFAP, and MCP-1 was able to discriminate favorable versus unfavorable outcome (AUC=0.83), and survival versus death (AUC=0.87), although not significantly better than S100B alone (AUC=0.82 and 0.86, respectively).

In another study, S100B levels at 24 h were a useful tool for predicting mortality at 1 month after TBI [327]. Best cut-offs for S100B were 0.461 and 0.025mg/l (serum and urine, respectively) with a sensitivity of 90%. In one small study [327] (n=28) in patients with DAI secondary to severe TBI, median levels of S100B (monomer of S100B) at 72 h (49.3 ng/l) were found to have 88% sensitivity and 100% specificity to predict unfavorable
outcome after 3 months ($p<0.0001$). S100B is being extensively studied as a prognosticator in severe TBI, but data in mild and moderate TBI are sparse.

GFAP is specific to glial cells in the CNS, and increased levels are detectable in the serum of patients with acquired brain injury. Studies suggest that serum GFAP levels correlate with clinical and neuroradiologic injury severity and are significantly higher in patients who die or have unfavorable outcome [323, 326, 330, 331]. The predictive value of other serum biomarkers, in particular tau protein, is still under investigation [332, 333].

Gullo [334], studying patients with severe TBI (n=84), reported that PTX3, a component of innate immunity) was observed to be predictive of hospital mortality. Patients who died showed a mean serum PTX3 level (mean of 18 h) of 9.95 mg/ml ($\pm$6.42) in comparison to 5.46 mg/ml ($\pm$4.87) in the survivor group ($p=0.007$). In patients with isolated TBI, elevated serum PTX3 levels remained significantly associated with mortality ($p=0.04$). An association between TBI and inflammatory cytokines has also been investigated. Elevated IL-2 levels ($>90$ pg/ml) at 10 hours after severe TBI were found to be six times more frequently associated with in-hospital mortality compared to levels less than 50 pg/ml (OR=6.2, 95% CI 1.2–25.1, $p=0.03$) in an observational study (n=93) by Schneider [335]. UCH-L1 is a novel gene product specific to neurons. Papa [336] reported that CSF UCH-L1 levels were elevated in patients (n=41 and 25 controls) with severe TBI who had intraventricular pressure monitoring. Mean UCH-L1 levels of 44.2 ng/ml ($\pm$7.9) at 6–18h after TBI were higher in patients with 6-week mortality and in those with an unfavorable 6-month outcome (GOS 1–4) compared with controls ($p<0.001$).

In summary, new serum biomarkers that represent cerebral tissue damage have been described. The current evidence demonstrates that there is an association of these biomarkers with the severity of brain injury and with outcome. Furthermore, these biomarkers may have the potential to improve prediction, but more research in this field is warranted.

1.4.6.6. Laboratory Parameters

Several routinely measured laboratory parameters have been linked to severity and adverse outcomes post brain injury and their association with outcome in TBI have been
investigated in several studies [211, 270, 337-341]. Coagulation disturbances, low platelet counts, anemia, hypoalbuminemia, elevated serum creatinine, and high serum glucose concentrations are the parameters mostly associated with adverse outcomes in both univariate and multivariate models. Given the adverse association of high glucose concentrations with outcome [342-345], recent RCTs have evaluated intensive insulin therapy to maintain normoglycemia in TBI patients. The question of whether hyperglycemia is simply a marker of more severe disease or actually causes worse outcome has not been definitively answered. However, the impact of interventions such as more aggressive control of blood sugar with intensive insulin therapy is being studied [346]. In surgical ICU patients, intensive insulin therapy to keep blood glucose between 80 and 110mg/dl compared to a blood glucose level of 180 to 215 mg/dl have shown to reduce mortality, morbidity, and ICU stay [347]. Overall in-hospital mortality rates were lower in the intensive insulin therapy group at 7.2% compared with 10.9% (p<0.01). Other studies using cerebral microdialysis have found that intensive insulin therapy may result in unfavorable trends in brain extracellular glucose and L/P ratios [348-350]. In a randomized crossover trial of intensive (80–110 mg/dL) vs. less intensive (120–150 mg/dL) glycemic control in 13 patients with severe TBI, critical reductions in brain interstitial glucose and elevations of L/P ratio were more frequent in patients allocated to intensive glycemic control and were associated with increased [18F]-deoxy-d-glucose uptake on PET [351]. Finally, a study by Green [346] demonstrated no benefit to intensive insulin therapy in patients with stroke and TBI, regarding their functional neurologic outcome. The authors concluded that intensive insulin therapy is not advantageous over the conventional control. Given data linking poor glycemic control and adverse head injury outcome [230, 341], additional studies are needed to evaluate whether increased brain glucose delivery via carefully titrated moderate hyperglycemia represents a viable strategy in severe TBI.

In summary, most tests are not specific to TBI, but they offer an overall idea of the global clinical situation of the patient. Determining blood sugar seems to be more important, as it seems that glucose level correlates with outcome more importantly.

1.4.6.7. Therapeutic Interventions
Evidence and good sense suggests that rigorous supportive management and enhancements of the process of care favourably influence outcome. Aggressive normalization of oxygenation, volume status, and blood pressure in the early stages of injury have been associated with improved outcome [352-354], as used in high-volume trauma centers with implemented clinical practice guidelines [355, 356]. However, studies favoring these interventions are associative in nature, and direct evidence of their causal effect on the biology and natural history of TBI still lacks. Hypothermia may have greater value in the management of high ICP. In a systematic review of hypothermia trials in TBI, four of five studies targeting ICP elevation reported a decrease in mortality or in the percentage of patients having a poor recovery, whereas none of the three studies in which hypothermia was assessed as a neuroprotectant showed any benefit [357].

In summary, although the evidence is not robust, it is instinctive that the care offered to the TBI patient, since the initial assessment and management in the trauma room and subsequently in the ICU, is of fundamental importance in determining outcome.

1.4.7. The Role of Catecholamines as Outcome Predictors after TBI

Many studies performed in animals and in humans have demonstrated an association between circulating catecholamine levels and severity of injury and with neurological outcome TBI [358]. The early hyperadrenergic response post TBI causes a rapid increase in the circulating E and NE levels in the blood and it appears that levels are directly linked to the severity of TBI [358]. Since catecholamine levels rise exponentially as a function of injury severity, they have been evaluated as prognostic biomarkers after TBI. Clinical studies show a significant association between high levels of admission E and NE in TBI patients and their GCS score, duration of mechanical ventilation, myocardial damage, endocrine abnormalities, length of hospital stay and neurological outcome [39, 42, 358-360].

Hamill [39] studied plasma catecholamines in 33 patients with TBI. A catecholamine gradient that reflected the extent of brain injury was demonstrated within 48 hours of the injury. In patients with a GCS of 3 to 4, NE and E levels increased four to fivefold and the DA level increased threefold above normal (NE, 1686+/−416 pg/mL; E, 430+/−172 pg/mL; DA, 236+/−110 pg/mL), while patients with mild brain injury (GCS
had either slightly elevated or normal levels. Patients with marked (GCS 5 to 7) and moderate (GCS 8 to 10) TBI had intermediate levels. Patients with severe and unchanging neurological impairment one week after injury had markedly elevated initial NE levels (2,176+/−531pg/mL), whereas initial NE levels (544+/−89 pg/mL) were only mildly elevated in patients who improved to a GCS of greater than 11. These authors concluded that markedly elevated NE levels predict outcome in patients with comparable neurological deficits.

Woolf [42] studied the catecholamine response in patients with multisystem trauma with and without TBI (n=124 and 82, respectively) and found that catecholamine concentrations, particularly NE levels, significantly correlated with severity of injury only in patients with TBI. The same authors [360] evaluated the catecholamine levels within 48 hours after TBI in 61 patients to determine whether their levels would provide reliable prognostic markers of outcome assessed by the GCS score. Those patients with NE less than 900pg/mL improved GCS while those with values greater than 900pg/mL remained with low GCS scores or died. It was also found that the catecholamine concentration correlated with the time of mechanical ventilation and length of stay. Woolf [361] also studied in 66 patients the relationship between changes in thyroid function tests and catecholamine concentrations measured on admission and after four days of TBI. They observed that T3 and T4 levels fell significantly within 24 hours of injury. They found highly significant correlations between day four T3 and T4 levels and admission and day four NE and E concentrations. The study concludes that patients with TBI exhibit a gradient of thyroid dysfunction that occurs promptly, is dependent on the degree of neurologic impairment, and reflects ultimate outcome. The significant association with catecholamine levels suggests a role for SNS activation in its causation, independent of a generalized stress response, since there is no correlation of thyroid test abnormality with the degree of adrenocortical secretion [362].

Rizoli [363] demonstrated that resuscitation with hypertonic saline plus dextran (HSD) significantly reduces catecholamine secretion in resuscitated hemorrhagic shock patients. In a prospective randomized controlled trial to evaluate prehospital hypertonic fluid resuscitation after severe TBI [104] it was demonstrated in a subgroup of 65 patients that mean admission levels of E (665.3±188.2) and NE (671.6±85.2) were elevated up to
18x in all patients compared to healthy volunteers (E 36.8±2.6; NE 311.8±21.0). These findings confirm the intense sympathetic surge after TBI and substantiate catecholamine levels as potential biomarkers for severity of TBI and consequently outcome. In this study, patients resuscitated with normal saline massively released E (1082.7±291.3) and NE (823.7±124.8) with levels up to 30x that of controls; whereas, HSD-resuscitated patients exhibited only modestly elevated E (195.8±60.1, 5x) and NE (500.5±85.9, 1.6x) levels, which normalized by 12h. Moreover, peak levels of E, NE and DA were significantly higher in patients with poor neurological outcome (GOS score of 1–3).

The catecholamine response in TBI patients seems to present a unique profile, being more robust and sustained compared to other neurologic insults. Woolf [43] investigated catecholamine response in four groups of patients: TBI (n=24), vascular brain injury (n=10), multi system trauma (n=7) and medical/surgical patients in an intensive care unit (ICU) (n=29). Despite significant 3 to 7-fold elevations in both free and total NE and E, the ratio of free to total NE:E remained constant over a very broad range of values. The proportion of free E was twice normal (30.1-33.5 vs. 16.2%) in all but patients with polytrauma, whereas the percentage of free NE was unchanged in all patients (43.0%). In the patients with traumatic or vascular brain injury, significant inverse correlations were present between free NE, E, and DA and total NE and DA levels and the degree of neurological dysfunction, as indicated by the concomitant GCS score. Thus, during conditions of intense and prolonged catecholamine release, the proportion of free catecholamine remains constant and the total as well as free catecholamine concentration is proportional to the GCS. The authors concluded that total as well as free NE and E may correlate only with the severity of brain injury.

In summary, the presented literature has important insights about the potential role of catecholamines in predicting outcome post brain injury. However, we found many limitations of the available studies, including small number of patients, lack of power to evaluate outcome, lack of adjustments for the main confounders, no clear determination of a cut-off value associated to outcome, variability in the population studied, and variability in the time that catecholamines were measured post injury.
CHAPTER 2 – The COMA-TBI Study

2.1. Rationale

Traumatic brain injury leads to an intense sympathetic nervous system discharge, where the catecholamine levels rise exponentially as a function of injury severity. Previous studies demonstrate that levels of catecholamines increase early in TBI, are associated with poor neurological outcome, and as consequence, could be used as predictors of outcome. Furthermore, recent evidence demonstrates that the suppression of catecholamine release, using beta blockers early post TBI may improve outcome, suggesting that high levels of catecholamines may have deleterious effects to the brain and systemically. However, the existing evidence is originated in studies with methodological limitations such as: (1) small sample sizes, (2) lack of adjustment for confounders, (3) delayed time to measure plasma catecholamine levels post injury, (4) heterogeneous population included in the studies.

2.2. Hypothesis and Aims

2.2.1. Hypothesis

We hypothesize that: Elevated admission catecholamine levels are independently associated with unfavorable outcome in moderate to severe isolated TBI patients and thus are useful biomarkers for prediction of outcome. To validate the hypothesis that there is an independent association between circulating plasma catecholamine levels on admission with neurological outcome in patients with TBI, we studied a cohort of 181 patients with isolated moderate to severe TBI in a prospective blinded fashion. We determined the association between elevated catecholamine levels on admission with the Glasgow Outcome Scale Extended (GOSE) score at 6 months. All analyses were adjusted for clinically important factors that can be associated with the outcome such as age, admission GCS, admission AIS, hypotension on admission, coagulopathy and the use of vasopressors.

2.2.2. Specific Aims

2.2.2.1. Specific Aims for the Initial Analysis (SAIA)

SAIA 1 – To describe patient enrollment flow in the study
SAIA 2 – To describe blood samples flow in the study

SAIA 3 – To describe and compare the association between demographic variables and patients with an unfavorable (GOSE 1-4) outcome

SAIA 4 – To describe and compare the association between the classification of severity of injury and patients with an unfavorable (GOSE 1-4) outcome

SAIA 5 – To describe and compare the association between clinical variables and patients with an unfavorable (GOSE 1-4) outcome

SAIA 6 – To describe and compare the association between laboratory variables and patients with an unfavorable (GOSE 1-4) outcome

SAIA 7 – To describe and compare the association between radiological variables and patients with an unfavorable (GOSE 1-4) outcome

SAIA 8 – To describe the distribution of patients across categories of moderate (GCS 9-12) and severe (GCS 3-8) head injury in the Glasgow Coma Scale (GCS)

SAIA 9 – To describe the distribution of patients across categories of 1 to 5 of the Abbreviated Injury Score (AIS)

SAIA 10 – To describe the distribution of patients across categories of I to VI of the Marshall Classification

SAIA 11 – To describe the distribution of patients across categories of 1 to 8 of the Glasgow Outcome Scale Extended (GOSE) at discharge

SAIA 12 – To describe the distribution of patients across categories of 1 to 8 of the Glasgow Outcome Scale Extended (GOSE) at 6 months

**2.2.2.3. Specific Aims for the Primary Outcome Analysis (SAPOA)**

SAPOA 1 – To describe the plasma catecholamine levels according to the Glasgow Outcome Scale Extended (GOSE) at 6 months in patients with favorable (GOSE 5-8) and unfavorable (GOSE 1-4) outcomes

SAPOA 2 – To estimate the unadjusted odds ratio of an unfavorable outcome at the 6-month follow up (GOSE1-4) for both E and NE
SAPOA 3 – To estimate the adjusted odds ratio of an unfavorable outcome at the 6-month follow up (GOSE 1-4) for both E and NE

SAPOA 4 – To estimate the adjusted odds ratio of an unfavorable outcome at the 6-month follow up (GOSE 1-4) using the median tertile levels of both E and NE

2.2.2.3. Specific Aims for the Secondary Outcome Analysis (SASOA)

SASOA 1 – To estimate and compare the hospital and ICU length of stay in both groups of patients with a favorable and an unfavorable outcome

SASOA 2 – To estimate and compare the ventilation days in both groups of patients with a favorable and an unfavorable outcome

SASOA 3 – To estimate the in-hospital and the 6-month mortality in patients with an unfavorable outcome

SASOA 4 – To estimate and compare the ventilator free days and ICU free days in both groups of patients with a favorable and an unfavorable outcome

2.2.2.1. Specific Aims for the Catecholamine Analysis (SACA)

SACA 1 – To describe the plasma catecholamine profile in pooled patients across all 4 time points and the baseline plasma catecholamine levels in healthy volunteers

SACA 2 – To describe the plasma catecholamine levels according to the Glasgow Coma Scale (GCS) in moderate (GCS 9-12) and severe (GCS 3-8) TBI patients

SACA 3 – To describe the plasma catecholamine levels according to the Abbreviated Injury Score (AIS) in patients with severe (AIS 4, 5) and non-severe (AIS 1, 2, 3) TBI

SACA 4 – To describe the plasma catecholamine profile across all 4 time points in all categories of the Marshall Classification

SACA 5 – To describe the plasma catecholamine profile across categories of 1 to 8 of the Glasgow Outcome Scale Extended (GOSE) at 6 months

SACA 6 – To analyse and compare the plasma catecholamine profile across all 4 time points in patients with favorable outcome (GOSE 1-4)
SACA 7 – To analyse and compare the plasma catecholamine profile across all 4 time points in patients with unfavorable outcome (GOSE 5-8)

SACA 8 – To describe the plasma catecholamine levels in patients with and without high ICP and compare both groups

SACA 9 – To describe the plasma catecholamine levels in patients with and without sepsis and compare both groups

SACA 10 – To describe the plasma catecholamine levels in patients with and without MODS and compare both groups

SACA 11 – To describe the plasma catecholamine levels in patients who died or survived and compare both groups
CHAPTER 3 – Methods

3.1. Setting

The COMA-TBI study was conducted in 3 Level I Trauma Centers in North America. Two centers are located in Toronto and affiliated with the University of Toronto (Sunnybrook Health Sciences Centre – SHSC and Saint Michael’s Hospital – SMH) and the third center is located in Los Angeles (LA), California (Los Angeles County Medical Center – LA County) affiliated with the University of South California (USC). The 2 Trauma Centers from Toronto capture all adult trauma cases in the Greater Toronto Area (over 5 million inhabitants) and surroundings. The LA County Hospital provides healthcare services for the region’s medically underserved areas serving around 4 million inhabitants and treats over 28 percent of the region’s trauma victims.

SHSC houses the Tory Regional Trauma Centre, which was the first designated Trauma Centre in Canada and remains the largest one. It receives approximately 1,200 adult severely injured patients annually. Sixty per cent of the patients arrive at Sunnybrook directly from the scene and most (80%) suffer blunt trauma. Sixty per cent have a head injury. Half of all trauma patients are admitted to the Intensive Care Unit (ICU) and 25% undergo surgery in the first 48 hours of admission. St. Michael’s Hospital serves over 800 trauma patients annually, 80% blunt and 20% penetrating. Seventy per cent have a head injury, 55% are admitted to the ICU and 35% undergo a surgical procedure. It has the Allen T. Lambert Trauma and Neurosurgery Intensive Care Unit (TNICU) which is one of only two of its kind in Canada, specializing in TBI, providing a unique environment to study the acute care of brain injury. Both centers have well established Research Institutes that function to create collaborations beyond divisions and departments reflecting the wide range of disciplines involved in trauma-related care and research. They support a range of activities from laboratory, clinical and broader team-based research activity. The LA County Hospital is a 600-bed public teaching hospital. It is jointly operated by Los Angeles County Department of Health Services and the USC and treats an average of 7000 trauma patients per year (86% blunt mechanism). It has a 18-bed Neuroscience ICU which is a state-of-the art unit allowing for the most advanced monitoring equipment. Trauma ICU
admissions reach 77% and 33% go under a surgical procedure.

In the trauma room of the three institutions, patients are assessed and treated by a trauma team led by a trauma team leader who can be a trauma surgeon, emergency physician, anesthesiologist or orthopedist. Acutely, care may also be provided by attending staff from Critical Care Medicine, Anesthesia, Vascular Surgery and Neurosurgery. For massively bleeding trauma patients, transfusion medicine specialists may also be contacted, and are available for assistance with transfusion decisions.

3.2. Study design

This is a multi-site prospective blinded cohort study of all consecutive adult trauma patients with isolated moderate to severe TBI. The core laboratories at each hospital centrifuged, froze, and stored the samples which were sent in batches every three months to the laboratory facility at Defense Research and Development Canada (DRDC) for analyses. The clinical and research teams did not have access to the data until the end of the study, thereby ensuring that all those involved with the trial remained blinded to the results until after the 6-month follow-up assessments were completed.

3.3. Study Definitions

The Injury Severity score (ISS) (Table 2). Patients included in the study were classified according to their severity of injury using the ISS. The score is an anatomic trauma scoring system which takes into account the squared values of the three mostly injured body areas based on the AIS and correlates strongly with mortality [364-367]. It varies from minor traumas with practically no significant injuries (score point = 1) to unsalvageable injuries (score point = 75). Mortality increases significantly for scores of 15 or higher, which is commonly used to define severe trauma [364-367].
Table 2 – The Injury Severity Score.

<table>
<thead>
<tr>
<th>Regions</th>
<th>AIS</th>
<th>AIS meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head, neck and C-spine</td>
<td>1</td>
<td>Minor</td>
</tr>
<tr>
<td>Face including nose, mouth, eyes, ears</td>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td>Thorax, thoracic spine, diaphragm</td>
<td>3</td>
<td>Serious</td>
</tr>
<tr>
<td>Abdomen and lumbar spine</td>
<td>4</td>
<td>Severe</td>
</tr>
<tr>
<td>Extremities including pelvis</td>
<td>5</td>
<td>Critical</td>
</tr>
<tr>
<td>External soft tissue injury</td>
<td>6</td>
<td>Maximal (untreatable)</td>
</tr>
</tbody>
</table>

Calculate AIS for most severely injured body part in each region. ISS is calculated as sum of square of AIS for the 3 most injured body regions. Maximum score is 75. If any body region is assigned a 6, the overall ISS is automatically 75.

Legend: AIS – abbreviated injury scale

**The Glasgow Coma Scale – GCS (Table 3): mild, moderate and severe head injury.** Since 1974, the Glasgow coma scale has been providing a practical method for bedside assessment of the level of consciousness, the clinical hallmark of acute brain injury [220]. The GCS score has been validated in the literature and is widely used for classification of the severity of the brain injury and the association with outcome [228-232]. We used the GCS score to classify study patients on admission. Patients are classified in three different groups, according to the score: severe head injury includes patients with a score from 3 to 8, moderate head injury includes patients with a score from 9 to 12 and mild head injury includes patients with a score from 13 to 15. The score is determined by the trauma team leader during the primary assessment of patients with isolated TBI, and is subsequently recalculated as needed for continuous follow-up.
### Table 3 – The Glasgow Coma Scale.

<table>
<thead>
<tr>
<th>Eye opening</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Spontaneous</td>
</tr>
<tr>
<td>3</td>
<td>To speech</td>
</tr>
<tr>
<td>2</td>
<td>To pain</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Verbal response</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Orientated</td>
</tr>
<tr>
<td>4</td>
<td>Confused</td>
</tr>
<tr>
<td>3</td>
<td>Inappropriate words</td>
</tr>
<tr>
<td>2</td>
<td>Incomprehensible sounds</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Best motor response</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Obeys commands</td>
</tr>
<tr>
<td>5</td>
<td>Localizes pain</td>
</tr>
<tr>
<td>4</td>
<td>Flexion, withdrawals to pain</td>
</tr>
<tr>
<td>3</td>
<td>Flexion, abnormal to pain</td>
</tr>
<tr>
<td>2</td>
<td>Extension to pain</td>
</tr>
<tr>
<td>1</td>
<td>No response</td>
</tr>
</tbody>
</table>

### The Glasgow Outcome Scale Extended – GOSE (Table 4):

The Glasgow Outcome Scale (GOS) was created in 1975 by Jennett [368] and has become the most widely used scale for assessing functional outcome after head injury and non-traumatic acute brain insults [8, 9]. The initial GOS was increasingly recognized to have important limitations. Shortcomings of GOS were addressed by adopting a standard format for the interview used to assign outcome. A structured interview (Table 5) was created for both the 5-point GOS and an extended eight-point GOS (GOSE), which yielded a high inter-rater reliability, turning the scale to a more practical and reliable tool [10, 11].

In our study the patients were evaluated at hospital discharge and at 6 months post TBI by
an experienced physician unaware of any catecholamine values. At 6 months the patients were evaluated via a telephone call with the structured interview questionnaire, with the patients themselves, a family member and/or a caregiver. Patients were classified according to the GOSE categories to assess primary outcome where 1 = dead and 8 = totally recovered. We dichotomized the scale to unfavorable outcome (GOSE 1 to 4) and favorable outcome (GOSE 5 to 8), as this is frequently used in the brain injury [369-371] literature. Dead patients, patients in vegetative state or with severe disability were considered as having unfavorable outcome and patients with moderate disability and good recovery were considered as having favorable outcome.

Table 4 – The Glasgow Outcome Scale Extended (GOSE).

<table>
<thead>
<tr>
<th>Score</th>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (D)</td>
<td>Death</td>
<td></td>
</tr>
<tr>
<td>2 (VS)</td>
<td>Persistent vegetative state</td>
<td>Condition of unawareness with only reflex responses but with periods of spontaneous eye opening</td>
</tr>
<tr>
<td>3 (SD-)</td>
<td>Lower severe disability</td>
<td>Patient who is dependent for daily support for mental or physical disability, usually a combination of both. If the patient can be left alone for more than 8 hours at home it is upper level of SD, if not then it is lower level of SD</td>
</tr>
<tr>
<td>4 (SD+)</td>
<td>Upper severe disability</td>
<td></td>
</tr>
<tr>
<td>5 (MD-)</td>
<td>Lower moderate disability</td>
<td>Patients have some disabilities such as aphasia, hemiparesis or epilepsy and/or deficits of memory or personality but are able to look after themselves. If they are able to return to work even with special arrangements it is upper level of MD, if not then it is lower level of MD</td>
</tr>
<tr>
<td>6 (MD+)</td>
<td>Upper moderate disability</td>
<td></td>
</tr>
<tr>
<td>7 (GR-)</td>
<td>Lower good recovery</td>
<td>Resumption of normal life with the capacity to work even if pre-injury status has not been achieved. Some patients have minor neurological or psychological deficits. If these deficits are not disabling then it is upper GR, if disabling then it is lower level of GR</td>
</tr>
<tr>
<td>8 (GR+)</td>
<td>Upper good recovery</td>
<td></td>
</tr>
</tbody>
</table>

The structured interview used post discharge for assessment of outcome in the GOSE scale is represented below in Table 5.

Table 5 – The Post Discharge Structured Interview for GOSE.

<table>
<thead>
<tr>
<th>POST DISCHARGE STRUCTURED INTERVIEW FOR GOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Glasgow Outcome Scale Extended)</td>
</tr>
</tbody>
</table>

**Consciousness:**

1 – Is the head-injured person able to obey simple commands or say any words?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No (VS)</th>
</tr>
</thead>
</table>

Note: anyone who shows the ability to obey even simple commands or utter any word or communicate specifically in any other way is no longer considered to be in vegetative state. Eye movements are not reliable evidence of meaningful responsiveness. Corroborate with nursing staff and/or other caretakers. Confirmation of VS requires full assessment.

**Independence at home:**

2a – Is the assistance of another person at home essential every day for some activities of daily living?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No (VS)</th>
</tr>
</thead>
</table>

Note: for a NO answer they should be able to look after themselves at home for 24 hours if necessary, though they need not actually look after themselves. Independence includes the ability to plan for and carry out the following activities: getting washed, putting on clean clothes without prompting, preparing food for themselves, dealing with callers and handling minor domestic crises. The person should be able to carry out activities without needing prompting or reminding and should be capable of being left alone overnight.

2b – Do they need frequent help of someone to be around at home most of the time?

<table>
<thead>
<tr>
<th>Yes (lower SD)</th>
<th>No (upper SD)</th>
</tr>
</thead>
</table>

Note: for a NO answer they should be able to look after themselves at home up to eight hours during the day if necessary, though they need not actually look after themselves.

2c – Was the patient independent at home before the injury?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

**Independence outside home:**

3a – Are they able to shop without assistance?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No (upper SD)</th>
</tr>
</thead>
</table>
Note: this includes being able to plan what to buy, take care of money themselves and behave appropriately in public. They need not normally shop, but must be able to do so.

3b – Were they able to shop without assistance before?
   Yes  No

4a – Are they able to travel locally without assistance?
   Yes  No (upper SD)
Note: they may drive or use public transport to get around. Ability to use a taxi is sufficient, provided the person can phone for it themselves and instructs the driver.

4b – Were they able to travel locally without assistance before the injury?
   Yes  No

Work:
5a – Are they currently able to work (or look after others at home) to their previous capacity?
   Yes  If yes, go to 6  No

5b – How restricted are they?
   a – Reduced work capacity?
   b – Able to work only in a sheltered workshop or non-competitive job or currently unable to work?
      a. (Upper MD)  b. (Lower MD)

5c – Does the level of restriction represent a change in respect to the pre-trauma situation?
   Yes  No

Social and Leisure activities:
6a – Are they able to resume regular social and leisure activities outside home?
   Yes  If yes, go to 7  No
Note: they need not have resumed all their previous leisure activities, but should not be prevented by physical or mental impairment. If they have stopped the majority of activities because of loss of interest or motivation, then this is also considered a disability.

6b – What is the extent of restriction on their social and leisure activities?
   a – Participate a bit less: at least half as often as before
   b – Participate much less: less than half as often
   c – Unable to participate: rarely, if ever, take part
      a. (lower GR)  b. (upper MD)  c. (lower MD)
6c – Does the extent of restriction in regular social and leisure activities outside home represent a change in respect or pre-trauma
   Yes                      No

**Family and friendships:**

7a – Has there been family or friendship disruption due to psychological problems?
   Yes                      No                          If no, go to 8

Note: typical post-traumatic personality changes are: quick temper, irritability, anxiety, insensitivity to others, mood swings, depression and unreasonable or childish behaviour.

7b – What has been the extent of disruption or strain?
   a – Occasional - less than weekly
   b – Frequent - once a week or more, but not tolerable
   c – Constant - daily and intolerable
      a. (lower GR)         b. (upper MD)         c. (lower MD)

7c. Does the level of disruption or strain represent a change in respect to pre-trauma situation?
   Yes                      No

Note: if there were some problems before injury, but these have become markedly worse since the injury then answer yes to question

Return to normal life:

8a – Are there any other current problems relating to the injury which affect daily life?
   Yes (Lower GR)           No (Upper GR)

Note: other typical problems reported after head injury: headaches, dizziness, sensitivity to noise or light, slowness, memory failures and concentration problems.

8b – If similar problems were present before the injury, have these become markedly worse?
   Yes                      No

9 – What is the most important factor in outcome?
   a – Effects of head injury
   b – Effects of illness or injury to another part of the body
   c – A mixture of these

Note: extended GOS grades are shown beside responses on the CRF. The overall rating is based on the lowest outcome category indicated. Areas in which there has been no change with respect to the pre-trauma situation are ignored when the overall rating is made
The Head Abbreviated Injury Scale – AIS (Table 6): severe and non-severe head injury. The AIS is the most widely used anatomic injury severity rating system in the world [267]. It was developed in 1971 by the American Medical Association Committee on Medical Aspects of Automotive Safety to provide researchers with an accurate method for rating and comparing injuries from automotive crashes, as well as to establish a common language when describing injuries (Committee on Medical Aspects of Automotive Safety, 1971, 1972) [372, 373]. A subsequent number of revisions followed its original version: (1) 1981, several non-anatomic components were included (Committee on Injury Scaling, 1981) [374]; (2) 1985, the scale body regions were expanded from five to nine and expanded sections on thoracic, abdominal and pelvic regions were added [375]. New entries for penetrating, vascular and skin injuries were incorporated (Committee on Injury Scaling, 1985) [375]; (3) 1990, more descriptors of penetrating injuries, specific codes for pediatric trauma and a different classification for the body regions were included (Association for the Advancement of Automotive Medicine, 1990) [376]. The head AIS was used to classify the severity of the brain injury, which was rated from minor (AIS=1) to unsalvageable (AIS=6). Severe head injury was considered a Head AIS >3 as previously defined in the literature [364-367].

**Table 6 – The Abbreviated Injury Scale (AIS).**

<table>
<thead>
<tr>
<th>Score</th>
<th>Severity of injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIS 1</td>
<td>Minor</td>
</tr>
<tr>
<td>AIS 2</td>
<td>Moderate</td>
</tr>
<tr>
<td>AIS 3</td>
<td>Serious but not life threatening</td>
</tr>
<tr>
<td>AIS 4</td>
<td>Severe, life threatening, survival probable</td>
</tr>
<tr>
<td>AIS 5</td>
<td>Critical, survival uncertain</td>
</tr>
<tr>
<td>AIS 6</td>
<td>Unsurvivable</td>
</tr>
</tbody>
</table>

The Marshall Classification (Table 7): diffuse injury, diffuse injury with intracranial hypertension, mass lesions. The Marshall classification uses the findings from CT scans on the status of the mesencephalic cisterns, the degree of midline shift and the presence or absence of local lesions to categorize patients into six different groups
[206]. It was used in our study for the correlation of catecholamine levels with severity of tomographic injury as demonstrated by the classification. Please refer to the item 1.4.5.2.: “Classification of TBI According to CT Findings” for detailed information about origin, validation and utilization of the Marshall Classification. The Classification is summarized on Table 7.

**Table 7 – The Marshall Classification.**

<table>
<thead>
<tr>
<th>Score</th>
<th>CT scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse injury I (no visible pathology)</td>
<td>No visible intracranial pathology seen on CT scan</td>
</tr>
<tr>
<td>Diffuse injury II</td>
<td>Cisterns are present with midline shift of 0-5 mm and/or lesions densities present; no high or mixed density lesion &gt;25cm³; may include bone fragments or foreign bodies</td>
</tr>
<tr>
<td>Diffuse injury III (swelling)</td>
<td>Cisterns compressed or absent with midline shift 0-5 mm; no high or mixed density lesion &gt;25cm³</td>
</tr>
<tr>
<td>Diffuse injury IV (shift)</td>
<td>Midline shift &gt;5mm; no high or mixed density lesion &gt;25cm³</td>
</tr>
<tr>
<td>Evacuated mass lesion</td>
<td>Any lesion surgically evacuated</td>
</tr>
<tr>
<td>Non evacuated mass lesion</td>
<td>High or mixed density lesion &gt;25cm³; not surgically evacuated</td>
</tr>
</tbody>
</table>

**Inclusion Criteria**

Patients were included if they had isolated moderate to severe TBI. Isolated TBI was defined by any head AIS plus a non-head AIS ≤2. Moderate and severe TBI were defined by a GCS 9-12 and GCS 3-8, respectively. To be included in the trial the patient had to meet both definitions of suffering an isolated TBI that was moderate or severe.

**Exclusion Criteria**

Patients were excluded if they had any of the following: elapsed time between the trauma and admission to hospital exceeding 3 hours; age less than 16 years; pregnancy;
absence of vital signs on admission or clinical evidence of brain death on hospital admission.

**Sample Size Calculation**

The sample size was determined based on previous data [1] of moderate/severe TBI patients showing that 25% had an unfavorable outcome (GOSE=1-4). To detect a statistically significant difference of 300ng/ml in E between patients with favorable (GOS=5-8) versus unfavorable outcome (GOSE 1-4), the study required 113 patients with favorable and 39 patients with unfavorable outcome, for 80% power at 5% significance level. Assuming losses in follow-up from 20% to 30%, we initially estimated that 200 patients were needed.

**Informed Consent**

Trauma team was responsible to enroll the patients who meet the criteria above after obtaining an informed consent from substitute decision makers for participation and continuation in the study. For patients without a substitute decision maker, the informed consent was delayed in accordance with the Tri-Council Policy Agreement for Research in Emergency Health Situations (Article 2.8). A delayed written consent for participation in the study was subsequently obtained from the next-of-kin. If the patient recovered sufficiently to provide written informed consent for continuation in the study, then the patient’s consent was also obtained (Appendix 1).

**3.4. Data Acquisition**

Each Trauma Center had a dedicated full-time team of research coordinators on-site during regular working hours Mon-Fri 9am-5pm who were available to respond to trauma pages and ensure that the blood samples were collected and processed, to obtain informed consent from the substitution decision makers or patients, fill out the Case Report Forms (Appendix 2) while the patient was still in-hospital, and complete the hospital discharge and 6-month follow-up assessments. In addition, given the unpredictable and 24/7 nature of trauma patient arrival to the trauma centers, the participating sites have implemented on-call programs for trauma clinical research assistants for off-hours. Once paged, the research coordinators were directed to the trauma room to confirm eligibility and collect the blood samples. The coordinators also arranged for the subsequent blood sample collections.
Clinical and laboratory data were retrieved from the hospital chart and electronic patient records.

**Clinical Data Collected Upon Hospital Admission (Appendix 2)**

- **Demographics**: age and gender.
- **Trauma**: mechanism of injury, elapsed time from the scene to the Emergency Room, injury.
- **Injury Severity Scores**: ISS, head Abbreviated Injury Score (head AIS).
- **Neurological status**: level of consciousness as categorized by the GCS, pupil size and reactivity, lateralization signs, seizures, alcohol level.
- **Clinical status**: blood pressure, heart rate, respiratory rate, tracheal intubation, spontaneous vs. mechanical ventilation, oxygen saturation, temperature, sedation, paralysis.
- **Past medical history and present medications**: including beta-blockers and anticoagulants.

**Routine Laboratory and Imaging on Admission**

- Complete blood count, potassium (K), glucose, arterial blood gas analysis, INR, aPTT, lactate and troponin, sodium (Na), chest radiography, electrocardiogram and head CT scan classified as Marshall CT score.

- Blood samples for the study of catecholamines, neuro biomarkers and inflammatory biomarkers were collected on admission and at 6, 12 and 24 hours post trauma. Neuro and inflammatory biomarkers were used for another arm of the COMA-TBI study that will study the association of these inflammatory biomarkers with outcome.

- Blood samples for routine laboratory exams and imaging studies were subsequently performed when clinically indicated as per standard of care. Na, K, pH, BD, glucose, troponin, lactate, platelet count, hemoglobin, aPTT and INR were measured each time point: admission and 6h, 12h, 24h post admission (Appendix 2).
First 24 Hours Events

All significant clinical/surgical events during the first 24 hours were recorded, including any treatment with vasoactive drugs, neurosurgical procedures, hypotension episodes, respiratory failure, high ICP (determined in the ED by the trauma team leader or neurosurgeon according to clinical signs, head CT scan (Marshall Classification) or in the intensive care unit/operating room by clinical signs, a subsequent CT scan or ICP monitoring). In case the patient had an ICP monitor, we considered the measurement to determine whether there was intracranial hypertension or not, instead of relying on clinical signs or the Marshall Classification. As per hospital protocol, high ICP was defined as one episode greater than 25mmHg persisting for at least 5 minutes. We also documented changes in head CT scan, chest radiography or electrocardiogram. Any treatment with vasoactive drug, doses and timing were recorded as we anticipate that the use of vasopressors likely would be given to a number of patients to help optimize cerebral perfusion pressure and could interfere with the catecholamine levels.

Follow up During Hospitalization

In addition to clinical, laboratory, and image data collected on admission and during the first 24 hours after admission, patients were followed during the course of their hospital stay, recording any new surgical procedure, including tracheostomy, duration of mechanical ventilation, development of infection, sepsis or a new organ failure, length of ICU and hospital stay. For those who died, the cause of death was recorded and classified as directly related to the TBI or not. Sepsis was defined following current guidelines from the Surviving Sepsis Campaign [377]: presence (probable or documented) of infection together with systemic manifestations of infection. Severe sepsis was defined as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion [377].

Follow up Post Discharge (GOSE at 6 months)

Using previous experience with studies of long-term outcome in patients with acute lung injury, we took the following steps to ensure high follow-up rates: (a) Prior to hospital discharge we obtained multiple contact numbers from family members; (b) We sent a study reminder card at 3 months post-enrollment to maintain contact with the participant; (c) We
sent a reminder card in advance of the 6-month follow-up; (d) We checked survival status of any participants lost to follow up from public provincial registries.

GOSE was recorded at discharge and at 6 months post trauma, by an expert physician, through a telephone structured interview with the patient and/or with their caregiver, as defined before (Table 5). The GOSE categories are from 1 = dead to 8 = good recovery (Table 4). We dichotomized the scale to unfavorable outcome (GOSE 1 to 4) and favorable outcome (GOSE 5 to 8), as this is being consistently used in the brain injury literature [369-371]. As for this dichotomization, patients who died, were in vegetative state or with severe disability were considered to have an unfavorable outcome and patients with moderate disability and good recovery, a favorable outcome.

The COMA-TBI study flow is highlighted in Figure 4.
Blood Sample Collection and Preservation

For the measurement of plasma circulating catecholamines, the blood samples were drawn into either 10-mL K₂EDTA (with 4 mM sodium metabisulfite [Na₂S₂O₅]) or 10mL sodium heparin vacutainers (Vacutainer, Becton Dickinson, Rutherford, NJ). Specimens were immediately centrifuged at 1600 x g for 15 minutes the plasma was separated into six
(1-2 mL) aliquots and frozen at -70°C until the analyses at DRDC.

**Measurement of Circulating Catecholamines**

Quantification of plasma catecholamines concentrations (pmol/L) was performed using commercially available solid-phase competitive enzyme immunoassay (EIA) kits (Rocky Mountains Diagnostics, Colorado Springs, CO), as per the manufacturer’s protocol. Briefly, plasma samples were extracted, derivitized and diluted with assay buffer; standards, samples, controls, and the solid-phase bound analytes compete for a fixed number of antiserum binding sites, which are incubated in precoated 96-well microtiter plates. Optical density with wavelength correction, were read using an automated Synergy 2 microplate photometer (BioTek, Winooski, VT). The reference values for E were 50pmol/L and for NE were 380pmol/L as per manufacturer information.

### 3.5. Study Outcomes

**3.5.1. Primary Study Outcome**

PSO 1 – Neurological function determined by GOSE score at 6 months.

PSO 2 – Independent association between admission catecholamine levels and unfavorable outcome

PSO 3 – Independent association between the tertiles of levels of both E and NE and an unfavorable outcome

**3.5.2. Secondary Study Outcomes**

SSO 1 – Length of ICU and hospital stay in both groups of patients with a favorable and an unfavorable outcome

SSO 2 – Ventilation days in both groups of patients with a favorable and an unfavorable outcome

SSO 3 – In-hospital and the 6-month mortality in patients with an unfavorable outcome

SSO 4 – Ventilator free days and ICU free days in both groups of patients with a favorable and an unfavorable outcome
SSO 5 – Plasma catecholamine profile across all 4 time points in pooled patients
SSO 6 – Plasma catecholamine levels in healthy volunteers
SSO 7 – Plasma catecholamine levels according to the Glasgow Coma Scale (GCS) in moderate (GCS 9-12) and severe (GCS 3-8) TBI patients
SSO 8 – Plasma catecholamine levels according to the Abbreviated Injury Score (AIS) in patients with severe (AIS 4, 5) and non-severe (AIS 1, 2, 3) TBI
SSO 9 – Plasma catecholamine profile across all 4 time points in all categories of the Marshall Classification
SSO 10 – Plasma catecholamine profile in pooled patients across categories of 1 to 8 of the Glasgow Outcome Scale Extended (GOSE) at 6 months
SSO 11 – Plasma catecholamine profile across all 4 time points in patients with favorable outcome (GOSE 1-4)
SSO 12 – Plasma catecholamine profile across all 4 time points in patients with unfavorable outcome (GOSE 5-8)
SSO 13 – Plasma catecholamine levels in patients with and without high ICP
SSO 14 – Plasma catecholamine levels in patients with and without sepsis
SSO 15 – Plasma catecholamine levels in patients with and without MODS
SSO 16 – Plasma catecholamine levels in patients who died or survived

3.6. Statistical Analysis

Descriptive statistics are presented as proportions, medians and interquartile ranges, Univariate associations between characteristics at time of hospital admission and unfavorable outcome were determined using the Wilcoxon two-sample test for continuous measures, and the chi-square or Fisher’s exact test for categorical variables. To test for the independent association of catecholamine levels at time of hospital admission and unfavorable outcome at 6 months (GOSE at 180 days =1 [dead], or 2 [vegetative state] or 3 [low severe disability] or 4 [upper severe disability]), we estimated unadjusted and adjusted odds ratios and 95% CI from two multivariate logistic regression models: Model 1 included
NE in units of 380pmol/L (mean basal levels among healthy individuals) and Model 2 included E in units of 50pmol/L (mean basal levels among healthy individuals). The clinical prognostic factors on admission used for the adjustment in the 2 models were: age (in decades), presence of hypotension (SBP<100mmHg), severe TBI (GCS 3-8), severe head injury (AIS 4-5), INR (<1.2), and the use of vasopressors. To facilitate interpretation we also reported the final model using admission tertiles of NE (≤6,540.5 [reference], 6,540.5-17,708.7, >17,708.7) and admission tertiles of E (≤1,368.7 [reference], 1,368.7-4,019.9, >4,019.9).

In addition to the independent association, the C-Statistic was estimated to provide the descriptive measure of discrimination between unfavourable and favourable outcome groups, with the estimation of AUC.

The Hosmer-Lemeshow goodness of fit assessed adequacy of the final model.

We estimated the association of both E and NE levels in categories III/IV of the Marshall Classification (brain edema with intracranial hypertension), with the other categories in the Classification, across all 4 time points (admission, 6, 12 and 24 hours). We hypothesized that patients with scores of III or IV have consistently higher levels of both E and NE over the 24-hour period, what was initially inferred in Figure 15. We compared categories III/IV with V/VI (edema/high ICP vs. collections), III/IV with II (edema with high ICP vs. just edema), I with II (normal vs. edema), and I with V/VI (normal vs. mass lesions). Therefore, we had 4 comparisons by time point resulting in a total of 16 comparisons, overall. To test the 16 comparisons and because of the catecholamines are measured at multiple time points, we performed a model that could accommodate the correlations among the repeated measurements. As we already noted, the distribution of catecholamines is skewed, although this aspect did not matter for the univariate analysis (using Wilcoxon or Kruskall-Wallis, non-parametric tests), or the multivariate analyses (using the logistic regression model). However, to analyze the longitudinal catecholamine data, we used repeated measures analyses using the mixed model framework. This technique requires that the model residuals, adjusting for the Marshall categories, follow a normal distribution, which is not possible if catecholamines are analyzed on their original scale. Therefore, we applied a log base-10 transformation to
E and NE before fitting the model, which included Marshall categories and time as factors, as well as their interaction. The correlation among repeated measures was assumed to have a compound symmetry structure. As we were interested in 16 comparisons, we used the Bonferroni approach to adjust the significance level for multiple comparisons, that is, for each catecholamine, a comparison was considered statistically significant if $p < 0.05/16 = 0.003125$. 
CHAPTER 4 – Results

4.1. Initial Analysis

4.1.1. Centers and Study Participants Enrollment

Figure 5 illustrates the study enrollment process and follow-up. From September 2011 to June 2013, 3,264 patients were screened in the 2 trauma centers in Toronto (2,216 at SHSC, 1,048 at SMH) and from January 2013 to March 2013, 1,750 patients were screened at the LA County). Two thousands and ninety five patients did not meet the inclusion criteria at SHSC, 978 at SMH and 1,737 at LA County. The final cohort was of 189 patients with isolated moderate to severe TBI enrolled in the study (121 patients from SHSC, 55 patients from SMH and 13 patients from LA County). One patient was excluded later after enrollment at SHSC as it was noticed that the age was less than 15 years old. Three patients at SHSC and four patients at LA County were removed from the cohort due to withdrawal of consent post enrollment, by the patient’s power of attorney. During the post hospital follow up period, we lost only 2 patients from SHSC, who unfortunately were not located using previous contact information and within the provincial registries.
4.1.2. Blood Samples Flow in the Study

Figure 6 illustrates the blood samples flow in the study. For each time point (admission, 6, 12 and 24 hours post injury), 181 blood samples should have been collected, totalizing 724 samples. Unfortunately, due to technical problems during the specimen acquisition, laboratory manipulation and preservation, we lost 101 vials (16 at the admission time point, 8.8% of the total number of admission samples). For the subsequent time points, samples were not collected or were discarded due to patients being discharged (n=9), dead (n=11) and the consent being withdrew post inclusion in the study (n=7). We
had a total of 596 samples sent to the laboratory at DRDC for circulating plasma catecholamine analysis.

Figure 6 – Flow diagram of blood samples collection and manipulation.

Legend: WC – withdrawal of consent.

4.1.3. Association between Demographics, Clinical, Laboratory and Radiological Characteristics with Unfavorable Outcome

We summarized all demographics, clinical, laboratory, and radiological variables, and checked whether there was significant difference in both groups of favorable (n=66 patients) and unfavorable outcome (n=113 patients) at 6 months (Table 8), using appropriate univariate statistical analysis as described previously.

Demographic Variables
The median/IQR age across all patients was 47 (29-64) years. Male gender was predominant (137 patients, 75.6%). Patients with an unfavorable outcome were older (51 [36-72]) years, compared to patients with a favorable outcome (33.5 [21-51]), \( p<0.0001 \). Most patients had a blunt mechanism as the cause of injury (176 patients, 97.2%).

**Classification of Injury Severity**

The scores of injury severity (GCS, AIS, ISS and Marshall) and the APACHE score were measured in all patients included in the study. The median/IQR ISS score was 25 (17-30) and the median/IQR APACHE score was 21 (17-26), across all patients. Patients with an unfavorable outcome had higher scores compared to patients with a favorable outcome (\( p<0.0001 \) for all scores: GCS, AIS, ISS and APACHE). Patients with an unfavorable outcome had worse Marshall classification, with 105 patients (92.9%) distributed in categories II to VI and only 5 patients (4.4%) in category I. Conversely, 55 patients (83.8%) with a favorable outcome were distributed in categories I and II and only 11 patients (16.6%) in the other categories (\( p<0.0001 \)).

**Clinical Variables**

At least one episode of hypotension occurred in 26 patients (14.3%), mostly in patients with unfavorable outcome (22 patients, 84.6%, \( p=0.01 \)). At least one episode of desaturation occurred in 4 patients (2.2%), mostly in patients with an unfavorable outcome (3 patients, 75%) but with a non-statistically significant difference due to the number of desaturations (\( p=1.00 \)). The median temperature/IQR across all patients was \( 36^0 \text{C} \) (35.1-37\(^0 \text{C} \)), and no statistical difference was found between both groups (\( p=0.35 \)). Vasopressors were administered to 54 patients (29.8%) and more frequently (48 patients, 88.8%) in patients with unfavorable outcome (\( p<0.0001 \)). No patients received a drip of vasopressors before the admission blood sample was collected. We unfortunately did not collect information about boluses of vasopressors received before admission to our ED. Most subjects (135 patients, 74.5%) were intubated on admission. Ninety six patients (84.9%) in the unfavorable outcome group were intubated on admission, compared to 39 patients (59.1%) in the favorable outcome group (\( p<0.0001 \)). Patients with an unfavorable outcome had more frequently unequal and unreactive pupils (\( p=0.0005 \) and \( p<0.0001 \), respectively)
and underwent more neurosurgical procedures (42 patients, 79.2%, $p=0.003$). ICP was measured in 42 patients (23.2%) and of these, 20 patients (47.6%) had a high ICP (at least one episode of ICP≥25mmHg persistently for 5 minutes or more). Patients with an unfavorable outcome had measured high ICP more commonly (27 patients, 64.3%, compared to patients with a favorable outcome [$p=0.004$]). Patients with high ICP were mostly from the group with an unfavorable outcome (16 patients, 80%, $p=0.02$). Signs of high ICP on CT scan (Marshall Classification) was seen in 46 patients (25.4%), the majority in the unfavorable outcome group (41 patients, 89.1%, $p=0.001$). Sepsis occurred in 48 patients (26.5%), more commonly in the unfavorable outcome group (38 patients, 79.1%, $p=0.007$). Multi organ dysfunction syndrome was diagnosed in 20 patients (11%), all from the unfavorable outcome group ($p=0.0003$). No patients with a favorable outcome at 6 months had MODS during their hospitalization period. Only 10 patients (5.5%) were using beta blockers prior to hospital admission, 3 in the favorable and 7 in the unfavorable outcome groups, respectively, but without statistical significance ($p=0.26$). Fifty seven patients (31.5%) had known comorbidities, which were similarly distributed between both groups (18/66, 27.3% and 39/113, 34.5%) in the favourable and unfavourable outcome groups, respectively ($p=0.44$).

**Laboratory Variables**

Laboratory parameters that reached statistical difference between those patients with unfavourable and favourable 6-month outcome were INR ($p=0.002$), platelet counts ($p=0.001$), hemoglobin ($p=0.0001$), glucose ($p=0.0008$), troponin ($p=0.02$) and K ($p=0.02$). However, a meaningful clinical difference was not observed in none of the parameters, as their values were mostly within normal ranges or mildly abnormal. The electrocardiogram was performed in 116 patients and was abnormal in 69 (59.5%), mostly in patients with an unfavorable outcome (47 patients, 68.1%, $p=0.014$).

**Radiological Variables**

A subsequent head CT scan was performed in 135 patients and was worse compared to the admission CT in 56 patients (41.5%). Patients with an unfavorable outcome had a worse subsequent CT scan in 41.6%, while the CT was worse in favorable
outcome patients in only 13.6% (p=0.0002). Pulmonary edema was diagnosed in only 7 patients (3.8%), mostly in the unfavorable outcome group (6 patients, 85.7%), which was not statistically significant (p=0.26). Only 22 patients (16.6%) had evidence of a worse chest X-ray during hospital admission, and no statistical difference was found between both groups (p=0.95).

Table 8 – Association between demographics, clinical, laboratory and radiological characteristics with unfavorable outcome

<table>
<thead>
<tr>
<th>Variable (median, IQR)</th>
<th>All trauma patients (n=181)</th>
<th>Favourable outcome (n=66)</th>
<th>Unfavourable outcome (n=113)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>47 (29-64)</td>
<td>33.5 (21-51)</td>
<td>51 (36-72)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male gender</td>
<td>137 (75.7%)</td>
<td>54 (81.8%)</td>
<td>81 (71.7%)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Clinical Variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanism of injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blunt</td>
<td>176 (97.2%)</td>
<td>65 (98.5%)</td>
<td>109 (96.5%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Penetrating</td>
<td>5 (2.7%)</td>
<td>1 (1.5%)</td>
<td>4 (3.5%)</td>
<td></td>
</tr>
<tr>
<td>Time to ED</td>
<td>60 (34-105)</td>
<td>60 (34-120)</td>
<td>50 (33-90)</td>
<td>0.13</td>
</tr>
<tr>
<td>ISS (1-75)</td>
<td>25 (17-30)</td>
<td>18 (10-25)</td>
<td>26 (25-33)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GCS (3-15)</td>
<td>5 (3-8)</td>
<td>7.5 (50-10)</td>
<td>4 (3-7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AIS severe head (&gt;3)</td>
<td>136 (75.1%)</td>
<td>40 (60.6%)</td>
<td>96 (84.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>APACHE (0-71)</td>
<td>21 (17-26)</td>
<td>18 (12.5-22)</td>
<td>24 (19-29)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Marshall Score</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>I</td>
<td>29 (16.0%)</td>
<td>19 (28.7%)</td>
<td>8 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>83 (45.8%)</td>
<td>36 (54.5%)</td>
<td>47 (41.6%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>15 (8.3%)</td>
<td>3 (4.5%)</td>
<td>12 (10.6%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>31 (17.1%)</td>
<td>2 (3.0%)</td>
<td>29 (25.6%)</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>22 (12.1%)</td>
<td>6 (9.0%)</td>
<td>16 (14.1%)</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>1 (0.5%)</td>
<td>0</td>
<td>1 (0.9%)</td>
<td></td>
</tr>
<tr>
<td>HR (60-80bpm)</td>
<td>88 (72-108)</td>
<td>88 (73-102)</td>
<td>88 (70-110)</td>
<td>0.91</td>
</tr>
<tr>
<td>SBP (90-119mmHg)</td>
<td>135 (120-159)</td>
<td>135 (120-156)</td>
<td>136 (120-160)</td>
<td>0.91</td>
</tr>
<tr>
<td>RR (12-20breaths/min)</td>
<td>18 (16-20.5)</td>
<td>18 (16-20)</td>
<td>18 (16-22)</td>
<td>0.61</td>
</tr>
<tr>
<td>Hypotension (≤100mmHg)</td>
<td>26 (14.4%)</td>
<td>4 (6.1%)</td>
<td>22 (19.5%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Desaturation (&lt;90%)</td>
<td>4 (2.2%)</td>
<td>1 (1.5%)</td>
<td>3 (2.6%)</td>
<td>1.00</td>
</tr>
<tr>
<td>O2 Sat (90-100%)</td>
<td>99 (97-100)</td>
<td>100 (97-100)</td>
<td>99 (97-100)</td>
<td>0.66</td>
</tr>
<tr>
<td>Temperature (36.5-37.5°C)</td>
<td>36.0 (35.1-37.0)</td>
<td>36 (35.3-37)</td>
<td>36 (35-36.65)</td>
<td>0.35</td>
</tr>
<tr>
<td>Variable (median, IQR)</td>
<td>All trauma patients (n=181)</td>
<td>Favourable outcome (n=66)</td>
<td>Unfavourable outcome (n=113)</td>
<td>p</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------</td>
<td>---------------------------</td>
<td>-------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>Vasopressors</td>
<td>54 (29.8%)</td>
<td>6 (9.1%)</td>
<td>48 (42.5%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>7 (3.9%)</td>
<td>1 (1.5%)</td>
<td>6 (5.3%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Intubation</td>
<td>135 (74.6%)</td>
<td>39 (59.1%)</td>
<td>96 (84.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sedation</td>
<td>141 (77.9%)</td>
<td>47 (71.2%)</td>
<td>94 (83.2%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Paralysis</td>
<td>86 (47.5%)</td>
<td>28 (42.4%)</td>
<td>58 (51.3%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Ventilation</td>
<td>135 (74.6%)</td>
<td>43 (65.1%)</td>
<td>92 (81.4%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>ICP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measured</td>
<td>42 (23.2%)</td>
<td>15 (35.7%)</td>
<td>27 (64.3%)</td>
<td>0.004</td>
</tr>
<tr>
<td>High ICP</td>
<td>20 (47.6%)</td>
<td>4 (20.0%)</td>
<td>16 (80.0%)</td>
<td>0.02</td>
</tr>
<tr>
<td>High ICP on CT</td>
<td>46 (25.4%)</td>
<td>5 (10.9%)</td>
<td>41 (89.1%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Seizures</td>
<td>18 (9.9%)</td>
<td>10 (15.1%)</td>
<td>8 (7.1%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Pupils unequal</td>
<td>46 (25.4%)</td>
<td>7 (10.6%)</td>
<td>39 (34.5%)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Pupils unreactive</td>
<td>72 (39.8%)</td>
<td>14 (21.2%)</td>
<td>58 (51.3%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sepsis/infection</td>
<td>48 (26.5%)</td>
<td>10 (15.1%)</td>
<td>38 (33.6%)</td>
<td>0.007</td>
</tr>
<tr>
<td>MODS</td>
<td>20 (11.0%)</td>
<td>0</td>
<td>20 (17.7%)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Neurosurgery</td>
<td>53 (29.3%)</td>
<td>11 (16.7%)</td>
<td>42 (37.2%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>10 (5.52%)</td>
<td>3 (4.54%)</td>
<td>7 (6.2%)</td>
<td>0.26</td>
</tr>
<tr>
<td>ETOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>64 (35.3%)</td>
<td>32 (48.5%)</td>
<td>32 (28.3%)</td>
<td>0.004</td>
</tr>
<tr>
<td>No</td>
<td>117 (64.6%)</td>
<td>34 (51.5%)</td>
<td>83 (73.4%)</td>
<td></td>
</tr>
<tr>
<td>Comorbidities present</td>
<td>57 (31.5%)</td>
<td>18 (27.3%)</td>
<td>39 (34.5%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Laboratory variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR (0.90-1.10)</td>
<td>1.05 (0.98-1.1)</td>
<td>1.02 (0.97-1.1)</td>
<td>1.08 (1-1.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>aPTT (24-36sec)</td>
<td>27.9 (25.6-30.7)</td>
<td>27.7 (25.2-28.7)</td>
<td>28.2 (25.9-34.5)</td>
<td>0.08</td>
</tr>
<tr>
<td>PLT (150-400.000/mm3)</td>
<td>219 (170-269)</td>
<td>240 (191-284)</td>
<td>199 (164-244)</td>
<td>0.001</td>
</tr>
<tr>
<td>HGB (130-180g/L)</td>
<td>134.5 (121.5-149)</td>
<td>143 (131-151)</td>
<td>130 (115-142)</td>
<td>0.0001</td>
</tr>
<tr>
<td>BD (0-4mmol/L)</td>
<td>4.15 (2-7)</td>
<td>4.2 (2.8-6.4)</td>
<td>4.2 (2-8)</td>
<td>0.79</td>
</tr>
<tr>
<td>Lactate (0.5-2.0mmol/L)</td>
<td>2.75 (1.9-4)</td>
<td>2.6 (1.5-3.6)</td>
<td>2.9 (1.9-4.2)</td>
<td>0.35</td>
</tr>
<tr>
<td>pH (7.35-7.45)</td>
<td>7.33 (7.28-7.4)</td>
<td>7.31 (7.28-7.4)</td>
<td>7.33 (7.27-7.4)</td>
<td>0.75</td>
</tr>
<tr>
<td>Glucose (4.0-8.0mmol/L)</td>
<td>7.8 (6.4-9.8)</td>
<td>7.15 (5.9-8.1)</td>
<td>8.4 (6.8-10.4)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Troponin (&lt;15ng/L)</td>
<td>7 (5-16)</td>
<td>5 (5-9)</td>
<td>8 (5-23)</td>
<td>0.02</td>
</tr>
<tr>
<td>ETOH level (&lt;2mmol/L)</td>
<td>43 (15.4-59.5)</td>
<td>43.95 (14.5-68)</td>
<td>42 (15.4-57.7)</td>
<td>0.47</td>
</tr>
<tr>
<td>K (3.5-5.0mmol/L)</td>
<td>3.7 (3.2-4)</td>
<td>3.8 (3.4-4.2)</td>
<td>3.6 (3.10-3.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Na (135-147mmol/L)</td>
<td>139 (136.5-141.5)</td>
<td>139 (137-141)</td>
<td>139 (136-142)</td>
<td>0.86</td>
</tr>
<tr>
<td>ECG abnormal *</td>
<td>69 (59.5%)</td>
<td>22 (33.3%)</td>
<td>47 (41.6%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Variable (median, IQR)</td>
<td>All trauma patients (n=181)</td>
<td>Favourable outcome (n=66)</td>
<td>Unfavourable outcome (n=113)</td>
<td>p</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------------------</td>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>----</td>
</tr>
<tr>
<td>Worse ECG**</td>
<td>4 (3.4%)</td>
<td>0</td>
<td>4 (3.53%)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

### Radiological variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>All trauma patients (n=181)</th>
<th>Favourable outcome (n=66)</th>
<th>Unfavourable outcome (n=113)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worse chest X-ray §</td>
<td>22 (16.7%)</td>
<td>7 (10.6%)</td>
<td>15 (13.3%)</td>
<td>0.95</td>
</tr>
<tr>
<td>Worse CT head †</td>
<td>56 (41.5%)</td>
<td>9 (13.6%)</td>
<td>47 (41.6%)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Data presented as numbers, percentage and median (interquartile ranges) when appropriate; p values generated by Wilcoxon rank sum or Fisher’s exact/Chi square tests when appropriate. Two-sided test performed and p<0.05 considered significant.


* ECG was performed in 116 patients.

** A subsequent ECG was performed in 116 patients.

§ A subsequent chest X-ray was performed in 132 patients.

† A subsequent head CT scan was performed in 135 patients.

### 4.1.4. Distribution of Patients According to Injury Severity Scores (GCS, AIS, Marshall) and GOSE.

Figure 7 illustrates the distribution of patients according to GCS score. Severe head injury (GCS 3-8) was diagnosed in 143 patients (79%), with 71 (49.6%) having a score of 3 and 72 (50.3%) distributed across scores 4 to 8. Within the severe head injury score range, 91 patients (63.6%) had scores 3 to 5 and 52 (36.4%) had 6 to 8. Only 38 patients (21%) were diagnosed with moderate TBI using GCS score. The median/IQR GCS across all patients was 5 (3-8), highlighting that the majority of patients had a very low score.
Figure 8 illustrates the distribution of patients according to the AIS score. The majority of subjects (141 patients, 77.9%) had a severe head injury (AIS 4-5) and only 40 patients (22.1%) had a non-severe injury (AIS 1-3). More than half of the subjects had an AIS 5 (92 patients, 55.7%). Patients with an AIS 4 were 49 (29.7%) and with an AIS 3 were 24 (14.5%).
Figure 9 illustrates the distribution of patients according to the Marshall classification. Only 29 patients (16%) had a normal initial head CT. Eighty three patients (45.8%) had an injury class II (brain edema with midline shift less than 5mm). In the more severe categories, with brain edema and compressed cisterns (class III) and a midline shift larger than 5mm (class IV), there were 15 (8.3%) and 31 (17.1%), respectively. In categories V (evacuated mass lesions) and VI (non-evacuated mass lesions), there were 22 patients (12.1%) and 1 patient (0.5%), respectively. Most subjects (129 patients, 71.3%) were distributed in the category with edema (category II) and in categories with edema and signs of intracranial hypertension (categories III and IV). Only 23 patients (12.7%) had mass lesions (categories V and VI).

Figure 9 – Distribution of patients according to Marshall score on admission CT scan.

Figure 10 illustrates the distribution of patients across the GOSE categories. Note that the in-hospital mortality was 28.7% (52 patients). At 6 months, 2 other patients died, resulting in 54 patients dead (30.2% mortality). There were no patients in vegetative state (GOSE 2) at discharge or at the 6-month follow-up. At discharge, patients with an unfavorable outcome were 145 (80.1%) and at 6 months they were 113 (63.1%). This reduction is represented by 30 patients (16.6%) from the severe disability category that
improved neurologically and moved to the categories of moderate disability and good recovery. The number of patients with a favorable outcome (moderate disability and good recovery, GOSE 5-8) at hospital discharge was 36 (19.9%) and increased to 66 (36.9%) at the 6-month follow up. Patients with a moderate disability at discharge were 14 (7.7%) and at 6 months they were 25 (14%). Patients with a good recovery at discharge were 22 (12.3%) and at 6 months they were 41 (22.9%).

Figure 10 – Distribution of patients according with GOSE categories at discharge and at 6 months post injury.

Legend: DC - discharge, GOSE - Glasgow outcome scale, GR - - lower good recovery, GR + - upper good recovery, MD - - lower moderate disability, MD + - upper moderate disability, SD - - lower severe disability, SD + - upper severe disability.

4.2. Primary Study Outcome

The GOSE was dichotomized into favorable (GOSE 5-8), and unfavorable (GOSE 1-4) outcomes (Figure 11), and both E and NE levels were judged on their ability to distinguish between these two groups. Sixty-six patients were classified as a having a “favorable” outcome at 6-month GOSE, and 113 patients were characterized as having an “unfavorable” outcome. The admission median levels of both E and NE were elevated in both groups of patients with favorable and unfavorable outcomes, compared to the levels of healthy volunteers. In patients with an unfavorable outcome, levels were substantially more elevated when compared to the levels in patients with a favorable outcome. Baseline
median levels of E/NE were 3,357.5pmol/L/17,210.7pmol/L in unfavorable outcome and 1,159.4pmol/L/6,276.6pmol/L in favorable outcome patients, respectively (p<0.0001 for both E and NE). Healthy volunteers had substantially lower median levels for both E and NE (E = 223.4pmol/L and NE = 2,000.4pmol/L).

After analysing the levels of catecholamines on admission in both groups of favorable and unfavorable outcomes, we conducted a logistic regression to estimate the unadjusted OR/CI of an unfavorable outcome at 6-month follow up (GOSE 1-4). In all models that used E and NE, we used the median admission level among healthy individuals for E (50pmol/L) and for NE (380pmol/L). The unadjusted OR/CI for E was 1.01 (CI 1.0-1.02) and a ROC/AUC = 0.73. This means that for every increase in 50 units of E, the odds of an unfavorable outcome increases by 1.1%. For NE, the unadjusted OR/CI was 1.03 (1.01-1.04) and a ROC/AUC = 0.76, meaning that for every increase in 380 units of NE, the odds of an unfavorable outcome increases by 3.0%.

Subsequently, to test for the independent association of catecholamine levels on admission with an unfavorable outcome at 6 months (GOSE 1-4) we estimated adjusted odds ratios and 95% CI from five multivariate logistic regression models (Table 9). We
included in these 5 models several predictors extracted from the univariate analysis which were associated with an unfavorable outcome and are known to be clinically relevant. The variables included were: severe TBI (GCS 3-8), severe head injury (AIS 4-5), age (in decades), hypotension (SBP<100mmHg), INR (>1.2) and the use of vasopressors. Model 1 included only the described variables without E or NE, and all patients (including patients who had missing values [n=16]) of admission catecholamines. The discriminatory power of predicting an unfavorable outcome in model 1 was high (ROC AUC 0.84). In model 2 we performed a sensitivity analysis, excluding the 16 patients without admission values of E and NE. The model estimated a ROC/AUC = 0.85. Model 3 included the 6 variables and E, which generated an OR = 1.01 (CI 1.0-1.01) and a ROC/AUC = 0.86. This means that for every increase in 50 pmol/L of E, the adjusted odds of an unfavorable outcome increases by 1%. The fourth model, including NE and the 6 variables, estimated an adjusted OR of 1.02 (CI 1.01-1.04) and an AUC of 0.8. This means that for every increase in 380 units of NE, the adjusted odds of an unfavorable outcome increases by 2.4%. Finally, in model 5 we included the 6 variables, E and NE what estimated an OR = 1.01 (CI 1.0-1.01) for E and an OR = 1.02 (CI 1.01-1.04) for NE. The model estimated a ROC/AUC = 0.87. We tested the calibration of the 5 models with the Hosmer-Lemeshow goodness-of-fit test, and a very good calibration was found, demonstrating that the models fit well the data.
Table 9 – Multivariate logistic regression models for predicting unfavorable outcome (GOSE 1-4) at 6 months.

<table>
<thead>
<tr>
<th>Models</th>
<th>Units (pmol/L)</th>
<th>Odds ratio</th>
<th>95% Wald confidence intervals</th>
<th>ROC AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 (variables only)</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>0.84</td>
</tr>
<tr>
<td>Model 2 (- missing values)</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>0.85</td>
</tr>
<tr>
<td>Model 3 (+ Epinephrine)</td>
<td>50</td>
<td>1.01</td>
<td>1.0 – 1.01</td>
<td>0.86</td>
</tr>
<tr>
<td>Model 4 (+ Norepinephrine)</td>
<td>380</td>
<td>1.02</td>
<td>1.01 – 1.04</td>
<td>0.88</td>
</tr>
<tr>
<td>Model 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+ Epinephrine)</td>
<td>50</td>
<td>1.01</td>
<td>1.0 – 1.01</td>
<td>0.87</td>
</tr>
<tr>
<td>(+ Norepinephrine)</td>
<td>380</td>
<td>1.02</td>
<td>1.0 – 1.04</td>
<td></td>
</tr>
</tbody>
</table>

Model 1 – Included age (in decades), hypotension (<100mmHg), severe TBI (GCS 3-8), severe head injury (AIS 4-5), coagulopathy (INR >1.2) and use of (*) vasopressors; model 2 – All 6 variables included in model 1 excluding patients with missing admission catecholamine levels; model 3 – Model 1 added median E admission level; model 4 – all 6 variables included in model 1 added to median NE admission level; model 5 – All 6 variables added E and NE.

(*) When a drip of vasopressors were used, they were administered after the determination of admission catecholamines

Hosmer and Lemeshow goodness-of-fit test demonstrated adequacy of the models (p=0.84)


We conducted another multivariate logistic regression analysis (Table 10) to facilitate interpretation, where we also reported the adjusted OR/CI in a model using median tertiles of E on admission (≤1,368.7pmol/L [used as reference], 1,368.7-4,019.9pmol/L, >4,019.9pmol/L) and NE on admission (≤6,540.5pmol/L [used as reference], 6,540.5-17,708.8pmol/L, >17,708.8pmol/L). For E, the third tertile (>4,019.9pmol/L) demonstrated an adjusted OR of 3.5 (CI 1.03-12.2). This means that
patients with admission median E level >4,019.9 pmol/L have an odds of an unfavorable outcome of 3.546 times higher compared to level in the first tertile. For NE, the third tertile (>17,708.8 pmol/L) demonstrated an adjusted OR of 4.61 (CI 1.12-18.8), what means that patients with an admission median NE level >17,708.8 pmol/L have an odds of an unfavorable outcome of 4.6 times higher compared to the level of the first tertile. We also conducted the Hosmer-Lemeshow goodness-of-fit test which showed a good calibration, demonstrating that the models fit well the data.

Table 10 – Multivariate logistic regression for tertiles of E and NE admission levels, including age (in decades), hypotension (<100 mmHg), severe TBI (GCS 3-8), severe head injury (AIS 4-5), coagulopathy (INR >1.2) and vasopressors (*) for predicting unfavorable outcome (GOSE 1-4) at 6 months.

<table>
<thead>
<tr>
<th>Tertiles (pmol/L)</th>
<th>Odds ratio</th>
<th>95% Wald confidence intervals</th>
<th>ROC AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine 2nd T (1,368.7 – 4,019.9)</td>
<td>1.77</td>
<td>0.6 – 4.9</td>
<td>0.86</td>
</tr>
<tr>
<td>Epinephrine 3rd T (&gt;4,019.9)</td>
<td>3.55</td>
<td>1.0 – 12.1</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine 2nd T (6,540.5 – 17,708.8)</td>
<td>1.43</td>
<td>0.5 – 3.8</td>
<td>0.87</td>
</tr>
<tr>
<td>Norepinephrine 3rd T (&gt;17,708.8)</td>
<td>4.61</td>
<td>1.1 – 18.8</td>
<td></td>
</tr>
</tbody>
</table>

(*) When vasopressors were used, they were administered after the determination of admission catecholamines. Hosmer and Lemeshow goodness-of-fit test demonstrated adequacy of the model (p=0.29)


4.3. Secondary Study Outcomes

4.3.1. Hospital/ICU Length of Stay, Ventilation and Mortality

Table 11 illustrates some of the secondary outcomes. Patients with unfavorable outcome had a median/IQR ICU stay of 7 (3-20) days, which was significantly longer compared to patients with a favorable outcome, who had a median/IQR of 3.5 (2-10) days, p=0.001. Length of hospital stay did not reach a statistical significance (p=0.08) between the two groups of favorable and unfavorable outcomes. Survivors had a longer hospital stay.
(median/IQR 18 [7-37] days) compared to patients who died (median/IQR 3 [1-8.2] days). As expected, patients with an unfavorable outcome required longer period of mechanical ventilation, with a median/IQR of 5 (2-16) days, compared to patients with a favorable outcome with 2 (1-6) days (p<0.0001). The median/IQR ICU free days and ventilator free days across all patients was 3 (0-14.2) and 1 (0-4), respectively.

Fifty two patients died in hospital (29%). Head injury was the cause of death in 47 patients (90.4%), followed by MODS in 4 patients (7.7%) and myocardial infarction in 1 patient (1.9%). Of the 47 patients who died of TBI, 18 (38.3%) had brain death declared and 8 (17%) had their life support removed after discussion with family. Post discharge, 2 additional patients died, totaling 54 patients (30.2%), both due to pneumonia and respiratory failure.

Table 11 – Association between secondary outcome variables and unfavorable outcome.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All trauma patients (n=181)</th>
<th>Favourable outcome (n=66)</th>
<th>Unfavourable outcome (n=113)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital stay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survivors</td>
<td>13 (4-30)</td>
<td>10.5 (4-22)</td>
<td>16 (3-37)</td>
<td>0.08</td>
</tr>
<tr>
<td>Dead</td>
<td>18 (7-37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (1-8.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU stay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 (2-17)</td>
<td>3.5 (2-10)</td>
<td>7 (3-20)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ventilation days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (1-12)</td>
<td>2 (1-6)</td>
<td>5 (2-16)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mortality in-hospital:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain injury</td>
<td>52 (28.7%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Brain death</td>
<td>47 (90.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WLS</td>
<td>18 (38.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MODS</td>
<td>8 (17%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>4 (7.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1 (19%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality at 6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>54 (30.2%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>2 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data presented as numbers, percentage and median (interquartile ranges) when appropriate; p values generated by Wilcoxon rank sum or Fisher’s exact/Chi square tests when appropriate. Two-sided test performed and p<0.05 considered significant. Legend: ICU – intensive care unit, IQR – interquartile range, MI – myocardial infarction, MODS – multiple organ dysfunction syndrome, WLS – withdrawal of life support.
4.3.2. Catecholamine Levels in Pooled TBI Patients

Figure 12 illustrates that the hospital admission median levels of catecholamines were significantly higher in TBI patients compared to healthy controls. Epinephrine levels were over 11 times higher (median/IQR=2,593.7pmol/L [986.9-5,753.6]) than healthy individuals (median/IQR=223.4pmol/L [145.2-299.7], p<0.0001), while NE levels were over 5 times higher (median/IQR=10,274.9pmol/L [5,256.5-28,959.8]) than their healthy counterparts (median/IQR=2,005.4pmol/L [477.7-2,561.5], p<0.0001). Although the circulating catecholamine levels for both E and NE demonstrated a decrescendo across all 4 times points, they remained significantly higher compared to the healthy control levels, across all-time points. As illustrated in Figure 12, NE median levels are substantially more elevated and the decrescendo is more pronounced across all 4 time points, differently from the E, whose initial median levels are smaller tending to a plateau in the last 2 time points (12 and 24 hours).

4.3.3. Catecholamine Levels According to Severity of Injury (GCS, AIS and Marshall)
Another step in the analysis was to study the association between the levels of catecholamines and the severity of head injury (GCS, AIS and Marshall Classification), and demonstrate whether there was an association between high levels with a worse injury.

As illustrated in Figure 13, in patients with a severe head injury (GCS3-8) or a moderate head injury (GCS 8-12), the median levels of both E and NE on hospital admission were significantly higher in those patients with severe injuries ($p<0.0001$ for NE and $p=0.001$ for E, respectively). Admission median/IQR levels for E were 3 times higher in severe TBI patients compared to moderate TBI (3,119.5pmol/L vs. 908.6pmol/L, $p=0.001$). For NE, the levels were almost 2 times higher (12,559.9pmol/L vs. 6,967.9pmol/L, $p<0.0001$) for severe and moderate TBI, respectively.

Figure 14 illustrates a similar association of admission median catecholamine levels when head AIS was used. Patients with a severe head injury (AIS 4-5) had baseline median/IQR levels for E also 3 times higher (3,141.8pmol/L vs. 988.5pmol/L, $p=0.005$) for severe injury compared to patients with non-severe injury (AIS 1-3). For NE, the levels were almost 2 times higher for severe head injury compared to non-severe head injury (13,092.4pmol/L vs. 6,850.9pmol/L, $p<0.0001$).
Patterns and severity of injury were also assessed according to the Marshall classification. The only patient in category VI was removed and considered in category V for analytical reasons. Differently from the previous analysis, we used the median levels of both E and NE in each time point to study the profile of both E and NE across all Marshall`s categories in each time point (Figure 15). Both E and NE had median levels increasing progressively from categories I to V/VI in all 4 time points, but more pronounced in the first 2 time points (first 6 hours post admission) with a median/IQR E 2,869.2pmol/L (2,063.5-4,084.1) on admission and 1,456.6pmol/L (878.8-3,026.7) at 6 hours, and a median/IQR NE 13,071.6pmol/L (9,618.1-19,925.4) on admission and 14,005.9pmol/L (5,041.1-24,350.7) at 6 hours. The median E level was smaller than the median NE level in each time point, across all categories of the Marshall classification. For E, the median/IQR was 878.80pmol/L (632.7-2,264.9) and for NE it was 9,976.2pmol.L (4,939.5-13,394.1), and both median values were statistically different when compared to the median values of healthy volunteers (p<0.0001).
As illustrated in Figure 15, median levels of both E and NE seem to be more elevated across all time points in the categories with tomographic signs of high ICP (Marshall III and IV). However, the levels are even more elevated in the first 2 time points (admission and 6 hours) when compared to the subsequent time points. Interestingly, the median NE level at the first time point (hospital admission) has a noticeable high peak associated with the category III. Smaller median peaks of NE at the 6-hour time point are also noticed in categories III and IV generating a small plateau. This denotes a visible association between high catecholamine levels and brain edema, especially when there are signs of high ICP (categories III and IV), compared to categories without high ICP (category II) or focal mass lesions (categories V and VI).

We conducted a subsequent step in the analysis of the profiles of both E and NE across all 4 time points in association with the Marshall Classification. Our hypothesis was that patients with scores III and IV had consistently higher levels of both E and NE vs. others over the 24-hour period (across all 4 time points). Categories III & IV (cerebral edema with signs of high ICP) and V & VI (focal mass lesions) were paired together for
the analyses. We compared categories III/IV with V/VI (cerebral edema and high ICP with focal mass lesions), III/IV with II (cerebral edema and high ICP with just cerebral edema), I with II (normal CT with just cerebral edema), and I with V/VI (normal CT with focal mass lesions). We conducted repeated measures with a mixed model framework using a log base-10 transformation to E and NE before fitting the model.

For E (Table 12), the comparisons between categories III/IV vs. V/VI did not show a statistical significance over the 4 time points, highlighting that the median E levels were not significantly different between those 2 categories. On admission, the comparisons of the median levels in categories II vs. I, V/VI vs. I and III/IV vs. II were all statistically significant ($p=0.0009$, $p<0.0001$, $p<0.0001$, respectively) (Table 12). At 6 hours, the comparisons between the median levels in categories V/VI vs. I and III/IV vs. II were also statistically significant ($p<0.0001$ for both comparisons). Finally, at 12 hours the statistically significant comparisons were in categories V/VI vs. I ($p<0.0001$) and in III/IV vs. II ($p=0.0009$). In conclusion, we found that patients with edema and signs of high ICP have higher levels of E consistently over the 4 time points.

### Table 12 – Statistically significant comparisons of the log base-10 scale of median E levels according with each time point in the Marshall Classification.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Result</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>I &lt; II</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>I &lt; V/VI</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>II &lt; III/IV</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 hours</td>
<td>I &lt; V/VI</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>II &lt; III/IV</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>12 hours</td>
<td>I &lt; V/VI</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>II &lt; III/IV</td>
<td>0.0009</td>
</tr>
<tr>
<td>24 hours (*)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mixed model framework with application of a log base-10 transformation to E and NE, including Marshall categories and time as factors, as well as their interaction. Bonferroni
Mixed model framework with application of a log base-10 transformation to E and NE, including Marshall categories and time as factors, as well as their interaction. Bonferroni approach was used to adjust the significance level for multiple comparisons (for each catecholamine, a comparison was considered statistically significant if $p < 0.05/16 = 0.003125$).

For NE (Table 13), the comparisons between categories III/IV vs. V/VI did not show a statistical significance in time points 6, 12, 24 hours. However, on admission there was a marginal significance ($p=0.03$). In all 4 time points, the comparisons between categories III/IV vs. II were statistically significant ($p<0.0001$ for all comparisons). The comparisons between categories V/VI vs. I were statistically significant in the 6, 12 and 24-hour time points ($p<0.0001$, $p=0.0002$ and $p=0.0006$, respectively), but not significant on admission. Corroborating with the findings for E, the levels of NE in patients with signs of edema and high ICP were consistently more elevated compared to the levels in patients without signs of high ICP.

Table 13 – Statistically significant comparisons of the log base-10 scale of median NE levels according with each time point in the Marshall Classification.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Result</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>II &lt; III/IV</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 hours</td>
<td>I &lt; V/VI</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>II &lt; III/IV</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>12 hours</td>
<td>I &lt; V/VI</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>II &lt; III/IV</td>
<td>0.0010</td>
</tr>
<tr>
<td>24 hours</td>
<td>I &lt; V/VI</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>II &lt; III/IV</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>


(*) – At the 24-hour time point no comparison was statistically significant.
After demonstrating that the levels of both E and NE progressively increase according to the severity of TBI (associating with GCS, AIS and Marshall), we studied whether there was an association between catecholamine levels and functional outcome (including mortality), using the GOSE at 6 months. Figure 16 illustrates the baseline median levels on admission of both E and NE according to the categories of GOSE at 6 months. Notably, median plasma levels increase progressively from severe disability to death (categories of unfavorable outcome, GOSE 1-4). There were no patients in vegetative state. We found a statistical difference for both E and NE in at least one category ($p<0.0001$) and a statistical difference when comparing severe disability with death ($p<0.0001$) for both E and NE. Median levels of both E and NE reach a plateau within the categories of moderate disability and good recovery, with a mild NE elevation in moderate disability + (MD+) category and a mild E elevation in good recovery - (GR-) category.

**Figure 16 – Profile of median E and NE admission levels across the GOSE categories.**

Data presented as median (interquartile ranges); $p$ values generated by Kruskal-Wallis test. Two-sided test performed and $p<0.05$ considered significant. $p<0.0001$ for both E and NE.

E – epinephrine, GOSE – Glasgow outcome scale, GR – - lower good recovery, GR + - upper good recovery, MD – - lower moderate disability, MD + - upper moderate disability, NE – norepinephrine, SD – - lower severe disability, SD + - upper severe disability. No patients in were classified in vegetative state (category 2).
In Figures 17 and 18 we plotted the median values in all 4 time points (admission, 6, 12 and 24 hours) to demonstrate the profile of both E and NE within the 24 hours post injury. The E profile (Figure 17) shows an important difference between the median levels on admission ($p<0.0001$) and at 6 hours ($p=0.001$), with levels, much higher in patients with an unfavorable outcome. The subsequent time points did not show a statistical difference. Conversely, the NE profile (Figure 18) demonstrates a much higher level across all time points with a statistical significance ($p<0.0001$) in each admission, 6, 12 and 24 h time points post admission.
4.3.4. Catecholamine Levels According to Death, MODS, Sepsis and ICP

We analysed plasma levels of both E and NE according to death, survival, presence or absence of sepsis, MODS and high ICP and checked whether there was a statistical difference in each group. As stated previously, diagnosis of high ICP was conducted in the ED by the trauma team leader and the neurosurgeon according to clinical (unilateral or bilateral pupils non-reactivity or Cushing response, for example) and/or CT scan findings (Marshall Classification). In the ICU the diagnosis was conducted by the intensive care attending, according to clinical signs, ICP monitoring, CT scan or MRI. Sepsis and MODS were confirmed by the ICU physician during ICU stay according to current guidelines mentioned previously. ICP was measured in 42 patients and 20 (47.6%) had high pressures. Forty eight patients (26.5%) were diagnosed with sepsis and 4 patients (11%) diagnosed with MODS.

In Figure 19, patients who had a diagnosis of elevated ICP, had higher median plasma levels of both E and NE with statistical significance (median E level = 3,956.6pmol/L vs. 2,317pmol/L [p=0.04]) and median NE level = 33,837.7pmol/L vs.
10,084.5pmol/L, \( p = 0.02 \). Patients with evidence of MODS had higher E levels when compared to patients without MODS (median E level = 4,027.1pmol/L vs. 2,115.3pmol/L, \( p = 0.03 \)). For NE, although patients with MODS had a higher median level, statistical significance was not achieved (median NE level = 17,352.9pmol/L vs. 10,003.3pmol/L \([p = 0.19]\)). For patients who died, the levels of both E and NE were markedly increased when compared to survivors (median E level = 6997.3pmol/L vs. 1,769.4pmol/L, \( p < 0.0001 \)) and median NE level = 22,269.7pmol/L vs. 7,798.5pmol/L, \( p < 0.0001 \)). A statistical significance was not found for patients with and without sepsis (\( p = 0.17 \) for E and \( p = 0.15 \) for NE).

Figure 19 – Median admission E and NE levels according to ICP, sepsis, MODS and death.

Data presented as median (interquartile ranges); \( p \) values generated by Wilcoxon two-sample Test. Two-sided test performed and \( p < 0.05 \) considered significant. ICP – \( p = 0.04 \) for E and \( p = 0.02 \) for NE; Sepsis – \( p = 0.17 \) for E and \( p = 0.15 \) for NE; MODS – \( p = 0.03 \) for E and \( p = 0.19 \) for NE; Death – \( p < 0.0001 \) for both E and NE.

CHAPTER 5 – Discussion and Future Directions

5.1. Discussion

5.1.1. Summary of Key Findings

The growing interest in modulating the adrenergic response initiated post brain injury, with the use of adrenergic medications, has increased the body of literature on this subject recently. Several studies have demonstrated that adrenergic agonists and antagonists administered to animals and patients with brain injury may blunt catecholamine release and the inflammatory response to injury. It appears that modulation of catecholamine release and the inflammatory response to brain injury improve cerebral edema, ischemia, and functional neurological outcome. Several studies in animals and in humans [70-71, 147-156] have addressed the administration of different types of β-blockers and alpha agonists or alpha antagonists and have demonstrated that they may offer a protective effect. Furthermore, several studies have demonstrated that high levels of catecholamines released after brain injury may be associated with a poor neurological outcome [39, 45] highlighting that catecholamines may take part in the pathophysiology of brain injury. However, molecular and cellular processes involved in this pathophysiology are still to be elucidated.

The COMA-TBI study was designed to replicate and extend the findings of those previous studies that demonstrated an association between high levels of circulating plasma catecholamines with severity of brain injury, and poor neurological outcome. No robust conclusions from these previous studies can be drawn proving that the association is independent due to lack of a better prospective design, power and adjustment for confounders, among other methodological problems. The primary objective of the COMA-TBI study was to determine whether patients with isolated moderate to severe TBI have high circulating plasma levels on admission, compared to basal catecholamines levels of healthy volunteers, and that the levels are independently associated with neurological outcome as determined by the GOSE scale at 6 months post injury. The approach was to conduct a powered prospective observational blinded cohort study examining the levels and their independent association with neurological outcome. We looked for factors that
were associated with an unfavorable outcome in the univariate analysis, and conducted a multivariate logistic regression model, generating adjusted ORs and CIs for an unfavorable outcome. Other secondary exploratory objectives of the COMA-TBI study were to: (1) Examine ICU and hospital length of stays in both groups of patients with unfavorable and favorable outcomes; (2) Examine ventilation days in both groups; (3) Estimate in-hospital and 6 months post discharge mortality; (4) Examine the profile of median circulating plasma catecholamine levels within 24 hours post admission (across 4 time points [admission, 6, 12 and 24 hours]), in pooled patients, and in both groups; (5) Describe the association of median catecholamine levels with severity of injury, using several scores, such as GCS, AIS, Marshall Classification, and the functional outcome scale GOSE; and lastly (6) Describe the levels in patients who had or did not have MODS, sepsis or high ICP, and in patients who died or survived.

Consistent with the hypothesis, patients with isolated moderate to severe TBI demonstrated a significant elevation of both E and NE on admission, compared to the basal levels in healthy volunteers. The COMA-TBI study is the first powered observational study to demonstrate the natural history of both E and NE release in the peripheral circulation early post moderate and severe isolated TBI. Furthermore, we found an independent association between admission levels and an unfavorable outcome at 6 months, after adjusting for age, severity of TBI (GCS, AIS), hypotension, use of vasopressors, and coagulopathy. Patients exhibit high levels early post injury, have a stepwise pattern, and a decrescendo profile over the first 24 hours post trauma. Patients who have worse indices of severity of injury (GCS, AIS and Marshall Classification), and worse neurological recovery by GOSE, have higher levels of both E and NE. Patients with severe head injury classified by GCS and AIS had higher median levels of both E and NE which were statistically different when compared with the median levels in patients with moderate TBI and non-severe head injury, respectively. As for the association with the categories of Marshall Classification, it became evident that the levels raise progressively across categories I to VI. There is also a substantial elevation of both E and NE across all 4 time points post trauma in the categories associated with brain edema and signs of intracranial hypertension (Marshall III and IV). As for GOSE, the levels progressively decrease across categories 1 to 8, with levels substantially increased in patients with unfavorable outcome compared to
patients with a favorable outcome. Finally, the association of median plasma catecholamine levels with death, MODS and high ICP is statistically significant compared to patients without those complications. A statistically significant median level was not found when analysing patients with and without sepsis.

5.1.2. Primary Study Outcome and Previous Data

Our study indicates that E and NE, two of the major components of the sympathoadrenomedullary axis, are biomarkers of the stress response to brain trauma and may be part of its pathophysiology. Within 24 hours of brain injury, the admission median plasma levels of E and NE increased eleven-fold and five-fold respectively, compared to the basal levels of both E and NE in healthy individuals. Since we enrolled patients directly from the scene and our median time to the first assessment and catecholamine determination (time to ED) was 60 minutes, we were able to measure E and NE levels early post injury. We have shown that the admission levels are the highest levels within the 24 hours post injury and that these levels are independently associated with an unfavorable outcome as measured by GOSE at 6 months, after adjusting for the main factors associated with unfavorable outcome (age, hypotension, GCS score, AIS score, coagulopathy and use of vasopressors).

The sympathoadrenomedullary activation in multisystem trauma and in cerebral injury, whether traumatic or nontraumatic, is well known [37, 38, 359, 378-381]. During the first week after TBI, about 25 to 30% of patients with severe brain injury exhibit periodic symptoms of the hyperadrenergic state, such as tachypnea, tachycardia, systolic hypertension, and hyperpyrexia [37]. A hyperdynamic state is present and characterized by an increased cardiac output, cardiac work, pulmonary shunting, and oxygen delivery and consumption [48]. The association between these responses and the severity of brain injury and outcome is not entirely explained. Furthermore, the harm that this exacerbated sympathetic response cause to the already injured brain is not completely understood. The non-neurological consequences of this exaggerated catecholamine surge, such as the cardiovascular dysfunction and the neurogenic pulmonary edema are substantially better described but still underdiagnosed and undertreated. However, the pathophysiology of the tissue damage to the already injured brain needs a more robust comprehension.
Earlier studies have reported that the degree of sympathoadrenomedullary activation correlates with the extent of brain injury and ICP [38, 358]. Although elevated NE levels may correlate with severity of brain injury [358] and elevated circulating catecholamine levels may underlie such cardiovascular dysfunction [378], as well as mediate the myocardial infarction and necrosis observed after head trauma [378, 382], there is not a proved association between prognosis and presence of autonomic abnormalities [379]. Animal and human studies demonstrate that a local accumulation of catecholamines causes vasoconstriction in the microcirculation, with low perfusion leading to ischemia, further edema, high intracranial pressure and worse outcomes. Damage of the BBB following brain injury was demonstrated by Schoultz [66] who found that trauma to the spinal cord possibly leads to the accumulation of catecholamines in the CNS from the circulation affecting local microcirculation and cellular function [67]. It appears that persistent elevated levels of NE promotes a leaky barrier and may worsen cerebral edema and ischemia [68]. Furthermore, with the injury to the BBB, circulating catecholamines produced peripherally may reach the already injured brain and promote even more damage.

Studies with small numbers of patients and without adjustment for confounders have demonstrated the association of the SNS activation and neurological outcome. Hamil [39] reported a better functional recovery after TBI in patients with lower circulating catecholamine levels. The study concluded that the elevated levels appear to reflect the extent of brain injury and may predict the recovery. In our study, patients with the highest levels had the worst neurological outcomes (GOSE 1-4), demonstrating a dose-effect association. We also identified an association of high levels with other severity scores, such as GCS, AIS and the Marshall Classification, what corroborates with the findings in the current literature that associate levels with the extent of brain injury. We could not identify a clear cut-off value of E or NE with high sensitivity/specificity that would predict an unfavorable outcome. Conversely, we demonstrated that the highest the E/NE levels, the highest is the adjusted odds of an unfavorable outcome (dose-effect response). In the current literature, the only study that found a cut-off value was conducted by Woolf [45] who evaluated catecholamine levels within 48 hours after TBI to determine whether their levels would provide reliable prognostic markers of outcome assessed by the GCS score. Those patients with NE less than 900 pg/mL improved GCS while those with values
greater than 900 pg/mL remained with low GCS scores or died. However, this study was not powered and included patients with multisystem trauma with or without brain injury and did not include patients with isolated TBI, and the catecholamine measurement was not early post trauma, as performed in our study.

Although current literature lacks robustness to definitively conclude, it is suggested that catecholamines may participate in the pathophysiology of brain injury. The normal and fundamental stress response post trauma may be exacerbated in some cases, leading to deleterious consequences. Due to the association with severity of injury and the outcome, plasma catecholamine levels may help to predict clinical outcome and may represent useful prognostic markers in patients with TBI. Catecholamine levels on admission may predict patients who will have a worse outcome or die and may be added to the current panel of biomarkers of brain injury severity. The feasibility, predictive value in relation to other indices, and the costs of measuring E and NE on admission are still to be addressed, though.

Outcome is influenced by many other factors in patients with severe TBI, such as age, GCS, pupillary response to light, abnormal computed tomography, and evoked potentials [177, 178, 251, 379, 383]. Our data indicate that high catecholamine levels are important biochemical variables that may be added to the existing panel of predictors of unfavorable outcome in severe TBI. In the future, it will be important to compare the predictive value of catecholamines with the traditional predictors, such as pupillary responses and brainstem reflexes. In addition, it will be important to investigate whether the exaggerated catecholaminergic storm is directly responsible for the cardiovascular collapse that appears to be a terminal event in many patients with severe TBI. Regardless of the final answers to these questions, it appears that sympathetic activation as reflected by plasma levels of E and NE is associated with the level of coma and may predict outcome.

Another important question that needs to be addressed is whether the augmented catecholamine response is maladaptive or is simply a reflection of injury severity. Currently the body of evidence lacks studies with a good methodological quality addressing these issues. However, the existing data suggests that, in addition to reflecting the severity of injury, the enhanced sympathetic response is deleterious. For example,
studies show that a hypermetabolic and hyperdynamic state is apparent early in trauma patients [384, 385]. High plasma glucose and lactate levels are directly associated with the concentrations of circulating catecholamines [386]. Increased cardiac output and cardiac work, hypertension, tachycardia, decreased or normal systemic and pulmonary vascular resistance, and increased oxygen delivery have been found after injury to the brain [387]. Finally, subendocardial infarction [378] and arrhythmias [388] are thought to be directly caused by excessive catecholamine discharge.

In brief, our results show that the exaggerated catecholamine surge that occurs early post moderate and severe isolated brain injury is associated with the severity of injury, mostly with the severity of TBI, in a dose response pattern. Patients with a worse initial severity of injury have higher levels and the levels decrease as the severity of injury decreases. We demonstrated that high levels are independently associated with neurological outcome at 6 months. Our findings are corroborated by previous animal and human studies. However, these previous studies have methodological limitations, such as small sample sizes and lack of adjustment for confounders. Catecholamine storm has systemic effects and is possibly harmful to cardiovascular, respiratory, metabolic, immune and coagulation systems, what may contribute to a worse outcome in patients with TBI. It is also demonstrated that the release of catecholamines in the injured brain activates local and systemic inflammatory pathways, with further cell damage, SIRS, infection and MODS. In general, animal and human studies report that the initial exaggerated catecholamine response in the brain may increase vasoconstriction of the microcirculation, leading to more edema, ischemia, higher intracranial pressures and worse outcomes. It is proposed that there is damage to the BBB, with further increase of catecholamine levels in the brain due to the circulating catecholamines in the blood, what promotes a vicious cycle of ongoing cell damage. However, a more robust knowledge of the pathophysiology related to this processes is still to be demonstrated. Finally, another critical question that needs to be addressed is whether the augmented catecholamine response is maladaptive or is simply a reflection of injury severity. Currently, the evidence demonstrates that the catecholamine surge related to moderate and severe TBI is not only a stress response geared towards re-establishing homeostasis, but is a harmful response that has local and systemic deleterious effects.
5.1.3. Secondary Outcomes and Previous Data

The profile of catecholamine response across all 4 time points in pooled patients demonstrated a stepwise pattern for both E and NE, with admission levels substantially elevated for both catecholamines and a subsequent decrescendo across the 6, 12 and 24h time points, especially for NE. On admission, E levels increased five-fold and NE levels increased eleven-fold, compared to the basal levels of healthy volunteers. Hamil [39] has reported in 33 patients with severe TBI that for both E and NE the levels increased four- to five-fold on admission. The stepwise pattern was not identified in other studies found in the literature. However, these studies did not measure catecholamine levels sequentially in the first hours post admission to the ED as performed in our study, and most patients in those studies had multisystem trauma, including or not TBI. Clifton [358] measured catecholamines on admission and daily at 8am and 8pm, for 1 week. The levels remained elevated over the entire week, without a specific trend, but the strongest association between the levels with neurological outcome occurred within the first 48 hours post admission. According to our findings, a substantial catecholamine surge is initiated early post injury and this is crucial information to be considered when studies addressing the modulation of the sympathetic activity post TBI are conducted. We believe that if the administration of adrenergic antagonists/agonists is conducted for modulation of the hyperadrenergic state, it should begin soon after injury.

Corroborating with our findings, Hamil [39] demonstrated an association between low GCS scores with the highest levels of catecholamines. High AIS scores, as demonstrated in our data, had a substantial association with high levels of both E and NE. Patients with a non-severe head injury, had lower E and NE levels compared to patients with a severe head injury as classified per AIS. We did not find information in the current literature addressing the association with AIS specifically. However, in the study by Clifton [358] a strong association between a high ISS score (that uses AIS for its determination) with higher levels of both E and NE was found.

Catecholamines are released by the sympathetic tissue in the brain. Sympathetic neurons secrete only NE, and not E, and NE is produced by noradrenergic neurons in the locus coeruleus in the brain stem. It appears that NE is produced in larger amounts
compared to E, which has been demonstrated by our results. However, even with smaller levels, E is consistently elevated and is also associated with the severity of brain tissue damage. Abercrombie [24] reported that NE levels measured in the microdialysate from the hippocampus remain normal until >50% of the neurons are destroyed. When simulation of excitotoxicity was induced, even when <50% of the cells were depleted, higher release of NE was achieved. This implicates the locus coeruleus as the primary source for NE elevation after TBI. Currently it is unclear whether a specific type of lesion influences the release of catecholamines within the CNS, such as DAI, that is associated with the autonomic storm, or whether certain critical diencephalic and/or brainstem lesions are present. We did not find the association between the location of cerebral injury with catecholamine levels. However, we were able to demonstrate an association between the profile response of both E and NE with the Marshall Classification. We noted that the highest levels of catecholamines were associated with increased edema and signs of high ICP (Marshall categories III and IV). Norepinephrine was substantially more elevated in the first 2 time points (admission and 6 hours) than in the 2 other time points. Epinephrine followed the same pattern. However, the E median levels were less elevated across all 4 time points. Furthermore, we found that patients who had their ICP measured by an ICP monitor and had at least one episode of ICP ≥25mmHg in the first 24 hours, had higher median E and NE levels. Our data emphasizes that there is an association between cerebral edema and intracranial hypertension with high levels of both E and NE within 24 hours post hospital admission. We believe that a vicious cycle may happen, involving: (1) Brain injury and damage to the BBB; (2) Production and release of central and peripheral catecholamines; (3) Activation of local and systemic inflammatory responses; (4) Local vasoconstriction of the microcirculation; (5) Subsequent necrosis; (6) Further edema; (7) Higher intracranial pressures, with further tissue damage, leading to worse clinical outcomes.

A stepwise pattern was observed when analysing the response profile of both E and NE according to the GOSE at 6 months. Patients in the categories of unfavorable outcome (GOSE 1-4) had higher levels with a decrescendo pattern and the highest levels in patients who died demonstrating and dose response pattern. The levels in patients with a favorable outcome were less elevated and there was a trend to a plateau. Clifton [358] described the
response profile of catecholamines in patients with multisystem trauma with brain injury, but not in patients with isolated brain injury. In his study, the levels were substantially higher in patients who died and showed a decrescendo pattern, but less pronounced than what was found in our study. We believe that this difference is due to the fact that we included isolated brain injury patients and there appears to be a stronger association between the levels of both E and NE in this population compared to patients with multisystem trauma without TBI.

In the analysis of the response profile of E and NE median levels according to the outcome (favorable or unfavorable), our data shows that for NE, the median plasma levels are significantly different across all time points. Patients with an unfavorable outcome have higher median NE levels in all measures, demonstrating a good ability to discriminate between both groups within the first 24 hours post injury. The response profile for E is less discriminatory as the median plasma levels are statistically different only in the first 2 time points (admission and 6 hours). Currently there is no data in the literature estimating the differences in the profiles for both E and NE according to the outcome as measured by GOSE. In general the ability of NE to discriminate between the 2 groups seems to more robust, compared to E, as demonstrated in our study.

We identified that levels of both E and NE in patients with MODS were substantially higher compared to patients who did not develop this complication. The current literature on inflammation demonstrates that the exacerbated catecholamine release triggers the local inflammatory response and SIRS [124]. SIRS causes the disruption or dysfunction of one or more organ systems [124]. For example, in patients with severe TBI, the presence of at least one organ system dysfunction occurred in 89% of subjects [125]. SIRS results from the release of inflammatory mediators that cause early and delayed systemic effects, including subsequent complement deficit and coagulopathy and can detrimentally self-propagate [124]. SIRS causes tissue damage, further inflammation and more damage, leaving the body in a vicious cycle of hyperinflammation. Therefore, important inflammatory mediators such as IL-1 beta, IL-6 and TNF alpha, are targeted in a compensatory anti-inflammatory response syndrome, in an attempt to control the development of SIRS. However, the activation of this anti-inflammatory response syndrome often leads to immunosuppression and subsequent sepsis and MODS [124].
In our data, we noticed higher levels of both E and NE in patients with sepsis, but it did not reach statistical significance, possibly due to the small number of patients that developed the complication. Studies [124, 126-129] have shown that after brain injury, the catecholamine surge can render the immune system anergic, and lead to an inability to fight sepsis, possibly culminating in MODS. As mentioned previously, the exaggerated release of catecholamines can alter the production of multiple inflammatory mediators in peripheral blood immune cells and in various organs (spleen, pancreas, lungs and the diaphragm). These immunomodulatory effects have increasingly received attention, especially due to the potential for pharmacological intervention. For example, patients with sympathetic storm post TBI have higher levels of IL-10 and severely depressed monocytic HLA-DR expression, 62% of whom develop severe infections [128]. Another example is shown in a study using isolation of splenic macrophages of a polymicrobial sepsis model, in which further adrenergic stimulation inhibits TNF and IL-6 production by the macrophages [129].

In brief, the COMA-TBI study demonstrates that both E and NE are substantially elevated early post isolated brain injury, and have a stepwise pattern with a progressive decrescendo within the first 24 hours post hospital admission, demonstrating a dose-response pattern. This pattern corroborates partially with the current literature, but previous studies have methodological limitations. Elevated median plasma catecholamine levels are consistently associated with indices of severity of injury, such as GCS and AIS, which also is documented in the current literature. Brain edema with signs of intracranial hypertension (Marshall Categories III and IV) is the pattern of injury that is markedly associated with the highest levels of both E and NE. We believe that the high circulating median catecholamine levels found specially in this pattern of injury, is part of a vicious cycle of brain injury, production and release of central and peripheral catecholamines, activation of local and systemic inflammatory responses, local vasoconstriction of the microcirculation and subsequent necrosis, further edema, and higher intracranial pressures, with further tissue damage, leading to worse clinical outcomes. Norepinephrine is the amine that has the best power to discriminate between groups of unfavorable and favorable outcome. The profiles of median NE levels within the first 24 hours in patients with unfavorable and favorable outcomes are consistently different, showing a discriminatory power of NE in
distinguishing both groups. High median levels of both E and NE seem to be associated with SIRS, sepsis and MODS. We believe that large production and release of catecholamines may lead to these complications due to the temporal relationship. SIRS, sepsis and MODS occur later post brain injury and are associated with the initial (on admission) high levels of both E and NE. High levels of catecholamines trigger local inflammation, with subsequent SIRS/MODS and the anti-inflammatory syndrome CARS. CARS leads to immunodepression and subsequent sepsis, with worse outcomes.

5.2. Future Directions

The COMA-TBI study enabled us to identify several areas that deserve further investigations:

5.2.1. Pathophysiology

(i) The pathophysiology of the hyperadrenergic storm in the CNS of patients with brain injury is not completely understood. We were able to demonstrate that there is an independent association between the production and release of catecholamines in the peripheral blood stream, with the severity of brain injury and with an unfavorable neurological outcome 6 months post injury. These elevated levels of both E and NE may be harmful to the brain itself, causing further tissue damage due to vasoconstriction, ischemia, necrosis and subsequent further edema leading to an increased ICP, and initiation/perpetuation of a local inflammatory process what contributes to more injury. The stress response post traumatic brain injury geared towards re-establishing homeostasis seems to be exaggerated in patients with a higher severity of TBI. Large amounts of E and NE seem to be deleterious systemically and have the potential to initiate non-neurological effects such as the activation of systemic inflammatory/anti-inflammatory systems, and effects on the neuroendocrine, immune, coagulation and metabolic systems. A fuller understanding of the pathophysiological processes at molecular and cellular level will undoubtedly help in developing strategies for early diagnosis and therapy, contributing to a decrease in morbidity and in mortality of patients with brain injury. A fundamental aspect that still needs to be elucidated is whether the catecholamine response is maladaptive or is simply a consequence of the severity of injury. In addition, it will be important to
investigate whether, in fact, the sympathetic storm is directly responsible for the peripheral cardiovascular and pulmonary collapses that appear to be a terminal event in many patients with severe brain injury.

5.2.2. Tools for Prediction of Outcome

(i) Age is one of the most important predictors of outcome in brain injury as described previously. However, some questions still need to be addressed. Future studies should use age as a continuous variable in their designs. Furthermore, analysis of the effects of confounding variables such as pre-existing medical conditions should be considered. Additionally, the biology of the aging brain and its vulnerability to injury still warrants further detailed investigation.

(ii) Although the impact on outcome of hypotension as a secondary brain insult is well established, future investigations must prospectively collect accurate and frequent physiologic data on the occurrence of hypotension as well as the actual blood pressure values throughout resuscitation. Critical physiologic threshold values and the efficacy of various therapeutic manipulations in decreasing secondary brain insults and improving outcome must be derived from such data after controlling for factor-factor interactions. Investigation into the prevention or elimination of secondary brain insult might well represent the area of early brain injury treatment with the greatest potential for improving outcome.

(iii) Currently, the measurement of pupil size and reactivity has many limitations. Future work should consider studying the association between those 2 parameters (appropriately measured) to outcome, as well as the duration of pupillary dilation or fixation. A standardized method of measuring pupil size and reactivity to light would decrease inter-observer variability. Additionally, a definition of the size of dilated pupils is lacking. Causative agents should be considered when assessing pupils in trauma patients, such as orbital trauma, hypotension, and repeat evaluation after evacuation of intracranial hematomas.

(iv) When considering the use of the GCS score for prognosis, the two most important problems are the reliability of the initial measurement and its lack of precision
for prediction of a good outcome if the initial GCS score is low. Future work should address are the optimal time after injury for determining the initial GCS, when to assess the GCS score for those who have received paralytic or sedative medication and the reliability of the prehospital GCS score acquisition.

(v) A strong correlation exists between the different types of intracranial lesions post TBI and outcome. However, there are still questions to be assessed. Future work should address the complicated issue of how optimally to combine CT characteristics for prognostic purposes and how to improve on currently used CT classifications to predict outcome more accurately. There is a need for improved definition for intraparenchymal lesions. A more detailed recording of surgical indications is required in future studies. Standardized reporting of indications for surgery (clinical deterioration, CT, results of ICP monitoring), time to operation and involving lesions are a prerequisite for comparison of different series and determination of prognostic value. Also reasons for not operating, such as poor prognosis or local “conservative” policy, should be explicitly stated.

(vi) The serum biomarkers and interstitial neurometabolites that represent cerebral tissue damage are associated with severity of brain injury and/or with neurological outcome. Further work for defining their specific role in predicting outcome in TBI is still warranted, though.

(vii) The strong and independent association between the levels of both E and NE with severity of brain injury and with neurological outcome may add important information to other pre-existing tools during the primary assessment/management of patients with moderate to severe TBI, and facilitate diagnosis, early and late management, prediction of outcome and family counselling. In the future, it will be important to compare the predictive value of the activation of the sympatoadrenomedullary axis with more traditional indices of brain injury, such as pupillary responses and brainstem reflexes.

5.2.3. Modulation of the Hyperadrenergic State

Modulation of the hyperadrenergic state is a new field in the management of brain injury. However, the evidence is growing substantially in the past years. Questions still to be solved in the near future are:
(i) Relevant pharmacological properties with regard to penetrance of specific β-blockers into the brain should be taken into account. Caution is advised in extrapolating pharmacokinetic properties in this regard from healthy subjects to patients with acute brain injury because permeability of the BBB is probably much higher in the latter.

(ii) As the evidence reports a potential benefit in reducing mortality with the use of β-blockers in patients with brain injury, it may be justifiable to proceed to phase II studies in humans.

(iii) Several important choices have to be made reflecting the uncertainties that still exist on the clinical significance and pathophysiology of sympathetic overstimulation. For example: 1) the type of β-blocker to be tested (selective versus nonselective, penetration of blood-brain barrier or not); 2) the precise selection criteria for study participation based on the presence or absence of risk factors for sympathetically mediated complications; 3) the primary outcome to be chosen (stress cardiomyopathy incidence, functional outcome, mortality); 4) dosing issues; 5) initiation and duration of treatment; 6) fixed dose treatment or titration to, for instance, a physiological variable such as heart rate, or even assessment of individual sympathetic/parasympathetic tone.

5.3. Limitations

Our study has several limitations as follows:

5.3.1. Risk of Information Biases

(i) Sixteen patients had no catecholamine measure available on admission due to technical problems during specimen acquisition. This represents 8.83% of the total admission samples obtained (181 samples). In further analysis of the 16 patients, we did not find a trend or pattern of association with patient`s characteristics. Patients who had their samples lost had their characteristics distributed randomly across all patients. We don’t expect introduction of bias due to this random loss. Imputation of data was not required due to the small number of admission samples lost.

(ii) Misclassification of the GCS on admission could possibly have happened in some cases. We did not retrieve information about when GCS was captured in patients who
arrived intubated, sedated and paralysed on admission. Misclassification of GCS may have caused an overestimation of coma and consequently misclassification bias. A factor that possibly minimized this problem was that the trauma team leader always tries to retrieve information from EMS personnel about the GCS before the patient’s sedation, intubation and paralysis. The research personnel were oriented to register GCS before sedation and paralysis (in case the patient was intubated at the scene or in route), or during the primary assessment in the trauma room, in case the patient was not sedated, intubated and paralysed pre admission.

(iii) Troponin and ECG were performed in a small number of patients. Both tests are not considered standard of care and are requested depending on the clinical indication. Although both tests were in our study protocol and were expected to be performed in all patients, the concerning about delaying care was the reason why they were performed only when necessary. Further analysis of both troponin and ECG could not be conducted due to the small number of measures.

5.3.2. Risk of Confounding

(i) We did not account for the administration of drugs that could have affected levels of catecholamines, before the acquisition of the admission blood sample, such as E or NE boluses for example. Other drugs such as Ketamine, Ephedrine and Cocaine are known to release catecholamines in the circulation. These drugs may act as confounders, as they are related to the independent and dependent variables, but not part of the causal pathway. Higher levels of catecholamines caused by these drugs may have caused bias to both directions, depending on what patient’s characteristics these higher levels were associated with. If the patient has a higher level caused by these confounders and had an unfavorable outcome, there will be an overestimation of the effect of catecholamine in patients with unfavorable outcome. Conversely, if a patient with a favorable outcome has higher levels due to these confounders, the effect of catecholamine levels in unfavorable outcome patients will be underestimated.

(ii) We did not account for patients who were receiving anticoagulants before hospital admission. However, we measured INR and platelets in all patients and a meaningful clinical difference was not observed, as their median values were mostly within
normal ranges or mildly abnormal. However, as coagulopathy is known as a factor that worsens outcome in trauma and in TBI, and as on univariate analysis we found INR associated with unfavorable outcome, we adjusted in the multivariate analysis for INR > 1.2.

5.3.3. Dichotomization of Variables

(i) We dichotomized some continuous variables to add them to the multivariate logistic regression model. We understand that dichotomization of continuous variables may create methodological problems such as loss of power and residual confounding [389], but we argue that these cut-off values used are clinically relevant and have been used in the previous and current literature. They have a strong association with unfavorable outcome in brain injury. Age was stratified according to decades and we understand that stratification was a better choice. Hypotension is one of the most important predictors of unfavorable outcome in brain injury. We used the cut-off of <100mmHg, which is widely used in the literature for general and brain trauma. Coagulopathy is also known as a predictor of unfavorable outcome in trauma. The cut-off of INR>1.2 was previously demonstrated as a clinically relevant factor for this population. Dichotomization of vasopressors could possibly have caused bias as we did not capture doses and types of vasopressors and they are known to have a dose-response pattern. Patients with severe TBI (GCS 3-8) and severe head (AIS 4-5) have an unfavorable outcome in their majority. Patients with a GCS 3 to 8 and with AIS 4 to 5 are classified in the same diagnostic category, are managed equally in their majority, and mostly have the same neurological outcome. We believe that these factors have minimized possibility of bias in our study.

5.3.4. Practice Variability

(i) The 3 sites followed the Brain Trauma Foundation guidelines [390] for the insertion of ICP monitors in moderate and severe brain injury patients. However, we noticed some variability in practice based on the attending neurosurgeon. In order to overcome this variability, we used the criteria previously described. In patients who did not have an ICP monitor high ICP was captured with clinical or tomographic signs. These criteria may have introduced bias to our analysis. If ICP is not being measured through a
monitor, a delay in capturing an elevated ICP or a mild elevated ICP is possible. If patients are considered to have a normal ICP, and in fact they have a high ICP, bias against showing a difference may have happen. Conversely, if the classification includes patients in the high ICP group, but in fact these patients have a normal ICP, bias favouring a difference may have happened.

(ii) LA County Hospital captured patients only in the end of the period of enrollment (13 patients). The number of patients was small and no large variability in the management of patients with TBI is expected to occur in the LA County Hospital compared to the 2 Canadian centers. We believe that introduction of bias have unlikely happened due to this reason.

5.4. Conclusions

The COMA-TBI study demonstrates that catecholamines are substantially elevated early post isolated brain injury and, furthermore, that there is a strong independent association between high levels of both E and NE with the severity of brain damage and with an unfavorable neurological outcome at 6 months post injury. Both E and NE are biomarkers of unfavorable outcome patients with isolated moderate to severe TBI and may be added to the panel of tools used to predict outcome in brain injury. They may help facilitating future research, improving quality of care and assisting with goals of care, and end-of-life decisions.

Our data demonstrates that there is a hyperadrenergic state in patients with isolated moderate to severe brain injury, and that this state is initiated early post injury, with high levels initially and a decrescendo stepwise profile thereafter. We were able to demonstrate the natural history of catecholamine release early post TBI. This early exaggerated storm is associated with the severity of injury in a dose-response fashion and may be part of the pathophysiology of TBI. It may be harmful to the brain itself, and possibly contribute to a vicious cycle of injury. High levels of central and peripheral catecholamines may cause vasoconstriction of the microcirculation, ischemia and further tissue damage, leading to subsequent worse edema and increased ICP. However, this hypothesis still needs to be further delineated as the molecular and cellular processes related to the hyperadrenergic
surge are still to be elucidated. Among other factors, future studies should address whether the catecholamine response is maladaptive or is simply a consequence of the severity of injury.

We believe that an important step in this field is to further address the modulation of the hyperadrenergic state in patients with brain injury, even before a better understanding of the pathophysiology of the hyperadrenergic state. As demonstrated in the current literature, the use of adrenergic antagonists to blunt catecholamine release may improve the injury itself by ameliorating inflammation, coagulopathy, ischemia, tissue damage, edema and ICP, with subsequent improvement in neurological outcome and mortality. However, current literature lacks studies with better methodological quality in this field.

8. Disclaimer

The Coma study was funded by the Physicians1 Services Incorporation Foundation and the Defense Research and Development Canada.

9. Conflicts of Interest

Sandro Rizoli received honorarium and speaker’s fees (as a member of the Scientific Advisory Board) from NovoNordisk S/A, manufacturer of NovoSeven (recombinant factor VII). The other authors have no conflict of interest relevant to the subject matter of this publication.


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11. Appendices

Appendix 1 – INFORMED CONSENT TO PARTICIPATE IN A RESEARCH STUDY – CATECHOLAMINES AS OUTCOME MARKERS IN TRAUMATIC BRAIN INJURY STUDY

INVESTIGATORS

Sandro Rizoli, MD, PhD
Trauma and Critical Care Medicine
St. Michael's Hospital 3-074 Donnelly Wing
30 Bond Street
Toronto, ON • M5B 1W8
Telephone: 416-864-5284

Leo Dante da Costa, MD
Neurosurgery
Sunnybrook Health Sciences Centre, Room A137
Toronto, ON • M4N 3M5
Telephone: 416-480-6100, ext. 6819

Gordon Rubenfeld, MD, MSc
Critical Care Medicine
Sunnybrook Health Sciences Centre, Room D503
Toronto, ON • M4N 3M5
Telephone: 416-480-6100, ext. 89657

INFORMED CONSENT

You are being asked to consider participating in a research study. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood.

This form explains the purpose of this research study, provides information about the study, the tests and procedures involved, possible risks and benefits and the rights of participants.

Please read this form carefully and ask any questions you may have. You may have this form and all information concerning the study explained to you. If you wish, someone may be available to verbally translate this form to you in your preferred language.

Please ask the study staff or one of the investigators to clarify anything you do not understand or would like to know more about. Make sure all your questions are answered to your satisfaction before deciding whether to participate in this research study.

Participating in this study is your choice (voluntary). You have the right to choose not to participate or to stop participating in this study at any time.

This research study is being conducted by Dr. Leo Da Costa, Dr. Sandro Rizoli, Dr. Martin Chapman, and Dr. Gordon Rubenfeld, conducted by the Departments of Critical Care, and Neurosurgery Teams at Sunnybrook Health Sciences Centre. Before you decide whether you will participate, it is important for you to understand why the research study is being done and what it will involve.
INTRODUCTION

You are being asked to consider participating in this study to serve as a comparison to the traumatic brain injured patients and because you will be undergoing a neurological surgical procedure. In patients who undergo these procedures there is some suggestion from other research that there may be catecholamines (stress hormones) released, which may have some relationship to other neurological scales that are routinely used to measure state of consciousness, and recovery outcome.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to help us understand the levels of these with TBI severity and outcome from the injury. If we find a relationship between these, then measuring catecholamine’s on a routine basis could help doctors better treat these TBI patients and possibly improve their outcome.

The two scales that will be used in this study are the Glasgow Coma Scale (GCS) and the Extended Glasgow Outcome Scale (GOS-E). The GCS is a neurological scale that is used to evaluate the conscious state of a person, for initial and follow-up assessment. A patient is assessed against criteria on the scale, and the resulting points give a patient a score between 3 (indicating deep unconsciousness) and 15 (indicating normal consciousness). The first goal of our study is to prove that there is a relationship between the amount of stress hormones in the blood and the relationship that it has with the Glasgow Coma Scale.

The outcome of a patient is evaluated using another similar scale to the GCS, called the Extended Glasgow Outcome Scale (GOS-E), which also a neurological scale, but it is an 8-point score used to assess the recovery of a patient after a traumatic brain injury (TBI). It is a general assessment that can be done over the telephone. For this study, a member of the study team will call you or a member of your family if you do not feel well, and ask some general questions about how recovery is going 6-months after surgery. This telephone call will take 5-10-minutes, and doesn’t require a special trip to the hospital.

WHAT WILL HAPPEN DURING THIS STUDY

If you consent to partake in the study, blood samples will be collected for special research tests that will measured the amount of stress hormones, brain injury markers, and inflammation and immune system markers that are present in your body. The blood collection will be done at admission, at 6-hours, 12-hours and 24-hours after your surgery. These blood samples are taken at the same time that other routine blood work will be done, so no additional punctures will be necessary. Each research blood test will require an additional 20-mL of blood (equals about 1-tablespoon). The total amount of blood required for the entire study is 60-mL (or approx. 4 tablespoons). This amount of blood is considered small, and should cause no adverse effects to you.

Other information about your stay in hospital will be recorded, such as vital signs (blood pressure, heart rate, breathing rate, and body temperature) and whether or not you were on a breathing machine will be recorded. Information about you surgery, treatment, and any other procedures will be recorded. Your condition during participation will be followed very closely by the study investigators and research coordinators. On discharge the Extended Glasgow Outcome Scale will be assessed, and also will be repeated by telephone at 6-months post-surgery. The 6-month follow-up assessment can be done in 5-10-min by telephone, and doesn’t require an extra trip to hospital.
HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY

Approximately 200 trauma patients will be enrolled from Sunnybrook Health Sciences Centre and St. Michael's Hospital, and 20 neurosurgical patients will be enrolled in this research study at Sunnybrook Health Sciences Centre. We will try and show a relationship between catecholamine levels on admission and over the first 24-hours after trauma or surgery, with the Extended Glasgow Outcome Score being performed at discharge from hospital, and again at 6-months. We expect this study to take 24-months to complete the enrollment period.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?

If you decide to participate in this study, you will be asked to do the following:
- Sign and date the consent form for the study. You will be given a copy to keep for your records.
- Blood samples will be taken at admission, at 6-hours, at 12-hours and at 24-hours after your surgery at the same time as other routine blood work.
- On discharge, the Extended Glasgow Outcome Score will be assessed.
- Six months post-surgery, the Extended Glasgow Outcome Score will again be assessed by telephone.

WHAT ARE THE RISKS OR HARMS OF PARTICIPATING IN THIS STUDY?

The risks of participating in this study are the same as those that might occur when standard routine blood sampling is performed. Since the research blood samples are collected during routine blood sampling times, there is no additional risk to participating in this research study.

You will be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to the study staff. This may include new information about the risks and benefits of being a participant in this study.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

There is no known direct benefit for you to participating in this study at this time. The information obtained during this study may help future trauma patients with traumatic brain injury, and future patients undergoing neurosurgical procedures.

WHAT OTHER CHOICES ARE THERE?

If you decide not to participate in this study, you will receive standard medical treatment for your neurosurgical procedure. You do not have to participate in this research study to receive treatment for your condition.

CAN PARTICIPATION IN THIS STUDY END EARLY?

The investigator(s) may decide to remove you from this study without your consent for any of the following reasons:
- If you are unable or unwilling to follow the study procedures.
- If there is evidence that the study should be stopped due to safety reasons or lack of treatment effect.

If you are removed from this study, the investigator(s) will discuss the reasons with you.

You can also choose to end your participation at any time without having to provide a reason. If you choose to withdraw, your choice will not have any effect on your current or future medical
treatment or health care. If you withdraw from the study, you are encouraged to contact Sandy Trpcic, Trauma Research Manager at (416) 480-6100 x 7322 immediately.

If you leave the study, the information about you and your blood work that was collected before you left the study will still be used. No new information about you will be collected and no further testing of your samples will be done without your permission.

WHAT ARE THE COSTS OF PARTICIPATING IN THIS STUDY?

Participation in this study will not involve any additional costs to you.

ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY

You will not be paid to participate in this study.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?

You have the right to have any information about you and your health that is collected, used or disclosed for this study to be handled in a confidential manner.

If you decide to participate in this study, the investigators and study staff will look at your personal health information and collect only the information they need for this study.

You have the right to access, review and request changes to your personal health information.

The following people may come to the hospital to look at your personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines:

- Representatives of the Sunnybrook Research Ethics Board

Access to your personal health information will take place under the supervision of the Principal Investigator.

Study data is health information about you that is collected for the study, but that does not directly identify you. Any study data about you that is sent outside of the hospital will have a code and will not contain your name or address or any other information that directly identifies you.

Study that is sent outside of the hospital will be used for the research purposes explained in this consent form.

The investigator, study staff and the other people listed above will keep the information they see or receive about you confidential, to the extent permitted by applicable laws. Even though the risk of identifying you from the study data is very small, it can never be completely eliminated.

The Principal Investigator will keep any personal health information about you in a secure and confidential location for five years and then destroy it according to Sunnybrook policy.

When the results of this study are published, your identity will not be disclosed.

You have the right to be informed of the results of the study once the entire study is complete. If you would like to be informed of the results of this study, please contact Sandy Trpcic, Trauma Research Manager at (416) 480-6100 x 7322.

DO THE INVESTIGATORS HAVE ANY CONFLICTS OF INTEREST?
The investigators have no conflict of interest to disclose. Neither your physician, nor the investigator will be paid a fee for enrolling you in this study.

WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?

You have the right to receive all significant information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction, before you make any decision. You also have the right to ask questions and to receive answers throughout the study.

If you have any questions about this study, you may contact the person in charge of this study, Dr. Sandro Rizoli at (416).864-5284

The Sunnybrook Research Ethics Board has reviewed this study. If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with someone not directly involved with the study, you may call Dr. Philip C. Hébert, Chair of the Sunnybrook Research Ethics Board at (416) 480-4276.

PATIENT DOCUMENTATION OF INFORMED CONSENT

You will be given a copy of this informed consent form after it has been signed and dated by you and the study staff.

Catecholamines as Outcome Markers in Traumatic Brain Injury Study

Name of Participant: _____________________________________________________________

Participant/Substitute decision-maker:

By signing this form, I confirm that:

- This study has been fully explained to me and all of my questions answered to my satisfaction
- I understand the requirements of participating in this research study
- I have been informed of the risks and benefits, if any, of participating in this research study
- I have been informed of any alternatives to participating in this research study
- I have been informed of the rights of research participants
- I have read each page of this form
- I authorize access to my personal health information (medical record) and research study data as explained in this form
- I have agreed, or agree to allow the person I am responsible for, to participate in this research study.

________________________________________     ___________________________     _________
Name of participant/Substitute decision-maker (print)                Signature                              Date

Person Obtaining Consent:

By signing this form, I confirm that:

- This study and its purpose has been explained to the participant named above
All questions asked by the participant have been answered
I will give a copy of this signed and dated document to the participant

Name of Person obtaining Consent (print)          Signature          Date

ASSISTANCE DECLARATION
Was the participant/substitute decision-maker assisted during the consent process? ☐ Yes ☐ No

☐ The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant/substitute decision-maker.

☐ The person signing below acted as a translator for the participant/substitute decision-maker during the consent process. He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that that participant/substitute decision-maker has understood the information translated.

Name of Person Assisting (print)          Signature          Date

Statement of Investigator:
I acknowledge my responsibility for the care and wellbeing of the above participant, to respect the rights and wishes of the participant as described in this informed consent document, and to conduct this study according to all applicable laws, regulations and guidelines relating to the ethical and legal conduct of research.

Name of Investigator (print)          Signature          Date

Further Contact Information
Sandy Trpcic
Trauma Research Manager
Sunnybrook Health Sciences Centre
2075 Bayview Avenue, Rm H113
Toronto, Ontario M4S 3M5
Telephone: 416-480-6100 ext. 7322

Appendix 2 – CASE REPORT FORM – CATECHOLAMINES AS OUTCOME MARKERS IN TBI STUDY (REB PIN: 068-2011)

STUDY NUMBER: __________________________
Hosp. File Number: __________________________ Arrival Date: __________________________
Time: __________________________ Elapsed Time (trauma to ER): __________________________
Age: __________________________ Sex: M ☐ F ☐ Weight (kg): __________________________ Height (cm) __________________________
Trauma: Blunt ☐ Penetrating ☐ Combined ☐
Fall from level □  Fall from stairs □  MVA □  Pedestrian hit by car □  Bike □  Assault □  
□ Elective OR patient  Type of OR _______________________

Other ___________________________

AIS non-Head, Assessed at Admission: ________________________

Past Medical History: DM □  COPD □  CAD □  PVD □  CRF □  CHF □  HTN □
PVD □  CVA □  Cancer □  Other: ___________

Concomitant Medications: B-Blocker _____ ASA _____ Plavix _____ Coumadin _________
Steroids ___________________________
Other Meds: _________________________________________

ISS: _______ AIS Head: _______ AIS non-Head: ________________________

**On Admission**

Neurological Status: GCS (E___ M___ V___) Intubated: Y □  N □  Sedated: Y □  N □
Pupils: Equal: Y □  N □  Reactive: Y □  N □  Seizures: Y □  N □  U □
Alcohol: Y □  N □  U □  Dosage: _______________________ Other Intoxication: ________________________
Neuromuscular Blockers: N □  Y □
Clinical Status: BP: ___________ HR: ___________ RR: _______ Temp: ___________
SatO2: ___________ Mech. Ventilation: Y □  N □
Chest Radiography: Pulmonary Edema: Y □  N □
Head CT Scan: Marshall Classification ___________ Description: ________________________
ECG: Normal □  Abnormal __________________________

**First 24 hours**

Neurosurgery: N □  Y □
Use of vasopressors: Y □  N □ If yes, please write all doses with dates and times
Dose: __________________ Date: ___________ Time: ___________
Dose: __________________ Date: ___________ Time: ___________
Dose: __________________ Date: ___________ Time: ___________
Dose: __________________ Date: ___________ Time: ___________
Episodes of: Hypotension (Max ≤90 mm Hg) Y □  N □
Desaturation < 90% Y □  N □
Intracranial Hypertension > 20 mmHg: Y □  N □  U □
Changes in Head CT Scan: __________________________
Changes in CXR: __________________________
Changes in ECG: __________________________
APACHE Score: _______ Other: __________________________

**Follow up**

Surgical Procedures: __________________________
Duration of Mechanical Ventilation: __________________________
Sepsis / Infections Y □  N □
Organ / System Failure: Y □  N □
Length of ICU Stay: CrCU: ___________ B5 ICU: ___________ Other: __________________________
Length of Hospital Stay: __________________________
GOS-E: At Hospital Discharge: ___________ At 6 Months: __________________________
Patient Died: Y □  N □
Cause of Death: Non-Brain Injury Y □  N □  Brain Injury: Y □  N □
**Laboratory Results**

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<th>12 h</th>
<th>24h</th>
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<td>Other:</td>
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**Marshalls Class**

| Diffuse Injury I | No visible intracranial pathology |
| Diffuse Injury II | Midline shift < 5 mm |
| Diffuse Injury III | Midline shift > 5 mm/cisterns compressed |
| Diffuse Injury IV | Midline shift > 5 mm |
| Evacuated Mass Lesion | Any evacuated mass |
| Non Evacuated Lesion | Mass lesion > 25 mL |
12. Copyright Acknowledgements
