

McGill University
Montreal

Assessment of Physiological Markers of Anxiety and Pain
as a Proxy of Perioperative Pain
in Adolescents Undergoing Spinal Fusion Surgery

Shajenth Premachandran
Department of Experimental Surgery
Faculty of Medicine
McGill University, Montreal
May 2021

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree
of Master of Science

© Shajenth Premachandran, 2021

TABLE OF CONTENTS

<u>ABSTRACTS</u>	<u>3</u>
English.....	3
Français.....	5
<u>ACKNOWLEDGMENTS</u>	<u>7</u>
<u>CONTRIBUTION OF AUTHORS</u>	<u>9</u>
<u>INTRODUCTION</u>	<u>10</u>
Objective & Rationale.....	10
Literature Review.....	12
Pain.....	12
Assessment of Post-Surgical Pain.....	26
Adolescent Idiopathic Scoliosis.....	30
Research Questions, and Hypotheses of M.Sc. Project.....	33
<u>METHODOLOGY</u>	<u>35</u>
<u>RESULTS</u>	<u>37</u>
Manuscript 1: Assessing Interleukin-6 as a proxy of Perioperative Pain Intensity in Adolescents with Idiopathic Scoliosis Undergoing Spinal Fusion Surgery: An Exploratory Study.....	37
Manuscript 2: Salivary Alpha-Amylase Assessment as a proxy of Preoperative Anxiety in Adolescents with Scoliosis.....	54
Manuscript 3: Systemic Modulation of Perioperative Monoamines in Pediatric Patients Undergoing Surgery.....	78
<u>DISCUSSION</u>	<u>109</u>
<u>CONCLUSION</u>	<u>120</u>
<u>BIBLIOGRAPHY</u>	<u>121</u>

ABSTRACT (ENG)

Suboptimal treatment of pediatric postoperative pain can negatively impact a child's recovery, and lead to chronic post-surgical pain. Therefore, appropriate treatment is needed, based on the mechanisms that affect a patient's respective pain experience, such as the inflammatory, stress, and nociceptive response systems. Following a surgical insult, the inflammatory response releases inflammatory mediators to sensitize nerve endings for nociception, and the nociceptive response mediates an inhibitory pain response through endogenous monoamine neurotransmitters. Psychological stress response activation is characterized by anxiety, in anticipation of surgery, which can facilitate pain postoperatively. Assessing markers of these main pain processes may allow for a more informed decision on the interventions required to achieve optimal pain management for pediatric patients undergoing invasive surgery.

This project aimed to evaluate plasma Interleukin-6 (IL-6), a pro-inflammatory cytokine, as a proxy of perioperative pain, assess salivary Alpha-Amylase (sAA) activity as a marker/predictor of perioperative anxiety and postoperative pain intensity, and to identify the relationship between the perioperative modulation of plasma monoamines and self-reported pain intensity, in a pediatric cohort undergoing invasive surgery. It was hypothesized that increasing plasma IL-6 levels would correlate with increasing self-reported pain intensity, increasing sAA activity levels would be associated with increasing self-reported anxiety, increasing preoperative sAA activity levels would be associated with increasing levels of self-reported postoperative pain intensity, and analyzing the perioperative trajectories of plasma monoamines in relation to self-reported pain would be able to identify groups of patients with differences in the postoperative descending control of pain.

Adolescent Idiopathic Scoliosis patients, undergoing corrective surgery, were recruited for this project. Patients' blood and saliva samples, and self-reported pain/anxiety scores, were collected perioperatively. Plasma IL-6 levels were analyzed with cytokine magnetic 30-plex assays, sAA activity was analyzed with kinetic enzyme assays, and plasma monoamines' levels were analyzed with liquid chromatography-tandem mass spectrometry. Spearman's rank correlation was used to assess perioperative associations between plasma IL-6 levels and self-reported pain scores, and between sAA activity levels and self-reported anxiety/postoperative pain scores. Growth mixture modelling followed by multiple correspondence analysis was used

to identify clusters of patients with similar perioperative monoamines and self-reported pain trajectories.

In 21 patients, there was no association between plasma IL-6 levels and pain intensity, postoperatively, but rather, an association between plasma IL-6 levels and pain intensity, preoperatively. In 31 patients, no associations were observed between perioperative sAA activity, and self-reported anxiety or postoperative pain intensity. In 85 patients, two clusters of patients were identified; where one cluster was mainly characterized by patients with increasing plasma monoamine levels perioperatively, and the second cluster was largely characterized by patients with constant plasma monoamines levels perioperatively. However, no differences in pain trajectory membership were observed between the two clusters.

Pain is a complex experience, and evaluating markers of the inflammatory, stress, and nociceptive response as proxies of perioperative pain, individually, remains an incomplete representation of a patient's actual physiological response to postoperative pain. Analyzing the perioperative trajectories of multiple markers of these different pain processes, all at once, in relation to self-reported pain, may provide a more accurate perspective into the relationship between these physiological response systems and postoperative pain.

ABRÉGÉ

Un traitement sous-optimal de la douleur post-opératoire pédiatrique peut entraîner une douleur post-chirurgicale chronique. Par conséquent, un traitement approprié de la douleur basé sur les réponses inflammatoire, de stress et nociceptive est nécessaire. Suite à une chirurgie, des médiateurs inflammatoires sont libérés pour sensibiliser les terminaisons nerveuses libres, et la réponse nociceptive en découle. L'activation de la réponse psychologique au stress est caractérisée par l'anxiété, par anticipation de la chirurgie, qui peut amplifier la douleur post-opératoire. L'évaluation de marqueurs de ces principaux processus de douleur permettrait une décision éclairée sur les interventions nécessaires pour mieux gérer la douleur.

Ce projet visait à évaluer la cytokine pro-inflammatoire interleukine-6 (IL-6) plasmatique en tant que proxy de la douleur péri-opératoire, à évaluer l'activité de l'alpha-amylase salivaire (sAA) en tant que marqueur d'anxiété péri-opératoire et d'intensité de douleur post-opératoire, puis à identifier la relation entre la modulation péri-opératoire des monoamines plasmatiques et l'intensité de la douleur de patients pédiatriques subissant une chirurgie invasive. Les hypothèses étaient qu'une augmentation d'IL-6 serait corrélée à une augmentation de l'intensité de la douleur, une augmentation de sAA serait associée à une augmentation de l'anxiété, et qu'une augmentation de sAA préopératoire serait associée à une augmentation des niveaux de l'intensité de la douleur, et l'analyse des trajectoires péri-opératoires des monoamines plasmatiques par rapport à la douleur permettrait d'identifier des groupes de patients présentant des différences dans le contrôle de la douleur.

Des adolescents ayant une scoliose idiopathique et subissant une chirurgie correctrice ont été recrutés pour ce projet. Les échantillons de sang et de salive et les scores de douleur et d'anxiété ont été prélevés durant la période péri-opératoire. Les niveaux d'IL-6 ont été analysés avec des dosages quantitatifs multiplex de cytokines, l'activité sAA avec des dosages enzymatiques et les niveaux de monoamines par chromatographie liquide-spectrométrie de masse. La corrélation de rang de Spearman a été utilisée pour évaluer les associations péri-opératoires entre les taux d'IL-6 et les scores de douleur, et entre les niveaux d'activité sAA et les scores d'anxiété et de douleur post-opératoire. Une modélisation du mélange de croissance suivie d'une analyse de correspondance multiple a été utilisée pour identifier des groupes de patients présentant des quantifications de monoamines péri-opératoires et des trajectoires de douleur similaires.

Chez 21 patients, aucune association entre les taux d'IL-6 et l'intensité de la douleur en période post-opératoire n'a été identifiée, mais plutôt une association entre le niveau d'IL-6 et l'intensité de la douleur préopératoire. Chez 31 patients, aucune association n'a été observée entre l'activité de sAA péri-opératoire et l'anxiété ou l'intensité de la douleur post-opératoire. Chez 86 patients, deux groupes de patients ont été identifiés : un sous-groupe principalement caractérisé par des patients présentant des taux plasmatiques de monoamines croissants durant la période péri-opératoire, et un second sous-groupe présentant des taux plasmatiques de monoamines constants. Aucune différence dans l'appartenance à la trajectoire péri-opératoire de la douleur n'a été observée entre les deux groupes.

La douleur est un phénomène complexe, et l'évaluation de marqueurs individuels de la réponse inflammatoire, de stress et nociceptive en tant que substituts de la douleur péri-opératoire ne peut que suggérer une représentation incomplète de la réponse physiologique réelle d'un patient par rapport à la douleur post-opératoire. L'analyse simultanée des trajectoires péri-opératoires de plusieurs marqueurs des différents processus physiologiques en lien avec la douleur pourrait fournir une perspective plus précise.

ACKNOWLEDGEMENTS

I would like to begin by saying a huge thank you to my supervisors, Dr. Catherine E. Ferland and Dr. Jean A. Ouellet, without whom I would have been unable to achieve all the work I have managed to do over the past two years as a M.Sc. student. Since I first started working as a student under Dr. Ferland's supervision, as a second year undergraduate student, I knew I had found a lab that would be a perfect fit for me to complete my Master's degree. Dr. Ferland, without your tutelage and constructive criticism over these past couple of years, I know I would not be half the researcher that I am today. You have provided me with many opportunities to present my research at conferences and network at such events, and also be involved in multiple projects, that has ended up in three separate first-author manuscripts for my thesis, along with some other manuscripts where I am a co-author. However, above all else, you have instilled in me a deep desire to continue doing research in the pain field, which I hope will motivate me in my future studies/career, and this is something I cannot thank you enough for. Thank you, Dr. Ouellet, for giving your perspective on my project during lab meetings, and also giving me advice on how I can move the project forward effectively. Your encouragement during these past couple of years has pushed me to strive for my best, and your expertise on the surgical experience has played a crucial part in the writing of my thesis. I'm sure that I would not have had the success I have had, as a M.Sc. student, without your help, as well.

I would also like to thank Ljiljana Nikolajev for taking me under her wing, as a second year undergraduate student, and teaching me the techniques, protocols, and methodologies I would come to use in my M.Sc. thesis project. I am sure without your guidance over the last few years, I would not be so comfortable with working in a lab, as I am now today. I would also like to give a huge thank you to Dr. Alisson R. Teles for sharing with me his knowledge of statistical analysis, and for looking through and giving feedback on my work, no matter how busy it gets for him. Thank you, Dee-Ann Naylor, your help with writing protocols, IRB approvals, consent forms, etc., and being on top of all of these documents, was beyond helpful in maintaining a sense of calm in the lab. Thank you, Sheila Bote, for being so organized with the scheduling of patient follow-ups, and lending a helping hand with the saliva processing early in the mornings. Your help was fundamental in having the sample collections, for my thesis project, run so smoothly week in, week out.

Last, but certainly not least, I want to give one huge thank you to the SPINE research group. Don Daniel Oca, Maxime St-Georges, Diana-Luk Ye, Natalie Betinjane, Alexandre Jolicoeur, Cynthia Larche, Kelsey Vickers, Mandy Li, Jeffrey Liao, Alice Bruneau, and Alexandre Castan, you have all played such an important role in my experience as a graduate student, with your support both in and out of work. You all made the environment in the lab so much more friendlier, and it was so easy to share opinions and discuss matters with all of you. I truly believe I have made some real friendships over the past couple of years, and I believe having colleagues, like you all, is so important when it comes to dealing with the stresses that can accompany graduate studies.

Thank you all for making these past few years unforgettable, and such an enjoyable experience. My thesis project would not have turned out the way it did without all of your inputs and help. I was so fortunate to have been a part of the SPINE research group, working with friends, enthusiastic colleagues, and supportive supervisors. I will cherish the memories I have made during my time in this wonderful lab, and once again, thank you all from the bottom of my heart.

CONTRIBUTION OF AUTHORS

The entirety of the thesis was reviewed by both of my supervisors, Dr. Catherine E. Ferland and Dr. Jean A. Ouellet, and they were both involved in developing the methodology for this project, as well.

I completed the writing and redaction of the following chapters:

- Abstracts
- Introduction
- Literature review
- Methodology
- Discussion
- Conclusion

The following chapters were completed with the help of co-authors, as detailed below:

- Results:
 - Manuscript 1 was written by me, and was co-authored by Ljiljana Nikolajev and Jun Jie Liao for their participation in the sample and data analysis, as well as the manuscript review, by Dr. Jean A. Ouellet for his patients and his expertise in the surgical experience, and by Dr. Catherine E. Ferland who oversaw all parts of the project, from the recruitment of patients to the sample and data analysis.
 - Manuscript 2 was written by me, and was co-authored by Kelsey Vickers and Dr. Alisson R. Teles for data analysis and manuscript review, by Dr. Jean A. Ouellet and Dr. Neil Saran for their patients and their expertise in the surgical experience, and by Dr. Catherine E. Ferland who oversaw all parts of the project, from the recruitment of patients to the sample and data analysis.
 - Manuscript 3 was written by me, and was co-authored by Dr. Alisson R. Teles for data analysis and manuscript review, by Dr. Pablo M. Ingelmo for his expertise in anesthesiology and perioperative pain, by Drs. Philippe Sarret, Alexandre J. Parent and Serge Marchand for their expertise in pain mechanisms, by Dr. Jean A. Ouellet and Dr. Neil Saran for their patients and their expertise in the surgical experience, and by Dr. Catherine E. Ferland who oversaw all parts of the project.

INTRODUCTION

Over one thousand children undergo orthopedic surgery each year at the Shriners Hospitals for Children-Canada alone. These procedures are often invasive, and can lead to tissue damage, which causes a cascade of physiological responses that lead to pain in the acute postoperative period for patients. This pain can have negative effects on a patient's postoperative outcomes if inadequately treated, such as poor recovery, delaying physical rehabilitation, and in some cases, this pain can persist, and lead to the development of chronic post-surgical pain. Thus, the need to accurately assess pain in the postoperative period is of the utmost importance, in order to provide proper pain management to these patients based on their individual needs. Furthermore, since preoperative anxiety in children has been associated with greater pain intensity experienced in the acute postoperative, the patient's anxiety before surgery needs to be accurately assessed as well in order to identify the patients who may be at risk of poor pain management in the postoperative periods. However, the current gold standards of assessing pediatric pain and anxiety in the clinic come in the form of self-reported measures. These subjective self-reported measures are known to have limitations when it comes to their use in non-verbal patients. Thus, there is a need to identify objective measures of pain and anxiety in pediatric patients who are scheduled to undergo major orthopedic surgery. To address this need, this project was developed in order to evaluate physiological molecular markers of the nociceptive and inflammatory response, as biomarkers of pain, and anxiety response, as biomarkers of anxiety, in a pediatric cohort.

The aims of this project were to:

1. Quantify the levels of interleukin-6 (IL-6), a marker of the inflammatory response, across the perioperative period, in pediatric patients scheduled for orthopedic surgery.
2. Quantify the levels of salivary alpha amylase (sAA), a salivary marker of anxiety, across the perioperative period.
3. Quantify the levels of serotonin (5-HT), dopamine (DA), epinephrine (EPI), norepinephrine (NE), and the respective metabolites of EPI and NE: metanephrine (ME) and normetanephrine (NME), as markers of the nociceptive response, across the perioperative period.

4. Assess for associations between the regulation of the physiological marker levels, and self-reported pain and anxiety scores throughout the perioperative period, accordingly.

The hypotheses were:

1. Plasma IL-6 concentrations will increase in the acute postoperative period, before returning to baseline levels (preoperative levels) six weeks after the surgery.
2. sAA activity levels will increase preoperatively, and then decrease in the postoperative period.
3. Plasma monoamines' levels will increase in the postoperative period, in comparison to baseline level.
4. Increased postoperative plasma IL-6 levels will be associated with an increase in postoperative self-reported pain scores. Increased preoperative sAA activity levels will be associated with an increase in preoperative self-reported anxiety scores, and an increase in postoperative self-reported pain scores in the acute postoperative period. Increasing postoperative plasma levels of the monoamines, and their respective metabolites, will be associated with increasing postoperative self-reported pain scores.

Identifying objective biomarkers of pain and anxiety in this study will have the benefit of highlighting the possible underlying mechanisms of perioperative pain in pediatric patients. This would then allow clinicians to better identify patients at risk of poor pain management, and provide personalized pain management.

LITERATURE REVIEW

1. PAIN

The International Association for the Study of Pain defines pain as “an unpleasant sensory or emotional experience associated with, actual or potential tissue damage” [1]. Pain plays an important role in humans as a warning system for actual or potential injury, and is useful in initiating protective mechanisms in response to such injury [2]. However, the problem arises when this pain becomes chronic, which is “defined as persistent or recurrent pain lasting longer than 3 months” [3], and is no longer acting as a protective mechanism, since there is no physiological need for it [2]. Pain is one of the most prevalent reasons people seek care from medical professionals [4, 5]. In fact, it is estimated that 11% to 38% of children and adolescents are affected by chronic pain [6], with approximately 1-3 millions of these children and adolescents living in Canada with chronic pain [6, 7].

Despite the great economic burden presented as a result of chronic pain in the pediatric population [8, 9], chronic pain has also been shown to have negative effects on their quality of life, affecting them on a social, psychological, and physical level [10]. Children and adolescents with chronic pain were reported to have less friends, and found themselves more isolated when compared to their peers who are healthy [11]. Disturbances in sleep were also commonly reported in children and adolescents with chronic pain [12], and proper sleep plays a crucial role in adolescent development, whereby it affects their cognition and behaviour during the day [13]. Furthermore, children and adolescents reporting greater severity of pain were associated with absences from school, increased pressure related to school, and more experiences of being bullied [14], which can adversely impact their cognitive and social development [15]. Lastly, pediatric chronic pain may eventually add to the societal and economical costs of chronic pain in the adult population, as well, since pediatric chronic pain can persist into adulthood [16, 17]. Thus, the need for adequate pediatric pain management is of the utmost importance.

1.1 Surgical Pain

Considering over a million children will undergo surgery per year, in the U.S. alone [18, 19], clinicians are constantly challenged with providing effective postoperative pain management, which is especially important for these patients not only in the short term period, but also affects their long term outcomes, such as the potential development of chronic post-

surgical pain (CPSP) [20]. The prevalence of this issue was highlighted in a prospective study conducted by Rosenbloom *et al.* (2019), where the authors identified an incidence of moderate-to-severe chronic pain in 35% of a pediatric cohort 6 months following either orthopedic or general surgery, and an incidence of 38% 12 months after surgery [21]. Ocaý *et al.* (2020) also observed that pediatric patients who experienced moderate to severe pain in the acute postoperative period, following orthopedic surgery, also had a greater probability of reporting higher levels of pain severity, more pain medication use, and a greater number of missed school or work days 6 months after their surgery [22]. Thus, the acute postoperative period appears to be a time at which proper pain management can positively impact the long-term outcomes of a patient following surgery, especially if the patient is at risk of experiencing a greater severity of acute postoperative pain.

1.1.1 Predictors of Postoperative Pain

Identifying predictors of postoperative pain has important clinical value since it would allow for an earlier implementation of pain management strategies, and help identify a more appropriate pain management strategy based on the predictive factors for postoperative pain identified in the patient [23]. In regard to this, a study conducted by Kain *et al.* (2006) highlighted increased levels of preoperative anxiety, in children, as a factor that is associated with greater pain experienced in the acute postoperative periods, as well as increased analgesic intake during their recovery at home, within the first 14 days after surgery [24]. This link between preoperative anxiety and postoperative pain was also replicated in a pediatric population undergoing orthopedic surgery, where higher levels of preoperative anxiety was shown to predict poorer improvements in postoperative pain [25]. However, along with preoperative anxiety, preoperative pain intensity was highlighted as a predictor of poorer improvements in postoperative pain in this study, as well [25]. Preoperative pain catastrophizing, which is defined as a “set of exaggerated and negative cognitive and emotional schema brought to bear during actual or anticipated painful stimulation” [26], was also shown to be linked to the intensity of acute postoperative pain in pediatric patients undergoing orthopedic surgery, with patients experiencing moderate-to-severe acute postoperative pain having an increased likelihood of being pain catastrophizers in relation to the patients reporting mild acute postoperative pain [22]. Thus, identifying the psychological (preoperative anxiety and pain catastrophizing) and

physiological (preoperative pain) factors affecting a patient before surgery may be crucial to providing optimal postoperative pain management in a pediatric population. This is especially important since preoperative pain, anxiety, and pain catastrophizing have been identified as risk factors for the development of CPSP, as well [27, 28].

1.1.2 Treatment of Postoperative Pain

Currently, the treatment of postoperative pain largely involves the use of opioid analgesics, as it has been shown to be one of the more effective drugs used in pain management, for a variety of situations [20]. In the acute clinical setting, the opioid analgesic, morphine, is commonly administered either orally or intravenously [29]. With low lipid solubility, morphine's onset of action is made slower since it passes through the blood-brain barrier slowly [29]. The blood-brain barrier acts as a barrier between the brain tissue and the circulatory system, and thus, is an anatomical structure that needs to be crossed if a drug needs to be delivered to the central nervous system (CNS) [30]. Thus, with morphine passing through the blood-brain barrier relatively slowly, it will also take a bit of time for morphine to reach its peak analgesic effect [29]. Furthermore, with an elimination half-life of approximately 2.5 hours, morphine will need to be given to patients in relatively frequent doses in order to maintain and optimize analgesia [29]. However, fentanyl and remifentanyl are other opioid analgesics provided in the clinical setting, that differ in their pharmacokinetic properties when compared to morphine, as these drugs are more lipid soluble and have a faster onset of action [29]. Remifentanyl is also rapidly metabolized in the body, which makes it especially useful as a short-acting opioid analgesic, when a rapid clearance of the opioid is preferred [29]. However, despite opioids being the gold standard to which other analgesic medication is compared [29], the usefulness of these drugs in achieving analgesia is also met with certain risks, such as drug abuse and opioid-related adverse drug events (e.g. nausea, vomiting, constipation, dizziness, and negative effects on cognitive function, balance, alertness and psychomotor coordination) [20]. These opioid-related adverse drug events then require treatment as well, which raises health care costs, and affects the patient's recovery period following surgery [20]. The risk of experiencing such adverse events greatly increases with the usage of higher opioid doses, and therefore, strategies, such as multimodal postoperative recovery protocols, have been implemented in the postoperative

setting in order to balance out the reduction in opioid usage, with providing effective analgesia [20].

A type of non-opioid analgesic agent used in the acute postoperative period are nonsteroidal anti-inflammatory drugs (NSAIDs), since surgery can lead to inflammation and pain [31, 32]. NSAIDs are preferably administered intravenously, in the postoperative period, if the patient already has an intravenous line, but intramuscular and oral administration are also considered to be effective, depending on whether the pediatric patient is sedated, or conscious, nauseated or unable to swallow, respectively [31]. NSAIDs and opioids are often used in conjunction, as a part of multimodal analgesia, since it can reduce postoperative opioid usage, and minimize the risk of any opioid-related adverse drug events [31]. The NSAIDs, ketorolac, ibuprofen, and diclofenac, have all been shown to be effective at achieving analgesia in pediatric patients, having undergone orthopedic surgery, when used alongside opioid analgesics [31]. Another non-opioid drug that NSAIDs are used alongside, as a part of multimodal analgesia, is acetaminophen, which can be administered orally or intravenously [31, 33]. Acetaminophen is commonly known as an effective agent for acute pain relief [33]. In the context of a surgery, although its mechanism of action is not well understood, thanks to its analgesic and antipyretic effects, acetaminophen administration has also been shown to have opioid-sparing effects postoperatively [33, 34].

Currently, the multimodal approach to the treatment of postoperative pain is an important step in providing effective postoperative pain management for patients, all the while minimizing any potential adverse events [20]. However, there is still a need to provide better targeting postoperative management [35], and therefore, understanding the underlying mechanisms of a patient's postoperative pain in order to provide a more personalized pain treatment would prove to be invaluable.

1.2 Nociceptive Response

The term “nociception” is used to refer to an organism's ability to detect and respond to a noxious stimuli [36], whereas pain refers to the perceived somatic sensation resulting from the higher centres of the brain processing the information conveyed from the periphery in response to noxious stimuli [36, 37]. The nociceptive response is separated into 4 sub-sections in this

literature review: transduction, transmission, processing of pain, and the descending control of pain.

1.2.1 Transduction

Transduction in the nociceptive response is the process by which “external stimuli are converted to electrical signals that can be perceived as pain” [37]. External noxious stimuli can come in the form of thermal, mechanical, and chemical stimuli [38]. Thermal noxious stimuli is often detected at temperatures greater than 43 degrees Celsius (heat), or at temperatures lower than 25 degrees Celsius (cold) [38]. Mechanical noxious stimuli often comes in the form of intense punctate or pinch pressure [39]. Lastly, chemical noxious stimuli can refer to certain foods (e.g. chilli peppers), environmental toxins, and inflammatory mediators that are released during tissue damage [38].

In the early 1900s, Charles Sherrington proposed that certain nerve endings in the skin had the specific function of detecting noxious stimuli, which would eventually be coined the term “nociceptors” [40]. The peripheral nerve fibers with these free nerve endings are classified into subtypes based on their physical properties, such as the diameter and myelination of the fibers, as well as their conduction velocity of nerve impulses [2, 41]. The diameter and myelination of these primary afferent fibers have a positive correlation with the conduction velocity, for example, as the diameter and myelination of the nerve fiber increases, so does its conduction velocity [41]. Thus, these nociceptive afferent fibers are separated and labelled as A β fibers, A δ fibers, and C fibers [41].

1.2.1.1 A β fibers

A β fibers have the largest diameter (6-12 μ m) and most myelination of the peripheral nerve fibers [2, 41]. Thus, A β fibers have a rapid conduction velocity (35-75 m/s) for nerve impulses [41]. A β fibers are not known to conduct nociceptive inputs, and rather, are known to be largely involved in detecting vibration, and light touch, as well as conducting proprioceptive inputs [2, 41]. However, the A β fibers are involved in the inhibition of nociceptive inputs [41], where the activation of peripheral nerve fibers by an innocuous stimulus can have an inhibitory effect on the nociceptive inputs from the same area of the skin [41, 42].

1.2.1.2 A δ fibers

A δ fibers have a smaller diameter (1-5 μm) than A β fibers and are lightly myelinated [2, 41]. Thus, A δ fibers have a conduction velocity (5-30 m/s) slower than A β fibers, and faster than C fibers [41]. A δ fibers are involved in detecting mechanical, thermal and chemical nociceptive inputs [2, 41]. The A δ fibers can even be further classified into two separate subtypes of nociceptive nerve fibres: 1) nociceptor nerve fibers involved primarily in the detection of intense noxious mechanical stimuli, and 2) nociceptor nerve fibers that detect noxious mechanical, thermal and chemical stimuli [2, 41]. A single noxious stimuli will evoke two distinct pain sensations referred to as first and second pain [43]. The initial first pain is brief, pricking, and well localized, and the second pain that follows is longer lasting, less localized, and provides a more diffuse, dull and aching pain sensation [41, 43]. Therefore, with a relatively fast conduction velocity compared to C-fibers, and a small receptive field allowing for a more well-localized pain sensation, A δ fibers mediate the first pain sensation [41, 43, 44].

1.2.1.3 C fibers

C fibers have the smallest diameter (0.2-1.5 μm) when compared to A β and A δ fibers, and are also unmyelinated [2, 41]. Therefore, C fibers have a conduction velocity (0.5-2 m/s) slower than both A β and A δ fibers, as well [41]. C fibers are involved in detecting noxious mechanical, thermal and chemical stimuli [2], but they also play a role in detecting certain non-nociceptive inputs as well, such as itch for example [41, 45]. In the context of first and second pain, C-fibers will mediate the second pain sensation, due to the fact that it has a slower conduction velocity than A δ fibers, and C-fiber branches have a broad distribution, which leads to the less localized pain sensation associated with second pain [39, 41, 43, 44].

1.2.1.4 Action Potentials

In the nociceptive response, transduction is dependent on the generation of action potentials [46]. In most neurons, the resting potential is -70mV, due to a greater concentration of Na⁺ externally to the neuron compared to the internal concentration of K⁺, and the threshold potential of these neurons is approximately -55mV [46]. When a nociceptor detects a noxious stimulus, either inhibitory postsynaptic potentials or excitatory postsynaptic potentials are generated, and the summation of these potentials can increase a neuron's membrane potential to

the threshold in order to create an action potential (Figure 1) [46]. An action potential begins with an influx of Na^+ , through voltage-gated Na^+ channels, and the membrane potential is depolarized until the peak potential of the neuron (+40mV) [46].

Following this, the Na^+ channels close, and voltage-gated K^+ channels are opened, causing an efflux of K^+ that will cause membrane potential hyperpolarization [46]. In the end, the membrane potential of the neuron reverts back to the resting potential when the K^+ channels close and through Na^+/K^+

transporters, the Na^+ and K^+ concentrations external to the neuron, and internally, are restored [46]. Thus, the action potentials are carried through the axon to the axon terminal, where the influx of Ca^{2+} through voltage-gated Ca^{2+} channels allow for synaptic transmission [46, 47]. This synaptic transmission is what allows the transmission of the nociceptive signals from one neuron to another. With the influx of Ca^{2+} into the presynaptic neuron, neurotransmitter vesicles release neurotransmitters into the synaptic cleft, and these neurotransmitters bind to ligand-gated ion channels on the postsynaptic neuron membrane to begin action potentials in the second neuron [46]. One such neurotransmitter that plays a pivotal role in sending nociceptive signals to higher centres in the brain is glutamate [41, 48]. However, neuropeptides, such as substance P (SP) and calcitonin-gene related peptide (CGRP), have also been shown to mediate and modulate this process by working in synergy with neurotransmitters, such as glutamate [49].

1.2.2 Transmission

Once a noxious stimuli is detected by nociceptors, the primary afferent fibres transmit the nociceptive signal from the periphery to the dorsal horn of the spinal cord before sending these

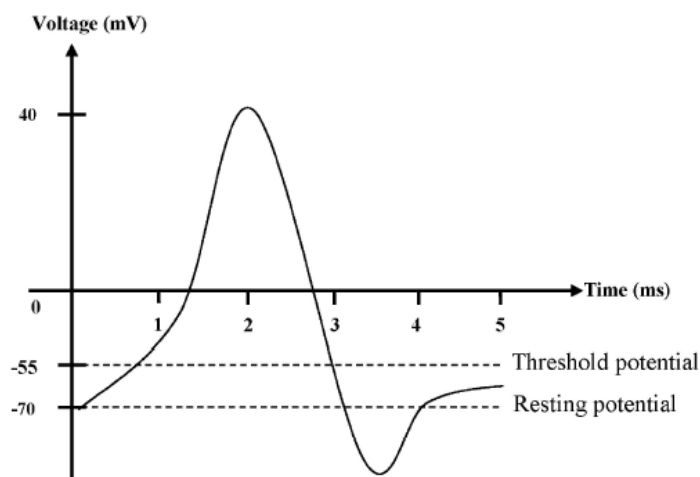


Figure 1. Action Potential of a Neuron. Once the threshold potential (-55mV) is achieved, an action potential is generated until the membrane potential is depolarized to the peak potential (+40mV). The neuron then undergoes hyperpolarization before returning to the resting potential (-70mV). Image taken from Yam, M.F., et al., *General pathways of pain sensation and the major neurotransmitters involved in pain regulation*. International journal of molecular sciences, 2018. **19**(8): p. 2164.

signals up to the brain to process the pain (Figure 2) [41, 50]. At the site of the dorsal horn, the peripheral nociceptive fibers will synapse with more neurons tasked with transmitting the nociceptive signals to higher centers [2, 41, 50, 51]. However, at the synaptic contact between the primary afferent fibers and the second-order neurons, there is a complex network of excitatory and inhibitory interneurons that modulate the nociceptive inputs being sent from the periphery up to the higher centers [41, 50]. One of these inhibitory mechanisms is proposed to occur through the recruitment and activation of inhibitory interneurons at the dorsal horn of the spinal cord, while the A β fibers are conducting non-nociceptive inputs [41]. Following this, the nociceptive signal is projected up to the thalamus, through the spinothalamic and spinoreticular tracts, in order for the higher brain centers to begin the processing and perception of pain [41, 50, 51].

1.2.3 Processing of Pain

The nociceptive signal is carried from the periphery to the thalamus, eventually reaching the cortical regions of the brain [41, 50, 52, 53]. The cortex can be separated to different regions based on its activity in processing pain, and these regions are the primary and secondary somatosensory cortices, insular cortex, anterior cingulate cortex, and the prefrontal cortex [41, 54]. The primary and secondary somatosensory cortices are involved in the processing of sensory information related to the nociception, such as location of the stimuli, as well as intensity and quality discrimination [41, 54-56], whereas the insular and anterior

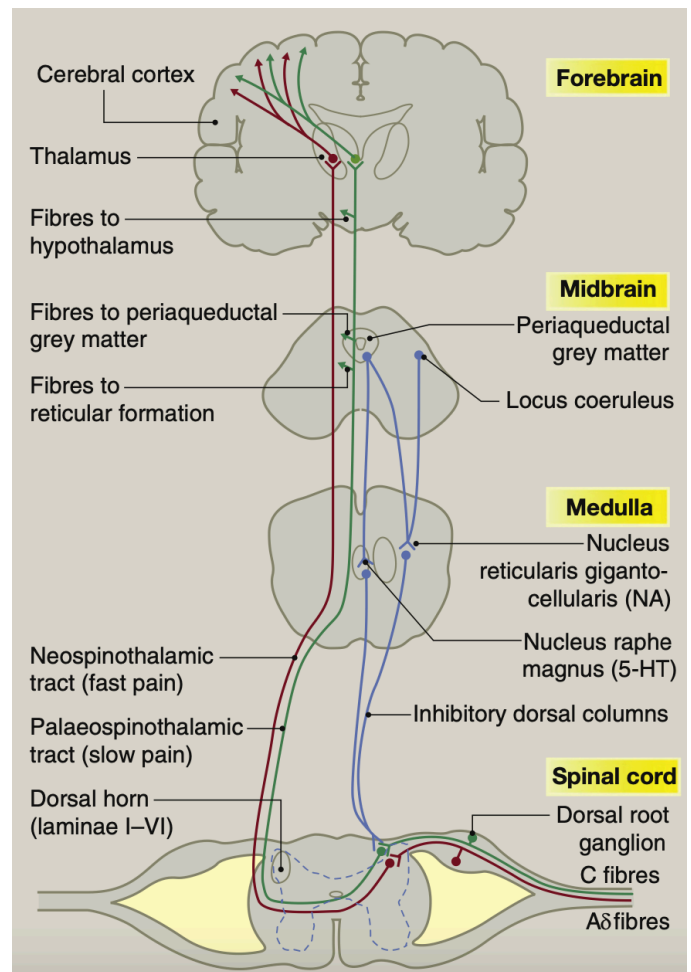


Figure 2. Nociception of Pain. The peripheral nociceptive nerve fibers (A δ fibers and C fibers) transmit nociceptive signals from the periphery to the spinal cord. The peripheral nerve fibers synapse with second-order neurons at the level of the dorsal horn before transmitting nociceptive signals up to the brain. Image taken and adapted from Steeds, C.E., *The anatomy and physiology of pain*. Surgery (Oxford), 2009. **27**(12): p. 507-511.

cingulate cortices are largely involved in the affective processing of the pain experience [41, 54, 57-59]. The prefrontal cortex is also proposed to be involved in the processing of pain in relation to certain cognitive variables such as attention and memory [54, 60, 61]. Thus, the differential processing of pain in the cortical regions highlight the complexity of pain interpretation, and why proper pain management would require an understanding of all of the variables involved in the patient's pain experience, such as the patient's sensory, emotional, and cognitive experience [41].

Although pain processing mainly occurs in the cortical regions of the brain, the activation of the thalamus has also shown to play a role in the pain experience [41, 54, 62]. When imaged, the thalamus has shown to be one of the most activated regions of the brain in response to noxious stimuli [62]. In fact, the stimulation of specific regions of the thalamic somatosensory nucleus has been shown to evoke memories of the affective and sensory information of pain that patients no longer experience, but had experienced previously [41, 63]. Therefore, it is possible that specific neurons in the thalamus are involved in preserving previous experiences of pain, which in turn, may affect one's current perceptions of pain [41].

1.2.4 Descending Control of Pain

Once pain is perceived, the brain sends signals through descending tracts to modulate and reduce pain [41, 50, 64]. The two regions of the brain involved in the descending inhibition of pain are the periaqueductal gray (PAG) and the nucleus raphe magnus (NRM), which are situated in the brainstem [41, 50]. The PAG receives information regarding the perception of pain from the cortical regions of the brain and the thalamus prior to modulating the pain [50, 64]. The PAG also has bidirectional connections to the amygdala [64], which is important due to the neurons in the amygdala that are involved with stress related changes to nociception [65, 66]. Thus, a neural circuit has been proposed, consisting of the amygdala, PAG and the rostral ventromedial medulla (RVM), that causes the inhibition of nociceptive inputs when activated directly by the amygdala through a stressor [65]. The PAG also receives collateral projections from the spinothalamic tract [50], and ascending nociceptive signals from the parabrachial nuclei, which is involved in relaying these signals from the periphery to higher brain centers involved in the processing of pain [64, 67, 68]. With all of these nociceptive inputs, and inputs from brain regions involved in pain interpretation, the activation of neurons in the PAG exerts its

anti-nociceptive properties by sending excitatory signals to neurons in the RVM, that includes the NRM [50, 64, 69].

The activation of the NRM then results in pain inhibition through the complementary activation of inhibitory interneurons in the spinal cord [41, 50]. Another mechanism by which the stimulation of the NRM results in analgesia is through projections down the spinal cord to inhibit dorsal horn neurons involved in pain transmission [50, 70]. However, the descending control of pain is not just inhibitory, but can be facilitatory as well [64, 71]. A study conducted by Fields *et al.* (1983) highlighted the increased activity of “on-cells” during a noxious stimuli, and the inhibition of “off-cells” during this period, at the level of the RVM [41, 64, 71, 72]. On the other hand, the activation of these “off-cells” and inhibition of the “on-cells” is sufficient to provide an analgesic effect [64, 71, 72]. Thus, the brainstem is crucial in the descending modulation of pain since the RVM directly, and the PAG indirectly, modulates both the facilitatory and inhibitory mechanisms for nociception.

Once again, neurotransmitters play a very important role in sending these signals for the descending control of pain [41, 46]. The neurotransmitters serotonin (5-HT) and norepinephrine (NE) are implicated in the descending inhibition of pain from the PAG and NRM, following a painful experience [41]. The neurotransmitter dopamine has also shown to be involved in these inhibitory descending pathways [41, 73, 74]. Furthermore, with opioid receptors present in the PAG and RVM, opioid-mediated analgesia is achieved through the stimulation of these descending inhibitory pathways, as well [29].

1.3 Inflammatory Response

The inflammatory response in the pain experience begins with any tissue injury, such as one would experience in an invasive surgical procedure, which causes the release of a variety of chemical mediators from the cells that have been damaged during tissue injury (Figure 3) [53, 75]. These chemical mediators can then either directly activate peripheral nociceptors to initiate the nociceptive response, or some chemical mediators can recruit immune cells that can further release more mediators that activate nociceptors [53, 75]. Furthermore, the released inflammatory mediators also contribute towards peripheral sensitization, which is an increased responsiveness of the peripheral nociceptive nerve fibers for further noxious stimulation [53, 75].

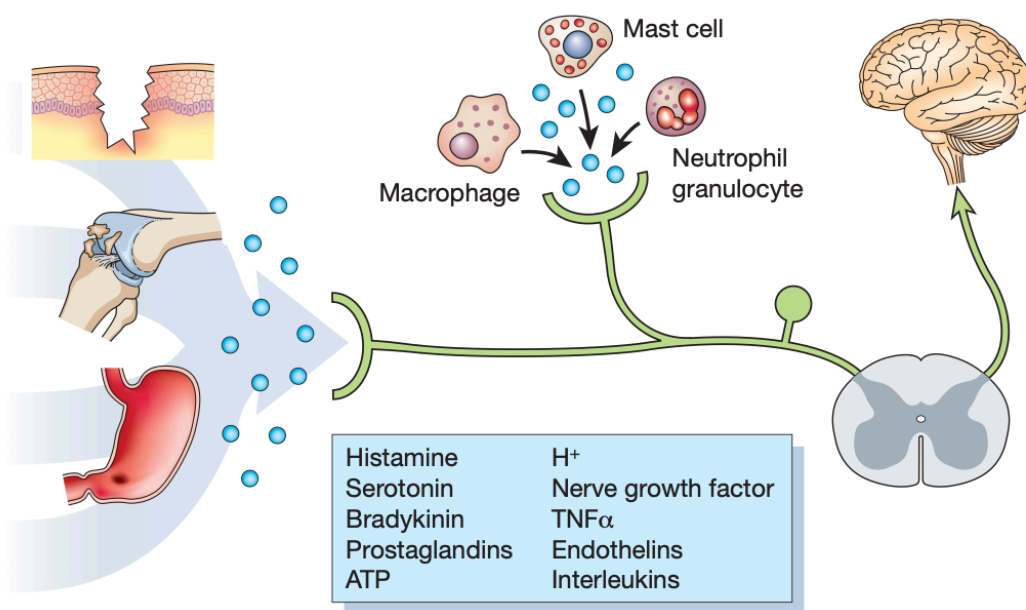


Figure 3. Inflammatory Pain. Upon tissue injury, inflammatory mediators are released from damaged cells to activate nociceptors, recruit immune cells to release more inflammatory mediators, and sensitize the nerve endings for further nociception. Image taken and adapted from Scholz, J. and C.J. Woolf, *Can we conquer pain?* Nature neuroscience, 2002. **5**(11): p. 1062-1067.

One of the inflammatory mediators released during the inflammatory response to tissue injury is bradykinin [53, 75]. Bradykinin has been shown to bind to two different types of bradykinin receptors, B₁ and B₂ [75], but the direct activation of nociceptors in acute pain is proposed to occur through the activation of the B₂ receptor subtype [76, 77]. Furthermore, the bradykinin receptors are localized at the nerve endings of nociceptive neurons [77, 78]. Thus, in a concentration-dependent manner, bradykinin has been shown to be capable of activating these nociceptors, providing evidence for bradykinin as an algogenic mediator [76]. Another set of important inflammatory mediators involved in the acute pain response are cytokines [53, 75]. Cytokines have shown to be largely involved in the peripheral sensitization of nociceptors [75, 79]. A study conducted by Oprée and Kress (2000) highlighted the thermal sensitizing effect of the pro-inflammatory cytokines, interleukin-6 (IL-6), interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), on peripheral nociceptors, in an *in vitro* model [80]. Even in *in vivo* models, the injection of the pro-inflammatory cytokine IL-1 β is also shown to be capable of producing enhanced pain sensitivity [79, 81]. However, when studying the sensitizing effect of TNF- α , prostaglandins were identified as possible mediators of this process [79, 82]. Prostaglandins are also important inflammatory mediators in the acute pain response, as they commonly take part in

the peripheral sensitization of nociceptors, but in certain cases, can directly activate nociceptors, as well [75]. These prostaglandins are produced through the cyclo-oxygenase-2 (COX-2) enzyme in response to pro-inflammatory stimuli from cytokines such as IL-1 and TNF- α [75, 83, 84]. The mechanism of action for NSAID-mediated analgesia involves the inhibition of the COX-2 enzyme activity, and thereby, inhibits prostaglandin synthesis [85]. The enzyme COX-1 is also capable of synthesizing prostaglandins, but in this case, the prostaglandins produced by COX-1 are involved in maintaining the mucosal epithelium of the stomach and intestines [83]. In conclusion, following a surgical insult, a large network of inflammatory molecules would be linked to a patient's postoperative pain experience, as all of these inflammatory mediators are working in conjunction to achieve peripheral sensitization and activate the nociceptive response [75].

1.4 Stress Response

Stress is defined as “a state of real or perceived threat to homeostasis” [86]. Anxiety, on the other hand, can be defined as a state of alarm that is characterized by vigilance, in response to a threat in the near future [87]. The neural circuitry involved in assessing a potential anxiety-inducing situation as threatening requires the coordination of the amygdala, the bed nucleus of the stria terminalis, the ventral hippocampus, and the prefrontal cortex [88]. Of these structures in the brain, the amygdala plays a crucial role in the determination of external stimuli as threatening because it is capable of associating sensory stimuli with emotion [88]. However, another reason the amygdala is important in the face of aversive stimuli is due to its role in the overlapping function between the neural circuitry of fear and anxiety, and the stress response [89]. When faced with a stressor, the amygdala can process this information, and then modulate the hypothalamus to initiate a physiological response [90, 91].

This physiological stress response begins with the activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to the stressful situation (Figure 4) [86]. Initially, neurons from the paraventricular nucleus of the hypothalamus synthesize and release corticotropin-releasing factor (CRF) into hypophyseal portal vessels [86]. Through these vessels, CRF reaches the anterior pituitary gland, which in turn, causes the secretion of adrenocorticotrophic hormone (ACTH) into the circulation [86]. The ACTH then reaches the adrenal cortex, which responds by synthesizing and releasing cortisol into the circulation [86]. In

return, cortisol is involved in modulating a range of physiological functions [86], as well as taking part in cognitive functions such as decision-making and alertness [92]. Lastly, cortisol also plays an important role in the inhibition of the stress response through negative feedback loops in the HPA axis [86, 93].

A second component of the physiological stress response involves the activation of the sympathetic nervous system, but more specifically, the sympathetic-adrenal-medullary system (SAMS) [94, 95]. The activation of the SAMS is associated with certain physiological changes as well, such as

increased heart rate, increased respiration, and increased blood glucose levels [95]. These physiological changes occur in preparation for a “fight or flight” response from the body, which is largely mediated through the release of catecholamines such as norepinephrine, from the nerve endings of sympathetic fibers, and epinephrine, from the adrenal medulla [96]. Thus, the activation of both the HPA axis and the sympathetic nervous system prepares an individual, both physiologically and cognitively, to respond to anxiety-inducing stimuli.

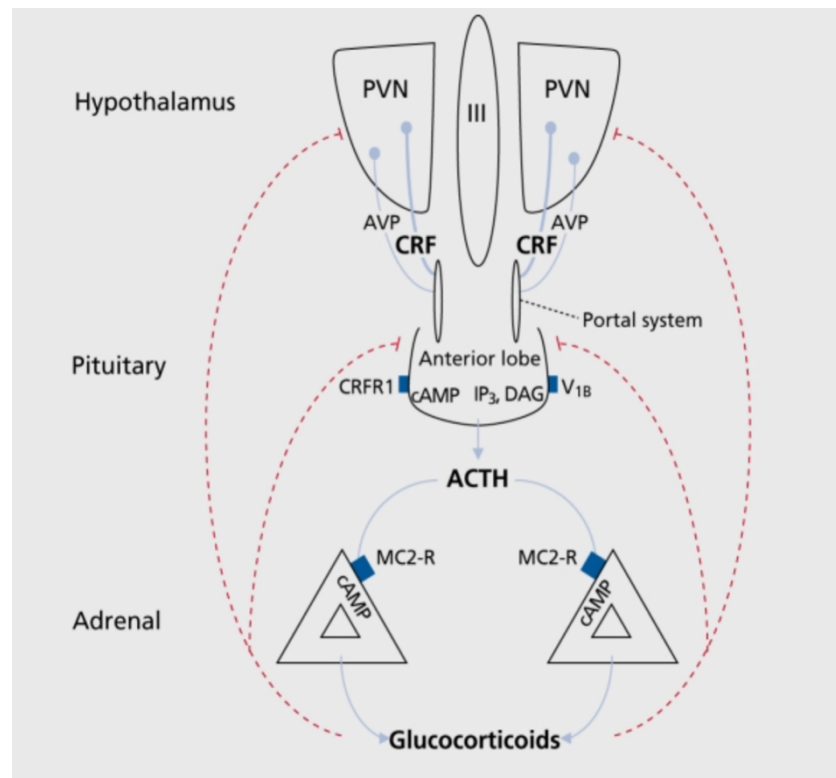


Figure 4. Hypothalamic-pituitary-adrenal (HPA) Axis. In response to stress, the HPA axis is activated; causing corticotropin-releasing factor (CRF) to be secreted from hypothalamic neurons. The CRF travels to the anterior pituitary gland to then cause the release of adrenocorticotrophic hormone (ACTH) into the systemic circulation. The ACTH targets the adrenal cortex to cause the release of glucocorticoids such as cortisol into the circulation. Through a series of negative feedback loops, cortisol is then able to inhibit further activation of the HPA axis, and the secretion of more cortisol. Image taken from Smith, S.M. and W.W. Vale, *The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress*. Dialogues in clinical neuroscience, 2006. 8(4): p. 383-395.

1.5 Central Sensitization

Central sensitization refers to the alterations that occur in the CNS, leading to the sensation of pain in the absence of noxious stimuli, or where the pain experienced is no longer representative of the intensity and/or duration of the peripheral stimuli [97]. The alterations in the CNS refer to an abnormal responsiveness of the nociceptive system, with a shift from “high-threshold nociception to low-threshold pain hypersensitivity” [97]. Repeated C fiber activation has shown to be able to produce central sensitization through altered membrane excitability of the dorsal horn neurons that synapse with the primary afferent neurons, resulting in their increased responsiveness and spontaneous activity [41, 98]. N-methyl-d-aspartate (NMDA) receptors play a key part in the nociceptive signalling pathways as they are activated by the excitatory neurotransmitter, glutamate [48, 99]. Continued activation of NMDA receptors also results in transcriptional changes at the level of the dorsal horn neurons, such as the increased expression of pronociceptive genes, leading to sensitization of the nociceptors [41, 100, 101]. Central sensitization also develops in response to a reduction of inhibitory interneurons and inhibitory transmitter action, leading to disinhibition, and hypersensitivity to pain [100]. Thus, central sensitization is involved in maintaining chronic pain, which is characterized by pain hypersensitivity [101].

1.6 Pain Psychology

Research has shown a significant interaction between pain and an individual’s emotional state [102]. For example, in the case of fear and anxiety, fear has an inhibitory effect on pain, whereas anxiety can increase the pain perceived by an individual [102, 103]. However, continuous exposure to experiences eliciting fear can result in anxiety, in anticipation of said experience, which can then contribute to persistent pain [102]. Similarly, psychological stress and trauma has been shown to be linked to persistent pain, and therefore, is thought to be a possible predisposing factor for persistent pain [102]. One such study identified the combination of childhood abuse and Post-Traumatic Stress Disorder to be a significant risk factor for pain in adulthood [102, 104]. Therefore, understanding the neurobiology that connects pain and psychological state can help identify how an individual would respond to a painful experience [102].

2. ASSESSMENT OF POST-SURGICAL PAIN

Since the personal pain experience has both a sensory and emotional component, the assessment of pain becomes very complex [42, 105]. Furthermore, pain can come in the form of acute pain, such as surgical pain, or it can come in the form of chronic pain, as well as pain as a symptom of another disease [105]. Thus, to begin with, the assessment of pain in the clinic becomes quite difficult when trying to analyze the personal experience of the patients and how it relates to the type of pain they have [105]. However, in the surgical context, being able to accurately assess an individual's pain postoperatively is extremely valuable when trying to provide them with the optimum pain management strategies.

2.1 Tools to Assess Pain

Currently, the assessment of pediatric pain in the clinic is largely accomplished through subjective self-reported measures [106]. Although self-report measures of pediatric pain appear to be the gold standard in assessing the pain experience of children, it does not come without its set of disadvantages and limitations. There are currently over 30 different self-report measures to assess pediatric pain, but of these self-report measures, the Pieces of Hurt Tool [107], the Faces Pain Scale [108] and Faces Pain Scale Revised [109], the Wong-Baker FACES Pain Scale [110], the Oucher [111], and visual analogue scales provide the greatest evidence of reliability and validity [110, 112-118]. However, a systematic review conducted by Stinson *et al.* (2006) saw that in these six self-report measures for pediatric pain, there was not one that was “reliable and valid across age groups and pain types”, especially in the case of pre-school children, for most of these self-report measures [112]. To further this point, one of the major limitations of using self-reported measures for pain becomes apparent in younger children who have difficulty understanding and expressing their pain experience [105]. This limitation would also extend to children who are non-verbal, or have developmental delays, as well [105]. Thus, there is a need to find an objective means of assessing pain in children since an accurate assessment of pain has diagnostic utility, and helps with decision-making in terms of a patient's pain treatment [105].

The most frequent assessment of self-reported pediatric anxiety in the clinic is accomplished through the State-Trait Anxiety Inventory for Children (STAI-C), which has shown to be reliable and valid [119, 120]. This self-report instrument, however, does have its drawbacks when it comes to its use in children who have limited linguistic competency, and

those who have difficulty understanding the items presented on the form [119, 121]. Other self-report measures of anxiety and fear have been developed and validated for the pediatric population [119], such as the Children's Fear Scale [122], the Revised Child Anxiety and Depression Scale [123], and the Visual Analog Anxiety Scale [124]. However, as self-report measures, these tools would also face the same limitations as the STAI-C, and the self-report measures of pediatric pain previously mentioned. Thus, there is also a need to find an objective means of assessing pediatric anxiety, especially in the context of a patient's perioperative experience. Since high levels of preoperative anxiety have been associated with greater postoperative pain experienced, among a list of other poor postoperative outcomes, in the pediatric population [24], the accurate assessment of anxiety would prove to be valuable in identifying patients who may be at risk of poor pain management, and may find benefit in an intervention prior to surgery to reduce their anxiety, for example.

2.2 Biomarkers of Pain

Biomarkers are “objective, quantifiable characteristics of biological processes” that are measured externally and in a reproducible manner [125]. The use of biomarkers are already integrated in the clinical setting; such as when a clinician takes an individual's pulse or blood pressure [125]. Therefore, biomarkers are especially useful in the diagnosis of diseases, and in some cases, also allow clinicians to effectively follow the progress of, and assess the effectiveness of treatments against, these diseases [126]. Thus, biomarkers can be effectively used as diagnostic, prognostic, and predictive tools [126]. In the case of clinical trials, biomarkers are even utilised as surrogate endpoints, based on the evidence that points to the biomarker being able to correctly predict a clinical outcome pertaining to the trial [125]. Current methods in identifying and analyzing biomarkers takes a more proteomic approach by looking at specific molecules in blood, saliva, cerebrospinal fluid, urine, etc. samples [126]. Thus, analyzing biomarkers of pain in these samples can provide a glimpse into the underlying mechanisms of the patient's perceived pain, and this information can help clinicians provide more personalized treatments to patients based on whether the patient is experiencing more inflammatory pain or nociceptive pain, for example.

Due to the involvement of the inflammatory response in the pain experience [53, 75], inflammatory mediators, especially cytokines, have been studied in association with pain in a

variety of painful conditions [127-131]. The pro-inflammatory cytokines TNF- α and IL-6 have been shown to have elevated serum levels in adult patients with chronic low back pain, in comparison to healthy controls [132]. A pilot study conducted by Ang *et al.* assessed the use of cytokines, specifically the chemokines: monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8), as biomarkers of pain in fibromyalgia [133]; which is a chronic pain syndrome [134]. In this study, both MCP-1 and IL-8 plasma concentrations had longitudinal associations to pain severity in fibromyalgia [133].

Neurotransmitters are another class of molecules that are implicated in the nociceptive response [41, 46]. Platelet serotonin (5-HT) levels have been shown to be significantly elevated in headache patients compared to controls, but the monoamine neurotransmitters, dopamine (DA), epinephrine (EPI) and norepinephrine (NE), had plasma levels that were significantly decreased in the headache patients when compared to the controls [135]. Furthermore, there was a negative correlation between plasma EPI levels and headache severity [135], suggesting a degree of variability in the modulation of monoamine neurotransmitters in a pain condition. Catechol-O-methyltransferase, the enzyme responsible for metabolising EPI, NE, and DA, was also discovered to lead to pain sensitivity with the inhibition of its activity, suggesting increased levels of EPI and NE play an important role in pain hypersensitivity [136].

Thus, these studies highlight the potential utility of pro-inflammatory cytokines, and monoamine neurotransmitters as potential markers of pain.

2.2.1 Biomarkers of Pain in the Surgical Context

In the context of acute pain, the gene expressions of the cytokines IL-6, IL-8, and MCP-1 were found to be upregulated following oral surgery, and were correlated to patient pain intensity, except in the group of patients who received the NSAID ketorolac [137]. Even following an orthopedic procedure, the serum inflammatory mediators IL-6, prostaglandin E₂ (PGE₂), and C-reactive protein (CRP) were shown to be positively correlated with self-reported pain scores in an adult population [138]. Therefore, the cytokine IL-6 appears to be a promising inflammatory mediator to assess as a potential biomarker of pain in a pediatric cohort undergoing orthopedic surgery, as well.

In a study looking at the effect of acute postoperative pain on plasma EPI and NE levels in an adult population undergoing an orthopedic or plastic surgery, there was no significant

association between plasma EPI or NE, and self-reported pain severity [139]. On the other hand, when the researchers then separated the self-reported pain scores according to whether the patients experienced no pain, mild pain, moderate pain or severe pain, plasma levels of NE were significantly higher in the patients reporting severe pain, compared to patients reporting no pain or mild pain in the acute postoperative period [139]. A study published from our lab also highlighted preoperative plasma NE and its metabolite, normetanephrine (NME), as well as cerebrospinal fluid NE, to be possible predictors of postoperative pain intensity in AIS patients undergoing corrective surgery [140]. However, in the two aforementioned studies, the researchers assessed these monoamine neurotransmitter levels at one time point, in correlation with self-reported pain scores at a single time point, as well. Perhaps, by assessing the modulation of these monoamine neurotransmitters, and their respective metabolites, across the perioperative period, in conjunction with the perioperative modulation of the patients' pain scores, may provide greater insight into pediatric perioperative pain. Furthermore, the differing perioperative modulation of these monoamines, between patients, could be a viable marker of their postoperative pain outcomes.

2.3 Biomarker of Anxiety

The identification of a biomarker of anxiety, in pediatric patients undergoing major surgery, holds great importance and clinical value. With the analysis of a biomarker of anxiety, preoperatively, clinicians can be made aware of patients who may experience greater pain after surgery, allowing the medical staff to monitor these patients more closely in the acute postoperative periods. The enzyme salivary alpha-amylase (sAA) has been characterized as a marker of SNS activity [141], and elevated sAA levels have been observed in generalized social anxiety disorder [142]. Furthermore, elevated sAA levels have shown to be correlated to increased anxiety in athletes, 3 minutes prior to competition [143]. However, there is conflicting evidence on the association between sAA activity and anxiety, as seen in a study that found no associations between sAA levels and self-reported anxiety in children with and without temporomandibular disorders [144]. Similarly, no associations between sAA levels and dental anxiety were observed in a cohort of adult participants [145]. Therefore, sAA needs to be further studied, in association with anxiety, to validate its possibility as a biomarker of anxiety,

especially with the scarcity in literature looking at correlations between sAA activity and preoperative anxiety, in a pediatric cohort.

3. ADOLESCENT IDIOPATHIC SCOLIOSIS

Scoliosis is defined as a “three dimensional deformity of the spine” with the spine curving laterally at an angle greater than 10° in the coronal plane [146, 147]. The three major subtypes of scoliosis are congenital, syndromic and idiopathic scoliosis [146]. Congenital scoliosis occurs as a result of improper development of the vertebrae, which in turn, may be caused by damage to the fetus during the development of the spine [146, 148]. Syndromic scoliosis refers to any scoliosis that arises in conjunction with a systemic disease [149], and these systemic diseases are often related to disorders that can affect the neuromuscular, skeletal and connective tissue systems [146], such as Ehler Danlos syndrome, Marfan syndrome, Prader-Willi syndrome and Down syndrome [150]. As its name suggests, the cause of idiopathic scoliosis is not known [146]. However, the subtypes of idiopathic scoliosis are separated by the age at which one develops scoliosis, and the diagnosis of said idiopathic scoliosis depends on if the other major types, and causes, of scoliosis have been excluded, as well [146]. If a patient develops a case of idiopathic scoliosis between the ages of 0-3 years, this is referred to as infantile idiopathic scoliosis, and similarly, if the idiopathic scoliosis develops between the ages of 4-10 years, this is known as juvenile idiopathic scoliosis [146]. However, the most common form of scoliosis that is seen in clinics is adolescent idiopathic scoliosis [151], which refers to patients who develop idiopathic scoliosis at an age between 11-18 years [146, 152].

Adolescent idiopathic scoliosis (AIS) is a common disease, with data from several studies putting the prevalence of AIS between 0.47% to 5.2% in children [152-159]. Furthermore, these studies also indicate a prevalence of AIS in females compared to males [155, 158], as well as higher Cobb angles in females [154, 156]. The Cobb angle is measured by making parallel lines from the top of the top vertebra and the bottom of the bottom vertebra which are involved in the curve, and then measuring the angle at which these parallel lines would intersect (Figure 5) [160]. This measurement of the Cobb angle is the gold standard for assessing the severity of the spinal curve [161]. The Cobb angle provides a good indication of how a patient's AIS is managed; where a Cobb angle less than 25° only calls for observation every four to six weeks to assess the curve progression of the patient [146]. However, should the patient's Cobb angle

progress to a 25°-45° curve, brace treatment is often offered to these patients in order to limit and delay curve progression [146]. In the case that a patient's Cobb angle curve exceeds 45°-50°, a surgical intervention would then be required to stop the curve progression [146]. In the case of AIS, the corrective surgery is a spinal fusion surgery that requires screws to be inserted into the spine, and two metal rods are then attached to these screws, at either side of the spine, and tightened (Figure 6) [146].

Although an agreed cause of AIS is not known, there is a consensus that “AIS is a multifactorial disease” with certain genetic factors that could put an individual at risk of developing AIS [146, 160, 162]. These genetic factors, such as polymorphisms in the *CHD7* gene, may affect the normal growth patterns of an adolescent, and in the process, be predisposing them to spinal deformities [162]. Other factors such as neurophysiological dysfunctions [163], and even abnormal modulation of metabolomic and

hormonal factors have also been proposed to play a role in the pathogenesis of AIS [160, 164]. In the end, a better understanding of the combined effects of these various factors, that could have a link to the aetiology of AIS, may provide greater insight into the preventative measures that can be taken, as well as ways to improve current treatment options, for patients diagnosed with AIS.

3.1 Adolescent Idiopathic Scoliosis and Pain

A study conducted by Makino *et al.* (2015) highlighted the prevalence of low back pain (LBP) in AIS patients, who had yet to undergo surgery, to be approximately 35% [165]. Similarly, a study published from our lab also observed that 50% of patients experienced pain prior to their spinal fusion surgery, and furthermore, 40% of them reported mild pain, while 10%

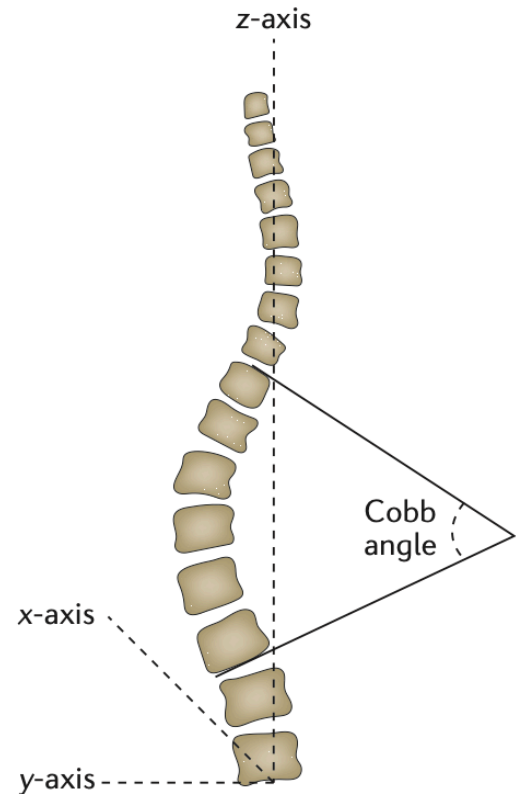


Figure 5. Measuring the Cobb Angle. The Cobb angle is measured by making lines parallel to the top of the top vertebra and the bottom of the bottom vertebra and measuring the angle at which these lines intersect. Image taken and adapted from Cheng, J.C., et al., *Adolescent idiopathic scoliosis*. Nature reviews disease primers, 2015. 1(1): p. 1-21.

of these patients reported moderate to severe pain [140]. However, even though there is a significant prevalence of pain in AIS patients, the role of AIS in causing this pain is not well understood [166]. In trying to understand the relationship between AIS and pain, Theroux *et al.* (2017) identified an association between the severity of main thoracic or lumbar scoliotic curves and the level of pain intensity experienced by

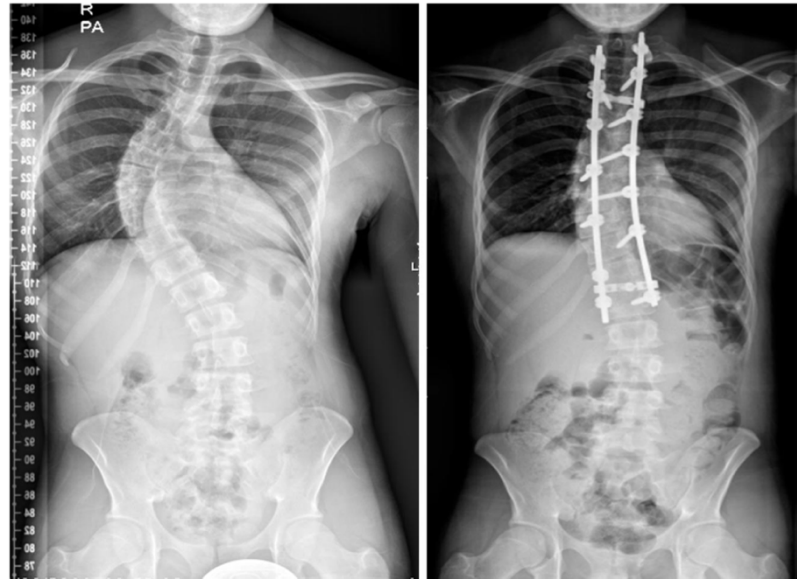


Figure 6. Spinal Fusion Surgery for AIS. Radiograph of patient with AIS before surgery (left) and after spinal fusion surgery (right). Image taken from Altaf, F., et al., *Adolescent idiopathic scoliosis*. Bmj, 2013. **346**.

patients [167]. Furthermore, Teles *et al.* (2020) showed an association between the location of back pain and the location of a patient's major curve [168]; the major curve being the greatest structural curve in a scoliotic patient [169]. Teles *et al.* also identified pelvic asymmetry and decreased thoracic kyphosis (referring to the curve of the thoracic spine [170]) as possible risk factors for back pain in these AIS patients [168]. However, these morphological variables may not be the only driving force of pain in AIS patients, as some psychological variables, such as pain catastrophizing and poor mental health, were also identified as risk factors for back pain in this study [168]. Furthermore, on a molecular level, the activation of Toll-like receptors (TLR) have also been suggested to contribute towards facet joint degeneration in AIS patients, which in turn, may play a role in the development of back pain due to the downstream inflammatory response from TLR activation [171]. Thus, a better understanding of the aetiology of pain in AIS is required in order to provide more targeted pain management for these patients.

3.2 Perioperative Pain in Adolescent Idiopathic Scoliosis Patients

Spinal fusion surgery also introduces another variation of pain for the AIS patients who require corrective surgery. Due to the invasiveness of the surgical procedure, the acute postoperative period following a spinal fusion surgery has also been characterized by the

presence of pain for AIS patients [140, 172]. In some cases, the pain severity experienced in the acute postoperative period has detrimental long-term effects for AIS patients [172]. In some cases, the postoperative pain of these patients can even develop into chronic post-surgical pain [173, 174] which, in turn, could result in poor health and functional disability in the long-term period [175]. Thus, the need for proper analgesia for these patients undergoing such an invasive surgical procedure is evident, but as the etiology of pain can differ between patient to patient [176], assessing the various response systems involved in perioperative pain may provide a means of identifying better targeting pain treatments in the future.

4. RESEARCH QUESTIONS, AND HYPOTHESES OF M.Sc. PROJECT

The research questions posed in this M.Sc. project are:

1. Can plasma IL-6 levels be utilised as a valid biomarker of pain to assess the perioperative pain of AIS patients scheduled to undergo spinal fusion surgery?
2. Can sAA activity be utilised as a valid marker of preoperative anxiety in pediatric patients, and would the level of sAA activity preoperatively be associated with the patient's pain postoperatively?
3. How are plasma monoamines, and their respective metabolites, modulated in the perioperative period, for pediatric patients scheduled to undergo a major orthopedic procedure?
4. Can the perioperative modulation of plasma monoamine levels be utilised as a valid marker of pediatric perioperative pain?

Thus, these research questions were addressed in this M.Sc. project through three sub-projects. In this thesis, they are presented in distinct manuscripts. The objective of the first sub-project was to assess the pro-inflammatory cytokine IL-6 as a proxy of perioperative pain in pediatric patients undergoing a major orthopedic surgery, by analyzing the relationship between plasma IL-6 levels and self-reported pain across the perioperative period. It was hypothesized that self-reported pain scores and plasma IL-6 concentration would increase in the acute postoperative period, before returning to baseline levels (preoperative levels) six weeks after the surgery. It was also hypothesized that higher self-reported pain scores would correlate with higher plasma levels of IL-6 across the perioperative period. The objective of the second sub-project was to assess sAA as a marker of preoperative anxiety in a pediatric population

scheduled to undergo major orthopedic surgery. The hypotheses in this sub-project were that patients would self-report higher levels of anxiety prior to surgery, and greater sAA activity would correlate to an increase in self-reported anxiety levels. Furthermore, increasing levels of sAA activity before surgery would be associated to increasing levels of self-reported postoperative pain intensity. The objective of the third sub-project was to analyze the modulation of perioperative plasma monoamines levels' in a pediatric population scheduled to undergo major orthopedic surgery, and to then assess the perioperative modulation of these plasma monoamines as a marker of self-reported perioperative pain. We hypothesized that the plasma monoamines', and their respective metabolites', levels would increase in the postoperative period, and elevated plasma monoamines' levels would be associated with higher self-reported pain scores across the perioperative period.

METHODOLOGY

The methodology used in this thesis project is described in each of the manuscripts presented in the Results section. The experimental design of these sub-projects (manuscripts) is summarized below:

Patient Recruitment and Consenting Process

The patients recruited for this thesis project agreed to participate in an ongoing longitudinal study aiming to assess the perioperative pain experience of AIS patients undergoing corrective surgery, which had received ethics approval from the Research Ethics Board of McGill University. The AIS patients, aged 12-18 years, were recruited from the outpatient spine clinic of the Shriners Hospitals for Children-Canada, where the surgeon would bring up the study to the patient, if the choice is made to undergo orthopedic surgery. Following this, a research assistant would be tasked with explaining the study in great detail, and obtaining a written informed consent/assent from the patient and/or parents if an agreement has been made by the patient to participate in the study.

General Design

This thesis project was split into three separate manuscripts based on the assessment of plasma IL-6 as a proxy of perioperative pain (manuscript 1), the assessment of sAA as a marker of preoperative anxiety, and as a predictor of postoperative pain (manuscript 2), and the assessment of the perioperative modulation of plasma monoamine levels as a marker of the perioperative modulation of pain intensity (manuscript 3), in AIS patients scheduled to undergo orthopedic surgery. In all three manuscripts, the study design was that of a prospective longitudinal study.

In manuscript 1 and 3, the study design consisted of four timepoints; one week before surgery (baseline), postoperative day 1 (POD1, 24 hours after surgery), postoperative day 2 (POD2, 48 hours after surgery), and six weeks after surgery. At each of these four timepoints, ten ml of blood was collected from the patients for molecular analysis, along with their self-reported pain scores. In manuscript 2, the study design consisted of five timepoints; baseline, the morning of the surgery, POD1, POD2, and six weeks after surgery. At each of these five timepoints, saliva was collected from patients for molecular analysis through the passive drooling method.

Patients' self-reported anxiety scores were taken at baseline, on the morning of surgery, and six weeks after surgery, and patients' self-reported pain scores were taken at baseline, POD1, POD2, and six weeks after surgery.

Outcome Measures

The primary outcome measures of this thesis project were the plasma IL-6 concentrations, sAA activity levels, and the plasma concentrations of EPI, NE, ME, NME, DA, and 5-HT. Plasma IL-6 concentrations were analyzed with human cytokine magnetic 30-plex assays (cat. #LHC6003M, Life Technologies, Vienna, Austria), and sAA activity levels were quantified through α -amylase kinetic enzyme assay kits (cat. #1-1902, Salimetrics, Carlsbad, United States). Plasma concentrations of the monoamines, and their respective metabolites, were analyzed with liquid chromatography-tandem mass spectrometry by Phenoswitch Bioscience (Sherbrooke, QC, Canada).

The secondary outcome measures were the patients' self-reported numerical pain rating scores, on a scale from 0 (no pain at all) to 10 (worst pain imaginable), using the Faces Pain Scale-Revised, the patients' plasma C-reactive protein (CRP) concentrations (in manuscript 1) to confirm the presence of inflammation, which was quantified using enzyme-linked immunosorbent assay kits (cat. #10011236, Cayman Chemicals, Ann Arbor, United States), the patients' self-reported anxiety scores measured using the STAI-C, and patient perioperative medication intake, which was extracted from patients' electronic medical charts.

RESULTS

(Manuscript 1 – Published)

TITLE PAGE

Title: Assessing Interleukin-6 as a proxy of Perioperative Pain Intensity in Adolescents with Idiopathic Scoliosis Undergoing Spinal Fusion Surgery: An Exploratory Study

Shajenth Premachandran¹⁻³, Ljiljana Nikolajev², Jun Jie Liao², Jean A. Ouellet²⁻⁴, Catherine E. Ferland^{2,3,5,6}

¹ Department of Experimental Surgery, McGill University, Montreal, Qc, Canada

² Shriners Hospitals for Children-Canada, Montreal, Qc, Canada

³ McGill Scoliosis and Spine Research Group, Montreal, Qc, Canada

⁴ Division of Orthopedic Surgery, McGill University, Montreal, Qc, Canada

⁵ Research Institute - McGill University Health Centre, Montreal, Qc, Canada

⁶ Department of Anesthesia, McGill University, Montreal, Qc, Canada

Corresponding author

Catherine E. Ferland

Shriners Hospitals for Children-Canada

1003, Decarie Blvd, Montreal, Canada, H4A 0A9

Telephone number: +1 (514) 842-4464 extension 7177

Fax number: +1 (514) 842-8664

E-mail address: catherine.ferland@mcgill.ca

This manuscript is published in the Journal of Community Medicine and Public Health (January 8, 2020) and has been reproduced for the purpose of this thesis with permission granted by Gavin Publishers. DOI: 10.29011/2577-2228.100068

Abstract

Introduction/Aim: The aim of this preliminary study was to assess the pro-inflammatory cytokine interleukin-6 (IL-6) as a physiological proxy of perioperative pain to allow for better targeting pharmacological intervention to adolescents with idiopathic scoliosis that do not respond well to the current standard of care.

Materials and Methods: Twenty-one patients scheduled for an elective spinal fusion surgery participated in the study. Patients reported their pain intensity and provided a blood sample before surgery, one day and two days after surgery (postoperative day 1 and 2 (POD1, POD2)), and 6 weeks after surgery. Concentrations of plasma IL-6 were quantified using a magnetic 30-plex assay (Life Technologies), in parallel to the C-reactive protein (CRP), a marker of inflammation. Repeated measure ANOVAs were used to identify changes in pain and IL-6 levels over time. Correlation analyses were performed to identify associations between the IL-6 concentration and the patient pain intensity at each timepoint. Mann Whitney test was used to see if patients reporting pain preoperatively had higher IL-6 levels than patients without pain.

Results: Forty-seven percent of patients reported having back pain before surgery. There was a significant effect of time on pain level and IL-6 levels, with an increase on POD1 and POD2 ($p < 0.0008$) compared to baseline, and returned to baseline levels 6 weeks after surgery. An association was found between the pain intensity reported before surgery and the preoperative IL-6 levels ($r=0.614$, $p=0.003$), but not after the surgery ($p > 0.05$). IL-6 concentration was significantly higher in patients reporting pain prior to surgery in comparison to patients reporting no pain (Mann-Whitney $U=12.50$, $p=0.0016$).

Discussion: These preliminary findings suggest that circulating IL-6 levels is not a quantifiable proxy for pain intensity after surgery but should be investigated furthermore as a proxy of pain in adolescents with idiopathic scoliosis.

Keywords

Adolescent idiopathic scoliosis; Cytokine; Inflammation; Interleukin-6; Perioperative pain

Introduction

Adolescent Idiopathic Scoliosis (AIS) is a three dimensional deformity of the spine affecting 1-3% of children between 10-16 years [1, 2]. Patients with lateral curvature of a Cobb angle greater than 50° are recommended for spinal fusion surgery with instrumentation to correct the spinal deformity [3]. Spinal fusion surgeries are invasive orthopaedic procedures that cause a significant increase in acute postoperative pain intensity [4, 5] that persists in 64% of patients 2 years after surgery along with 29.5% reporting continued analgesic use for back pain [6]. Furthermore, 35% of patients diagnosed with AIS experience significant pain prior to surgery [7]. The presence of preoperative pain has been observed to predict greater postoperative pain intensity in AIS patients [5], and greater preoperative pain intensity also predicted slower improvements in pain following spinal fusion surgery [8]. Previous studies have shown that inadequate postoperative pain relief can result in persistent post-surgical pain, significantly impairing quality of life [6, 9].

Postoperative standard of care has now turned towards a multimodal approach to achieve proper analgesia through the synergistic properties of different analgesic classes [10]. It has been shown that the combination of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in combination with opioid analgesics provided great efficacy in treating acute postoperative pain by targeting inflammatory processes [11]. This is due to the fact that pro-inflammatory cytokines are direct facilitators for the occurrence of surgical pain [12]. Following tissue injury, cytokines are released by immune cells in the local environment of afferent nerve fiber endings, thus sensitizing nociceptors and contributing to hyperalgesia [13]. Among the pro-inflammatory cytokines, Interleukin-6 (IL-6) has been shown to have an up-regulatory effect during acute inflammation and has been associated to surgical pain [14, 15]. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are currently used to decrease pain, and their anti-inflammatory mechanism of action is mainly targeting the selective inhibition of the enzyme Cyclo-Oxygenase (COX) to reverse inflammation [16-18]. However, current postoperative pain management is still too often insufficient to alleviate the patient's pain, and it may result from the unspecificity of the treatment in targeting molecular mechanisms directly involved in the endogenous nociceptive processes.

The objective of this study was to assess IL-6 as a potential proxy of pain in adolescents undergoing spinal fusion surgery by analyzing its relationship with the self-reported perioperative pain intensity. We hypothesized that pain intensity and IL-6 levels would increase following a surgical insult and return to baseline levels 6 weeks after the surgery. We further hypothesized that high plasma levels of IL-6 would correlate with high self-reported pain intensity over the perioperative period. Such preliminary results were hypothesized to provide clinical significance to improve the perioperative pharmacological interventions proposed to our patients by investigating furthermore IL-6 specific targeting.

Materials and Methods

This study was conducted after obtaining ethics approval from the Research Ethics Board of McGill University (A05-M57-11B and A08-M71-14B). Patients were recruited from the outpatient clinic of the Shriners Hospitals for Children-Canada. Written informed consents were obtained prior to the beginning of the study.

Study participants

Patients aged between 12 and 18 years old, diagnosed with an Adolescent Idiopathic Scoliosis (AIS) and scheduled to undergo an elective spine surgery participated in the study. The exclusion criteria included adolescents who could not speak English or French, patients unable to complete the self-report measurement of pain intensity as a result of a diagnosed developmental delay (e.g. cognitive impairment), and patients with major chronic medical conditions (American Society of Anesthesiology status III or higher) [19].

Pain assessment and molecular analysis

Study variables were assessed at baseline (7-10 days prior to surgery), at postoperative day 1 (POD1, 24 hours after surgery) and postoperative day 2 (POD2, 48 hours after surgery), as well as at six weeks after surgery matching the standard of care follow-up visit with the treating surgeon. At each time point, patients were asked to report their pain intensity using a Numerical

Rating Scale (NRS) from 0 (no pain) to 10 (worst pain imaginable) which has been validated for use in children [20]. Scores ranging from 1 to 3 indicate mild pain intensity, 4 to 6 indicate moderate pain intensity and 7 to 10 indicate severe pain intensity [21].

Subsequently, 10 ml of venous blood were collected in EDTA-coated tubes. The time of collection was recorded and blood samples were immediately centrifuged at 1200 *g* for 10 minutes at 4 °C. Plasma was isolated and stored in microtubes at -80 °C pending analysis. The IL-6 levels were assessed in triplicate and quantified using human cytokine magnetic 30-plex assay (cat. #LHC6003M, Life Technologies, Vienna, Austria), with a sensitivity of <0.5 pg/ml. The mean was calculated for each sample at each time point. To confirm the presence of inflammation, C-reactive protein (CRP) plasma concentrations were also quantified using commercially available enzyme-linked immunosorbent assay kit (cat. #10011236, Cayman Chemicals, Ann Arbor, United States) with a detection range of 46.9-3000 pg/ml. Both procedures were conducted following the manufacturer's instructions.

Anesthetics and analgesics in postoperative pain management

All patients undergoing spinal fusion surgery followed the institutional perioperative anesthesia, surgical, and spinal cord monitoring protocols. The anesthesia protocol was standardized to include total intravenous anesthesia with propofol and remifentanyl or sufentanil, ketamine, and dexamethasone. After induction, all patients received a single injection of spinal morphine (0.005 mg/kg).

The postoperative pain management was standardized for the study purposes and included morphine and ketamine patient-controlled analgesia (PCA, bolus 1/1 mg) upon arrival to the post anesthesia care unit (PACU) with a starting bolus dose of 20 mg/kg, lockout 6 minutes and a 4 hours' maximum dose up to 0.4 mg/kg. The NSAID ketorolac was provided to the patients based on the individual decision of the treating anesthesiologist. Medication administration and time of administration were extracted from the patient medical charts.

Statistical Analysis

Normality of data was assessed using the Shapiro-Wilk test. Non-parametric repeated-measures ANOVA (Kruskal-Wallis test) followed by Dunn's multiple comparisons test to assess differences in time for pain intensity, IL-6 and CRP levels. Due to the non-parametric characteristics of IL-6 plasma levels, Spearman's Rho-rank statistics were used for correlative analyses between perioperative IL-6 concentrations and patient pain intensity. In order to control for the effect of NSAIDs on postoperative IL-6 levels and pain, partial correlations were used for correlative analyses between postoperative IL-6 levels and pain with NSAIDs dosage as a covariate in the analysis. IL-6 levels being a non-parametric variable, Mann-Whitney tests were used to analyze differences in IL-6 concentration between patients reporting pain and no pain preoperatively.

Power analysis was performed using G*Power 3.1 software. An estimated sample size of 19 patients revealed to provide 95% power and a two-sided alpha value of 0.05 for this preliminary study on AIS patients. Data analysis was performed using SPSS software package (IBM SPSS Statistics Version 24.0, Chicago, United States). A two-tailed p value of less than 0.05 was considered statistically significant.

Results

Patient Characteristics

Twenty-one participants were included in this exploratory study, with a majority of female patients. All patients completed the study measurements over the 4 timepoints and measures were collected, with the exception of 5 blood samples that could not be collected on POD1 due to coordination issues.

Almost half of the cohort reported experiencing back pain prior to their surgery. Patients reporting preoperative pain had an average pain score of 2.5 ± 1.1 with a range between 1.0 and 4.0 on the NRS at baseline. Non-parametric Spearman correlations revealed no associations between baseline IL-6 levels and age ($p=0.2400$) or weight ($p=0.1831$). Mann-Whitney tests revealed no significant difference in baseline IL-6 levels between gender ($p>0.9999$), as well. All

patients underwent a posterior spinal fusion with instrumentation either across the thoracic and/or the lumbar spine. Two patients received the Non-Steroidal Anti-Inflammatory Drug (NSAID) ketorolac on POD1 at least 6 hours before blood was collected. Six patients received ketorolac at least 6 hours before blood collection on the POD2 timepoint. None of the patients received ibuprofen prior to blood collection on either POD1 or POD2.

Pain intensity over the perioperative period

A mean score pain intensity of 1.2 ± 1.5 on the 0-10 numerical rating scale was observed with all 21 patients at baseline. Patient's pain intensity increased significantly on POD1 (4.0 ± 1.4) and POD2 (3.6 ± 1.8) from baseline ($p=0.0002$ and $p=0.0008$ respectively, Figure 1A). Six weeks after surgery, patient's average pain intensity decreased and returned to baseline levels, with no significant variation in pain intensity between the two time points ($p>0.05$).

Perioperative molecular levels

Baseline plasma IL-6 concentration was 3.5 ± 2.9 pg/ml. Plasma IL-6 increased significantly in all patients on POD1 (129.2 ± 69.3 pg/ml) and POD2 (114.5 ± 107.3 pg/ml) before returning to baseline levels 6 weeks after surgery ($p<0.0001$, Figure 1B). Baseline plasma CRP concentration was 1.3 ± 2.9 μ g/ml. Plasma CRP levels increased significantly on POD1 (144.8 ± 200.5 μ g/ml) and POD2 (313.2 ± 614.5 μ g/ml) before returning to baseline levels 6 weeks after surgery ($p<0.0001$, Figure 1C).

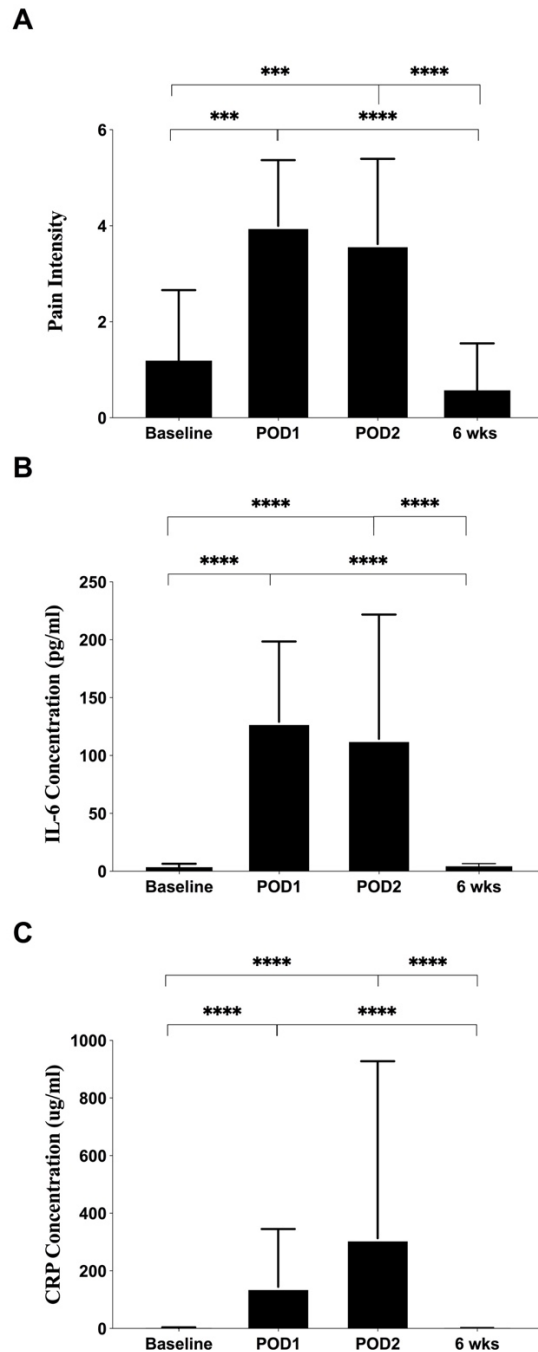


Figure 1: Average pain intensity, IL-6 and CRP levels measured throughout the perioperative period. A) A significant increase in average pain intensity is seen on POD1 ($p=0.0002$) and POD2 ($p=0.0008$) before decreasing to baseline levels 6 weeks after surgery ($n=21$). **B)** Plasma IL-6 levels were shown to increase significantly on POD1 ($p<0.0001$) and POD2 ($p<0.0001$) before returning to baseline levels 6 weeks after surgery ($n=21$). **C)** Plasma CRP levels were shown to increase significantly on POD1 ($p<0.0001$) and POD2 ($p<0.0001$)

before returning to baseline levels 6 weeks after surgery (n=21). (Data expressed as Mean $\pm$ SD. ***= $p<0.001$, ****= $p<0.0001$). POD1: Postoperative Day 1; POD2: Postoperative Day 2; 6 wks = 6 weeks after surgery.

Association between perioperative plasma IL-6 levels and pain intensity

A moderate positive association was found between baseline plasma IL-6 levels and baseline pain intensity ($r=0.614$, $p=0.003$, Figure 2). However, no other associations were observed at any of the postoperative time points, either at 24 hours ($r=-0.3820$, $p=0.1444$), 48 hours ($r=-0.0747$, $p=0.7476$) or 6 weeks ($r=0.2558$, $p=0.2631$) after surgery. Partial correlations, while controlling for ketorolac intake, also showed no association between IL-6 concentrations and pain intensity 24 hours ($r=-0.3510$, $p=0.1990$) or 48 hours ($r=-0.1010$, $p=0.6710$) after surgery. Mann-Whitney tests revealed a significant difference between patients with and without preoperative pain (Mann-Whitney $U=12.50$, $p=0.0016$). Patients with preoperative pain (score greater than 0) had higher levels of plasma IL-6 (5.4 ± 2.9 pg/ml) in comparison to patients without preoperative pain (1.8 ± 1.6 pg/ml) (Figure 3).

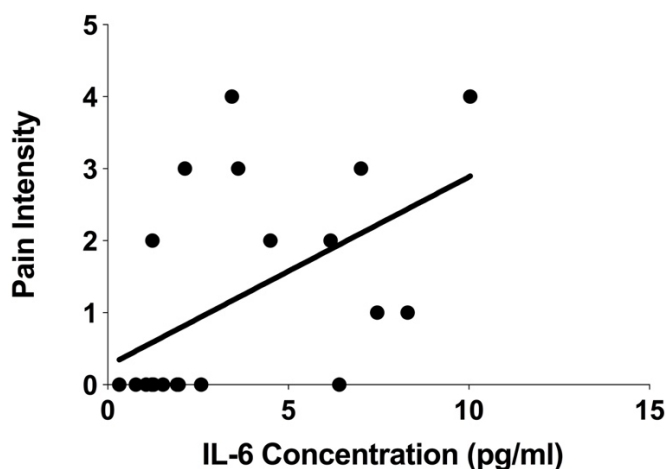


Figure 2: Association between baseline plasma IL-6 levels and pain intensity. A moderate, positive correlation ($r=0.614$, $p=0.003$) is observed between baseline plasma IL-6 levels and baseline average pain intensity (n=21).

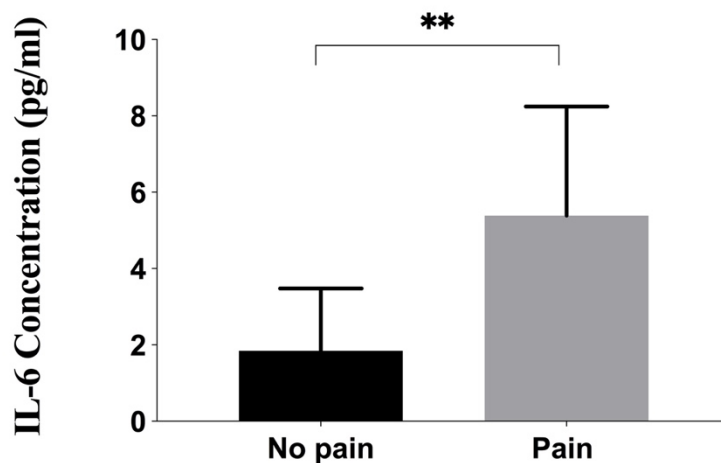


Figure 3: Baseline plasma IL-6 levels of patients with and without preoperative pain intensity. Patients with preoperative pain had higher levels of plasma IL-6 (5.4 ± 2.9 pg/ml) in comparison to patients without preoperative pain (1.8 ± 1.6 pg/ml, $p=0.0016$) ($n=21$). (Data expressed as mean $\pm$ SD. **= $p<0.01$).

Discussion

Adolescent patients who underwent spinal fusion surgery reported experiencing an increase in pain intensity 24 hours and 48 hours after surgery, which returned to baseline levels 6 weeks after surgery. The results confirmed the hypothesis that plasma IL-6 levels would rise following a surgical insult in the acute postoperative period (POD1 and POD2) before decreasing back to baseline levels six weeks after surgery. The inflammation process was also confirmed by a similar result for the CRP levels, suggesting that the invasive nature of the surgery causes an upregulation of pro-inflammatory processes involving IL-6.

We also hypothesized that high pain intensity would correlate with high levels of plasma IL-6. Baseline plasma IL-6 levels and preoperative pain intensity of the patients were associated, but no correlation was observed between IL-6 and pain intensity after surgery. This may have occurred due to the fact that two of the patients received ketorolac on POD1 and six on POD2 at least 4-6 hours before blood collection. Ketorolac has an elimination half-life of 4-6 hours, and in the postoperative period it has been shown to downregulate IL-6 production following surgical wounds from caesarean deliveries and oral surgeries involving the tooth removal [22-24]. This

may have skewed results because six patients received ketorolac prior to blood collection on POD2 and only one of these patients reported severe pain, three reported moderate pain and two reported mild pain on POD2. Following orthopaedic surgery in paediatric patients, ketorolac has been shown to cause a greater decrease in pain scores postoperatively in comparison to morphine alone [25], and thus, it is possible these patients reporting mild to moderate pain after ketorolac administration in our cohort may have had higher pain scores without ketorolac administration. We attempt to control for the effect of ketorolac on POD1 and POD2 on IL-6 concentrations and pain intensity by adding the doses of ketorolac given to patients at least 6 hours prior to blood collection as a co-variate in our partial correlations. However, the effect of the duration of time, within the 6 hour cut-off, between ketorolac administration and blood collection could not be accounted for in this analysis. Furthermore, the effect of ketorolac on the actual change in a patient's pain experience prior to and after ketorolac administration cannot be accounted for in this analysis, as well.

Although a correlation between plasma IL-6 and pain after surgery was not observed, there was a moderate positive correlation between baseline IL-6 levels and pain before surgery. These results are in agreement with the findings observed in a recent publication where preoperative IL-6 levels correlated with preoperative pain in adult patients scheduled to undergo total knee arthroplasty [26]. In their study, the authors also hypothesized that high postoperative serum IL-6 levels would be associated with high pain scores, and concluded that no associations were found. Therefore, it was suggested that the elevated cytokine levels may have a role in contributing to the chronic preoperative pain rather than the acute postoperative pain. In our cohort of AIS patients, we observed that 10 of the 21 patients came to their baseline timepoint with preoperative pain, and these patients had significantly higher levels of IL-6 in comparison to the patients with no preoperative pain. In a previous publication from our team, it was noted that 47% of AIS patients scheduled for spinal fusion surgery had reported sporadic episodes of back pain preoperatively [5]. A previous study has also shown that back pain affects three-quarters of AIS patients prior to surgery, and AIS has also been observed to be a possible risk factor in the development of paediatric low back pain [6, 27, 28]. Back pain is a common cause of chronic Musculoskeletal (MSK) pain in youth [29], and therefore, the role of IL-6 in the pathophysiology of paediatric chronic MSK pain may need to be explored further. Analyzing IL-6 levels in pediatric chronic MSK pain patients in comparison to a healthy pediatric cohort could

help further validate IL-6 as a possible proxy of chronic MSK pain to be pharmacologically targeted.

Lastly, this was an exploratory study and the sample size should be increased to draw any conclusion of the results. Another limitation of this preliminary study resides in the quantification of the circulating molecule, being affected by various covariates related to the surgical experience such as the anesthetics and analgesics intake, as well as the surgical stress induced. An alternative approach would be to test the same hypotheses but by assessing the mRNA levels of IL-6 in the peripheral blood mononuclear cells, along with the plasma IL-6 protein analysis. This may provide more accuracy in the results of the postoperative levels of these inflammatory mediators due to the fact that stable mRNAs usually have longer half-lives than the protein themselves [30]. In this study there was large variation in the plasma IL-6 protein levels on POD1 and POD2, and adding the IL-6 mRNA analysis would indicate if this large variation is replicated in the transcription of IL-6 as well. Thus, this would further validate the results obtained in this study. This would better highlight whether IL-6 is involved in the pathophysiology of chronic MSK pain in the pediatric population or whether it is just a marker of inflammation that is upregulated in response to the inflammatory processes involved with the invasiveness of orthopedic surgeries.

Conclusion

In conclusion, this study revealed that circulating IL-6 is not a proxy of perioperative pain in patients scheduled to undergo spinal fusion surgery and may not be a viable pharmacological target for pain postoperatively. However, an association between IL-6 and pain intensity before surgery was observed, with higher IL-6 in patients reporting back pain. Thus, more work should be done to assess the validity of IL-6 as a possible proxy of pain in adolescents with MSK pain compared to healthy controls.

Acknowledgement

The authors would like to thank the Strategies in Pain Intervention and Evaluation (SPINE) research group for their support and contribution, and the patients for participating in the study.

Disclosures

The authors have no conflicts of interest to declare. The McGill Scoliosis & Spine Research Chair and the Shriners Hospitals financially supported this study.

References

1. Stokes IA (1994) Three-dimensional terminology of spinal deformity. A report presented to the Scoliosis Research Society by the Scoliosis Research Society Working Group on 3-D terminology of spinal deformity. *Spine (Phila Pa 1976)* 19: 236-248.
2. Weinstein SL, Dolan LA, Cheng JC, Danielsson A, Morcuende JA (2008) Adolescent idiopathic scoliosis. *Lancet* 371: 1527-1537.
3. Bridwell KH (1999) Surgical treatment of idiopathic adolescent scoliosis. *Spine (Phila Pa 1976)* 24: 2607-2616.
4. Borgeat A, Blumenthal S (2008) Postoperative pain management following scoliosis surgery. *Curr Opin Anaesthesiol* 21: 313-316.
5. Ferland CE, Saran N, Valois T, Bote S, Chorney JM, et al. (2017) Preoperative distress factors predicting postoperative pain in adolescents undergoing surgery: a preliminary study. *J Pediatr Health Care* 31: 5-15.
6. Landman Z, Oswald T, Sanders J, Diab M (2011) Prevalence and predictors of pain in surgical treatment of adolescent idiopathic scoliosis. *Spine (Phila Pa 1976)* 36: 825-829.
7. Sieberg CB, Simons LE, Edelstein MR, DeAngelis MR, Pielech M, et al. (2013) Pain prevalence and trajectories following pediatric spinal fusion surgery. *J Pain* 14: 1694-1702.
8. Connelly M, Fulmer RD, Prohaska J, Anson L, Dryer L, et al. (2014) Predictors of postoperative pain trajectories in adolescent idiopathic scoliosis. *Spine (Phila Pa 1976)* 39: E174-E181.
9. Wong GT, Yuen VM, Chow BF, Irwin MG (2007) Persistent pain in patients following scoliosis surgery. *Eur Spine J* 16: 1551-1556.
10. Buvanendran A, Kroin JS (2009) Multimodal analgesia for controlling acute postoperative pain. *Curr Opin Anaesthesiol* 22: 588-593.
11. Moote C (1992) Efficacy of nonsteroidal anti-inflammatory drugs in the management of postoperative pain. *Drugs* 44: 14-30.
12. DeVon HA, Piano MR, Rosenfeld AG, Hoppensteadt DA (2014) The association of pain with protein inflammatory biomarkers: a review of the literature. *Nurs Res* 63: 51-62.

13. Stürmer T, Raum E, Buchner M, Gebhardt K, Schiltenswolf M, et al. (2005) Pain and high sensitivity C reactive protein in patients with chronic low back pain and acute sciatic pain. *Ann Rheum Dis* 64: 921-925.
14. Beilin B, Shavit Y, Trabekín E, Mordashev B, Mayburd E, et al. (2003) The effects of postoperative pain management on immune response to surgery. *Anesth Analg* 97: 822-827.
15. Esme H, Kesli R, Apiliogullari B, Duran FM, Yoldas B (2011) Effects of flurbiprofen on CRP, TNF- α , IL-6, and postoperative pain of thoracotomy. *Int J Med Sci* 8: 216-221.
16. Cashman J, McAnulty G (1995) Nonsteroidal anti-inflammatory drugs in perisurgical pain management. Mechanisms of action and rationale for optimum use. *Drugs* 49: 51-70.
17. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, et al. (1998) Cyclooxygenase in biology and disease. *FASEB J* 12: 1063-1073.
18. Vane JR, Botting RM (1998) Mechanism of action of nonsteroidal anti-inflammatory drugs. *Am J Med* 104: 2S-8S.
19. Daabiss M (2011) American Society of Anaesthesiologists physical status classification. *Indian J Anaesth* 55: 111-115.
20. von Baeyer CL, Spagrud LJ, McCormick JC, Choo E, Neville K, et al. (2009) Three new datasets supporting use of the Numerical Rating Scale (NRS-11) for children's self-reports of pain intensity. *Pain* 143: 223-227.
21. Jones KR, Vojir CP, Hutt E, Fink R (2007) Determining mild, moderate, and severe pain equivalency across pain-intensity tools in nursing home residents. *J Rehabil Res Dev* 44: 305-314.
22. Buckley MM, Brogden RN (1990) Ketorolac. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential. *Drugs* 39: 86-109.
23. Carvalho B, Lemmens HJ, Ting V, Angst MS (2013) Postoperative subcutaneous instillation of low-dose ketorolac but not hydromorphone reduces wound exudate concentrations of interleukin-6 and interleukin-10 and improves analgesia following caesarean delivery. *J Pain* 14: 48-56.
24. Singh P, Rastogi S, Bansal M, Kumar S, Singh R, et al. (2015) A prospective study to assess the levels of interleukin-6 following administration of diclofenac, ketorolac and tramadol after surgical removal of lower third molars. *J Maxillofac Oral Surg* 14: 219-225.

25. Vetter TR, Heiner EJ (1994) Intravenous ketorolac as an adjuvant to pediatric patient-controlled analgesia with morphine. *J Clin Anesth* 6: 110-113.
26. Azim S, Nicholson J, Rebecchi MJ, Galbavy W, Feng T, et al. (2018) Interleukin-6 and leptin levels are associated with preoperative pain severity in patients with osteoarthritis but not with acute pain after total knee arthroplasty. *Knee* 25: 25-33.
27. Kovacs FM, Gestoso M, Gil del Real MT, López J, Mufraggi N, et al. (2003) Risk factors for non-specific low back pain in schoolchildren and their parents: a population based study. *Pain* 103: 259-268.
28. Théroux J, Stomski N, Hodgetts CJ, Ballard A, Khadra C, et al. (2017) Prevalence of low back pain in adolescents with idiopathic scoliosis: a systematic review. *Chiropr Man Therap* 25: 10.
29. Clinch J, Eccleston C (2009) Chronic musculoskeletal pain in children: assessment and management. *Rheumatology* 48: 466-474.
30. Bendtsen KM, Jensen MH, Krishna S, Semsey S (2015) The role of mRNA and protein stability in the function of coupled positive and negative feedback systems in eukaryotic cells. *Scientific reports* 5: 13910.

Analyzing a Preoperative Predictor of Postoperative Pain

Plasma IL-6 levels were associated with patients' self-reported pain scores in the preoperative period, but this association was not observed in the acute postoperative period. Thus, further investigation is required into the association between plasma IL-6 levels and pain in AIS patients, as there was a significant increase in the plasma IL-6 concentration of patients reporting pain, when compared to patients reporting no pain, prior to surgery. However, plasma IL-6 levels were not a viable marker of postoperative pain in AIS patients undergoing orthopedic surgery, and there is still a need to identify an objective measure of pain intensity in this cohort of patients.

One of the confounding factors identified in the assessment of IL-6 as a marker of postoperative pain, was the pain medication use in the acute postoperative period. Although NSAID use in the acute postoperative period was added as a co-variate in our analyses, the extent to which the NSAID, ketorolac, has an effect on certain individuals' postoperative pain experiences could not be revealed through this statistical analysis. Therefore, medication intake remains an important variable to take into account when studying postoperative pain biomarker levels in association with postoperative pain.

However, preoperative anxiety has been previously shown to be associated with increased postoperative pain in the pediatric population [24], and therefore, identifying a preoperative biomarker of anxiety in our AIS cohort undergoing orthopedic surgery, may also be a viable predictor of postoperative pain intensity. Thus, sAA activity level was assessed as a proxy of preoperative anxiety, and also assessed for associations with postoperative pain intensity in an AIS cohort undergoing orthopedic surgery, in the following sub-project.

TITLE PAGE

Title: Salivary Alpha-Amylase Assessment as a proxy of Preoperative Anxiety in Adolescents with Scoliosis

Shajenth Premachandran^{1,2}, Kelsey Vickers^{2,7}, Alisson R. Teles^{2,3,5,8}, Jean A. Ouellet^{2,5,6}, Neil Saran^{2,6}, Catherine E. Ferland^{2-5,8}

¹ McGill University, Department of Experimental Surgery, Montreal, Qc, Canada

² Shriners Hospital for Children-Canada, Montreal, Qc, Canada

³ McGill University, Integrated Program in Neuroscience, Montreal, Qc, Canada

⁴ McGill University, Department of Anesthesia, Montreal, Qc, Canada

⁵ Alan Edwards Centre for Research on Pain, Montreal, Qc, Canada

⁶ McGill University, Department of Pediatric Orthopedics, Montreal, Qc, Canada

⁷ McGill University, Department of Anatomy and Cell Biology, Montreal, Qc, Canada

⁸ Research Institute-McGill University Health Centre, Montreal, Qc, Canada

Corresponding author:

Catherine E. Ferland

Shriners Hospitals for Children-Canada

1003, Decarie Blvd, Montreal, Canada, H4A 0A9

Telephone number: +1 (514) 842-4464 extension 7177

E-mail address: catherine.ferland@mcgill.ca

Abstract

Introduction/Aim: The evaluation of perioperative stress and anxiety in patients undergoing major surgery is key to their management and for an efficient recovery. As salivary Alpha-Amylase (sAA) has been shown to be a marker of anxiety, the aim of this study was to assess sAA as a biomarker of pediatric preoperative anxiety.

Materials and Methods: Thirty-one patients scheduled to undergo corrective surgery for adolescent idiopathic scoliosis were enrolled in the study. sAA activity was measured 1 week before surgery (baseline), on the morning of surgery, and 24 hours, 48 hours and six weeks after surgery. Self-reported state anxiety and worst pain intensity were recorded at each time point in parallel.

Results: A significant increase in sAA activity was observed from the morning of surgery to POD2 ($p=0.0259$). No associations were observed between sAA activity levels and self-reported state anxiety at baseline ($r=-0.2661$, $p=0.1985$) or on the morning of surgery ($r=-0.0081$, $p=0.9693$). Similarly, no main effect of sAA activity was observed as a predictor in the variation of patient's self-reported state anxiety over the perioperative period ($p=0.175$).

Discussion: These findings suggest that sAA is not a reliable marker of preoperative anxiety, and is not predictive of postoperative pain intensity, in pediatric patients undergoing major orthopedic surgery.

Keywords

Adolescent idiopathic scoliosis; Alpha-amylase; Anxiety; Orthopedic surgery; Perioperative pain

Introduction

About 50-70% of children undergoing surgery experience a significant amount of perioperative anxiety that can negatively affect their post-surgical recovery [1-3]. Anxiety is defined as an emotional state consisting of tension, apprehension, or nervousness, coupled with heightened activity of the autonomic nervous system [4]. Anxiety has been shown to have a significant negative impact on post-surgical recovery in children, where high anxiety states were found to be predictors of high postoperative pain intensity, higher incidences of postoperative sleep problems, and increased postoperative analgesic intake [3, 5-9]. In clinical settings, patient's anxiety is currently evaluated using self-report questionnaires [10-12]. While useful, these self-reported measures have limitations due to the necessity of the patient to read and comprehend the questions. Therefore, its use is impractical for patients that are either too young, non-verbal, and/or suffer from neurodevelopmental delay [13]. Finding new means to evaluate perioperative anxiety levels could help identify patients vulnerable to poor postoperative outcomes, and better manage their postoperative care.

Anxiety is regarded as a form of stressor that can elicit a physiological response by activating the autonomic nervous system, which consists of the sympathetic and parasympathetic divisions [14, 15]. The sympathetic nervous system (SNS) plays a key role in influencing anxiety state and subsequent pain levels by activating the flight-or fight response resulting in increased alertness of feelings and surroundings [16], thus increasing sympathetic response in anticipation of pain [17-19]. Salivary Alpha-Amylase (sAA), a digestive enzyme responsible for the breakdown of complex sugars into maltose and glucose, has been characterized as a biomarker of the SNS activity [20] and its enzymatic activity has been shown to increase in the presence of both psychological and physical stressors [21, 22]. In this present work, we measured perioperative sAA catalytic activity in adolescents undergoing spinal fusion surgery, a surgical procedure invasive in nature, that is characterized by preoperative anxiety and postoperative pain [23]. The objective of this study was to identify sAA as a potential biomarker of anxiety in adolescents scheduled to undergo a major surgery. We hypothesized that patients would self-report high levels of anxiety before their surgery, and an increase in self-reported anxiety would be associated with an increase in sAA catalytic activity levels. We also hypothesized that

increased preoperative sAA activity levels would be associated with increasing levels of postoperative pain experienced.

Methods

This study was conducted after obtaining ethics approval from the institutional Research Ethics Board (A05-M57-11B and A08-M71-14B). Patients were recruited from the outpatient clinic of our hospital, and written informed consents were obtained prior to the start of the study.

Study Participants

Patients between the ages of 12 and 18 years, diagnosed with adolescent idiopathic scoliosis (AIS) and scheduled to undergo corrective surgery (posterior spinal fusion with instrumentation), participated in the study. The exclusion criteria included adolescents who could not speak English or French, a diagnostic of any developmental delay that would interfere with study measurements completion, and diagnostic of a major chronic medical condition (American Society of Anesthesiology status III or higher) [24].

Study Protocol

Saliva samples from patients were collected through passive drool [25], one week before surgery (baseline), on the morning of surgery, 24 hours after surgery (POD1), 48 hours after surgery (POD2) and 6 weeks after surgery. All samples were obtained between 7am and 10am with at least an hour of fasting before the sample collection. The passive drool technique was implemented with a 1-inch straw that was connected to a sterile tube. The saliva samples were then centrifuged at 3000 x g for 5 min. Supernatant was aliquoted and stored at -80°C until further biochemical analysis.

Patients completed the validated State-Trait Anxiety Inventory-child (STAI-c) questionnaire at baseline, on the morning of the surgery, and six weeks after surgery. The STAI-c is a self-reported questionnaire based on 40 statements, that measures a patient's state and trait anxiety [10]. The state scale measures the child's anxiety during a particular situation, whereas

the trait scale is indicative of the individual differences in a child's proneness to anxiety [4]. Higher scores on the STAI-c represent higher levels of anxiety for the children. The STAI-c questionnaire consists of separate subsets of state and trait anxiety scales with 20 items each that are answered based on a 3-point Likert scale. As the experimental design of this study centres around a surgical insult at a given period of time, and the children's physiological responses to this particular moment, the total score of the state anxiety subscale was used to assess the patient's anxiety across all timepoints in association with sAA activity.

Patients were also asked about their average and worst pain intensity at baseline, POD1, POD2 and 6 weeks after surgery by providing a numerical rating score (NRS) on a scale of 0-10, where 0 is no pain at all and 10 is the worst pain ever.

Biochemical Analysis

Salivary alpha-amylase activity for all the samples were analyzed using the Salimetrics® α -Amylase Kinetic Enzyme Assay Kit and performed according to manufacturer's instructions (catalog number 1-1902, Salimetrics, Carlsbad, CA, USA). Briefly, samples were thawed and centrifuged at 1500 x g for 15 minutes and using a chromogenic substrate, enzymatic activity was measured after 1 minute and 3 minutes following substrate addition. All samples were assessed in triplicates, and changes in absorbances were read at 405nm using a microplate reader set at 37°C (Elx808, Biotek Instruments, Winooski, VT, USA).

Medication Intake Across the Perioperative Period

Medication intake before surgery were collected in an interview form by a research assistant. All patients undergoing spinal fusion surgery followed the institutional perioperative anesthesia, surgical and spinal cord monitoring protocols [23]. To summarize, the anesthesia protocol was standardized across all patients who received total intravenous anesthesia with propofol and remifentanyl or sufentanyl, ketamine, and dexamethasone during surgery. After induction, patients were given an injection of spinal morphine (0.005 mg/kg). Following surgery, all patients received morphine and ketamine patient-controlled analgesia (PCA, bolus 1:1) upon arrival to the post anesthesia care unit (PACU), with a starting bolus dose of 20 mg/kg, lockout

time of 6 minutes and a 4 hours maximum dose up to 0.4 mg/kg. Patients received PCA dose adjustments and additional medication when required (e.g. acetaminophen, naloxone, anti-inflammatory drugs). PCA data was recorded in the patients' electronic medical charts. Postoperative medication administration, and time of administration, were extracted from the patient medical charts.

Statistical Analysis

All analyses were performed using SPSS software version 22 (IBM Corporation, Armonk, NY), and GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA). Normality was assessed using the Shapiro-Wilk test. Associations between sAA activity and any of the patient's sociodemographic results (age, weight and gender) as confounding factors were assessed with Spearman's rank correlation and Mann-Whitney U tests, respectively. Non-parametric Friedman tests followed by Dunn's multiple comparisons tests were performed to assess differences of sAA activity levels and self-reported pain over time, with a significance level of 0.05. Differences in patient's self-reported anxiety over time were assessed with one-way ANOVAs followed by Tukey's multiple comparisons test with a 95% confidence interval (CI) of difference. Non-parametric Spearman rank correlations with a 95% CI were used to assess associations between preoperative sAA activity levels, preoperative anxiety levels, and postoperative levels of pain. Linear mixed models were used to assess the relationship between sAA activity levels, and pain and anxiety over time. Two different models were built, with one for pain and one for anxiety as dependent variables (DV). On both models, time was used as factor and sAA as covariate. Linear effects were applied and the model was built using a non-structured design. An interaction term between sAA and time was tested on both models. Mann-Whitney U tests were performed to identify differences in sAA activity between patients receiving medication, and those who did not receive medication, prior to saliva collection, so as to identify medication intake as a possible confounding factor in this study. All results are expressed as mean $\pm$ standard deviation (SD) unless otherwise stated. The number of study participants to be recruited in this study was determined through a power analysis based on a previous study that found a significant correlation ($r=0.589$, $p<0.01$) between the percent change in sAA levels and State anxiety scores [26]. The required sample size was calculated with

G*Power 3.1 for a univariate correlation. With an α of 0.05 and power of 0.95, the required sample size for this study was 27.

Results

Patient Characteristics

Thirty-one patients were consented to take part in the study, and completed their 6-week follow-up timepoint as scheduled (Table 1). All patients had their saliva samples collected across all timepoints. However, some patients' self-reported measures were not recorded across certain timepoints. Only twenty-five of the 31 participants had their self-reported state anxiety recorded from baseline to 6 weeks after surgery, as a result of coordination issues in filling the questionnaires on the morning of surgery for six of the participants. Furthermore, only twenty-nine of the 31 participants had their self-reported pain recorded at all time points starting from baseline to 6 weeks after surgery. This was a result of missing values in the questionnaires, at baseline and at their 6-week follow-up, for two participants.

Upon looking at the patient's sociodemographic results as confounding factors in the analysis of sAA activity, no associations were observed between preoperative sAA activity and age (CI95%:[-0.4221, 0.3027], $r=-0.06878$, $p=0.7131$, $n=31$) or weight (CI95%:[-0.4788, 0.2367], $r=-0.1391$, $p=0.4554$, $n=31$). No differences were observed in sAA activity preoperatively between female ($n=24$) and male ($n=7$) participants (CI95%:[-0.5012, 0.3153], Mann-Whitney $U=81$, $p=0.9081$).

Perioperative Salivary Alpha Amylase (sAA)

The sAA enzymatic activity from thirty-one saliva samples were measured at all time periods (Figure 1a). Significant change in sAA activity was observed across the perioperative period ($\chi^2(4)=10.81$, $p=0.0288$). The sAA activity levels did not vary from baseline (0.88 ± 0.53 nKat/L) to morning of surgery (0.69 ± 0.45 nKat/L, $p=0.8959$), POD1 (0.89 ± 1.01 nKat/L, $p>0.9999$), POD2 (1.40 ± 1.18 nKat/L, $p>0.05$) or 6 weeks after surgery (0.93 ± 0.60 nKat/L,

$p>0.05$). However, a significant increase in sAA activity was observed from the morning of surgery to POD2 ($p=0.0259$).

Perioperative State Anxiety

State anxiety of twenty-five patients was measured at baseline (1 week before surgery), on the morning of surgery as well as 6 weeks after surgery (Figure 1b). Significant differences in state anxiety were observed across the perioperative period ($F(2, 72)=22.52$, $p<0.0001$). Patient's state anxiety was significantly decreased 6 weeks after their surgery (Mean: 28.92 ± 3.99) in comparison to the baseline values (35.56 ± 5.47 , $CI_{95\%}: [2.922, 10.36]$, $p=0.0002$) and the morning of surgery (39.20 ± 6.68 , $CI_{95\%}: [6.562, 14.00]$, $p<0.0001$). No significant difference in state anxiety was observed between baseline and the morning of the surgery ($CI_{95\%}: [-7.358, 0.0777]$, $p=0.0563$).

Perioperative Pain

Patient self-reported pain was also monitored for twenty-nine participants throughout the postoperative period (Figure 1c and 1d). Significant differences in worst pain levels were observed throughout the perioperative period ($\chi^2(3)=50.12$, $p<0.0001$). Patients worst pain reported increased significantly from baseline to POD1 (4.78 ± 3.04 and 6.97 ± 2.20 respectively, $p=0.0191$). Patients' worst pain experienced decreased 6 weeks after surgery (2.00 ± 1.85) in comparison to baseline ($p=0.0015$), POD1 ($p<0.0001$) and POD2 (6.17 ± 2.02 , $p<0.0001$). Similarly, significant differences in average pain levels were observed throughout the perioperative period ($\chi^2(3)=51.75$, $p<0.0001$). A significant increase in average pain was observed between baseline (2.95 ± 2.14) and POD1 (4.50 ± 2.09 , $p=0.0362$). Significant decreases in average pain intensity were observed from baseline ($p=0.0033$), POD1 ($p<0.0001$) and POD2 (4.14 ± 1.50 , $p<0.0001$) to 6 weeks after surgery (0.93 ± 1.17).

Association Between Perioperative sAA Activity and State Anxiety

No association was observed between baseline sAA activity levels and baseline state anxiety (CI95%:[-0.6062, 0.1563], $r=-0.2661$, $p=0.1985$, $n=25$, Figure 2a). No association was also observed between sAA activity levels and state anxiety on the morning of surgery (CI95%:[-0.4123, 0.3987], $r=-0.0081$, $p=0.9693$, $n=25$, Figure 2b). Furthermore, no main effect of sAA activity or interaction between sAA activity and time were observed as predictors in the variation of patient state anxiety throughout the perioperative period ($p=0.175$, $n=31$).

Predictive Role of sAA Activity on Postoperative Pain

No association was observed between preoperative sAA activity levels at baseline and postoperative levels of worst pain on POD1 (CI95%:[-0.4697, 0.2477], $r=-0.1277$, $p=0.4937$, $n=31$), POD2 (CI95%:[-0.2569, 0.4730], $r=0.1249$, $p=0.5108$, $n=30$), and 6 weeks (CI95%:[-0.4013, 0.3377], $r=-0.0369$, $p=0.8467$, $n=30$) after surgery (Figure 3a-c). No association was also observed between preoperative sAA activity levels on the morning of surgery and postoperative levels of worst pain on POD1 (CI95%:[-0.5569, 0.1335], $r=-0.2421$, $p=0.1894$, $n=31$), POD2 (CI95%:[-0.5594, 0.1438], $r=-0.2389$, $p=0.2037$, $n=30$), and 6 weeks (CI95%:[-0.5982, 0.08614], $r=-0.2931$, $p=0.1159$, $n=30$) after surgery (Figure 4a-c). No association was observed between preoperative sAA activity levels at baseline and postoperative levels of average pain on POD1 (CI95%:[-0.2563, 0.4648], $r=0.1215$, $p=0.5151$, $n=31$), POD2 (CI95%:[-0.2696, 0.4623], $r=0.1114$, $p=0.5577$, $n=30$), and 6 weeks (CI95%:[-0.3833, 0.3564], $r=-0.0156$, $p=0.9348$, $n=30$) after surgery (Figure 3d-f). No association was observed between preoperative sAA activity levels on the morning of surgery and postoperative levels of average pain on POD1 (CI95%:[-0.5541, 0.1160], $r=-0.2485$, $p=0.1776$, $n=31$), POD2 (CI95%:[-0.4595, 0.2522], $r=-0.1189$, $p=0.5314$, $n=30$), and 6 weeks (CI95%:[-0.4560, 0.2563], $r=-0.1145$, $p=0.5468$, $n=30$) after surgery (Figure 4d-f). Furthermore, no main effect of sAA or interaction between sAA and time were observed as predictors in the variation of patient's self-reported pain throughout the perioperative period ($p=0.915$, $n=31$).

Effect of Medication Intake on Study Variables

Two out of the 31 patients received acetaminophen at least 2.5 hours prior to saliva collection on POD1. Eight patients also received acetaminophen at least 2.5 hours prior to saliva collection, and five patients received an increase in the rate of continuous infusion of naloxone at least 1 hour prior to saliva collection on POD2. Acetaminophen has an approximate half-life of 2-2.5 hours in humans [27], and naloxone has an approximate half-life of 1 hour in humans [28, 29].

Naloxone was found to have a significant effect on sAA activity levels, where an increase in enzyme function was observed in patients receiving an increase in the rate of continuous infusion of the drug, compared to the ones that did not on POD2 (2.519 ± 1.952 nKat/L, $n=5$ versus 1.185 ± 0.868 nKat/L, $n=26$ respectively (CI95%:[-1.979, -0.0036], Mann-Whitney $U=28$, $p=0.0476$)) (Figure 5). However, no differences in self-reported worst pain were observed between the patients who received acetaminophen and those who did not receive acetaminophen, at least 2.5 hours prior to self-reporting their pain scores, on POD1 (Mann-Whitney $U=23.5$, $p=0.6364$) or POD2 (Mann-Whitney $U=48.5$, $p=0.1175$). Similarly, no differences in self-reported average pain were observed between the patients who received acetaminophen and those who did not receive acetaminophen, at least 2.5 hours prior to self-reporting their pain scores, on POD1 (Mann-Whitney $U=17$, $p=0.4323$) or POD2 (Mann-Whitney $U=41.5$, $p=0.1121$).

Discussion

The goal of this study was to identify sAA as a potential biomarker of anxiety in adolescents undergoing a major orthopedic procedure, and to assess its relationship with postoperative pain. However, no changes in sAA activity were denoted between baseline and the morning of surgery, while sAA activity levels were greatly increased on POD2. Moreover, no associations were observed between sAA levels and self-reported anxiety or pain throughout the perioperative period. Interestingly, there was a significant increase in sAA activity between the patients who received an increase in the rate of continuous intravenous infusion of naloxone

compared to the patients who received no naloxone or had no changes in the rate of continuous infusion of naloxone at least 1 hour prior to saliva collection on POD2.

Previous studies assessing the relationship between anxiety and sAA have demonstrated an increase in this enzyme's activity in the presence of a stressor, both physical and psychological [30-32]. The number of studies that find significant increases in the enzyme's activity during a stressor, vastly outweigh the studies that indicate no significant changes in sAA activity [33, 34], suggesting sAA is a highly sensitive parameter that is influenced by stress [20]. Still, the assessment of sAA as a marker of anxiety in a surgical setting, with pediatric patients, is limited. In contrast to the previous literature, no significant changes in sAA activity were observed in this study from baseline to the morning of surgery, in anticipation of the surgical procedure. Therefore, the differences observed in the effect of stress on sAA activity may be a result of the different types of stressor and the population being studied [20]. Findings from a study conducted by Robles *et al.* (2012) highlighted a marked decrease in sAA levels during the surgical visit, in an adult population undergoing dental surgery, in comparison to the preoperative consultation and post-surgical visits, suggesting the patients' adrenergic responses may have peaked prior to arrival at the clinic. The anticipation of the surgery itself and the energy expended to reach the clinic on time can activate the stress response, that diminishes upon arrival to the surgical visit [35]. This may explain the lack of change observed in sAA activity from baseline to the morning of the surgery in our study, where patients have the initial anxiety towards the surgical procedure, that diminishes as patients arrive to the clinic and get accustomed to the new environment. Therefore, sAA may be too sensitive and influenced by external factors, that cannot be controlled for in adolescents undergoing a major orthopedic procedure, to identify changes in its activity in anticipation of a surgery, in this study. No associations were also observed between sAA activity levels and self-reported anxiety or pain throughout the perioperative period. However, it must be mentioned that the clinical use of these subjective scores does not hinge on the accuracy of the objective measures of a child's physiological response. Rather, the subjective scores are a tool on its own that may be complemented by an objective measure [36]. Furthermore, the sensitivity of response systems to a certain anxiety inducing stimuli may vary from individual to individual [37]; thus, this may explain the lack of a direct correlation between the sAA activity levels and self-reported anxiety in this study. Although the STAI-c is widely utilized in a multitude of studies, certain drawbacks

to the questionnaire are still present. One study highlighted the fact that children aged 5-18 years had difficulty understanding and completing the STAI-c, with only 60% of children having completed the entirety of the questionnaire in a sample size of five-hundred and seventy-two children [38]. Therefore, it is possible that the self-reported anxiety scores of certain individuals, used in this study, may not be the best representation of their actual anxiety levels when trying to associate these subjective scores with an objective physiological marker of anxiety.

However, a significant increase in sAA activity was noted on POD2, but no significant increase in sAA activity was observed on POD1 or 6 weeks after surgery. Morphine is used as an opioid analgesic in the postoperative periods following major surgery through PCA, and opioids are also a drug class commonly associated with causing dry mouth [39]. Therefore, with limited saliva production, the activity of sAA during sample collection, in the acute postoperative periods, may be abnormally declined. Thus, the administration of morphine in the postoperative periods can explain why no differences in preoperative sAA activity levels and sAA activity levels on POD1 are observed. Interestingly, naloxone was shown to have an effect on sAA activity on POD2, with a significant increase in sAA activity noted in the patients who had an increase in their rate of continuous intravenous infusion of naloxone within 1 hour prior to saliva collection. Naloxone is an opioid receptor antagonist used to reverse the effects of opioids, and has been shown to be effective in reducing opioid-induced pruritis [40, 41]. Naloxone has also been shown to increase sympathetic activity in multiple animal models [42, 43]. However, only 5 of 31 patients received an increase in the rate of continuous intravenous infusion of naloxone, due to opioid-induced pruritus, 1 hour prior to saliva collection on POD2, and no patients received naloxone or had a change in their rate of continuous infusion of naloxone 1 hour prior to saliva collection on POD1. Although this result may be due to the individual variability in sAA activity within a small sample size, this finding suggests that naloxone administration might also be responsible for the significant increase of sAA activity on POD2. Furthermore, the 5 patients who received an increased rate of continuous infusion of naloxone on POD2 had higher sAA activity than the median sAA activity on POD2. Therefore, in future studies, it would be interesting to look at the direct correlations that may exist between sAA activity and the administration of naloxone to patients, and further assessments should be made between the naloxone-sympathetic nervous system-sAA activity relationship. These results also highlight some of the difficulties that arise when identifying any salivary biomarkers in a post-surgical

setting. The effects of different drugs after surgery, on the physiological response, is difficult to account for, and the extent to which it may affect certain individuals is yet to be explored and quantified.

Certain limiting factors in this study have to be addressed. First, there is an approximate 3:1 sex ratio of our recruited participants, with 24 female and 7 male patients, as AIS is predominant in females [44]. This would limit the external validity of our findings to the general pediatric population, especially when taking into consideration the conflicting evidence on the sex differences in basal sAA levels [45, 46]. This study also does not have a control group to compare the sAA activity to the group undergoing surgery. Although the sample collection one week before surgery is meant to be the basal level of sAA activity, it is possible that the anticipation of the upcoming surgery, and the fact that the saliva collection is conducted in a clinical setting, may affect the sAA activity of these individuals even one week before surgery. Therefore, the addition of a control group in similar future studies, assessing sAA activity perioperatively, would allow for better understanding of a patient's stress response before a major surgery.

Conclusion

This study demonstrated that salivary alpha amylase is not a viable proxy of perioperative anxiety. Further investigation should be conducted to find means of evaluating stress and anxiety in children vulnerable to poor postoperative outcomes after major surgery. After surgery, naloxone administration may have an effect on the increase in patients' sAA activity, through the mediation of the sympathetic nervous system (SNS). Assessing such relationship would help clarify the viability of assessing sAA as a perioperative salivary biomarker in patients undergoing major orthopedic surgery.

Acknowledgements

The author would like to thank the Strategies in Pain INtervention and Evaluation (SPINE) research group for their continuous efforts, and the patients for participating in the study. The Shriners Hospitals financially supported this study.

References:

1. Erhaze, E.K., M. Dowling, and D. Devane, *Parental presence at anaesthesia induction: a systematic review*. International journal of nursing practice, 2016. **22**(4): p. 397-407.
2. Kain, Z.N. and L. Mayes, *Anxiety in children during the perioperative period*. Child development and behavioral pediatrics, 1996: p. 85-103.
3. Kain, Z.N., et al., *Preoperative anxiety, postoperative pain, and behavioral recovery in young children undergoing surgery*. Pediatrics, 2006. **118**(2): p. 651-658.
4. Spielberger, C.D., *State-Trait anxiety inventory*. The Corsini encyclopedia of psychology, 2010: p. 1-1.
5. Kain, Z., *Postoperative maladaptive behavioral changes in children: incidence, risks factors and interventions*. Acta Anaesthesiologica Belgica, 2000. **51**(4): p. 217.
6. Gouin, J.-P. and J.K. Kiecolt-Glaser, *The impact of psychological stress on wound healing: methods and mechanisms*. Immunology and Allergy Clinics, 2011. **31**(1): p. 81-93.
7. Chieng, Y.J.S., et al., *Perioperative anxiety and postoperative pain in children and adolescents undergoing elective surgical procedures: a quantitative systematic review*. Journal of advanced nursing, 2014. **70**(2): p. 243-255.
8. LaMontagne, L.L., J.T. Hepworth, and M.H. Salisbury, *Anxiety and postoperative pain in children who undergo major orthopedic surgery*. Applied Nursing Research, 2001. **14**(3): p. 119-124.
9. Logan, D.E. and J.B. Rose, *Is postoperative pain a self-fulfilling prophecy? Expectancy effects on postoperative pain and patient-controlled analgesia use among adolescent surgical patients*. Journal of Pediatric Psychology, 2005. **30**(2): p. 187-196.
10. Spielberger, C.D., *State-trait anxiety inventory for children*. 1973: Consulting Psychologists Press.
11. Reynolds, C.R. and B.O. Richmond, *What I think and feel: A revised measure of children's manifest anxiety*. Journal of abnormal child psychology, 1978. **6**(2): p. 271-280.

12. March, J.S., et al., *The Multidimensional Anxiety Scale for Children (MASC): factor structure, reliability, and validity*. Journal of the American academy of child & adolescent psychiatry, 1997. **36**(4): p. 554-565.
13. Nilsson, S., M. Buchholz, and G. Thunberg, *Assessing children's anxiety using the modified short state-trait anxiety inventory and talking mats: A pilot study*. Nursing Research and Practice, 2012. **2012**.
14. Sadi, H., M. Finkelman, and M. Rosenberg, *Salivary cortisol, salivary alpha amylase, and the dental anxiety scale*. Anesthesia progress, 2013. **60**(2): p. 46-53.
15. McCorry, L.K., *Physiology of the autonomic nervous system*. American journal of pharmaceutical education, 2007. **71**(4).
16. Romero, M.L. and L.K. Butler, *Endocrinology of stress*. International Journal of Comparative Psychology, 2007. **20**(2).
17. Walding, M.F., *Pain, anxiety and powerlessness*. Journal of Advanced Nursing, 1991. **16**(4): p. 388-397.
18. Jansen, A.S., et al., *Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response*. Science, 1995. **270**(5236): p. 644-646.
19. Seifert, F., et al., *Brain activity during sympathetic response in anticipation and experience of pain*. Human brain mapping, 2013. **34**(8): p. 1768-1782.
20. Nater, U.M. and N. Rohleder, *Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research*. Psychoneuroendocrinology, 2009. **34**(4): p. 486-496.
21. Nater, U.M., et al., *Stress-induced changes in human salivary alpha-amylase activity—associations with adrenergic activity*. Psychoneuroendocrinology, 2006. **31**(1): p. 49-58.
22. Tanaka, Y., et al., *Salivary alpha-amylase and cortisol responsiveness following electrical stimulation stress in major depressive disorder patients*. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2012. **36**(2): p. 220-224.
23. Ferland, C.E., et al., *Preoperative distress factors predicting postoperative pain in adolescents undergoing surgery: a preliminary study*. Journal of Pediatric Health Care, 2017. **31**(1): p. 5-15.
24. Daabiss, M., *American Society of Anaesthesiologists physical status classification*. Indian journal of anaesthesia, 2011. **55**(2): p. 111.

25. Salimetrics, L. and L. SalivaBio, *Saliva collection and handling advice*. State College, PA: Salimetrics LLC, SalivaBio LLC, 2011: p. 1-14.
26. Noto, Y., et al., *The relationship between salivary biomarkers and state-trait anxiety inventory score under mental arithmetic stress: a pilot study*. *Anesthesia & Analgesia*, 2005. **101**(6): p. 1873-1876.
27. Forrest, J.A., J. Clements, and L. Prescott, *Clinical pharmacokinetics of paracetamol*. *Clinical pharmacokinetics*, 1982. **7**(2): p. 93-107.
28. Ngai, S.H., et al., *Pharmacokinetics of naloxone in rats and in man: basis for its potency and short duration of action*. *Anesthesiology*, 1976. **44**(5): p. 398-401.
29. Rzasa Lynn, R. and J.L. Galinkin, *Naloxone dosage for opioid reversal: current evidence and clinical implications*. *Therapeutic advances in drug safety*, 2018. **9**(1): p. 63-88.
30. Ljungberg, G., et al., *Saliva and marathon running*. *Scandinavian journal of medicine & science in sports*, 1997. **7**(4): p. 214-219.
31. Rohleder, N., et al., *Psychosocial stress-induced activation of salivary alpha-amylase*. *Annals of the New York Academy of Sciences*, 2004. **1032**: p. 258-263.
32. Schoofs, D., R. Hartmann, and O. Wolf, *Neuroendocrine stress responses to an oral academic examination: No strong influence of sex, repeated participation and personality traits*. *Stress*, 2008. **11**(1): p. 52-61.
33. Morrison, W.E., et al., *Noise, stress, and annoyance in a pediatric intensive care unit*. *Critical care medicine*, 2003. **31**(1): p. 113-119.
34. Schaffer, L., et al., *Cardiac autonomic balance in small-for-gestational-age neonates*. *American Journal of Physiology-Heart and Circulatory Physiology*, 2008. **294**(2): p. H884-H890.
35. Robles, T.F., et al., *Utility of a salivary biosensor for objective assessment of surgery-related stress*. *Journal of oral and maxillofacial surgery*, 2012. **70**(10): p. 2256-2263.
36. Thyer, B.A., et al., *Autonomic correlates of the subjective anxiety scale*. *Journal of behavior therapy and experimental psychiatry*, 1984. **15**(1): p. 3-7.
37. Lang, P.J., *Physiological assessment of anxiety and fear*. *Behavioral assessment: New directions in clinical psychology*, 1977: p. 178-195.
38. Schisler, T., J. Lander, and S. Fowler-Kerry, *Assessing children's state anxiety*. *Journal of pain and symptom management*, 1998. **16**(2): p. 80-86.

39. Scully Cbe, C., *Drug effects on salivary glands: dry mouth*. Oral diseases, 2003. **9**(4): p. 165-176.
40. Sawynok, J., C. Pinsky, and F. LaBella, *On the specificity of naloxone as an opiate antagonist*. Life sciences, 1979. **25**(19): p. 1621-1631.
41. He, F., Y. Jiang, and L. Li, *The effect of naloxone treatment on opioid-induced side effects: A meta-analysis of randomized and controlled trails*. Medicine, 2016. **95**(37).
42. Koyama, S., et al., *Effect of naloxone on baroreflex, sympathetic tone and blood pressure in the cat*. European journal of pharmacology, 1983. **90**(4): p. 367-376.
43. Feria, M., et al., *Naloxone potentiation of cardiovascular responses to sympathomimetic amines in the rat*. Journal of Pharmacology and Experimental Therapeutics, 1990. **255**(2): p. 523-528.
44. Roberts, D.W., et al., *Male-female differences in Scoliosis Research Society-30 scores in adolescent idiopathic scoliosis*. Spine, 2011. **36**(1): p. E53-E59.
45. van Stegeren, A.H., O.T. Wolf, and M. Kindt, *Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex*. International journal of Psychophysiology, 2008. **69**(1): p. 33-40.
46. Nater, U.M., et al., *Determinants of the diurnal course of salivary alpha-amylase*. Psychoneuroendocrinology, 2007. **32**(4): p. 392-401.

Table 1. Patient Characteristics

Sociodemographic	n=31
Age, years (SD)	15.54 ($\pm$ 1.82)
Sex, female/male	24/7
Clinical Presurgical Indicators	
Worst Pain 0-10, Mean (SD)	4.78 ($\pm$ 2.99)
Average Pain 0-10, Mean (SD)	2.95 ($\pm$ 2.14)
Anxiety State, score (SD)	35.62 ($\pm$ 6.35)
sAA Activity, nKat/L (Range)	0.88 (0.30-2.91)

SD: Standard Deviation.

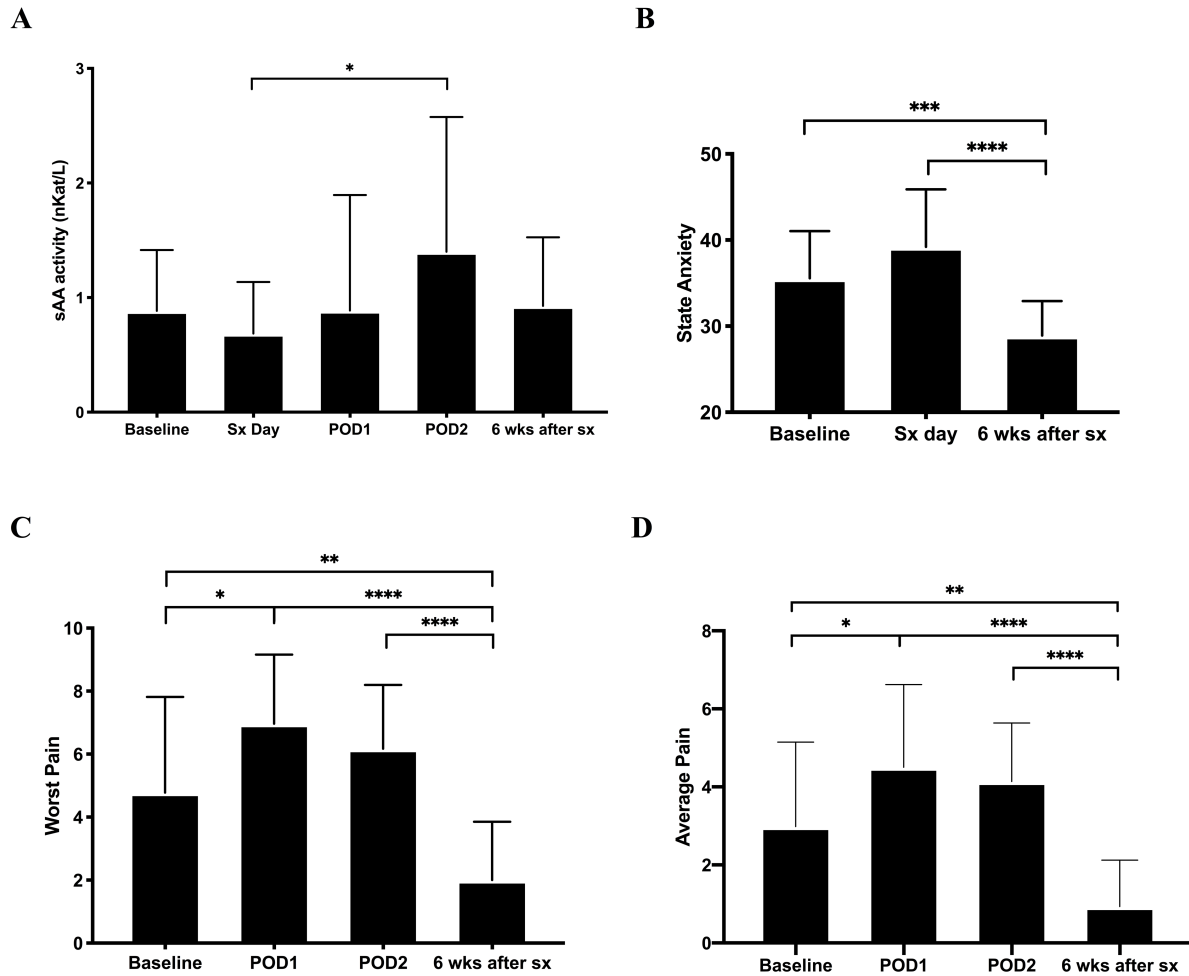


Figure 1. Salivary Alpha Amylase Activity, State Anxiety, Worst Pain and Average Pain Intensity throughout the perioperative period. A) A significant increase in sAA activity is observed from Sx day to POD2 ($p = 0.0259$) ($n=31$). **B)** A significant decrease in state anxiety is observed at 6 weeks after surgery, compared to values at Baseline ($p = 0.0002$) and Sx day ($p<0.0001$) ($n=25$). **C)** Worst Pain increased significantly from Baseline to POD1 ($p = 0.0191$), before decreasing during the 6 weeks after surgery. A significant decrease in worst pain is observed from Baseline ($p = 0.0015$), POD1 ($p<0.0001$), and POD2 ($p<0.0001$) to 6 weeks after surgery. ($n=29$) **D)** Average Pain increased significantly from Baseline to POD1 ($p=0.0362$) before decreasing during the 6 weeks after surgery. Average Pain decreases significantly from Baseline ($p=0.0033$), POD1 ($p<0.0001$), and POD2 ($p<0.0001$) to 6 weeks after surgery. ($n=29$) (Data expressed as Mean $\pm$ SD. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$).

Baseline = 1 week before surgery; Sx day = morning of surgery; POD1 = postoperative day 1 (24 hours after surgery); POD2 = (48 hours after surgery); 6 wks after sx = 6 weeks after surgery.

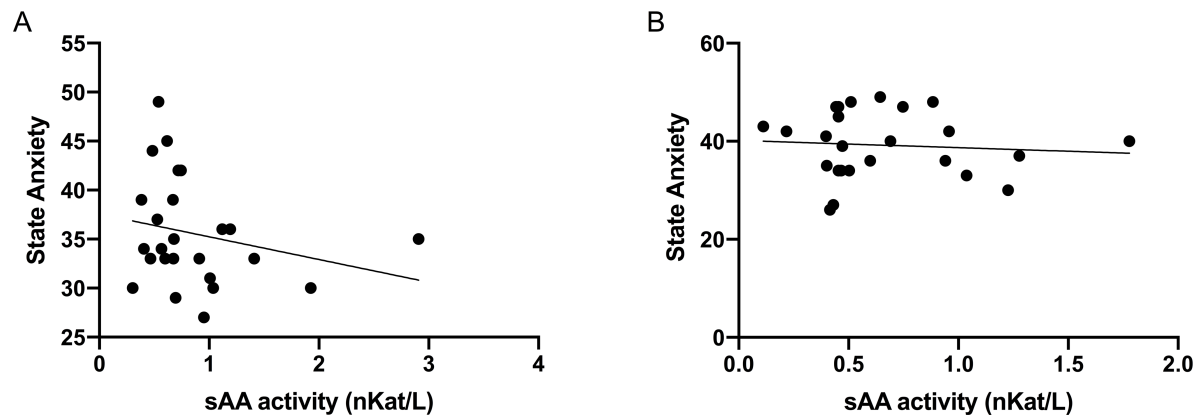


Figure 2. Associations between sAA activity and State Anxiety at baseline and on the morning of surgery. A) No significant correlation is observed between sAA activity and State anxiety at baseline ($r=-0.2661$, $p=0.1985$) ($n=25$). **B)** No significant correlation is observed between sAA activity and State anxiety on the morning of surgery ($r=-0.0081$, $p=0.9693$) ($n=25$). Baseline = 1 week before surgery.

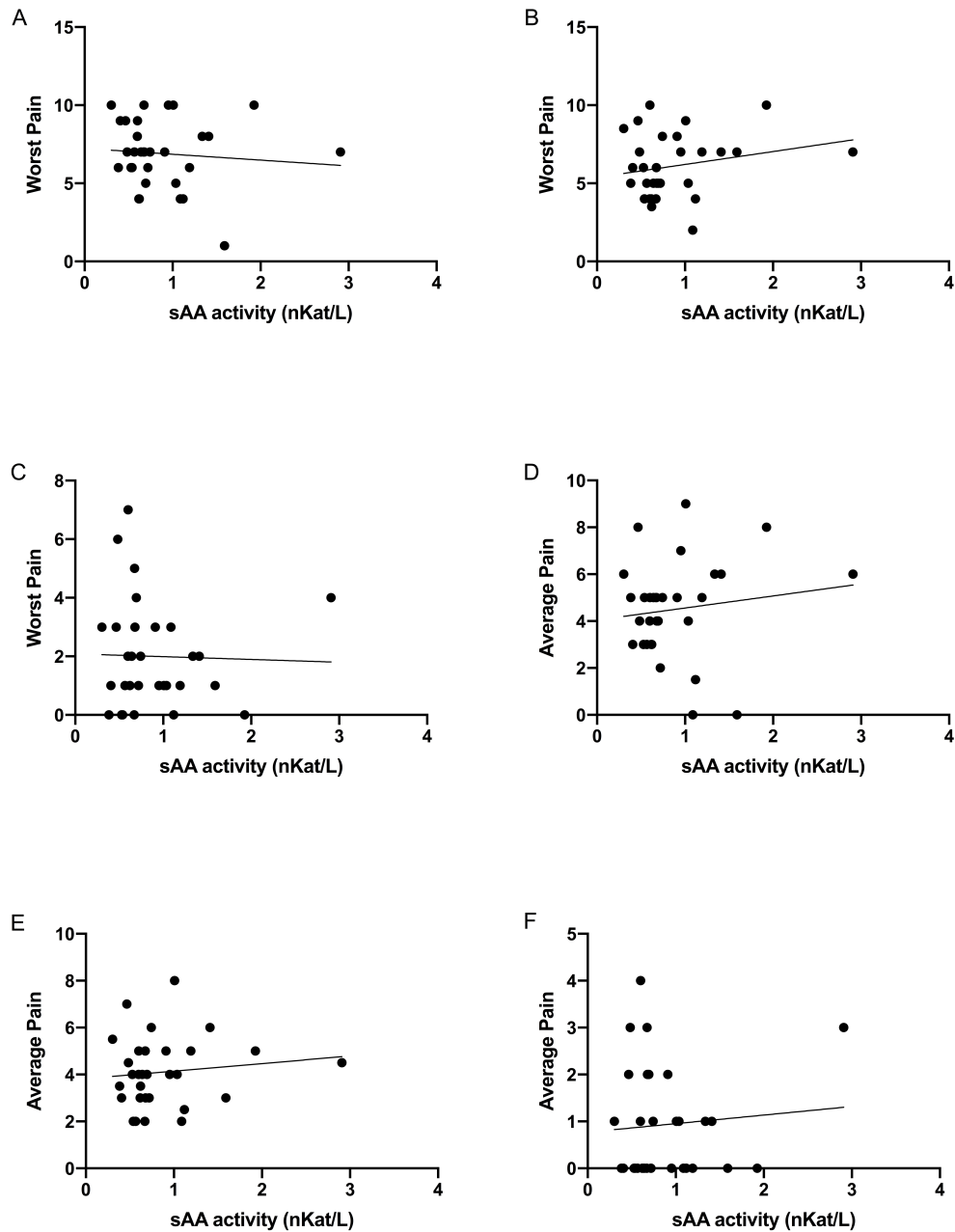


Figure 3. Associations between baseline sAA activity and postoperative worst and average pain intensity. **A)** No significant correlation is observed between baseline sAA activity and worst pain on POD1 ($r=-0.1277$, $p=0.4937$) ($n=31$). **B)** No significant correlation is observed between baseline sAA activity and worst pain on POD2 ($r=0.1249$, $p=0.5108$) ($n=30$). **C)** No significant correlation is observed between baseline sAA activity and worst pain 6 weeks after surgery ($r=-0.0369$, $p=0.8467$) ($n=30$). **D)** No significant correlation is observed between baseline sAA activity and average pain on POD1 ($r=0.1215$, $p=0.5151$) ($n=31$). **E)** No significant

correlation is observed between baseline sAA activity and average pain on POD2 ($r=0.1114$, $p=0.5577$) ($n=30$). F) No significant correlation is observed between baseline sAA activity and average pain 6 weeks after surgery ($r=0.0156$, $p=0.9348$) ($n=30$). Baseline = 1 week before surgery; POD1 = postoperative day 1 (24 hours after surgery); POD2 = (48 hours after surgery).

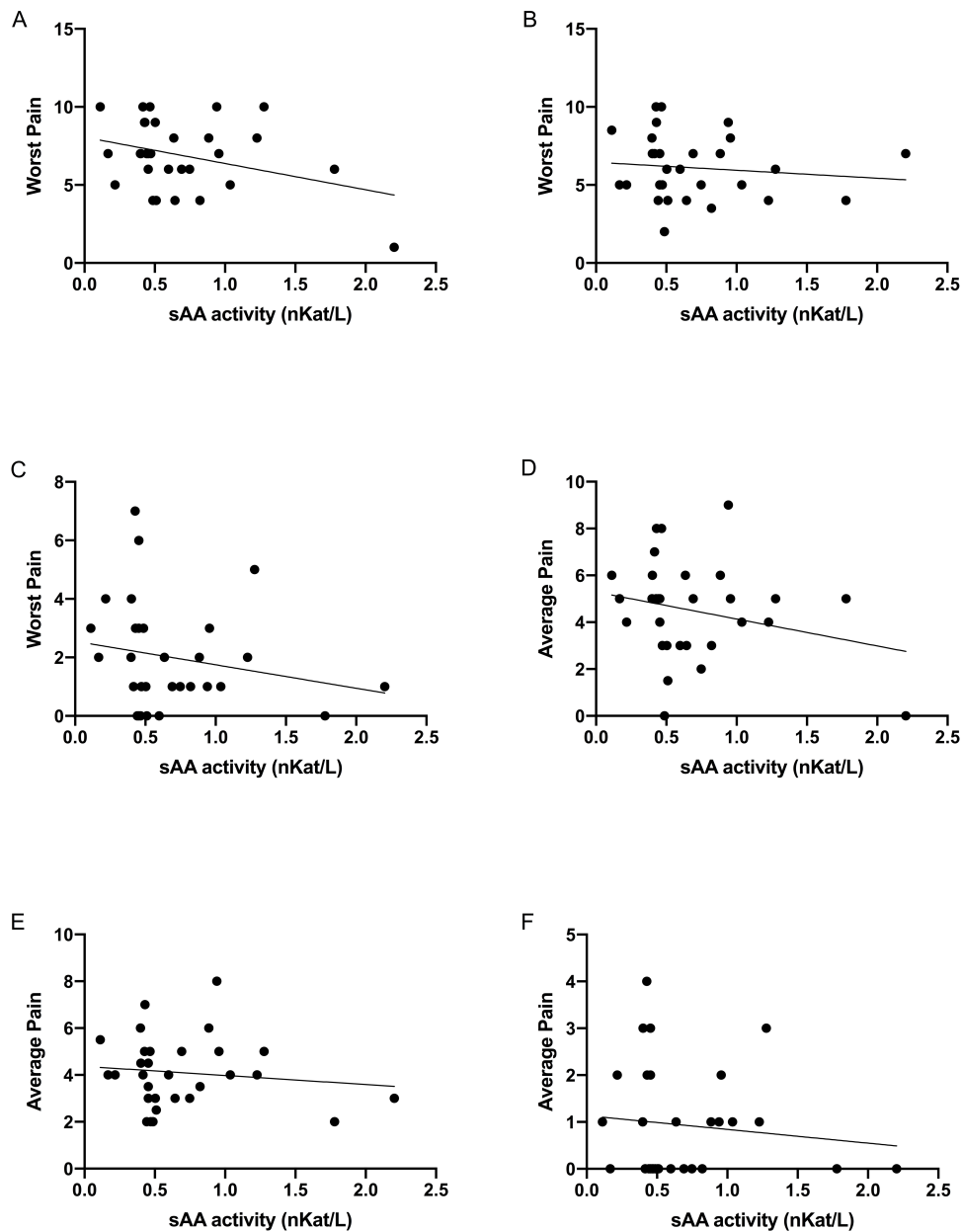


Figure 4. Associations between sAA activity on the morning of surgery and postoperative worst and average pain intensity. A) No significant correlation is observed between sAA

activity on the morning of surgery and worst pain on POD1 ($r=-0.2421$, $p=0.1894$) ($n=31$). **B)** No significant correlation is observed between sAA activity on the morning of surgery and worst pain on POD2 ($r=-0.2389$, $p=0.2037$) ($n=30$). **C)** No significant correlation is observed between sAA activity on the morning of surgery and worst pain 6 weeks after surgery ($r=-0.2931$, $p=0.1159$) ($n=30$). **D)** No significant correlation is observed between sAA activity on the morning of surgery and average pain on POD1 ($r=-0.2485$, $p=0.1776$) ($n=31$). **E)** No significant correlation is observed between sAA activity on the morning of surgery and average pain on POD2 ($r=-0.1189$, $p=0.5314$) ($n=30$). **F)** No significant correlation is observed between sAA activity on the morning of surgery and average pain 6 weeks after surgery ($r=-0.1145$, $p=0.5468$) ($n=30$). POD1 = postoperative day 1 (24 hours after surgery); POD2 = postoperative day 2 (48 hours after surgery).

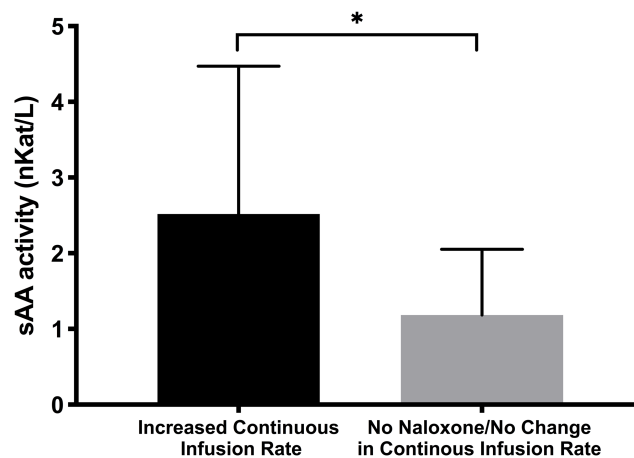


Figure 5. Differences in sAA activity between patients that received an increase in the rate of continuous naloxone infusion, or did not receive naloxone or any changes in the rate of continuous naloxone infusion before saliva collection on POD2. The sAA activity level is significantly increased in patients who received an increase in the rate of continuous naloxone infusion prior to the saliva collection ($n=5$), in comparison to those who did not receive naloxone or received no changes in the rate of continuous naloxone infusion ($n=26$, $p=0.0476$). (Data expressed as Mean $\pm$ SD. $*$ = $p<0.05$). POD2 = postoperative day 2 (48 hours after surgery).

Trajectory Analysis of Perioperative Biomarker Levels and Self-Reported Pain Scores

sAA activity levels were not associated either with self-reported preoperative anxiety scores, or self-reported postoperative pain scores in a pediatric cohort undergoing orthopedic surgery. Thus, sAA activity level is not a viable proxy of preoperative anxiety, or a predictor of postoperative pain intensity, in AIS patients undergoing orthopedic surgery. However, identifying associations between physiological markers and self-reported pain through correlation analyses may not be an accurate representation of the relationship between these markers and self-reported pain across the perioperative period. Future work in the identification of a biomarker of perioperative pain may need to focus on the differences observed between individuals of a cohort in their respective postoperative pain experiences, since the pain response after surgery can differ between groups of patients of the same cohort. This was highlighted in a recent study that identified 4 subgroups of patients, in a pediatric cohort undergoing orthopedic surgery, based off their acute postoperative pain intensity trajectories, where individuals belonging to the different subgroups had varying long-term postoperative outcomes in regards to their pain and medication use [22]. Therefore, analyzing the differences in the perioperative modulation of physiological markers of pain, in relation to the perioperative modulation of self-reported pain intensity, may reveal the associations between the markers and the postoperative pain experience between individuals in the same cohort, effectively. In the future, having the information on the differential modulation of multiple physiological markers of pain for an individual may eventually be integrated in personalized postoperative pain management for these patients, based on which pain mechanisms are stimulated or inhibited, and are influencing their acute postoperative pain experience.

In the following sub-project, a different statistical analysis approach was used in comparison to the first two sub-projects, where the perioperative modulation of the plasma monoamine neurotransmitters EPI, NE, DA, and 5-HT, and the respective metabolites of EPI and NE: ME and NME, were analyzed using trajectory analysis, in conjunction with the perioperative trajectory analysis of patients' self-reported pain scores. Finally, multiple correspondence analysis was used to identify the relationship between the perioperative modulation of monoamines and self-reported pain by assessing the differences in the monoamines' and pain trajectory memberships between sub-groups of AIS patients scheduled to undergo orthopedic surgery.

TITLE PAGE

Title: Systemic Modulation of Perioperative Monoamines in Pediatric Patients Undergoing Surgery

Shajenth Premachandran¹, Alisson R. Teles^{2,3}, Pablo M. Ingelmo^{2, 4}, Philippe Sarret^{5, 6}, Alexandre J. Parent⁵, Serge Marchand^{2,6}, Jean A. Ouellet^{1,2,3,7,8}, Neil Saran^{7,8}, Catherine E. Ferland^{1-4,7}

¹ McGill University, Department of Experimental Surgery, Montreal, Qc, Canada

² Alan Edwards Centre for Research on Pain, Montreal, Qc, Canada

³ McGill Scoliosis and Spine Research Group, Montreal, Qc, Canada

⁴ McGill University, Department of Anesthesia, Montreal, Qc, Canada

⁵ Université de Sherbrooke, Department of Pharmacology and Physiology, Sherbrooke, Qc, Canada

⁶ Centre de recherche du CHUS, Sherbrooke, Qc, Canada

⁷ Shriners Hospital for Children-Canada, Montreal, Qc, Canada

⁸ McGill University, Department of Orthopedic Surgery, Montreal, Qc, Canada

Corresponding author:

Catherine E. Ferland

Shriners Hospitals for Children-Canada

1003 Decarie Blvd, Montreal, Canada, H4A 0A9

Telephone number: +1 (514) 842-4464 extension 7177

Fax number: +1 (514) 842-8664

E-mail address: catherine.ferland@mcgill.ca

Abstract

Introduction/Aim: The descending monoaminergic pathway is involved in regulating the endogenous pain system. Understanding how plasma levels of monoamines change across the perioperative period in pediatric patients undergoing surgery can provide insight into the mechanisms underlying post-surgical pain, allowing for better targeted pharmacological treatment to be provided to patients. This study aimed to analyze the perioperative modulation of plasma monoamines in a pediatric cohort undergoing surgery, and to assess the modulation of plasma monoamines as a marker of patients' postoperative pain experiences.

Materials and Methods: One hundred and two patients scheduled to undergo corrective surgery for adolescent idiopathic scoliosis were enrolled in the study. Blood samples and self-reported pain scores of patients scheduled for surgery were collected one week before surgery (baseline), and 24, 48 hours and 6 weeks after surgery. Liquid chromatography-tandem mass spectrometry was used to analyze the plasma concentrations of four monoamines and metabolites (dopamine: DA, epinephrine: EPI, norepinephrine: NE, metanephrine: ME, normetanephrine: NME, and serotonin: 5-HT). Growth mixture modelling was performed to identify perioperative monoamine and pain trajectories, and multiple correspondence analysis to identify groups of patients displaying similar perioperative monoamine and pain trajectories.

Results: Three perioperative trajectories for plasma EPI were identified, with one trajectory reporting decreasing plasma levels (226.50 ± 60.93 pg/ml to 22.18 ± 37.24 pg/ml), the second trajectory reporting stable plasma levels (range: 162.38 ± 95.00 pg/ml – 194.56 ± 54.64 pg/ml), and the third trajectory reporting increasing plasma levels (191.50 ± 15.15 pg/ml to 488.31 ± 166.66 pg/ml). Three perioperative trajectories for plasma NE were identified, with the first trajectory reporting stable plasma concentrations (range: 347.29 ± 166.58 pg/ml – 584.40 ± 186.13 pg/ml), the second trajectory reporting an increase in plasma concentrations starting from POD2 (436.67 ± 227.88 pg/ml) to 6 weeks after surgery (1037.42 ± 169.17 pg/ml), and the third trajectory reporting increasing plasma concentrations across the perioperative period starting from baseline (535.06 ± 245.58 pg/ml) to POD1 (1117.84 ± 202.79 pg/ml). Three perioperative trajectories for plasma ME were identified, with one trajectory reporting increasing plasma

concentrations in the acute postoperative period (49.13 ± 14.49 pg/ml to 133.80 ± 51.53 pg/ml), the second trajectory reporting stable plasma concentrations (range: 49.00 ± 27.55 pg/ml – 57.96 ± 28.88 pg/ml), and the third trajectory also reporting increasing plasma concentrations in the acute postoperative period (73.34 ± 23.05 pg/ml to 220.10 ± 34.81 pg/ml). Three perioperative trajectories for plasma NME were identified, with one trajectory reporting stable plasma concentrations (range: 82.82 ± 69.38 pg/ml – 153.91 ± 120.67 pg/ml), the second trajectory reporting increasing plasma concentrations (128.03 ± 49.22 pg/ml to 745.83 ± 353.22 pg/ml), and the third trajectory also reporting increasing plasma concentrations (172.17 ± 61.57 pg/ml to 1240.25 ± 27.57 pg/ml). Two perioperative trajectories for plasma DA were identified, with one trajectory reporting stable plasma concentrations (range: 35.80 ± 18.73 pg/ml – 65.20 ± 54.88 pg/ml), and the second trajectory reporting an increase in plasma concentrations (46.49 ± 12.23 pg/ml to 334.09 ± 177.46 pg/ml). Two perioperative trajectories for plasma 5-HT were identified, with one trajectory reporting stable plasma concentrations (range: 43.38 ± 32.59 pg/ml – 162.78 ± 131.86 pg/ml), and the second trajectory reporting an increase in plasma concentrations (376.76 ± 214.99 pg/ml to 626.41 ± 184.30 pg/ml). Based on perioperative monoamines trajectory membership, two groups of patients were identified in this cohort, with one group mainly consisting of patients reporting increasing plasma levels of EPI, NE, ME, NME, and DA postoperatively, decreasing plasma levels of EPI postoperatively, and stable plasma levels of 5-HT. The second group mainly consisted of patients reporting stable plasma levels of EPI, NE, ME, NME, and DA, and increasing plasma levels of 5-HT postoperatively. No difference in pain trajectory membership or postoperative pain intensity was identified between the two groups.

Discussion: This study identified two groups of pediatric surgical patients, with increasing or stable plasma monoamine levels, perioperatively. The modulation of plasma monoamine levels between the two groups is not associated to postoperative pain intensity, and may be associated to minimal or increased sympathetic nervous system activity in response to the surgical insult.

Keywords

Monoamines; Adolescent idiopathic scoliosis; Orthopedic surgery; Perioperative pain; Trajectory analysis

Introduction

The descending biphasic modulatory systems involved in the sensory transmission of pain from the brain have been identified as being both facilitatory and inhibitory [1]. Among the brain regions involved in this descending modulation, the brainstem rostroventral medial medulla (RVM) is one of the key relay in the bulbo-spinal inhibitory and facilitatory circuit [1]. Importantly, the balance between inhibition and facilitation is dynamic and critical in the transition from acute to chronic pain which favors descending facilitation, and can therefore affect the therapeutic efficacy of analgesics [2]. The monoaminergic pathways are the main endogenous transmitter systems involved in descending pain controls [3-7]. Monoamine neurotransmitters include serotonin (5-hydroxytryptamine or 5-HT), dopamine (DA), epinephrine (EPI), and norepinephrine (NE) as well as their respective metabolites metanephrine (ME) and normetanephrine (NME). Through their binding to different G protein-coupled receptor subtypes, they play a key role in pain modulation by regulating the release of other neuromodulators from nociceptive afferents, such as glutamate and substance P, and by controlling the level of excitability of dorsal horn neurons [3, 8]. Although NE has been largely identified as having anti-nociceptive properties through its binding to alpha-2 adrenergic receptors at the level of the spinal dorsal horn, 5-HT and DA can exert both anti- and pro-nociceptive effects through interaction with serotonin and dopamine receptor subtypes [3].

The regulation of monoamines, mainly NE and EPI, has previously been assessed in adults undergoing various surgeries [9-14]. These studies found that monoamine levels significantly increased immediately after surgery and returned to preoperative levels 24 hours up to 5 days after surgery [10-12, 14]. Most of these studies have analyzed the modulation of monoamines, in the perioperative period, in relation to surgical stress and sympathetic activity [10, 11, 14]. Similarly, in neonates, perioperative plasma NE and EPI levels were shown to be significantly correlated to the degree of surgical stress experienced [15]. However, there is little information regarding the modulation of monoamines throughout the perioperative period of pediatric patients, and their relation to pain. Our group previously demonstrated that preoperative plasma NE and NME are potential predictors of postoperative pain intensity in pediatric patients undergoing spine surgery [16]. Given the relevance of monoamine neurotransmitters in the descending modulation of pain, we hypothesized that the analysis of the perioperative trajectories of plasma monoamines, and their

respective metabolites, will reveal trajectories indicating an increase in plasma monoamine levels in the acute postoperative period, and similarly, analyzing the perioperative trajectories of self-reported pain intensity will also reveal trajectories indicating an increase in pain intensity in the acute postoperative period. Furthermore, it was hypothesized that the patients with the trajectories highlighting a greater increase in the plasma monoamines' levels in the acute postoperative period would belong to the perioperative pain trajectories indicating greater levels of self-reported pain intensity in the acute postoperative periods, as well.

The main objective of this study was to observe the systemic modulation of perioperative monoamines in pediatric patients undergoing surgery and to assess the perioperative modulation of plasma monoamine levels as a marker of the patient's postoperative pain experience. To this aim, we studied the patients' perioperative plasma monoamine trajectories in relation to their perioperative self-reported pain score trajectories, and morphine intake in the acute postoperative period.

Methods

This study was conducted at the Shriners Hospitals for Children-Canada. Ethics approval was obtained prior to the beginning of the study from the Research Ethics Board of McGill University (A05-M57-11B and A08-M71-14B). Patients with Adolescent Idiopathic Scoliosis (AIS) aged between 12 and 18 years old and scheduled to undergo spinal fusion surgery were recruited from the outpatient clinic. Exclusion criteria included diagnosis of a cognitive and/or developmental disability that would interfere with completing clinical outcome measures, and major chronic medical conditions (ASA physical status III or higher before surgery). Written informed consents were obtained prior to the beginning of the study.

Sample Collection and LC-MS/MS Parameters (Multiple Reaction Monitoring)

To limit the effect of time, all samples were collected between 7:00-10:00 a.m. Five ml of blood were collected in EDTA-coated collection tube one week before surgery (baseline), in the acute postoperative period (POD1: 24 hours after surgery and POD2: 48 hours after surgery), and

at the first postsurgical follow-up visit at the hospital (6 weeks after surgery). After each collection, samples were centrifuged at 1200 *g* for 10 minutes and stored at -80°C pending biochemical analysis.

Samples were prepared and analyzed as previously described [16], and unless indicated otherwise, all manipulations were performed at room temperature. Briefly, 400 µl of plasma was first acidified, precipitated by the addition of 3 volumes of acetonitrile-containing internal standards, and then evaporated. 800 µl of derivation solution (0.37 M acetate buffer, 2.23 g/L cyanoborohydride and 1.42% acetaldehyde) was added and the samples were incubated for 1h at 37°C. The reaction was quenched by the addition of ammonium acetate (50 mM, pH 7.0). Samples were then purified by weak cation exchange (WCX) solid phase extraction (SPE) and dried down. Catecholamines were reconstituted with 0.2% formic acid-containing water before being processed by liquid chromatography coupled with tandem mass spectrometry using a TripleTOF 5600 mass spectrometer (AB Sciex, Foster City, CA, USA), equipped with a DuoSpray source (AB Sciex). Samples were introduced to the electrospray ionization source in a 50 µm electrospray ionization probe (Eksigent, Concord, On, CA), using a microLC200 system (Eksigent) equipped with a 150mmx150mm Synergi-Fusion-RPC18 4µm column (Phenomenex). Acquisition was performed with a ABSciex equipped with electrospray interface with a 25 µm iD capillary and coupled to an Eksigent µUHPLC (Eksigent, Redwood City, CA, USA). Analyst TF 1.6 software was used to control the instrument and for data processing and acquisition. The source voltage was set to 5.2 kV and maintained at 400°C; curtain gas was set at 27 psi; gas one at 12 psi and gas two at 10 psi. Separation was performed on a reversed phase HALO PFP column 0.3 µm i.d., 2.7 µm particles, 50mm long (Advance Materials Technology, Wilmington, DE) which was maintained at 40°C. Samples were injected by loop overfilling into a 5 µL loop. For the 2.5 min LC gradient, the mobile phase consisted of the following solvent A (0.2% v/v formic acid and 10mM ammonium formate in water) and solvent B (0.2% v/v formic acid in acetonitrile) at a flow rate of 30 µL/min. The gradient steps consisted of the following: hold 3% B from 0 to 0.2min, from 3% to 50% B from 0.2 to 1.85 min, from 50% to 100% B from 1.85 to 1.9 min, hold 100% B from 1.9 to 2.3 min, from 100% to 3% B from 2.3 to 2.35 min, hold 3% B from 2.35 to 2.5 min, followed by a 0.4 min post-flush at 30uL/min at final condition. Monoamine quantification was performed with MultiQuant software version 2.0 (AB Sciex) using the area under the curve. Analyses were performed by PhenoSwitch Bioscience (Sherbrooke, QC, CA).

Pain Intensity Measurements

For all time points throughout the study (baseline, POD1, POD2 and 6 weeks after surgery), the patient's current pain intensity was rated by using a numerical rating score (NRS) after being asked the following question: "How would you rate your level of pain on a scale from 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable?". A self-reported pain score between 1-3 represented mild pain, between 4-6 represented moderate pain, and a pain score between 7-10 represented severe pain [17].

Perioperative Care

The institutional perioperative anesthesia, surgical and spinal cord monitoring protocols were followed for the perioperative care of all patients undergoing corrective surgery for scoliosis. The standards of care remained unchanged for all patients. All patients received total intravenous anesthesia with propofol and remifentanyl, ketamine and dexamethasone. Following their induction, all patients received a single dose injection of spinal morphine (5 mcg/kg). Upon arrival to the post-anesthetic care unit, pain management was standardized and included patient-controlled analgesia (PCA) morphine and ketamine (bolus 1mg:1mg) with a starting bolus dose of 20 µg/kg, lockout 6 minutes and a 4 hours maximal dose up to 0.4 mg/kg, as well as acetaminophen and non-steroidal anti-inflammatory drugs. Patients also received subsequent dose adjustments to the PCA when necessary. PCA data were recorded in the patients' electronic medical charts and cumulative morphine intake was calculated per postoperative periods.

Statistical Analysis

All analyses were performed using SPSS software version 22 (IBM Corporation, Armonk, NY), SAS® version 9.3 (SAS Institute Inc, Cary, NC), GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA) or R version 3.2.1. Growth mixture modelling was used to perform trajectory analysis for perioperative levels of monoamines and pain intensity, as detailed in previous literature [18]. Patient's average plasma levels of monoamines and pain intensity at baseline,

POD1, POD2 and 6 weeks after surgery were used as the basis for this analysis. The heterogeneous linear mixed effects function of the latent class mixed model package in R version 3.2.1 was utilized to test five linear and five linear+quadratic trajectory models for the perioperative plasma monoamines' levels and perioperative pain intensity. Low values for Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), indicating a better fitting model, a minimum trajectory class size of 5% of the total sample size of the study cohort, and parsimony [18-20] were utilised to select the best trajectory model. The FactoMineR package in the R studio software was used to identify clusters of patients displaying similar trajectories through an unsupervised hierarchical clustering method [21]. Multiple correspondence analysis (MCA) was first conducted to identify relationships between the patient trajectory memberships for each of the monoamines and pain intensity in order to condense the information into principal components (PC). PCs with eigenvalues >0.2 were retained to explain the greatest amount of variation in our data, and variable loading >0.3 was considered significant, on each component [22]. The best grouping of clusters was based on the highest relative loss of inertia and parsimony [22]. Chi-squared tests were used to identify differences in patient trajectory memberships for each monoamine analyzed and pain intensity, between clusters. T-tests were used to identify differences in self-reported pain scores and morphine intake on POD1, POD2, and 6 weeks after surgery between patient cluster memberships. Results are presented as mean $\pm$ standard deviation (SD) unless otherwise stated.

Results

A total of 102 pediatric patients scheduled for spinal fusion surgery were recruited. Sixteen patients were removed from the plasma monoamine trajectory analysis: two patients were lost to follow-up, and fourteen patients were removed because their analytes could not be measured at any time points due to the absence of integrated peaks in the LC-MS/MS readings. Three patients were removed from the pain trajectory analysis: two patients were lost to follow-up, and one patient had most clinical variables missing. An additional time point (POD1) was added after the start of the study; therefore, blood sample collection on that day was done for 80 patients only. Patient characteristics, clinical and post-surgical variables are described in **Table 1**. In this study, the length of surgery, on average, was of 4.44 ± 1.29 hours and blood loss was of 807.7 ± 464.2 ml.

On average, the number of vertebrae fused per surgery was 10.77 ± 2.56 , and the length of stay in the hospital was 6.68 ± 1.42 days.

Monoamine Trajectories Across the Perioperative Period

The perioperative trajectories of the six monoamines analyzed for 86 patients are presented in **Figure 1**. The goodness of fit indices of the ten trajectory models tested for each monoamine, the description of the final trajectory models for each monoamine, and the predicted and raw plasma monoamine concentrations according to the selected trajectory model can be found in **Table 2**, **Table 3**, and **Table 4**, respectively. The simplest model with the best fit for EPI levels across the perioperative period ($AIC=3350.52$; $BIC=3379.97$) contained 3 trajectories (Figure 1a). Trajectory 1 ($n=20$) for EPI levels was characterized with decreasing plasma concentrations across the perioperative period, with a mean plasma EPI concentration of 226.50 ± 60.93 pg/ml at baseline, and a mean plasma EPI concentration of 22.18 ± 37.24 pg/ml 6 weeks after surgery. Trajectory 2 ($n=60$) for EPI levels was characterized with stable plasma concentrations across the perioperative period (range of mean plasma EPI levels: 162.38 ± 95.00 pg/ml – 194.56 ± 54.64 pg/ml). Trajectory 3 ($n=6$) for EPI levels was characterized with increasing plasma concentrations across the perioperative period, with a mean plasma EPI concentration of 191.50 ± 15.15 pg/ml at baseline, and a mean plasma EPI concentration of 488.31 ± 166.66 pg/ml 6 weeks after surgery. Through trajectory analysis, three groups of patients were identified in this cohort based on their differing perioperative modulations of plasma EPI levels, with 70% of patients reporting stable plasma EPI levels across the perioperative period, and 30% of patients reporting changes (increase or decrease) in the modulation of plasma EPI levels perioperatively. The simplest model with the best fit for NE levels across the perioperative period ($AIC=4138.2$; $BIC=4167.66$) had 3 trajectories (Figure 1b). Trajectory 1 ($n=50$) for NE levels was characterized with stable plasma concentrations across the perioperative period (range of mean plasma NE levels: 347.29 ± 166.58 pg/ml – 584.40 ± 186.13 pg/ml). Trajectory 2 ($n=16$) for NE levels was characterized with a visibly large increase in plasma concentrations starting from POD2 to 6 weeks after surgery, with a mean plasma NE concentration of 436.67 ± 227.88 pg/ml on POD2, and a mean plasma NE concentration of 1037.42 ± 169.17 pg/ml 6 weeks after surgery. Trajectory 3 ($n=20$) for NE levels was characterized with increasing plasma concentrations across the perioperative period, starting in the acute postoperative period from

baseline to POD1, with a mean plasma NE concentration of 535.06 ± 245.58 pg/ml at baseline, and a mean plasma NE concentration of 1117.84 ± 202.79 pg/ml on POD1. Therefore, three groups of patients were again identified in this cohort based on their differing perioperative modulations of plasma NE levels, with 42% of patients reporting an increase in plasma NE levels at some point postoperatively, and 58% of patients reporting stable plasma NE levels across the perioperative period.

The simplest model with the best fit for ME levels across the perioperative period (AIC=3110.54; BIC=3139.99) contained 3 trajectories (Figure 1c). Trajectory 2 (n=45) for ME levels was characterized as having stable plasma concentrations from baseline to 6 weeks after surgery (range of mean plasma ME levels: 49.00 ± 27.55 pg/ml – 57.96 ± 28.88 pg/ml). Trajectory 1 (n=18) and Trajectory 3 (n=23) for ME levels were characterized by increasing plasma concentrations in the acute postoperative period, with the greatest rate of increase in plasma ME concentrations belonging to Trajectory 3. Trajectory 1 contained patients with a mean plasma ME concentration of 49.13 ± 14.49 pg/ml at baseline, and a mean plasma ME concentration of 133.80 ± 51.53 pg/ml on POD2. Trajectory 3 contained patients with a mean plasma ME concentration of 73.34 ± 23.05 pg/ml at baseline, and a mean plasma ME concentration of 220.10 ± 34.81 pg/ml on POD2. Through trajectory analysis, three groups of patients were identified based on their differing perioperative modulations of plasma ME levels, with 48% of patients reporting an increase in ME levels in the postoperative periods, and 52% of patients reported stable plasma ME levels postoperatively. The simplest model with the best fit for NME levels across the perioperative period (AIC=4275.25; BIC=4304.71) had 3 trajectories (Figure 1d). Trajectory 1 (n=42) for NME levels was characterized as having stable plasma concentrations from baseline to 6 weeks after surgery (range of mean plasma NME levels: 82.82 ± 69.38 pg/ml – 153.91 ± 120.67 pg/ml). Trajectory 2 (n=35) and Trajectory 3 (n=9) for NME levels were characterized by increasing plasma concentrations across the perioperative period, with the greatest rate of increase in plasma NME concentrations belonging to Trajectory 3. Trajectory 2 contained patients with a mean plasma NME concentration of 128.03 ± 49.22 pg/ml at baseline, and a mean plasma NME concentration of 745.83 ± 353.22 pg/ml 6 weeks after surgery. Trajectory 3 contained patients with a mean plasma NME concentration of 172.17 ± 61.57 pg/ml at baseline, and a mean plasma NME concentration of 1240.25 ± 27.57 pg/ml 6 weeks after surgery. Three groups

of patients were identified in this cohort based on their differing perioperative modulations of plasma NME levels, with 51% of patients reporting an increase in NME levels in the acute postoperative period, and 49% of patients reported stable perioperative plasma NME levels.

The simplest model with the best fit for DA levels across the perioperative period (AIC=3505.44; BIC=3520.16) had 2 trajectories (Figure 1e). Trajectory 2 (n=27) for DA levels was characterized with an increase in plasma concentration in the acute postoperative period, up to 6 weeks after surgery, with a mean plasma DA concentration of 46.49 ± 12.23 pg/ml at baseline, and a mean plasma DA concentration of 334.09 ± 177.46 pg/ml 6 weeks after surgery. Trajectory 1 (n=59) for DA levels was characterized with stable plasma concentration across the perioperative period (range of mean plasma DA levels: 35.80 ± 18.73 pg/ml – 65.20 ± 54.88 pg/ml). In this case, two groups of patients were identified based on their differing perioperative modulation of plasma DA levels, with 31% of patients reporting an increase in DA levels in the acute postoperative period, and 69% of patients reported stable perioperative plasma DA levels.

The simplest model with the best fit for 5-HT levels across the perioperative period (AIC=3929.48; BIC=3944.21) contained 2 trajectories (Figure 1f). 5-HT Trajectory 2 (n=17) was characterized by increasing plasma concentrations from baseline to 6 weeks after surgery, with a mean plasma 5-HT concentration of 376.76 ± 214.99 pg/ml at baseline, and a mean plasma 5-HT concentration of 626.41 ± 184.30 pg/ml 6 weeks after surgery. 5-HT Trajectory 1 (n=69) was characterized by stable plasma 5-HT concentrations from baseline to 6 weeks after surgery (range of mean plasma 5-HT levels: 43.38 ± 32.59 pg/ml – 162.78 ± 131.86 pg/ml). Two groups of patients were again identified based on their differing perioperative modulation of plasma 5-HT levels, with 20% of patients reporting an increase in 5-HT levels in the acute postoperative period, and 80% of patients reported stable perioperative plasma 5-HT levels.

Pain Trajectories Across the Perioperative Period

The perioperative trajectories of self-reported pain intensity for 99 patients are presented in **Figure 2**. The quality of fit indices of the ten pain trajectory models tested can be found in **Table 5**. The description of the final pain trajectory model, and the predicted and raw perioperative pain intensity values according to the selected trajectory model can be found in **Table 6**. The simplest model with the best fit for pain intensity across the perioperative period (AIC=1380.68;

BIC=1422.20) contained 4 trajectories. Patients in Pain Trajectory 1 (n=35) reported mild pain consistently, from before surgery to 6 weeks after surgery. Patients in Pain Trajectory 2 (n=35) reported moderate pain before surgery and in the acute postoperative period before declining to mild pain 6 weeks after surgery. Patients in Pain Trajectory 3 (n=22) reported mild pain before surgery, which increased to moderate pain during the acute postoperative period, before declining to mild pain 6 weeks after the surgery. Patients in Pain Trajectory 4 (n=7) reported moderate pain before surgery, and severe pain in the acute postoperative period before declining to mild pain 6 weeks after surgery.

Multiple Correspondence Analysis of Monoamines and Pain Trajectory Membership

Multiple correspondence analysis on our data derived 2 principal components accounting for 35.85% of the total variation in data. Principal component 1 was summarized as representing the dimension of constant perioperative plasma EPI levels (EPI Trajectory 2), ME levels (ME Trajectory 2), NME levels (NME Trajectory 1), DA levels (DA Trajectory 1) and 5-HT levels (5-HT Trajectory 1), as well as increasing plasma ME levels (ME Trajectory 3), NME levels (NME Trajectory 2), DA levels (DA Trajectory 2) and 5-HT levels (5-HT Trajectory 2) from baseline to POD1, POD2 and 6 weeks after surgery. Principal component 2 was summarized as representing the dimension of an increase in plasma NE levels (NE Trajectory 3) across the perioperative period, starting at baseline.

Cluster Analysis of Monoamines and Pain Trajectory Membership

Based on the highest relative loss of inertia and parsimony, 2 clusters accounting for 27% of the total variation best represented the data (n=85). Results from Chi-squared tests revealed significant differences in patient trajectory memberships for all six monoamines between clusters (**Table 7**). Cluster 1 (n=38) displayed a greater distribution of patients with stable perioperative plasma levels of EPI ($\chi^2=28.64$, $p<0.001$), NE ($\chi^2=21.42$, $p<0.001$), ME ($\chi^2=48.74$, $p<0.001$), NME ($\chi^2=47.19$, $p<0.001$), and DA ($\chi^2=29.40$, $p<0.001$), in comparison to cluster 2 (n=47). On the other hand, cluster 2 displayed a greater distribution of patients with both increasing and decreasing plasma levels of EPI across the perioperative period, patients with an increase of plasma

NE levels in the acute postoperative period, patients with increasing plasma levels of ME across the perioperative period, patients with increasing plasma NME levels across the perioperative period, and patients with increasing plasma levels of DA perioperatively.

However, in the case of 5-HT, cluster 1 showed a greater distribution of patients with increasing perioperative plasma levels, whereas cluster 2 displayed a greater distribution of patients with stable plasma levels across the perioperative period ($\chi^2=23.56$, $p<0.001$). Furthermore, no significant difference in self-reported pain intensity trajectory membership was observed between clusters ($\chi^2=4.10$, $p=0.25$).

Differences in Postoperative Pain Intensity and Morphine Intake Between Cluster Membership

The differences in postoperative pain intensity on POD1, POD2 and 6 weeks after surgery between cluster membership are shown in **Figure 3**. However, no significant difference in average pain intensity was observed between patients in cluster 1 and cluster 2 on POD1 ($p=0.298$, Figure 3a), POD2 ($p=0.082$, Figure 3b), and 6 weeks after surgery ($p=0.328$, Figure 3c). The differences in acute postoperative morphine intake on POD1 and POD2 between cluster membership are shown in **Figure 4**. No significant difference in morphine intake was observed between patients in cluster 1 and cluster 2 on POD1 ($p=0.362$, Figure 4a), or POD2 ($p=0.132$, Figure 4b).

Discussion

The objectives of this study were to analyze the variation in plasma levels of monoamines in a pediatric population undergoing an invasive surgical procedure, and to assess for associations between the modulation of these monoamines, and the modulation of patients' self-reported pain intensities, across the perioperative period. We observed a variation in the monoamines', and their respective metabolites', modulations in pediatric patients, throughout the perioperative period. Through trajectory analysis, 3 distinct trajectories (groups of patients) were identified, based on the variations in the perioperative modulation of either plasma EPI, NE, ME, or NME. Similarly, 2 distinct trajectories were identified when based on the variations in the perioperative modulation of either plasma DA or 5-HT. The identification of multiple trajectories for the six monoamines

analyzed highlights the possibility of sub-groups of patients in our pediatric cohort with postoperative interindividual differences in their physiological responses to surgery. Similarly, self-reported perioperative pain trajectory analysis also revealed sub-groups of patients with four different trajectories identified. In a study conducted by Ocaý *et al.* (2020), acute postoperative trajectory analysis in AIS patients undergoing orthopedic surgery also revealed four distinct trajectories, where patients belonging to the trajectories indicating moderate-to-severe pain intensity experienced in the acute postoperative period, as opposed to mild pain intensity experienced in the acute postoperative period, were more likely to develop negative long-term postoperative outcomes, such as reporting greater levels of pain and pain medication 6 months after surgery [18]. Thus, the sub-groups of patients identified in our pediatric cohort through pain trajectory analysis may also represent patients with worse long-term postoperative outcomes, as the severity of pain experienced in the acute postoperative increases. This is especially concerning since three of the four perioperative pain trajectories identified in this study were characterized by patients experiencing moderate or severe pain in the acute postoperative period. Therefore, these differences in an individual's postoperative modulation of pain intensity may need to be taken into consideration when providing postoperative pain management strategies, and thus, the assessment of objective physiological markers of pain that are able to differentiate these sub-groups of patients, with varying postoperative pain experiences, would be valuable for clinicians in identifying patients at risk of poor pain management, and providing insight into the underlying mechanisms of their pain experience.

Following the multiple correspondence analysis to assess the associations between the perioperative modulations of all of the monoamines, and their respective metabolites, and self-reported pain intensity, two distinct groups (clusters) of patients were identified with significant differences in the distribution of perioperative monoamines' trajectory memberships. Major spinal surgeries cause a great degree of surgical trauma that leads to postoperative pain, and also activates the postoperative "stress response", which triggers sympathetic nervous system (SNS) activity [23]. The SNS activation plays an important role in pain inhibition by activating descending inhibitory pathways involving NE and 5-HT [5], also results in the secretion of EPI and NE from the adrenal medulla [24]. Furthermore, DA is stored in sympathetic nerve terminals alongside NE, since DA is a precursor molecule that is converted to NE by the enzyme dopamine- β -hydroxylase, and is released in conjunction with NE following SNS activity [25]. Therefore, increased surgical

trauma may have elicited greater SNS activity in the group of patients characterized by increasing plasma monoamine levels postoperatively, as opposed to the second group of patients characterized by stable plasma monoamine levels across the perioperative period. The postoperative “stress response”, when prolonged, leads patients into a hypermetabolic and hypercatabolic state that induces postoperative complications, such as impairments in the inflammatory response and wound healing, and organ dysfunction, leading to delayed recovery [26]. Therefore, following the group of patients characterized by increasing postoperative plasma levels of monoamines, and their respective metabolites, more closely in the acute postoperative period, and providing appropriate nutritional supplementation, exercise and pharmacological treatment, may help reduce the negative postoperative outcomes associated with the hypermetabolic and hypercatabolic responses [26].

One of the monoamine neurotransmitters that did not follow this above-mentioned trend between the two groups of patients identified was plasma 5-HT. Although the group of patients characterized by stable perioperative plasma monoamine levels also contained the 17 patients belonging to the increasing perioperative trajectory of plasma 5-HT levels, 21 of the patients belonging to the stable perioperative trajectory of plasma 5-HT were also part of this group of patients, and the rest of the 47 patients with the stable perioperative trajectory of plasma 5-HT belonged to the other group of patients characterized by increasing postoperative plasma levels of monoamines. However, when analyzing the mean plasma 5-HT levels between both perioperative trajectories identified in this study, there is a sharp decline in the plasma 5-HT concentration on POD1 and POD2. The largest source of peripheral 5-HT are platelets and mast cells [27], and the levels of these cellular sources for 5-HT may have been reduced significantly following the blood loss that occurs during major surgery. This may explain why the perioperative modulation of plasma 5-HT may be skewed in our results, and why there is a marked reduction in mean plasma 5-HT levels in the acute postoperative period. Therefore, future work studying the modulation of 5-HT perioperatively will benefit from analyzing the mRNA expression of 5-HT receptors from peripheral blood mononuclear cells, in order to bypass the complication that blood loss during surgery may have on peripheral 5-HT levels.

However, no significant differences were identified in the perioperative pain trajectory memberships, or mean self-reported pain intensity scores between the two groups of patients identified, suggesting a lack of relationship between the monoamine modulation and the

postoperative pain experience. As our results show, Ledowski *et al.* (2012) also found no association between blood levels of EPI and NE with the severity of acute postoperative pain in adult patients who had elective orthopedic or plastic surgery. Furthermore, they had noted the residual effect of drugs, such as volatile anesthetics and analgesics, provided to these patients in a surgical setting as a possible limitation to their study [28]. Similarly, this may have also been a confounding factor in our study, that could affect the associations we would expect to see between perioperative monoamine modulation and acute postoperative pain. Therefore, we analyzed the differences in morphine intake in the acute postoperative between the two groups of patient, since opioid-mediated analgesia is achieved through the stimulation of descending inhibitory pathways [29]. However, no significant differences in morphine intake were observed between the two groups of patients, as well. A study conducted by Li *et al.* (2019) observed five distinct opioid consumption trajectories, in AIS patients undergoing orthopedic surgery, where high opioid consumers experienced increased levels of acute postoperative pain intensity in relation to low opioid consumers [30]. Thus, using a similar trajectory analysis of the acute postoperative opioid consumption in the current study may elucidate any associations between the patients' trajectory memberships for the perioperative monoamines or self-reported pain intensity trajectories, and the acute postoperative opioid consumption trajectories, more effectively.

One of the limitations of this study is the lack of healthy age-matched controls for our baseline measurement. The average plasma EPI concentration and NE concentration in healthy control is averaged at 50 pg/ml and 210 pg/ml, respectively [31]. Thus, the mean baseline plasma concentrations, for EPI and NE, in our AIS cohort are elevated in comparison to these basal EPI and NE values in healthy controls. Since NE levels have been shown to be elevated in AIS patients [32], further investigation is required into monoamine neurotransmitter levels in relation to the pathology of AIS. This may provide an explanation for the decrease in plasma EPI levels observed in some patients towards 50 pg/ml, following the corrective surgery. Furthermore, another limitation of this study is that our cohort consists of only AIS patients, which represent a specific subset of pediatric surgical candidates. It is therefore important to take into consideration that our results limit us from extrapolating our conclusions to the general pediatric population. Lastly, increasing in the sample size will confirm the trends observed in our small cohort to a larger population, and validate the perioperative monoamines' trajectories with $n < 10$, in case they are affected by the variability in plasma monoamine concentrations observed in our data.

Conclusion

In the present work, we identified AIS patients that had increasing levels of monoamines following surgery, but this was not observed in all patients. Thus, these two groups of patients may represent individuals with greater or minimal activation of the SNS in response to the degree of surgical insult caused by major surgery. No association with patient's perioperative pain scores was observed between the two groups of patients, suggesting that the perioperative modulation of plasma monoamines, and their respective metabolites, is not a viable marker of patients' postoperative pain experiences. However, while limited to AIS patients, our results provide a glimpse at plasma monoamine modulation following surgery in adolescents, for which knowledge is scarce and underexplored.

Acknowledgements

The authors would like to thank the hospital patients and staff for their precious contribution, and the Strategies in Pain INtervention and Evaluation (SPINE) research group for their support and contribution. The Shriners Hospitals financially supported this study.

References

1. Zhuo, M., *Descending facilitation: From basic science to the treatment of chronic pain*. Molecular Pain, 2017. **13**: p. 1744806917699212.
2. Heinricher, M., et al., *Descending control of nociception: specificity, recruitment and plasticity*. Brain research reviews, 2009. **60**(1): p. 214-225.
3. Benarroch, E.E., *Descending monoaminergic pain modulation Bidirectional control and clinical relevance*. Neurology, 2008. **71**(3): p. 217-221.
4. Pertovaara, A., *Noradrenergic pain modulation*. Prog Neurobiol, 2006. **80**(2): p. 53-83.
5. Millan, M.J., *Descending control of pain*. Progress in neurobiology, 2002. **66**(6): p. 355-474.
6. Gebhart, G., *Descending modulation of pain*. Neuroscience & Biobehavioral Reviews, 2004. **27**(8): p. 729-737.
7. Ren, K. and R. Dubner, *Descending modulation in persistent pain: an update*. Pain, 2002. **100**(1-2): p. 1-6.
8. Kwon, M., et al., *The role of descending inhibitory pathways on chronic pain modulation and clinical implications*. Pain Practice, 2014. **14**(7): p. 656-667.
9. Halter, J.B., A.E. Pflug, and D. Porte Jr, *Mechanism of plasma catecholamine increases during surgical stress in man*. The Journal of Clinical Endocrinology & Metabolism, 1977. **45**(5): p. 936-944.
10. Benedict, C., D. Grahame-Smith, and A. Fisher, *Changes in plasma catecholamines and dopamine beta-hydroxylase after corrective surgery for coarctation of the aorta*. Circulation, 1978. **57**(3): p. 598-602.
11. Ogawa, K., et al., *Suppression of cellular immunity by surgical stress*. Surgery, 2000. **127**(3): p. 329-336.
12. Sametz, W., et al., *Perioperative catecholamine changes in cardiac risk patients*. European journal of clinical investigation, 1999. **29**(7): p. 582-587.
13. Joris, J.L., et al., *Hemodynamic changes and catecholamine release during laparoscopic adrenalectomy for pheochromocytoma*. Anesthesia & Analgesia, 1999. **88**(1): p. 16-21.
14. Chernow, B., et al., *Hormonal responses to graded surgical stress*. Archives of Internal Medicine, 1987. **147**(7): p. 1273-1278.

15. Anand, K.J. and A. Aynsley-Green, *Measuring the severity of surgical stress in newborn infants*. Journal of pediatric surgery, 1988. **23**(4): p. 297-305.
16. Ferland, C.E., et al., *Preoperative norepinephrine levels in cerebrospinal fluid and plasma correlate with pain intensity after pediatric spine surgery*. Spine deformity, 2017. **5**(5): p. 325-333.
17. Tsze, D.S., et al., *Validation of self-report pain scales in children*. Pediatrics, 2013. **132**(4): p. e971-e979.
18. Oca, D.D., et al., *Predicting Acute Postoperative Pain Trajectories and Long-Term Outcomes of Adolescents after Spinal Fusion Surgery*. Pain Research and Management, 2020. **2020**.
19. Ram, N. and K.J. Grimm, *Methods and measures: Growth mixture modeling: A method for identifying differences in longitudinal change among unobserved groups*. International journal of behavioral development, 2009. **33**(6): p. 565-576.
20. Pagé, M.G., et al., *Acute pain trajectories and the persistence of post-surgical pain: a longitudinal study after total hip arthroplasty*. Journal of anesthesia, 2016. **30**(4): p. 568-577.
21. Lê, S., J. Josse, and F. Husson, *FactoMineR: an R package for multivariate analysis*. Journal of statistical software, 2008. **25**(1): p. 1-18.
22. Hair, J.F., et al., *Multivariate data analysis: A global perspective (Vol. 7)*. 2010, Upper Saddle River, NJ: Pearson.
23. Ezhevskaya, A.A., S.G. Mlyavykh, and D.G. Anderson, *Effects of continuous epidural anesthesia and postoperative epidural analgesia on pain management and stress response in patients undergoing major spinal surgery*. Spine, 2013. **38**(15): p. 1324-1330.
24. Desborough, J., *The stress response to trauma and surgery*. British journal of anaesthesia, 2000. **85**(1): p. 109-117.
25. Bell, C., *Dopamine release from sympathetic nerve terminals*. Progress in neurobiology, 1988. **30**(2-3): p. 193-208.
26. Finnerty, C.C., et al., *The surgically induced stress response*. JPEN. Journal of parenteral and enteral nutrition, 2013. **37**(5 Suppl): p. 21S-9S.

27. Sommer, C., *Serotonin in pain and analgesia*. Molecular neurobiology, 2004. **30**(2): p. 117-125.
28. Ledowski, T., et al., *Effects of acute postoperative pain on catecholamine plasma levels, hemodynamic parameters, and cardiac autonomic control*. PAIN®, 2012. **153**(4): p. 759-764.
29. Pathan, H. and J. Williams, *Basic opioid pharmacology: an update*. British journal of pain, 2012. **6**(1): p. 11-16.
30. Li, M.M., et al., *Acute postoperative opioid consumption trajectories and long-term outcomes in pediatric patients after spine surgery*. Journal of pain research, 2019. **12**: p. 1673.
31. Christensen, N.J., *Plasma Epinephrine and Plasma Norepinephrine Concentrations in Early Diabetes*, in *Vascular and Neurological Changes in Early Diabetes*, R.A. Camerini-Dávalos and H.S. Cole, Editors. 1973, Academic Press. p. 227-234.
32. Morningstar, M., *Neurotransmitter patterns in patients with adolescent idiopathic scoliosis (AIS)*. Scoliosis and Spinal Disorders, 2013. **8**(Suppl 2).

Table 1. Patient Characteristics, Clinical and Post-Surgical Variables. Data are presented as Mean $\pm$ standard deviation, or otherwise stated.

Patient Characteristics	<i>n</i> = 102
Sex (Female: Male)	81:21
Age (years)	15.36 $\pm$ 2.02
Weight (kg)	54.90 $\pm$ 11.44
Clinical Variables	Mean (Range)
Epinephrine concentration at baseline (pg/ml)	201.20 (101.00-362.00)
Norepinephrine concentration at baseline (pg/ml)	515.80 (196.30-1195.00)
Metanephrine concentration at baseline (pg/ml)	56.37 (20.90-120.00)
Normetanephrine concentration at baseline (pg/ml)	120.70 (27.25-338.40)
Dopamine concentration at baseline (pg/ml)	38.26 (6.80-107.40)
Serotonin concentration at baseline (pg/ml)	212.80 (7.00-721.00)
Presence of preoperative pain (yes:no)	79:23
Post-Surgical Variables	
PCA total dose at POD1 (mcg/kg)	0.48 $\pm$ 0.35
PCA total dose at POD2 (mcg/kg)	0.88 $\pm$ 0.48

PCA: Patient-Controlled Analgesia. POD: Postoperative Day

Table 2. Goodness of Fit Indices for the Ten Trajectory Models Tested for Each Monoamine

Number of trajectories (EPI)	Linear			Linear + quadratic		
	AIC	BIC	SC (%)	AIC	BIC	SC (%)
1	3408.71	3416.08	100	3405.24	3415.06	100
2	3364.62	3379.64	7.0	3357.08	3376.71	9.3
3	3361.09	3383.18	7.0	3350.52*	3379.97*	7.0*
4	3345.27	3374.73	1.1	3358.52	3397.79	0
5	3351.27	3388.09	0	3336.07	3385.16	0
Number of trajectories (NE)	Linear			Linear + quadratic		
	AIC	BIC	SC (%)	AIC	BIC	SC (%)
1	4343.18	4350.54	100	4340.11	4349.93	100
2	4226.17	4240.89	24.4	4149.56	4169.2	24.4
3	4230.29	4252.38	15.1	4138.2*	4167.66*	18.6*
4	4236.29	4265.74	0	4146.2	4185.47	0
5	4242.29	4279.1	0	4143.27	4192.36	0
Number of trajectories (ME)	Linear			Linear + quadratic		
	AIC	BIC	SC (%)	AIC	BIC	SC (%)
1	3349.43	3356.79	100	3333.83	3343.65	100
2	3189.28	3204.01	31.4	3133.61	3153.24	29.1
3	3180.62	3202.71	20.9	3110.54*	3139.99*	20.9*
4	3186.62	3216.07	0	3118.54	3157.81	0
5	3185.43	3222.25	0	3077.86	3126.95	0
Number of trajectories (NME)	Linear			Linear + quadratic		
	AIC	BIC	SC (%)	AIC	BIC	SC (%)
1	4412.48	4419.84	100	4410.83	4420.65	100
2	4316.31	4331.03	48.8	4299.51	4319.14	50.0
3	4322.31	4344.4	0	4275.25*	4304.71*	10.5*
4	4303.72	4333.18	0	4283.25	4322.52	0
5	4309.72	4346.54	0	4291.25	4340.34	0
Number of trajectories (DA)	Linear			Linear + quadratic		
	AIC	BIC	SC (%)	AIC	BIC	SC (%)
1	3603.99	3611.36	100	3605.88	3615.7	100
2	3505.44*	3520.16*	31.4*	3508.83	3528.47	31.4
3	3511.44	3533.53	0	3516.83	3546.29	0
4	3468.59	3498.04	0	3432.32	3471.59	0
5	3474.59	3511.4	0	3440.32	3489.41	0
Number of trajectories (5-HT)	Linear			Linear + quadratic		
	AIC	BIC	SC (%)	AIC	BIC	SC (%)
1	3981.79	3989.15	100	3923.35	3933.17	100
2	3929.48*	3944.21*	19.8*	3770.76	3790.39	24.4

3	3935.48	3957.57	0	3778.76	3808.21	0
4	3941.48	3970.94	0	3740.82	3780.09	0
5	3938.22	3975.03	0	3748.82	3797.91	0

**indicates the model with the best fit. AIC: Akaike Information Criterion. BIC: Bayesian Information Criterion. SC: Smallest Class Size*

Table 3. Description of Final Plasma Monoamines' Trajectory Models

Monoamine		<i>n</i>	Intercept	Slopes	
				Linear	Quadratic
EPI	Trajectory 1	20	448.7	-272.01	42.7
	Trajectory 2	60	253.51	-70.09	13.44
	Trajectory 3	6	74.64	173.86	-20.2
NE	Trajectory 1	50	786.2	-380.77	81.77
	Trajectory 2	16	1300.38	-874.79	198.73
	Trajectory 3	20	-124.04	818.47	-122.12
ME	Trajectory 1	18	-46.31	118.62	-19.81
	Trajectory 2	45	42.1	11.81	-2.55
	Trajectory 3	23	-107.17	224.59	-36.49
NME	Trajectory 1	42	178.06	-85.42	19.91
	Trajectory 2	35	-312.86	570.7	-80.31
	Trajectory 3	9	-686.15	1091.76	-158.72
DA	Trajectory 1	59	24.31	10.97	-
	Trajectory 2	27	-26.8	85.33	-
5-HT	Trajectory 1	69	131.07	-14.01	-
	Trajectory 2	17	139.94	77.35	-

Table 4. Predicted and Raw Plasma Monoamine Concentrations According to Trajectory Model

Monoamine		<i>n</i>	Predicted values				Raw values, mean (SD)			
			Baseline	POD1	POD2	6wks	Baseline	POD1	POD2	6wks
EPI (pg/ml)	Trajectory 1	20	219.39	75.48	16.97	43.86	226.5 (60.93)	34.22 (45)	17.78 (28.87)	22.18 (37.24)
	Trajectory 2	60	196.86	167.09	164.2	188.19	194.56 (54.64)	179.79 (104.92)	162.38 (95)	190.83 (81.2)
	Trajectory 3	6	228.3	341.56	414.42	446.88	191.5 (15.15)	445.15 (67.42)	358.58 (169.59)	488.31 (166.66)
NE (pg/ml)	Trajectory 1	50	487.2	351.74	379.82	571.44	472.58 (119.08)	392.03 (191.07)	347.29 (166.58)	584.4 (186.13)
	Trajectory 2	16	624.32	345.72	464.58	980.9	644.72 (222.95)	417.87 (166.66)	436.67 (227.88)	1037.42 (169.17)
	Trajectory 3	20	572.31	1024.42	1232.29	1195.92	535.06 (245.58)	1117.84 (202.79)	1154.02 (199.15)	1232.87 (70.63)
ME (pg/ml)	Trajectory 1	18	52.5	111.69	131.26	111.21	49.13 (14.49)	115.47 (60.89)	133.8 (51.53)	116.84 (61.43)
	Trajectory 2	45	51.36	55.52	54.58	48.54	51.5 (18.23)	57.96 (28.88)	51.7 (30.64)	49 (27.55)
	Trajectory 3	23	80.93	196.05	238.19	207.35	73.34 (23.05)	219.59 (41.71)	220.1 (34.81)	214.71 (42.61)
NME (pg/ml)	Trajectory 1	42	112.55	86.86	100.99	154.94	107.11 (57.04)	105.85 (117.03)	82.82 (69.38)	153.91 (120.67)
	Trajectory 2	35	177.53	507.3	676.45	684.98	128.03 (49.22)	682.78 (365.52)	570.53 (315.93)	745.83 (353.22)
	Trajectory 3	9	246.89	862.49	1160.65	1141.37	172.17 (61.57)	1183.98 (149.62)	1023.19 (311.65)	1240.25 (27.57)
DA (pg/ml)	Trajectory 1	59	35.28	46.25	57.22	68.19	35.8 (18.73)	42.73 (35.94)	57.18 (55)	65.2 (54.88)
	Trajectory 2	27	58.53	143.86	229.19	314.52	46.49 (12.23)	197.67 (137.71)	184.04 (81.33)	334.09 (177.46)
5-HT (pg/ml)	Trajectory 1	69	117.06	103.05	89.04	75.03	162.78 (131.86)	45.62 (45.34)	43.38 (32.59)	115.36 (108.52)
	Trajectory 2	17	217.29	294.64	371.99	449.34	376.76 (214.99)	162.33 (132.41)	129.2 (82.39)	626.41 (184.3)

SD: Standard Deviation. POD: Postoperative Day. 6wks: 6 Weeks After Surgery

Table 5. Goodness of Fit Indices for the Ten Pain Trajectory Models Tested

Number of trajectories	Linear			Linear + quadratic		
	AIC	BIC	SC (%)	AIC	BIC	SC (%)
1	1719.61	1727.4	100	1502.65	1513.03	100
2	1721.01	1736.58	45.5	1444.73	1465.5	37.4
3	1727.01	1750.36	0	1393.91	1425.05	23.2
4	1733.01	1764.15	0	1380.68*	1422.2*	7.1*
5	1739.01	1777.93	0	1368.38	1420.29	6.1

**indicates the model with the best fit. AIC: Akaike Information Criterion. BIC: Bayesian Information Criterion. SC: Smallest Class Size*

Table 6. Description of Final Pain Trajectory Model, and the Predicted and Raw Perioperative Pain Intensity Values According to Trajectory Model

Pain Trajectory	<i>n</i>	Intercept	Slopes		Predicted values				Raw values, mean (SD)			
			Linear	Quadratic	Baseline	POD1	POD2	6wks	Baseline	POD1	POD2	6wks
1	35	-3.96	6.04	-1.23	0.85	3.2	3.09	0.52	0.93 (0.92)	2.85 (1.18)	3.32 (0.92)	0.36 (0.9)
2	35	1.4	4.27	-1.08	4.59	5.62	4.49	1.2	4.67 (1.31)	5.34 (1.12)	4.93 (1.16)	1.18 (1.16)
3	22	-9.53	13.03	-2.62	0.88	6.05	5.98	0.67	0.82 (0.85)	6 (1.19)	6.35 (0.95)	0.71 (0.72)
4	7	-3.05	9.35	-1.98	4.32	7.73	7.18	2.67	4.43 (1.9)	7.5 (0.55)	7.79 (0.7)	2.71 (1.11)

SD: Standard Deviation. POD: Postoperative Day. 6wks: 6 Weeks After Surgery

Table 7. Differences in Patient Monoamines and Pain Trajectory Memberships Between Cluster 1 and Cluster 2

Trajectories	Cluster 1 (n = 38)	Cluster 2 (n = 47)	χ^2 value	<i>P</i>-value
Epinephrine			28.64	<0.001
1	0	19		
2	38	22		
3	0	6		
Norepinephrine			21.42	<0.001
1	31	19		
2	7	9		
3	0	19		
Metanephrine			48.74	<0.001
1	2	15		
2	36	9		
3	0	23		
Normetanephrine			47.19	<0.001
1	34	7		
2	4	31		
3	0	9		
Dopamine			29.4	<0.001
1	38	20		
2	0	27		
Serotonin			23.56	<0.001
1	21	47		
2	17	0		
Pain			4.10	0.25
1	12	19		
2	13	17		
3	8	10		
4	5	1		

P-values from Chi-squared tests considered significant ($p < 0.05$) are bolded.

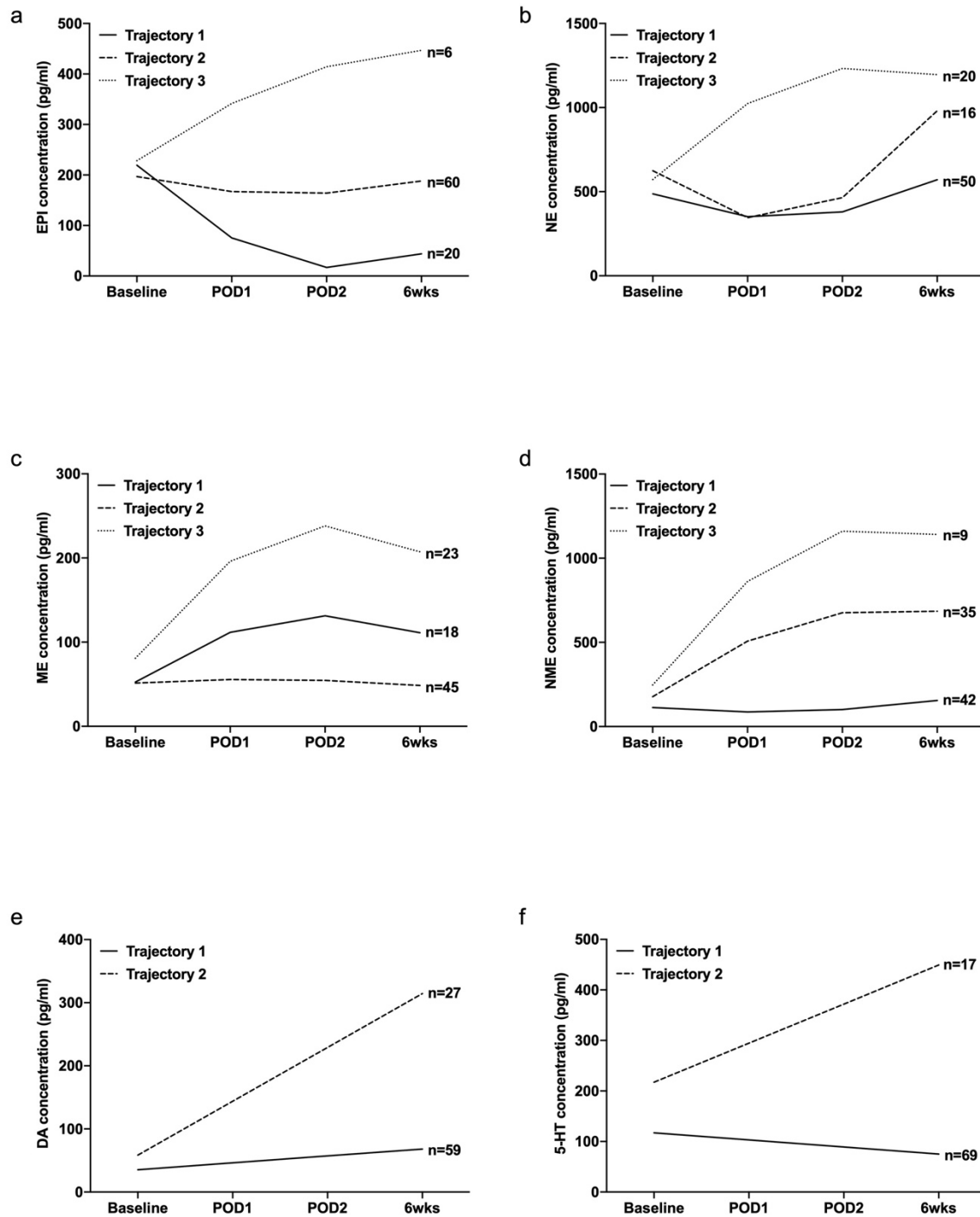


Figure 1. Perioperative Monoamine Trajectories. *a*, Perioperative plasma EPI trajectories: Trajectory 1 (n=20), Trajectory 2 (n=60), and Trajectory 3 (n=6). *b*, Perioperative plasma NE trajectories: Trajectory 1 (n=50), Trajectory 2 (n=16), and Trajectory 3 (n=20). *c*, Perioperative plasma ME trajectories: Trajectory 1 (n=18), Trajectory 2 (n=45), and Trajectory 3 (n=23). *d*, Perioperative plasma NME trajectories: Trajectory 1 (n=42), Trajectory 2 (n=35), and Trajectory

3 (n=9). *e*, Perioperative plasma DA trajectories: Trajectory 1 (n=59), and Trajectory 2 (n=27). *f*, Perioperative plasma 5-HT trajectories: Trajectory 1 (n=69) and Trajectory 2 (n=17). Baseline = 1 week before surgery; POD1 = postoperative day 1 (24 hours after surgery); POD2 = postoperative day 2 (48 hours after surgery); 6wks = 6 weeks after surgery.

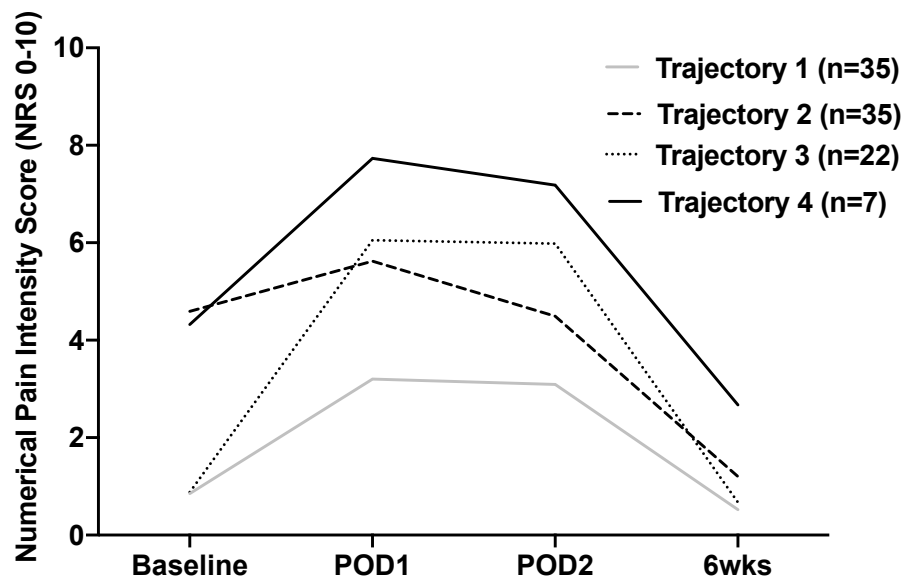


Figure 2. Perioperative Pain Trajectories. Perioperative pain trajectories: Trajectory 1 (n=35), Trajectory 2 (n=35), Trajectory 3 (n=22), and Trajectory 4 (n=7). Baseline = 1 week before surgery; POD1 = postoperative day 1 (24 hours after surgery); POD2 = postoperative day 2 (48 hours after surgery); 6wks = 6 weeks after surgery.

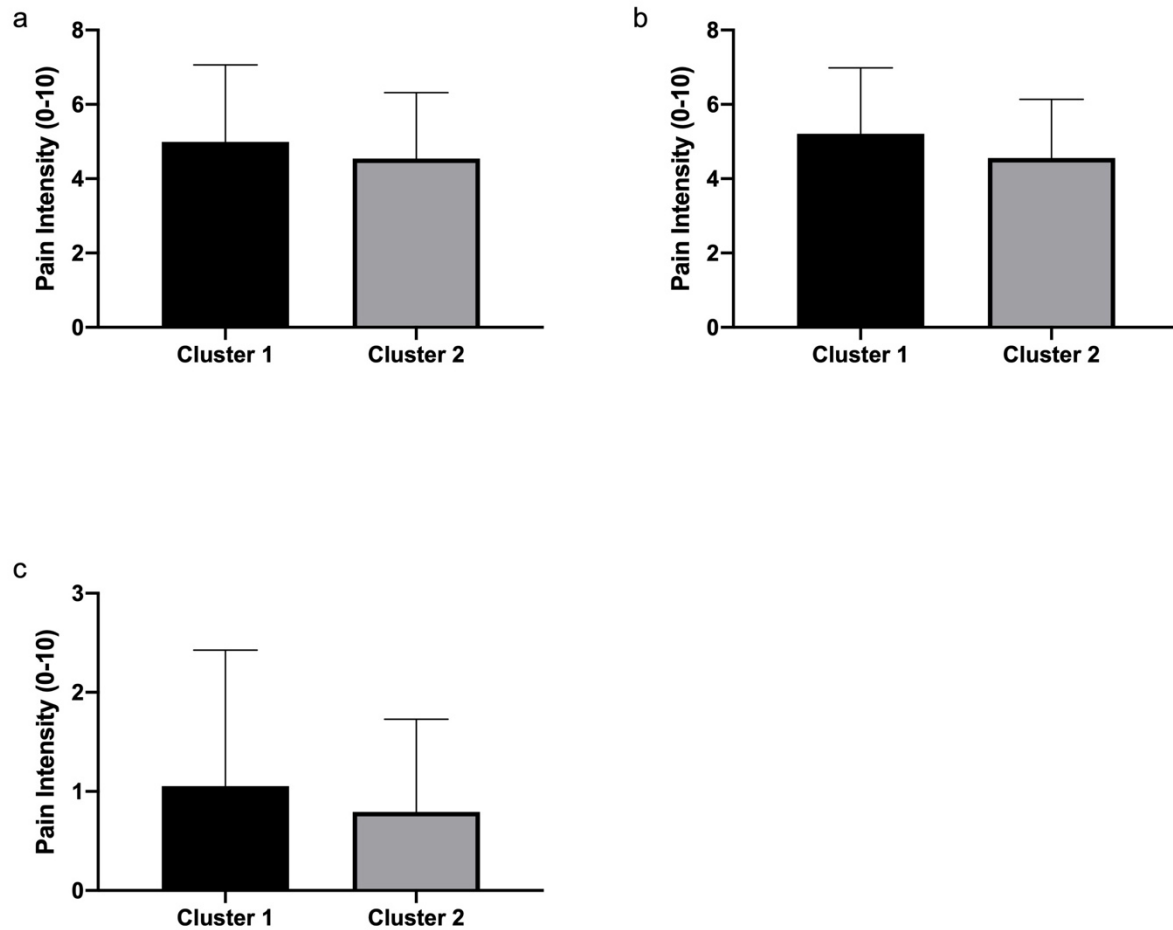


Figure 3. Differences in Postoperative Pain Intensity According to Cluster Membership. No significant difference in pain intensity observed between cluster 1 and 2 on POD1 ($p=0.298$; *a*), POD2 ($p=0.082$; *b*), and 6 weeks after surgery ($p=0.328$; *c*). (Data expressed as Mean $\pm$ SD). POD1 = postoperative day 1 (24 hours after surgery); POD2 = postoperative day 2 (48 hours after surgery); 6wks = 6 weeks after surgery.

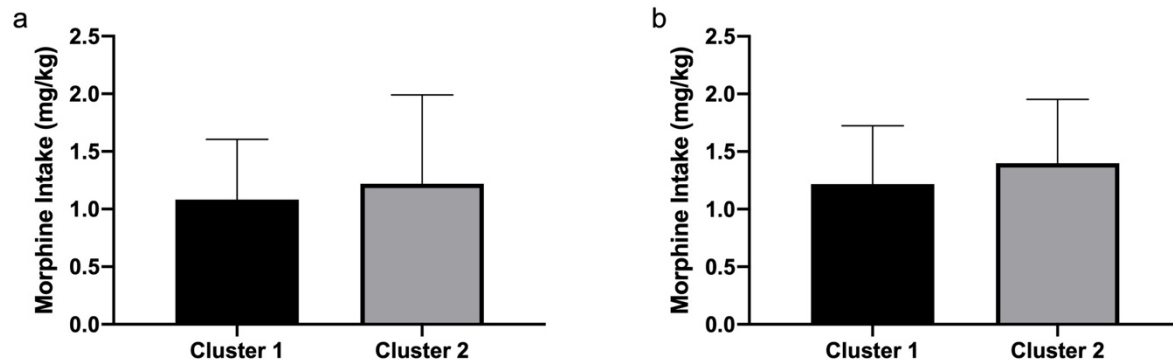


Figure 4. Differences in Acute Postoperative Morphine Intake According to Cluster Membership. No significant difference in morphine intake observed between cluster 1 and 2 on POD1 ($p=0.298$; **a**) and POD2 ($p=0.082$; **b**). (Data expressed as Mean $\pm$ SD). POD1 = postoperative day 1 (24 hours after surgery); POD2 = postoperative day 2 (48 hours after surgery).

DISCUSSION

Subjective self-reported measures of anxiety and pain, in the clinic, pose certain limitations when it comes to their use in children who are distressed, too young, non-verbal due to motor or cognitive impairments, and/or suffer from neurodevelopmental delays [119, 177, 178]. Therefore, the goal of this thesis project was to identify objective markers of pain and anxiety in a pediatric cohort scheduled to undergo spinal fusion surgery; a surgical procedure which has previously been characterized by postoperative pain [140], and for some patients, preoperative anxiety, as well [24]. In working towards this goal, the physiological markers assessed as proxies of pain and anxiety in this project were based off the response systems that would be active during the perioperative period, such as the inflammatory response, the stress response, and the nociceptive response, which were examined individually in separate manuscripts. The objective of the first sub-project was to assess plasma IL-6, a pro-inflammatory cytokine, as a marker of perioperative pain in AIS patients scheduled for corrective surgery. In the second sub-project, we assessed sAA as a proxy of preoperative anxiety and as a predictor of postoperative pain in another cohort of AIS patients undergoing spinal fusion surgery. The objective of the final sub-project was to assess the perioperative modulation of plasma monoamine levels as a marker of the perioperative modulation of pain intensity in AIS patients scheduled to undergo corrective surgery, as well. Although the external validity of the findings from this thesis project may be limited due to the cohorts used in each of these manuscripts only representing a subset of pediatric patients undergoing surgery and experiencing postoperative pain, this thesis project provides a closer look at the molecular mechanisms that could underly a pediatric patient's postoperative pain. Furthermore, to my knowledge, there is a scarcity of studies that examine the viability of using objective biomarkers of perioperative pain and anxiety in a pediatric population, and therefore, the results from this project can provide future directions to explore in the search for an objective means of measuring perioperative pain or anxiety in a pediatric cohort.

Perioperative Pain

In the first two sub-projects of this thesis, there was a significant difference in patients' self-reported pain scores across the perioperative period, with an increase in pain scores in the acute postoperative period, when compared to the scores at baseline. Then, it was observed that

the elevated acute postoperative pain scores were significantly decreased once more, 6 weeks after the surgery. Although the modulation of patients' self-reported pain appears uniform among the first two sub-projects, a change in the approach to the statistical analysis of perioperative self-reported pain scores, in the third sub-project, provided greater detail into the patients' perioperative pain. Trajectory analysis revealed four distinct trajectories for the modulation of self-reported pain in our pediatric cohort. Thus, there is significant variability in the modulation of patients' self-reported perioperative pain intensities between certain groups of patients. Similarly, Ocaý *et al.* (2020) also observed a total of four distinct trajectories for the pediatric patients' self-reported pain scores in the acute postoperative period [22]. In their study, the patient's trajectory membership was able to identify their long-term outcomes 6 months after surgery, with patients in the moderate pain trajectories being more likely to report greater pain severity and pain medication usage, when compared to the patients belonging to the mild pain trajectories in the acute postoperative period [22]. Therefore, using trajectory analysis for self-reported pain scores, across the perioperative period, is able to effectively identify sub-groups of patients with differing acute postoperative pain, that translate to varying long-term postoperative outcomes, and this may not be readily seen if assessing the patients' postoperative pain by only taking the mean of the self-reported pain scores at each timepoint.

In our pediatric cohort, in the third sub-project, two of the trajectories identified patients who reported moderate pain intensity in the acute postoperative period, and interestingly, one of the trajectories identified seven patients who reported severe pain intensity in the acute postoperative period (POD1 and POD2), and still reported pain 6 weeks after surgery. Therefore, in future studies, analyzing the perioperative levels of inflammatory mediators, such as IL-6, between the pain trajectory membership of these patients, similar to what was done with the monoamine neurotransmitters, may reveal the relationship between the postoperative pain and these potential inflammatory pain biomarker levels, more accurately. Furthermore, a study conducted by Pagé *et al.* (2016) also observed four distinct acute postoperative pain trajectories in patients undergoing orthopedic surgery, and found anxiety to be a significant predictor of pain trajectory membership in their cohort [179]. Thus, analyzing the preoperative self-reported anxiety scores (STAI-c), in conjunction with preoperative sAA activity levels, as predictors of pain trajectory membership in our pediatric cohort may also lead to the identification of sub-groups of patients whose postoperative pain are significantly affected by anxiety, when

compared to others. In addition, as a marker of anxiety, preoperative sAA activity levels can be assessed for associations to postoperative pain by analyzing its ability to objectively identify these sub-groups of patients based on their pain trajectory membership.

Variability of Biomarkers Across the Perioperative Period

Both plasma IL-6 levels, and plasma CRP levels, a known marker of the inflammatory response [180], were significantly elevated during the acute postoperative period, similar to the patients' self-reported average pain intensity scores. Similarly, when it came to the modulation of plasma monoamines perioperatively, one sub-group of patients were identified to have a greater distribution of individuals with increasing plasma levels of EPI, NE, ME, NME, and DA in the postoperative periods, with the exceptions of also having the greatest distribution of patients with decreasing plasma levels of EPI postoperatively, and patients with stable levels of plasma 5-HT perioperatively, when compared to the second sub-group of patients identified in our pediatric cohort, with the greatest distribution of individuals with stable plasma levels of EPI, NE, ME, NME, and DA perioperatively, as well as the greatest distribution of individuals with increasing plasma levels of 5-HT in the postoperative period. As a pro-inflammatory cytokine, IL-6 plays an important role in the peripheral sensitization of nociceptors following tissue injury, and has also been shown to affect the responsiveness of CNS neurons by playing a pronociceptive role through the reduction of inhibitory neurotransmission [53, 181]. Thus, following surgery, the increase in plasma IL-6 levels in the acute postoperative period can also be linked to an increase in the transmission of nociceptive signals to the brain, that eventually lead to the modulation of the surgical pain through the descending pathways, as seen in one sub-group of patients in our cohort, with the increase in plasma levels of EPI, NE, ME, NME, and DA in the acute postoperative periods, as well.

However, in this sub-group of patients with increasing plasma levels for most monoamines and their respective metabolites, plasma EPI levels were also observed to decline significantly for some of the patients, postoperatively. The plasma concentration of EPI in healthy individuals is approximately 50 pg/ml [182], but in a study conducted by Ledowski *et al.* (2012), in a cohort of patients scheduled to undergo orthopedic or plastic surgery, the range for the plasma levels of EPI were from 0-394 pg/ml for individuals who were reporting no pain [139]. Therefore, even though the trajectory for decreasing plasma EPI levels in this sub-group

of patients, in our cohort, stays within the previously mentioned range, from baseline to six weeks after surgery, the decline in plasma EPI levels does occur following the surgical insult, and approaches the 50 pg/ml concentration in the acute postoperative period. Seeing as NE has been shown to be elevated in AIS patients when compared to non-scoliotic individuals [183], and NE is converted into EPI using the enzyme phenylethanolamine-N-methyl transferase [184], future work should investigate the basal plasma levels of EPI in AIS patients, in relation to healthy age-matched individuals, which may reveal whether the pathology has an effect on increased plasma EPI levels, as was observed with these patients at the baseline timepoint.

Serotonin is a monoamine neurotransmitter that plays an important role in the descending modulation of pain as it has both algesic and analgesic properties depending on the subtype of receptors it binds to [185]. Much like the pro-inflammatory cytokine, IL-6, 5-HT is also involved in sensitizing peripheral nociceptors following tissue injury [185], suggesting that an increase in plasma 5-HT levels should be observed in our pediatric cohort following a surgical insult, as seen with IL-6. However, in the trajectory analysis of plasma 5-HT levels across the perioperative period, two trajectories were identified, where 69 of the 86 patients analyzed had stable levels of plasma 5-HT levels across the perioperative period, and only 17 patients had an increase in their plasma 5-HT levels in the postoperative period. Furthermore, when the patients were divided into two sub-groups based on their trajectory membership for the monoamines analyzed, one sub-group consisted of 47 patients belonging to the trajectory with stable plasma 5-HT levels across the perioperative period, and the second sub-group consisted of 21 patients belonging to the trajectory with stable plasma 5-HT levels across the perioperative period, as well as the 17 patients belonging to the trajectory with an increase in plasma 5-HT levels postoperatively. Therefore, there is a difference in the plasma 5-HT trajectory memberships between the two sub-groups, but both groups are mainly characterized by patients with no changes in their plasma 5-HT secretion following a surgical insult. This may be due to the fact that platelets and mast cells represent one of the largest sources of 5-HT in the periphery [185], and with an invasive procedure such as a spinal fusion surgery, the blood lost during the procedure can affect the platelet and mast cell count, with a decrease in platelets in the acute postoperative period also being caused by hemodilution and consumption of platelets in order to stop the bleeding caused during the surgery [186], thereby affecting peripheral 5-HT secretion.

In the second sub-group of patients identified, in our pediatric cohort, when analyzing the perioperative modulation of plasma monoamines, and their respective metabolites, there was a greater distribution of patients with stable levels of EPI, NE, ME, NME, and DA across the perioperative period. Surgery has been shown to cause several hormonal and metabolic changes in the body, which is referred to as the “stress response” to surgery [187]. These changes are initiated through the activation of the sympathetic nervous system which also plays a role in pain inhibition through the descending control of pain pathways [187, 188]. Since the magnitude and duration of this “stress response” to surgery is related to the degree of surgical injury and any surgical complications [189], this second sub-group of patients may have a reduced activation of the SNS in response to minimal surgical trauma, whereas the first sub-group of patients, with increasing plasma levels for most monoamines and their respective metabolites, may have increased SNS activity in response to experiencing greater surgical trauma. Therefore, it may be worth investigating certain surgical variables, such as number of vertebrae fused, blood loss during surgery, and duration of surgery, as predictors of patient trajectory membership in the perioperative plasma monoamines’ trajectories in our AIS cohort. This analysis would allow for the assessment of these surgical variables as factors that can help identify individuals at risk of poor postoperative pain management, if elevated postoperative levels of plasma monoamines are associated with increased postoperative self-reported pain intensity.

Lastly, no significant changes in sAA activity levels was observed across the perioperative period in our pediatric cohort, except for a sharp increase in sAA activity levels observed after the surgical insult, on POD2, in relation to the morning of surgery. It was initially hypothesized that the increase in sAA activity levels would have been observed preoperatively, presumably when these patients would be most anxious, and, as such, a significant decrease in self-reported anxiety was then observed between the preoperative period and 6 weeks after surgery, suggesting that these patients were more anxious prior to their surgery, rather than after. In our analysis, it was observed that naloxone, an opioid antagonist [190], had a significant effect on sAA activity levels on POD2, where patients receiving an increase in the continuous infusion rate of naloxone also had increased sAA activity levels. β -endorphins are endogenous peptides that belong to the opioid receptor agonist system, and have both an analgesic effect, and is involved in the reducing the activity of the stress response [191]. β -endorphin levels increase following surgery [191, 192], and SNS activity has been shown to result in the release of β -

endorphin [193]. Thus, an increase in β -endorphin levels in the acute postoperative period may have reduced any sAA activity in the acute postoperative period, seeing as sAA is a known biomarker of the SNS [141], which would be activated following surgery since it is also involved in the suppression of pain through descending noradrenergic and serotonergic pathways [188]. However, since naloxone antagonizes β -endorphin's effects [191], this may explain the increase in sAA activity levels observed on POD2 in our pediatric cohort. Therefore, quantifying the perioperative plasma levels of β -endorphin in our pediatric cohort would provide greater insight into the endogenous opioid system, and its effect on the patients' postoperative pain, as well as the SNS activity in these patients, following a surgical insult. This data would be important to compare and analyze for any associations when assessing the perioperative modulation of other physiological markers as proxies of postoperative pain.

Associations Between Biomarkers and Perioperative Pain

A positive correlation was observed between preoperative plasma IL-6 levels and preoperative pain scores in our cohort of AIS patients. This association between IL-6 levels and pain before surgery may be an indication of the mechanisms underlying the back pain experienced by 35-50% of AIS patients [140, 165]. Bisson *et al.* (2018) also observed an increased expression of inflammatory mediators in the facet joint cartilage of AIS patients, showing facet joint degeneration similar to osteoarthritis patients, and came to the conclusion that as in these osteoarthritis patients, this facet joint degeneration may be the cause of pain observed in certain AIS patients [194]. Thus, using inflammatory mediators, such as IL-6, as markers of back pain in AIS patients may provide an objective assessment of the patients who are experiencing pain prior to their surgery. Studying IL-6 levels, along with a number of other pro-inflammatory cytokines such as IL-8, IL-1 β and TNF- α , in AIS patients, could help develop new therapeutic approaches for providing analgesia in the patients experiencing back pain. Furthermore, identifying AIS patients with this increased inflammatory expression profile, and providing NSAIDs, prior to their corrective surgery, may be beneficial for these individuals' postoperative outcomes, since greater preoperative pain intensity is a predictor of poor postoperative recovery [25].

However, no associations were observed between plasma IL-6 levels and patient self-reported pain intensity in the acute postoperative period, or at 6 weeks after the surgery,

suggesting plasma IL-6 levels are not a viable proxy of perioperative pain in AIS patients undergoing major orthopedic surgery. Similarly, when analyzing patients' trajectory memberships for the four perioperative pain trajectories identified in the third sub-project, between the two sub-groups of patients identified, no significant difference in pain trajectory membership was observed. No significant difference in self-reported pain scores was also identified between these two sub-groups in the acute postoperative period, or 6 weeks after surgery, suggesting the perioperative modulation of plasma monoamines, and their respective metabolites, may not be a viable marker of perioperative pain in AIS patients undergoing orthopedic surgery, as well. One of the major caveats of studying perioperative pain that can affect or mask any associations between pain intensity and biomarkers levels, is the medication intake in the perioperative period. Although none of the patients in our cohort indicated any pain medication use preoperatively, and the variable of medication intake was standardized in the protocol for postoperative pain management, the extent to which a drug can affect one individual, in comparison to another, may not have been effectively accounted for in our statistical analysis. A study conducted by Housby *et al.* (1999) highlighted how the use of NSAIDs can inhibit the expression of genes encoding a number of cytokines, including IL-6, in a human monocytic cell line [195]. Furthermore, in a cohort of AIS patients undergoing spinal fusion surgery, Li *et al.* (2019) observed that high opioid consumers in the acute postoperative period were the ones reporting greater pain intensity, in comparison to low opioid consumers [196]. In our statistical analysis, acute postoperative NSAID intake was only used as a covariate in the assessment of associations between plasma IL-6 levels and self-reported pain, and only the differences in acute postoperative morphine intake was assessed between the two sub-groups of patients with differing trajectory memberships. Therefore, future work in the identification of a biomarker of perioperative pain may benefit from categorizing patients based on whether the patients are high pain medication users and experience mild postoperative pain, low pain medication users experiencing mild postoperative pain, high pain medication users experiencing moderate-to-severe postoperative pain, and low pain medication users experiencing moderate-to-severe postoperative pain. This type of categorization may help understand the individual differences in the postoperative pain experience in relation to the patient's reliance on pain medication use, and will also allow researchers to observe, more clearly, the extent to which pain medication affects the postoperative modulation of physiological markers of pain, by analyzing

the differences in the plasma levels of these markers between each of the above-mentioned categories.

Another variable, that is a part of pain medication intake, to take into account in our three sub-projects is the use of patient-controlled analgesia (PCA, morphine and ketamine) in the acute postoperative period. PCA refers to the patient's use of a programmable computerized pump to administer analgesics to themselves, and this route of administration reduces both the risks of an overdose, and pain intensity following surgery [197]. However, preoperative psychological characteristics of patients are highlighted as a factor to be taken into consideration when providing PCA in the postoperative periods [198]. Of these preoperative psychological characteristics, high levels of anxiety were associated with greater levels of postoperative pain and more frequent PCA demands in patients provided with postoperative PCA [198-200]. Since significantly increased levels of anxiety were observed in our pediatric cohort of AIS patients before surgery, in sub-project 2, there may have been a significant effect on postoperative PCA use, which, in turn, can significantly affect a patient's postoperative pain. However, no associations were observed between preoperative sAA activity levels and self-reported pain scores in our cohort, which may have been affected by the combined effects of PCA with other drugs given in the acute postoperative period, such as NSAIDs and acetaminophen, as well. Furthermore, sAA activity levels were also not observed to be associated to preoperative self-reported anxiety in our pediatric cohort. Thus, sAA activity levels are not a viable proxy of preoperative anxiety, or a predictor of postoperative pain intensity, in AIS patients scheduled to undergo orthopedic surgery. Although sAA activity levels have shown significant correlations to the STAI in healthy adult cohorts [201, 202], this relationship may not be reflected in a pediatric cohort with AIS, preparing to undergo corrective surgery, where the patients' self-reported anxiety is measured through the STAI-c questionnaire. Furthermore, using a control group at baseline, with healthy age-matched individuals, may reveal differences in sAA activity levels, when compared to the AIS patients anticipating major surgery. However, the relationship between sAA activity levels and the STAI-c questionnaire needs to be investigated further in pediatric cohorts, to assess the viability of studying sAA activity as a marker of anxiety in association with the self-reported anxiety scores from the STAI-c.

Of the physiological markers of pain studied in this thesis project, none of the markers were associated with the postoperative pain experience of AIS patients undergoing orthopedic

surgery. However, future work may require changes in the analysis of these physiological markers in relation to self-reported pain, and the type of physiological markers analyzed, as well. Perhaps, approaching the analysis of these physiological markers following orthopedic surgery from a genomic or genetic perspective, rather than having a proteomic approach, may provide a deeper understanding of the patient's physiological response following surgery. A study conducted by Wang *et al.* (2009) observed associations between IL-6 gene expression with postoperative pain following oral surgery in an adult cohort [137]. However, they found that this association between IL-6 gene expression and postoperative pain scores was only present in the placebo group of their study, and was not replicated in the group that received intravenous ketorolac preoperatively, highlighting the potent analgesic effect of ketorolac, since it was able to lower the patients' pain scores without affecting the gene expression of IL-6 in the acute postoperative period [137]. A study conducted by Henker *et al.* (2013) also highlighted that genetic variations in the catechol-o-methyltransferase (COMT) gene were associated with postoperative pain and opioid consumption in an adult cohort undergoing orthopedic surgery [203]. The COMT gene encodes the enzyme COMT which is responsible for inactivating the neurotransmitters EPI, NE, and DA [204], and COMT gene polymorphisms have been previously implicated in human pain sensitivity [205]. Thus, genomic/genetic approaches for pain biomarker identification in a pediatric population may yield a clearer picture of whether these markers are truly associated with self-reported postoperative pain scores, but medication intake remains a confounding variable in studies spanning the perioperative period.

Furthermore, other inflammatory mediators can also be analyzed as potential markers of postoperative pain in a pediatric cohort undergoing orthopedic surgery. The gene expression of the inflammatory mediators, Interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1), along with IL-6, were upregulated following surgery, and were observed to be correlated to pain intensity [137]. Bradykinin is another inflammatory mediator that is released following tissue trauma, and acts as an algogenic mediator through the acute activation of nociceptors [77]. In patients undergoing orthopedic surgery, cerebrospinal fluid (CSF) levels of prostaglandin E2 were also elevated, alongside CSF IL-6 levels, after surgical incision, and was associated with postoperative pain [206]. Therefore, analyzing the perioperative modulation of these above-mentioned inflammatory mediators through trajectory analysis, along with IL-6, in relation to the perioperative self-reported pain trajectories of AIS patients undergoing surgery, may reveal

associations between markers of the inflammatory response and postoperative pain more clearly, than identifying correlations between postoperative levels of plasma IL-6 and self-reported pain intensity.

The neuropeptides Substance P and CGRP are another set of physiological marker candidates to be assessed as viable proxies of perioperative pain in AIS patients undergoing surgery. Both of these neuropeptides are involved in modulating synaptic transmission of nociceptive signals from the periphery [207, 208]. Thus, adding these neuropeptides in the analysis of the perioperative modulation of monoamine neurotransmitters would give a more complete picture of the nociceptive response following surgery, rather than exclusively studying the descending control of pain, which can then be associated to the perioperative modulation of patients' self-reported pain scores. Future work may also need to focus on studying the markers of the inflammatory and nociceptive response together, in relation to the patients' postoperative pain. This analysis would be a more accurate representation of the patient's perceived pain, since all of these molecular networks and processes interact and work together to collectively influence postoperative pain, and the relationship of these various mechanisms to the perceived pain intensity may be lost when looking at each molecular network or process individually.

Limitations

One of the limitations of this thesis project is that our pediatric cohort in all three manuscripts consists largely of female patients since AIS is more prevalent in females [155, 158], and pain perception has been shown to be influenced by sex [209]. Thus, this limits the external validity of these findings to the general pediatric population. By separating our pediatric cohort by males and females, we may have observed differences in self-reported pain scores between the two groups, but this was not possible with a small sample size in some of our sub-projects, which comes to our second limitation in this thesis project.

A small sample size in the first and second sub-projects was also a limitation of the findings, as there was significant variation in plasma IL-6 concentration and sAA activity levels in the acute postoperative period. A larger sample size for these manuscripts may have compensated for this variability, since this variability in IL-6 levels and sAA activity would have had less of an impact on the results in a study with a larger sample size. This is due to the fact

that with a larger sample size, the standard deviation in our data would be reduced, and outliers in our data set can be more readily identified.

CONCLUSION

In conclusion, the objectives of the research were met, by assessing perioperative plasma IL-6 levels, and the perioperative modulation of the monoamine neurotransmitters EPI, NE, ME, NME, DA, and 5-HT, as markers of perioperative pain in a pediatric cohort diagnosed with AIS, scheduled to undergo spinal fusion surgery. Similarly, this project assessed sAA activity levels as a marker of preoperative anxiety and a predictor of postoperative pain in an AIS cohort undergoing corrective surgery, as well. Preoperative plasma IL-6 levels were identified as a possible marker of back pain in AIS patients, suggesting further investigation into the role of plasma IL-6 levels in chronic musculoskeletal (MSK) pain in the pediatric population. Understanding how plasma IL-6 levels contribute to pediatric MSK pain may provide insights into how we can approach the treatment of these patients in the future. Furthermore, two distinct clusters of patients were identified based on differing perioperative modulations of plasma monoamines, suggesting two different physiological responses to surgery were identified in this pediatric cohort. The cluster mostly characterized by increasing levels of plasma monoamines perioperatively would represent patients with elevated postoperative SNS activity, in comparison to the cluster largely characterized by stable levels of plasma monoamines perioperatively.

However, none of the physiological markers were found to be viable markers of postoperative self-reported pain in this pediatric population. The sAA activity levels were also found to have no association to preoperative self-reported anxiety. These results highlight the difficulties of associating objective physiological markers to subjective self-reported pain and anxiety scores in a pediatric surgical population, where postoperative medication intake is a prominent confounding variable. Furthermore, the results from this project provide a chance for future work, in the search of a biomarker of perioperative pain, to approach the subject from a more multifaceted approach. Analyzing the perioperative modulation of many physiological markers, belonging to different molecular networks and processes involved in the perception of pain (e.g. pro-inflammatory cytokines, prostaglandins, monoamine neurotransmitters, Substance P, CGRP), simultaneously, may provide a more realistic depiction of the underlying mechanisms of a pediatric patient's postoperative pain. From these findings, researchers can then develop and provide therapeutic strategies to achieve effective postoperative analgesia in pediatric patients undergoing major surgery in a more personalized manner, based on each patient's perioperative physiological response.

BIBLIOGRAPHY

1. Raja, S.N., et al., *The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises*. Pain, 2020. **161**(9): p. 1976-1982.
2. Julius, D. and A.I. Basbaum, *Molecular mechanisms of nociception*. Nature, 2001. **413**(6852): p. 203-210.
3. Treede, R.-D., et al., *A classification of chronic pain for ICD-11*. Pain, 2015. **156**(6): p. 1003.
4. Voscopoulos, C. and M. Lema, *When does acute pain become chronic?* British journal of anaesthesia, 2010. **105**(suppl_1): p. i69-i85.
5. Schmitt, P., *Rehabilitation of chronic pain: A multidisciplinary approach*. Journal of Rehabilitation, 1985. **51**(4): p. 72.
6. King, S., et al., *The epidemiology of chronic pain in children and adolescents revisited: a systematic review*. Pain, 2011. **152**(12): p. 2729-2738.
7. Birnie, K.A., et al., *Partnering for pain: a priority setting partnership to identify patient-oriented research priorities for pediatric chronic pain in Canada*. CMAJ open, 2019. **7**(4): p. E654.
8. Eccleston, C. and J. Clinch, *Adolescent chronic pain and disability: A review of the current evidence in assessment and treatment*. Paediatrics & child health, 2007. **12**(2): p. 117-120.
9. Palermo, T.M., *Impact of recurrent and chronic pain on child and family daily functioning: a critical review of the literature*. Journal of Developmental and Behavioral Pediatrics, 2000.
10. Liossi, C. and R.F. Howard, *Pediatric chronic pain: biopsychosocial assessment and formulation*. Pediatrics, 2016. **138**(5).
11. Forgeron, P.A., et al., *Social functioning and peer relationships in children and adolescents with chronic pain: A systematic review*. Pain Research and Management, 2010. **15**(1): p. 27-41.
12. Valrie, C.R., et al., *A systematic review of sleep in pediatric pain populations*. Journal of developmental and behavioral pediatrics: JDBP, 2013. **34**(2): p. 120.

13. Wolfson, A.R. and M.A. Carskadon, *Sleep schedules and daytime functioning in adolescents*. Child development, 1998. **69**(4): p. 875-887.
14. Vervoort, T., et al., *Severity of pediatric pain in relation to school-related functioning and teacher support: an epidemiological study among school-aged children and adolescents*. PAIN®, 2014. **155**(6): p. 1118-1127.
15. Dick, B.D. and R.P. Riddell, *Cognitive and school functioning in children and adolescents with chronic pain: a critical review*. Pain Research and Management, 2010. **15**(4): p. 238-244.
16. Walker, L.S., et al., *Functional abdominal pain patient subtypes in childhood predict functional gastrointestinal disorders with chronic pain and psychiatric comorbidities in adolescence and adulthood*. Pain, 2012. **153**(9): p. 1798-1806.
17. Henschke, N., S.J. Kamper, and C.G. Maher. *The epidemiology and economic consequences of pain*. in *Mayo Clinic Proceedings*. 2015. Elsevier.
18. Graves, E. and L.J. Kozak, *National Hospital Discharge Survey; annual summary, 1996*. 1999.
19. Rabbitts, J.A., et al., *Epidemiology of ambulatory anesthesia for children in the United States: 2006 and 1996*. Anesthesia & Analgesia, 2010. **111**(4): p. 1011-1015.
20. Oderda, G., *Challenges in the management of acute postsurgical pain*. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 2012. **32**(9pt2): p. 6S-11S.
21. Rosenbloom, B.N., et al., *Pediatric Chronic Postsurgical Pain And Functional Disability: A Prospective Study Of Risk Factors Up To One Year After Major Surgery*. Journal of pain research, 2019. **12**: p. 3079-3098.
22. Oca, D.D., et al., *Predicting Acute Postoperative Pain Trajectories and Long-Term Outcomes of Adolescents after Spinal Fusion Surgery*. Pain Research and Management, 2020. **2020**.
23. Ip, H.Y.V., et al., *Predictors of postoperative pain and analgesic consumption: a qualitative systematic review*. The Journal of the American Society of Anesthesiologists, 2009. **111**(3): p. 657-677.
24. Kain, Z.N., et al., *Preoperative anxiety, postoperative pain, and behavioral recovery in young children undergoing surgery*. Pediatrics, 2006. **118**(2): p. 651-658.

25. Connelly, M., et al., *Predictors of postoperative pain trajectories in adolescent idiopathic scoliosis*. Spine, 2014. **39**(3): p. E174-E181.
26. Quartana, P.J., C.M. Campbell, and R.R. Edwards, *Pain catastrophizing: a critical review*. Expert review of neurotherapeutics, 2009. **9**(5): p. 745-758.
27. Katz, J. and Z.e. Seltzer, *Transition from acute to chronic postsurgical pain: risk factors and protective factors*. Expert review of neurotherapeutics, 2009. **9**(5): p. 723-744.
28. Weinrib, A.Z., et al., *The psychology of chronic post-surgical pain: new frontiers in risk factor identification, prevention and management*. British journal of pain, 2017. **11**(4): p. 169-177.
29. Pathan, H. and J. Williams, *Basic opioid pharmacology: an update*. British journal of pain, 2012. **6**(1): p. 11-16.
30. Nicolazzo, J.A., S.A. Charman, and W.N. Charman, *Methods to assess drug permeability across the blood-brain barrier*. Journal of pharmacy and pharmacology, 2006. **58**(3): p. 281-293.
31. Kokki, H., *Nonsteroidal anti-inflammatory drugs for postoperative pain*. Pediatric Drugs, 2003. **5**(2): p. 103-123.
32. Lloyd-Thomas, A.R., *Modern concepts of paediatric analgesia*. Pharmacology & therapeutics, 1999. **83**(1): p. 1-20.
33. Garimella, V. and C. Cellini, *Postoperative pain control*. Clinics in colon and rectal surgery, 2013. **26**(3): p. 191.
34. Groudine, S. and S. Fossum, *Use of intravenous acetaminophen in the treatment of postoperative pain*. Journal of PeriAnesthesia Nursing, 2011. **26**(2): p. 74-80.
35. Gan, T.J., et al., *Incidence, patient satisfaction, and perceptions of post-surgical pain: results from a US national survey*. Current medical research and opinion, 2014. **30**(1): p. 149-160.
36. Kavaliers, M., *Evolutionary and comparative aspects of nociception*. Brain Research Bulletin, 1988. **21**(6): p. 923-931.
37. McEntire, D.M., et al., *Pain transduction: a pharmacologic perspective*. Expert review of clinical pharmacology, 2016. **9**(8): p. 1069-1080.

38. Renthall, W., *Chapter 23 - Pain genetics*, in *Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease (Sixth Edition)*, R.N. Rosenberg and J.M. Pascual, Editors. 2020, Academic Press. p. 397-410.
39. Dubin, A.E. and A. Patapoutian, *Nociceptors: the sensors of the pain pathway*. The Journal of clinical investigation, 2010. **120**(11): p. 3760-3772.
40. Sherrington, C.S., *Qualitative difference of spinal reflex corresponding with qualitative difference of cutaneous stimulus*. The Journal of physiology, 1903. **30**(1): p. 39.
41. Marchand, S., *The physiology of pain mechanisms: from the periphery to the brain*. Rheumatic disease clinics of North America, 2008. **34**(2): p. 285-309.
42. Melzack, R. and P.D. Wall, *Pain mechanisms: a new theory*. Science, 1965. **150**(3699): p. 971-979.
43. Ploner, M., et al., *Cortical representation of first and second pain sensation in humans*. Proceedings of the National Academy of Sciences, 2002. **99**(19): p. 12444-12448.
44. Marino, M.H. and T.K. Watanabe, *Chapter 12 - Posttraumatic Pain Management*, in *Rehabilitation After Traumatic Brain Injury*, B.C. Eapen and D.X. Cifu, Editors. 2019, Elsevier. p. 165-177.
45. Ständer, S., et al., *Neurophysiology of pruritus: cutaneous elicitation of itch*. Archives of dermatology, 2003. **139**(11): p. 1463-1470.
46. Yam, M.F., et al., *General pathways of pain sensation and the major neurotransmitters involved in pain regulation*. International journal of molecular sciences, 2018. **19**(8): p. 2164.
47. Schmidt-Nielsen, K., *Animal physiology: adaptation and environment*. 1997: Cambridge university press.
48. Chiechio, S. and F. Nicoletti, *Metabotropic glutamate receptors and the control of chronic pain*. Current opinion in pharmacology, 2012. **12**(1): p. 28-34.
49. Rogoz, K., et al., *Multimodal use of calcitonin gene-related peptide and substance P in itch and acute pain uncovered by the elimination of vesicular glutamate transporter 2 from transient receptor potential cation channel subfamily V member 1 neurons*. Journal of Neuroscience, 2014. **34**(42): p. 14055-14068.
50. Steeds, C.E., *The anatomy and physiology of pain*. Surgery (Oxford), 2009. **27**(12): p. 507-511.

51. Basbaum, A.I., et al., *Cellular and molecular mechanisms of pain*. Cell, 2009. **139**(2): p. 267-284.
52. Dostrovsky, J.O., *Role of thalamus in pain*. Progress in brain research, 2000. **129**: p. 245-257.
53. Scholz, J. and C.J. Woolf, *Can we conquer pain?* Nature neuroscience, 2002. **5**(11): p. 1062-1067.
54. Apkarian, A.V., et al., *Human brain mechanisms of pain perception and regulation in health and disease*. European journal of pain, 2005. **9**(4): p. 463-484.
55. Chen, J.-I., et al., *Differentiating noxious-and innocuous-related activation of human somatosensory cortices using temporal analysis of fMRI*. Journal of neurophysiology, 2002. **88**(1): p. 464-474.
56. Treede, R.-D., et al., *The cortical representation of pain*. PAIN, 1999. **79**(2): p. 105-111.
57. Rainville, P., et al., *Pain affect encoded in human anterior cingulate but not somatosensory cortex*. Science, 1997. **277**(5328): p. 968-971.
58. Craig, A.D., *How do you feel? Interoception: the sense of the physiological condition of the body*. Nature reviews neuroscience, 2002. **3**(8): p. 655-666.
59. Lu, C., et al., *Insular cortex is critical for the perception, modulation, and chronification of pain*. Neuroscience bulletin, 2016. **32**(2): p. 191-201.
60. Strigo, I.A., et al., *Differentiation of visceral and cutaneous pain in the human brain*. Journal of neurophysiology, 2003. **89**(6): p. 3294-3303.
61. Ong, W.-Y., C.S. Stohler, and D.R. Herr, *Role of the Prefrontal Cortex in Pain Processing*. Molecular neurobiology, 2019. **56**(2): p. 1137-1166.
62. Yen, C.-T. and P.-L. Lu, *Thalamus and pain*. Acta Anaesthesiologica Taiwanica, 2013. **51**(2): p. 73-80.
63. Lenz, F., et al., *Stimulation in the human somatosensory thalamus can reproduce both the affective and sensory dimensions of previously experienced pain*. Nature medicine, 1995. **1**(9): p. 910-913.
64. Ossipov, M.H., K. Morimura, and F. Porreca, *Descending pain modulation and chronification of pain*. Current opinion in supportive and palliative care, 2014. **8**(2): p. 143.

65. Helmstetter, F.J., et al., *Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla*. Brain research, 1998. **779**(1-2): p. 104-118.
66. Helmstetter, F.J., *The amygdala is essential for the expression of conditional hypoalgesia*. Behavioral neuroscience, 1992. **106**(3): p. 518.
67. Uddin, O., et al., *Amplified parabrachial nucleus activity in a rat model of trigeminal neuropathic pain*. Neurobiology of Pain, 2018. **3**: p. 22-30.
68. Gauriau, C. and J.F. Bernard, *Pain pathways and parabrachial circuits in the rat*. Experimental physiology, 2002. **87**(2): p. 251-258.
69. Behbehani, M.M. and H.L. Fields, *Evidence that an excitatory connection between the periaqueductal gray and nucleus raphe magnus mediates stimulation produced analgesia*. Brain research, 1979. **170**(1): p. 85-93.
70. Fields, H.L., et al., *Nucleus raphe magnus inhibition of spinal cord dorsal horn neurons*. Brain Research, 1977. **126**(3): p. 441-453.
71. Heinricher, M.M., et al., *Descending control of nociception: Specificity, recruitment and plasticity*. Brain research reviews, 2009. **60**(1): p. 214-225.
72. Fields, H.L., et al., *The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat*. J Neurosci, 1983. **3**(12): p. 2545-52.
73. Millan, M.J., *Descending control of pain*. Progress in neurobiology, 2002. **66**(6): p. 355-474.
74. Kwon, M., et al., *The role of descending inhibitory pathways on chronic pain modulation and clinical implications*. Pain Practice, 2014. **14**(7): p. 656-667.
75. Kidd, B. and L. Urban, *Mechanisms of inflammatory pain*. British journal of anaesthesia, 2001. **87**(1): p. 3-11.
76. Dray, A., et al., *Bradykinin-induced activation of nociceptors: receptor and mechanistic studies on the neonatal rat spinal cord-tail preparation in vitro*. British journal of pharmacology, 1992. **107**(4): p. 1129-1134.
77. Dray, A. and M. Perkins, *Bradykinin and inflammatory pain*. Trends Neurosci, 1993. **16**(3): p. 99-104.

78. Steranka, L.R., et al., *Bradykinin as a pain mediator: receptors are localized to sensory neurons, and antagonists have analgesic actions*. Proceedings of the National Academy of Sciences, 1988. **85**(9): p. 3245-3249.
79. Zhang, J.-M. and J. An, *Cytokines, inflammation and pain*. International anesthesiology clinics, 2007. **45**(2): p. 27.
80. Oprea, A. and M. Kress, *Involvement of the proinflammatory cytokines tumor necrosis factor- α , IL-1 β , and IL-6 but not IL-8 in the development of heat hyperalgesia: effects on heat-evoked calcitonin gene-related peptide release from rat skin*. Journal of Neuroscience, 2000. **20**(16): p. 6289-6293.
81. Watkins, L.R., et al., *Characterization of cytokine-induced hyperalgesia*. Brain research, 1994. **654**(1): p. 15-26.
82. Nicol, G.D., J.C. Lopshire, and C.M. Pafford, *Tumor necrosis factor enhances the capsaicin sensitivity of rat sensory neurons*. Journal of Neuroscience, 1997. **17**(3): p. 975-982.
83. Vane, J., Y. Bakhle, and R. Botting, *CYCLOOXYGENASES 1 AND 2*. Annual review of pharmacology and toxicology, 1998. **38**(1): p. 97-120.
84. Ballou, L.R., et al., *Nociception in cyclooxygenase isozyme-deficient mice*. Proceedings of the National Academy of Sciences, 2000. **97**(18): p. 10272-10276.
85. Cashman, J.N., *The Mechanisms of Action of NSAIDs in Analgesia*. Drugs, 1996. **52**(5): p. 13-23.
86. Smith, S.M. and W.W. Vale, *The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress*. Dialogues in clinical neuroscience, 2006. **8**(4): p. 383-395.
87. Davis, M., et al., *Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety*. Neuropsychopharmacology, 2010. **35**(1): p. 105-135.
88. Calhoon, G.G. and K.M. Tye, *Resolving the neural circuits of anxiety*. Nature Neuroscience, 2015. **18**(10): p. 1394-1404.
89. Shin, L.M. and I. Liberzon, *The neurocircuitry of fear, stress, and anxiety disorders*. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 2010. **35**(1): p. 169-191.

90. Hakamata, Y., et al., *Amygdala-centred functional connectivity affects daily cortisol concentrations: a putative link with anxiety*. Sci Rep, 2017. **7**(1): p. 8313.
91. Kim, J.J., et al., *Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning*. Journal of Neuroscience, 2001. **21**(14): p. 5222-5228.
92. Russell, G. and S. Lightman, *The human stress response*. Nature Reviews Endocrinology, 2019. **15**(9): p. 525-534.
93. Keller-Wood, M.E. and M.F. Dallman, *Corticosteroid inhibition of ACTH secretion*. Endocr Rev, 1984. **5**(1): p. 1-24.
94. McCarty, R., *Regulation of plasma catecholamine responses to stress*. Seminars in Neuroscience, 1994. **6**(4): p. 197-204.
95. McCarty, R. and P.E. Gold, *Catecholamines, stress, and disease: a psychobiological perspective*. Psychosomatic Medicine, 1996. **58**(6): p. 590-597.
96. Kopin, I.J., G. Eisenhofer, and D. Goldstein, *Sympathoadrenal Medullary System and Stress*, in *Mechanisms of Physical and Emotional Stress*, G.P. Chrousos, D.L. Loriaux, and P.W. Gold, Editors. 1988, Springer US: Boston, MA. p. 11-23.
97. Latremoliere, A. and C.J. Woolf, *Central sensitization: a generator of pain hypersensitivity by central neural plasticity*. The journal of pain, 2009. **10**(9): p. 895-926.
98. Li, J., D.A. Simone, and A.A. Larson, *Windup leads to characteristics of central sensitization*. Pain, 1999. **79**(1): p. 75-82.
99. Petrenko, A.B., et al., *The Role of N-Methyl-d-Aspartate (NMDA) Receptors in Pain: A Review*. Anesthesia & Analgesia, 2003. **97**(4).
100. Woolf, Clifford J., *Central Sensitization: Uncovering the Relation between Pain and Plasticity*. Anesthesiology, 2007. **106**(4): p. 864-867.
101. Ji, R.-R., et al., *Neuroinflammation and central sensitization in chronic and widespread pain*. Anesthesiology, 2018. **129**(2): p. 343-366.
102. Lumley, M.A., et al., *Pain and emotion: a biopsychosocial review of recent research*. Journal of clinical psychology, 2011. **67**(9): p. 942-968.
103. Rhudy, J.L. and M.W. Meagher, *Fear and anxiety: divergent effects on human pain thresholds*. Pain, 2000. **84**(1): p. 65-75.

104. Raphael, K.G. and C.S. Widom, *Post-traumatic stress disorder moderates the relation between documented childhood victimization and pain 30 years later*. *Pain*, 2011. **152**(1): p. 163-169.
105. Cohen, L.L., et al., *Evidence-based assessment of pediatric pain*. *Journal of pediatric psychology*, 2008. **33**(9): p. 939-955.
106. Brand, K. and A. Al-Rais, *Pain assessment in children*. *Anaesthesia & Intensive Care Medicine*, 2019. **20**(6): p. 314-317.
107. Hester, N.K.O., *The preoperational child's reaction to immunization*. *Nursing Research*, 1979.
108. Bieri, D., et al., *The Faces Pain Scale for the self-assessment of the severity of pain experienced by children: development, initial validation, and preliminary investigation for ratio scale properties*. *Pain*, 1990. **41**(2): p. 139-150.
109. Hicks, C.L., et al., *The Faces Pain Scale–Revised: toward a common metric in pediatric pain measurement*. *Pain*, 2001. **93**(2): p. 173-183.
110. Wong, D.L. and C.M. Baker, *Pain in children: comparison of assessment scales*. *Pediatr Nurs*, 1988. **14**(1): p. 9-17.
111. Beyer, J.E., M.J. Denyes, and A.M. Villarruel, *The creation, validation, and continuing development of the Oucher: a measure of pain intensity in children*. *Journal of pediatric nursing*, 1992. **7**(5): p. 335-346.
112. Stinson, J.N., et al., *Systematic review of the psychometric properties, interpretability and feasibility of self-report pain intensity measures for use in clinical trials in children and adolescents*. *PAIN*, 2006. **125**(1).
113. Gharaibeh, M. and H. Abu-Saad, *Cultural validation of pediatric pain assessment tools: Jordanian perspective*. *Journal of Transcultural Nursing*, 2002. **13**(1): p. 12-18.
114. Perrott, D.A., B. Goodenough, and G.D. Champion, *Children's ratings of the intensity and unpleasantness of post-operative pain using facial expression scales*. *European Journal of Pain*, 2004. **8**(2): p. 119-127.
115. Miró, J. and A. Huguet, *Evaluation of reliability, validity, and preference for a pediatric pain intensity scale: the Catalan version of the faces pain scale–revised*. *Pain*, 2004. **111**(1-2): p. 59-64.

116. Goodenough, B., et al., *Pain in 4-to 6-year-old children receiving intramuscular injections: a comparison of the Faces Pain Scale with other self-report and behavioral measures*. The Clinical journal of pain, 1997. **13**(1): p. 60-73.
117. Migdal, M., et al., *Rapid, needle-free delivery of lidocaine for reducing the pain of venipuncture among pediatric subjects*. Pediatrics, 2005. **115**(4): p. e393-e398.
118. Luffy, R. and S.K. Grove, *Examining the validity, reliability, and preference of three pediatric pain measurement tools in African-American children*. Pediatric nursing, 2003. **29**(1): p. 54.
119. Nilsson, S., M. Buchholz, and G. Thunberg, *Assessing children's anxiety using the modified short state-trait anxiety inventory and talking mats: A pilot study*. Nursing Research and Practice, 2012. **2012**.
120. Spielberger, C.D., *Manual for the state-trait anxiety inventory for children*. Consulting Psychologists' Press, Palo Alto, 1973.
121. Schisler, T., J. Lander, and S. Fowler-Kerry, *Assessing children's state anxiety*. Journal of pain and symptom management, 1998. **16**(2): p. 80-86.
122. McMurtry, C.M., et al., *Children's fear during procedural pain: preliminary investigation of the Children's Fear Scale*. Health Psychology, 2011. **30**(6): p. 780.
123. Chorpita, B.F., et al., *Assessment of symptoms of DSM-IV anxiety and depression in children: A revised child anxiety and depression scale*. Behaviour research and therapy, 2000. **38**(8): p. 835-855.
124. Bringuier, S., et al., *The perioperative validity of the visual analog anxiety scale in children: a discriminant and useful instrument in routine clinical practice to optimize postoperative pain management*. Anesthesia & Analgesia, 2009. **109**(3): p. 737-744.
125. Strimbu, K. and J.A. Tavel, *What are biomarkers?* Current Opinion in HIV and AIDS, 2010. **5**(6): p. 463.
126. Marchi, A., et al., *Pain biomarkers*. Clinical drug investigation, 2009. **29**(1): p. 41-46.
127. Uçeyler, N., et al., *Differential expression of cytokines in painful and painless neuropathies*. Neurology, 2007. **69**(1): p. 42-9.
128. Uçeyler, N., W. Häuser, and C. Sommer, *Systematic review with meta-analysis: cytokines in fibromyalgia syndrome*. BMC Musculoskelet Disord, 2011. **12**: p. 245.

129. Parkitny, L., et al., *Inflammation in complex regional pain syndrome: a systematic review and meta-analysis*. Neurology, 2013. **80**(1): p. 106-17.
130. White, A.T., et al., *Severity of symptom flare after moderate exercise is linked to cytokine activity in chronic fatigue syndrome*. Psychophysiology, 2010. **47**(4): p. 615-24.
131. DeVon, H.A., et al., *The association of pain with protein inflammatory biomarkers: a review of the literature*. Nursing research, 2014. **63**(1): p. 51-62.
132. Kraychete, D.C., et al., *Serum cytokine levels in patients with chronic low back pain due to herniated disc: analytical cross-sectional study*. Sao Paulo Medical Journal, 2010. **128**(5): p. 259-262.
133. Ang, D.C., et al., *MCP-1 and IL-8 as pain biomarkers in fibromyalgia: a pilot study*. Pain Medicine, 2011. **12**(8): p. 1154-1161.
134. Chakrabarty, S. and R. Zoorob, *Fibromyalgia*. American family physician, 2007. **76**(2): p. 247-254.
135. Castillo, J., et al., *Plasma monoamines in tension-type headache*. Headache: The Journal of Head and Face Pain, 1994. **34**(9): p. 531-535.
136. Nackley, A.G., et al., *Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both β 2-and β 3-adrenergic receptors*. Pain, 2007. **128**(3): p. 199-208.
137. Wang, X.-M., et al., *Upregulation of IL-6, IL-8 and CCL2 gene expression after acute inflammation: Correlation to clinical pain*. PAIN®, 2009. **142**(3): p. 275-283.
138. Si, H.-b., et al., *Correlations between inflammatory cytokines, muscle damage markers and acute postoperative pain following primary total knee arthroplasty*. BMC musculoskeletal disorders, 2017. **18**(1): p. 1-9.
139. Ledowski, T., et al., *Effects of acute postoperative pain on catecholamine plasma levels, hemodynamic parameters, and cardiac autonomic control*. PAIN, 2012. **153**(4).
140. Ferland, C.E., et al., *Preoperative norepinephrine levels in cerebrospinal fluid and plasma correlate with pain intensity after pediatric spine surgery*. Spine deformity, 2017. **5**(5): p. 325-333.
141. Nater, U.M. and N. Rohleder, *Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research*. Psychoneuroendocrinology, 2009. **34**(4): p. 486-496.

142. Van Veen, J., et al., *Elevated alpha-amylase but not cortisol in generalized social anxiety disorder*. Psychoneuroendocrinology, 2008. **33**(10): p. 1313-1321.
143. Lim, I.-S., *Comparative analysis of the correlation between anxiety, salivary alpha amylase, cortisol levels, and athletes' performance in archery competitions*. Journal of exercise nutrition & biochemistry, 2018. **22**(4): p. 69.
144. Kobayashi, F.Y., et al., *Salivary stress biomarkers and anxiety symptoms in children with and without temporomandibular disorders*. Brazilian oral research, 2017. **31**.
145. Sadi, H., M. Finkelman, and M. Rosenberg, *Salivary cortisol, salivary alpha amylase, and the dental anxiety scale*. Anesthesia progress, 2013. **60**(2): p. 46-53.
146. Altaf, F., et al., *Adolescent idiopathic scoliosis*. Bmj, 2013. **346**.
147. Committee, T., *Scoliosis Research Society. A glossary of scoliosis terms*. Spine, 1976. **1**: p. 57.
148. Arlet, V., T. Odent, and M. Aebi, *Congenital scoliosis*. European Spine Journal, 2003. **12**(5): p. 456-463.
149. Levy, B.J., et al., *Complications associated with surgical repair of syndromic scoliosis*. Scoliosis, 2015. **10**(1): p. 1-16.
150. Chung, A.S., et al., *Syndromic scoliosis: National trends in surgical management and inpatient hospital outcomes: A 12-year analysis*. Spine, 2019. **44**(22): p. 1564-1570.
151. Lonstein, J.E., *Adolescent idiopathic scoliosis*. The Lancet, 1994. **344**(8934): p. 1407-1412.
152. Konieczny, M.R., H. Senyurt, and R. Krauspe, *Epidemiology of adolescent idiopathic scoliosis*. Journal of children's orthopaedics, 2013. **7**(1): p. 3-9.
153. Kamtsiuris, P., et al., *Prevalence of somatic diseases in German children and adolescents. Results of the German Health Interview and Examination Survey for Children and Adolescents (KiGGS)*. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz, 2007. **50**(5-6): p. 686-700.
154. Suh, S.-W., et al., *Idiopathic scoliosis in Korean schoolchildren: a prospective screening study of over 1 million children*. European spine journal, 2011. **20**(7): p. 1087-1094.
155. Nery, L.S., et al., *Prevalence of scoliosis among school students in a town in southern Brazil*. Sao Paulo medical journal, 2010. **128**(2): p. 69-73.

156. Daruwalla, J., et al., *Idiopathic scoliosis. Prevalence and ethnic distribution in Singapore schoolchildren*. The Journal of bone and joint surgery. British volume, 1985. **67**(2): p. 182-184.
157. Wong, H.-K., et al., *Idiopathic scoliosis in Singapore schoolchildren: a prevalence study 15 years into the screening program*. Spine, 2005. **30**(10): p. 1188-1196.
158. Cilli, K., et al., *School screening for scoliosis in Sivas, Turkey*. Acta Orthop Traumatol Turc, 2009. **43**(5): p. 426-430.
159. Soucacos, P.N., et al., *School-screening for scoliosis. A prospective epidemiological study in northwestern and central Greece*. JBJS, 1997. **79**(10): p. 1498-1503.
160. Cheng, J.C., et al., *Adolescent idiopathic scoliosis*. Nature reviews disease primers, 2015. **1**(1): p. 1-21.
161. Zhang, J., et al., *Computer-aided Cobb measurement based on automatic detection of vertebral slopes using deep neural network*. International journal of biomedical imaging, 2017. **2017**.
162. Gao, X., et al., *CHD7 gene polymorphisms are associated with susceptibility to idiopathic scoliosis*. The American Journal of Human Genetics, 2007. **80**(5): p. 957-965.
163. Chen, Z., et al., *Comparison of somatosensory evoked potentials between adolescent idiopathic scoliosis and congenital scoliosis without neural axis abnormalities*. The Spine Journal, 2014. **14**(7): p. 1095-1098.
164. Lombardi, G., et al., *Biochemistry of adolescent idiopathic scoliosis*. Advances in clinical chemistry, 2011. **54**: p. 165-182.
165. Makino, T., et al., *Low back pain and patient-reported QOL outcomes in patients with adolescent idiopathic scoliosis without corrective surgery*. Springerplus, 2015. **4**(1): p. 1-6.
166. Balagué, F. and F. Pellisé, *Adolescent idiopathic scoliosis and back pain*. Scoliosis and spinal disorders, 2016. **11**(1): p. 1-15.
167. Thérout, J., et al., *Back pain prevalence is associated with curve-type and severity in adolescents with idiopathic scoliosis*. Spine, 2017. **42**(15): p. E914-E919.
168. Teles, A.R., et al., *Back pain in adolescents with idiopathic scoliosis: the contribution of morphological and psychological factors*. European Spine Journal, 2020. **29**(8): p. 1959-1971.

169. Ovadia, D., *Classification of adolescent idiopathic scoliosis (AIS)*. Journal of children's orthopaedics, 2013. **7**(1): p. 25-28.
170. Hinman, M.R., *Comparison of thoracic kyphosis and postural stiffness in younger and older women*. The Spine Journal, 2004. **4**(4): p. 413-417.
171. Bisson, D.G., et al., *Toll-like receptor involvement in adolescent scoliotic facet joint degeneration*. Journal of Cellular and Molecular Medicine, 2020. **24**(19): p. 11355-11365.
172. Ferland, C.E., et al., *Preoperative distress factors predicting postoperative pain in adolescents undergoing surgery: a preliminary study*. Journal of Pediatric Health Care, 2017. **31**(1): p. 5-15.
173. Chidambaran, V., et al., *Predicting the pain continuum after adolescent idiopathic scoliosis surgery: a prospective cohort study*. European journal of pain, 2017. **21**(7): p. 1252-1265.
174. Landman, Z., et al., *Prevalence and predictors of pain in surgical treatment of adolescent idiopathic scoliosis*. Spine, 2011. **36**(10): p. 825-829.
175. Rabbitts, J.A., et al., *Trajectories of postsurgical pain in children: risk factors and impact of late pain recovery on long-term health outcomes after major surgery*. Pain, 2015. **156**(11): p. 2383.
176. McGuire, D.B., *Comprehensive and multidimensional assessment and measurement of pain*. Journal of pain and symptom management, 1992. **7**(5): p. 312-319.
177. Breau, L.M. and C. Burkitt, *Assessing pain in children with intellectual disabilities*. Pain Research and Management, 2009. **14**(2): p. 116-120.
178. Schiavenato, M. and K.D. Craig, *Pain assessment as a social transaction: beyond the "gold standard"*. The Clinical journal of pain, 2010. **26**(8): p. 667-676.
179. Pagé, M.G., et al., *Acute pain trajectories and the persistence of post-surgical pain: a longitudinal study after total hip arthroplasty*. Journal of anesthesia, 2016. **30**(4): p. 568-577.
180. Du Clos, T.W., *Function of C-reactive protein*. Annals of Medicine, 2000. **32**(4): p. 274-278.
181. Kawasaki, Y., et al., *Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1 β , interleukin-6, and tumor necrosis factor- α in*

- regulating synaptic and neuronal activity in the superficial spinal cord.* Journal of neuroscience, 2008. **28**(20): p. 5189-5194.
182. Christensen, N.J., *Plasma Epinephrine and Plasma Norepinephrine Concentrations in Early Diabetes*, in *Vascular and Neurological Changes in Early Diabetes*, R.A. Camerini-Dávalos and H.S. Cole, Editors. 1973, Academic Press. p. 227-234.
 183. Morningstar, M., *Neurotransmitter patterns in patients with adolescent idiopathic scoliosis (AIS)*. Scoliosis, 2013. **8**(Suppl 2): p. O1-O1.
 184. Ciolkowski, E.L., et al., *Direct observation of epinephrine and norepinephrine cosecretion from individual adrenal medullary chromaffin cells*. Journal of the American Chemical Society, 1992. **114**(8): p. 2815-2821.
 185. Sommer, C., *Serotonin in pain and analgesia*. Molecular neurobiology, 2004. **30**(2): p. 117-125.
 186. Skeith, L., et al., *A practical approach to evaluating postoperative thrombocytopenia*. Blood advances, 2020. **4**(4): p. 776-783.
 187. Burton, D., G. Nicholson, and G. Hall, *Endocrine and metabolic response to surgery*. Continuing Education in Anaesthesia Critical Care & Pain, 2004. **4**(5): p. 144-147.
 188. Schlereth, T. and F. Birklein, *The sympathetic nervous system and pain*. Neuromolecular medicine, 2008. **10**(3): p. 141-147.
 189. Desborough, J., *The stress response to trauma and surgery*. British journal of anaesthesia, 2000. **85**(1): p. 109-117.
 190. Skolnick, P., *On the front lines of the opioid epidemic: Rescue by naloxone*. European Journal of Pharmacology, 2018. **835**: p. 147-153.
 191. Piloizzi, A., C. Carro, and X. Huang, *Roles of β -Endorphin in Stress, Behavior, Neuroinflammation, and Brain Energy Metabolism*. International journal of molecular sciences, 2020. **22**(1): p. 338.
 192. Dubois, M., et al., *Surgical stress in humans is accompanied by an increase in plasma beta-endorphin immunoreactivity*. Life Sciences, 1981. **29**(12): p. 1249-1254.
 193. Binder, W., et al., *Sympathetic activation triggers endogenous opioid release and analgesia within peripheral inflamed tissue*. European Journal of Neuroscience, 2004. **20**(1): p. 92-100.

194. G. Bisson, D., et al., *Facet joint degeneration in adolescent idiopathic scoliosis*. JOR spine, 2018. **1**(2): p. e1016.
195. Housby, J.N., et al., *Non-steroidal anti-inflammatory drugs inhibit the expression of cytokines and induce HSP70 in human monocytes*. Cytokine, 1999. **11**(5): p. 347-358.
196. Li, M.M., et al., *Acute postoperative opioid consumption trajectories and long-term outcomes in pediatric patients after spine surgery*. Journal of pain research, 2019. **12**: p. 1673.
197. Oca, D.D., et al., *Safety of patient-controlled analgesia after surgery in children and adolescents: concerns and potential solutions*. Frontiers in pediatrics, 2018. **6**: p. 336.
198. Macintyre, P., *Safety and efficacy of patient-controlled analgesia*. British journal of anaesthesia, 2001. **87**(1): p. 36-46.
199. Gil, K.M., et al., *Patient-controlled analgesia in postoperative pain: the relation of psychological factors to pain and analgesic use*. The Clinical journal of pain, 1990. **6**(2): p. 137-142.
200. Perry, F., et al., *Role of psychological factors in postoperative pain control and recovery with patient-controlled analgesia*. The Clinical journal of pain, 1994. **10**(1): p. 57-63; discussion 82.
201. Takai, N., et al., *Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults*. Archives of Oral Biology, 2004. **49**(12): p. 963-968.
202. Noto, Y., et al., *The relationship between salivary biomarkers and state-trait anxiety inventory score under mental arithmetic stress: a pilot study*. Anesthesia & Analgesia, 2005. **101**(6): p. 1873-1876.
203. Henker, R.A., et al., *The associations between OPRM1 and COMT genotypes and postoperative pain, opioid use, and opioid-induced sedation*. Biological research for nursing, 2013. **15**(3): p. 309-317.
204. Belfer, I., et al., *Pain modality-and sex-specific effects of COMT genetic functional variants*. PAIN®, 2013. **154**(8): p. 1368-1376.
205. Diatchenko, L., et al., *Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli*. Pain, 2006. **125**(3): p. 216-224.

206. Buvanendran, A., et al., *Upregulation of Prostaglandin E2 and Interleukins in the Central Nervous System and Peripheral Tissue during and after Surgery in Humans*. The Journal of the American Society of Anesthesiologists, 2006. **104**(3): p. 403-410.
207. Zieglgänsberger, W., *Substance P and pain chronicity*. Cell and tissue research, 2019. **375**(1): p. 227-241.
208. Urits, I., et al., *An evidence-based review of CGRP mechanisms in the propagation of chronic visceral pain*. Best Practice & Research Clinical Anaesthesiology, 2020. **34**(3): p. 507-516.
209. Paller, C.J., et al., *Sex-based differences in pain perception and treatment*. Pain medicine, 2009. **10**(2): p. 289-299.