

**THE EFFECT OF MASSAGE ON AUTONOMIC NERVOUS SYSTEM  
IN PATIENTS IN PEDIATRIC INTENSIVE CARE UNITS**

by

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## ABSTRACT

**Background:** Patients in Pediatric Intensive Care Units (PICU) face high stressors. The autonomic nervous system (ANS) plays an important role in modulating stress and inflammatory responses. Excess stress reaction includes SNS over-stimulation and PNS suppression. If prolonged, this situation may be source of severe acute and chronic inflammation. Massage therapy is a noninvasive intervention that may regulate stress-induced ANS dysfunction by stimulating PNS and decreasing SNS responses; therefore, acting toward homeostasis.

**Objectives:** Primary objective was to assess the massage effect on rebalancing the ANS function (measured by Heart Rate Variability, HRV). Secondary objective was to investigate the correlation between HRV and clinical severity (measured with Pediatric Logistic Organ Dysfunction (PELOD) Scores). Pilot study objective was to determine study feasibility.

**Methods:** All PICU patients were eligible for the study. 22 patients were randomly allocated into one or six massage sessions per day (30-min per session). HRV was extracted from PICU central station. Clinical information was collected from medical records. Descriptive statistics and graphs were used. Study feasibility (problems and solutions) was also assessed.

**Results:** 18 subjects were included in the analyses. logHF and logLF increased significantly during the first massage session compared to baseline values ( $p < 0.05$ ). HF increased by a mean of 75.06% (95% CI: 19.9~130.23%,  $p = 0.01$ ) and LF increased by a mean of 56.3% (95% CI: 20.31~92.29 %,  $p = 0.004$ ) during the first massage session; although HF and LF decreased in four subjects. After massage the increases in both HF and LF were not significant. LF/HF ratio, when out of the normal range, converged toward normal values during massage. In the six massage sessions ( $n = 7$ ), HF and LF increased in the initial three sessions and plateaued in the

remaining sessions. There was a positive relationship between HRV and clinical severity. Study feasibility analysis led us identifying problems and finding solutions.

**Conclusion:** ANS dysfunction was correlated with clinical severity. Massage therapy had an effect in improving ANS function with increasing PNS and decreasing SNS activities in PICU patients. The optimal number of massage sessions seemed to be three. However, massage could also be a stress for some patients in certain conditions.

## **PREFACE**

The overall study was designed and implemented under the leadership of my supervisor, Dr. Jean-Paul Collet. The study was also supported by Dr. Niranjan Kissoon, Dr. Peter Skippen, Dr. Mark Ansermino and Dr. Pascal Lavoie. Ethical approval was obtained from the University of British Columbia Children's & Women's Health Center Clinical Research Ethics Board (H10-00986, Sept 10, 2010)

PICU staffs (Rosella Jefferson and Gordon Krahn) at BC Children's Hospital were responsible for contacting patients and obtaining informed consent. Registered massage therapists led by Annette Ruitenbeek and Samantha Eibensteiner administered massage intervention on study subjects. The study coordinator, Nataliya Yuskiv, was responsible for organization and management. My contribution included: (1) developing part of the protocol under supervision; (2) designing the "Clinical information collection form" and "Massage information collection form" (3) organizing data collection and collecting data, including the HRV data (ECG recording) and clinical information (personal information, diagnosis, physiological indices, medication and follow up) (4) monitoring study subjects with video camera (5) designing analyses strategy and conducting all the data analyses and interpretation, including HRV analyses, PELOD scores analyses and study feasibility (6) performing all statistical analyses, including using different statistical tests and "R" software (7) writing up of all this thesis. Dr. Collet guided and supported me throughout the whole process. My committee members Dr. Niranjan Kissoon, Dr. Tim Oberlander and Dr. Rollin Brant gave me great assistance in understanding the important theoretical concepts, analyzing HRV and performing statistical analyses.

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## LIST OF ABBREVIATIONS

ACh	acetylcholine
ACTH	corticotrophin/adrenocorticotrophin
ANS	autonomic nervous system
CA	catecholamine
CNS	central nervous system
E	epinephrine
F&H massage	foot and hand massage
HF	high frequency
HPA axis	hypothalamus-pituitary-adrenal axis
HRV	heart rate variability
IL-1	interleukin-1
IL-6	interleukin-6
IL-10	interleukin-10
LF	low frequency
MODS	multiple organ dysfunction syndrome
NE	norepinephrine
NET	norepinephrine transporter
NK-cells	natural killer cells
PICU	pediatric intensive care units
PSD	power spectral density
TNF	tumor necrosis factor

PNS	parasympathetic nervous system
RSA	respiratory sinus arrhythmia
SIRS	systemic inflammatory response syndrome
SNS	sympathetic nervous system
TH	tyrosine hydroxylase
ULF	ultra-low frequency
VLF	very low frequency

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## DEDICATION

*To my dear dear families who live thousands of miles away  
but deliver love, concern, warm and belief to me every day; to my close friends  
Ding and Yu who have been by my side for more than half of my life;  
and to my boyfriend DP, who faces to my  
crazy, always returns back  
smile*

## **CHAPTER 1: INTRODUCTION**

Patients in pediatric intensive care units (PICU) face multiple stressors and at a high risk of developing systemic inflammatory responses and life-threatening organs failure (Alberti et al., 2002). The stress response (inflammation) under physiological conditions is an adaptive response to “combat” the stress triggers such as infection and trauma, and hence restores functionality and reestablishes homeostasis (Medzhitov, 2008). However, excessive inflammatory response and circulating cytokines may cause systemic organ impairment and even mortality (P J Godin & Buchman, 1996; Hahn et al., 1995). Inflammatory responses thus need tight and rapid control to maintain homeostasis and avoid overshoot.

The autonomic nervous system (ANS) plays an important role in modulating inflammatory responses. Physiologically, the two branches of ANS, the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) act synergistically to inhibit the synthesis and release of inflammatory cytokines, thus controlling the inflammation locally; especially for the PNS, which was shown to have powerful anti-inflammatory properties (Robertson, 2004). However, in severe acute stressful situations, the SNS is over-stimulated and PNS is suppressed. This may cause a “paradoxical pro-inflammatory action” by the over-activated SNS and a blunt anti-inflammatory pathway by the depressed PNS (Annane et al., 1999; Miksa, Wu, Zhou, & Wang, 2005; Tracey, 2002; Werdan et al., 2009). Many studies offered the supporting findings that in the situation of systemic inflammatory response syndrome (SIRS) or sepsis, the sympathetic activity was overwhelmingly increased while the parasympathetic activity was suppressed (Miksa et al., 2005; Rittirsch, Flierl, & Ward, 2008; H. Schmidt et al., 2005; Werdan et al., 2009). The ANS

dysfunction indicates severity and predicts mortality during severe stressful situations and, conversely, the recuperation of ANS activity predicts recovery (Ellenby, McNames, Lai, & McDonald, 2001). Therefore, evidence suggests that ANS dysfunction can aggravate the inflammatory responses and conversely, ANS improvement is beneficial to control the inflammatory response and avoid detrimental consequences.

Heart rate variability (HRV) has been applied to measure ANS function in several studies (Akselrod et al., 1981; ESC/NASPE Task Force, 1996; Pomeranz et al., 1985). It is a noninvasive and precise measurement that reflects different aspects of ANS function: the high frequency (HF) component which is solely mediated by the vagus nerve is an index of PNS activity; the low frequency (LF) component is controlled by both PNS and SNS; and the ratio of LF to HF (LF/HF) represents the balance between sympathetic and parasympathetic responses (ESC/NASPE Task Force, 1996; Rajendra Acharya, Paul Joseph, Kannathal, Lim, & Suri, 2006).

Among different interventions to modulate ANS, massage therapy is a noninvasive intervention that has been demonstrated to have positive effects in improving ANS functions with the stimulation on PNS activity and the suppression of the over-activated SNS. The literature on the effectiveness of massage has already been established, however, the complete evidence regarding duration of effects and the optimal number of massage sessions is still lacking.

The main aims of my thesis are two folds. The clinical research objectives are to collect HRV and clinical data of PICU patients (1 month to 18 year old) in order to (1) investigate the SNS and PNS functions under stressful conditions; (2) assess the effects of long-duration (30 min per

session) and multiple sessions (up to 6 sessions per day) of foot and hand massage on stimulating PNS and decreasing SNS; and (3) investigate the relationship between HRV (HF+LF) magnitude and patients' clinical severity. The pilot study objective is to evaluate the feasibility and practicability of conducting a future larger-scale study to assess the effect of massage in patients hospitalized in the ICU.

## **CHAPTER 2: BACKGROUND**

### **2.1 Stress**

#### **2.1.1 Stress in Intensive Care Units**

The term “stress” describes a state of threatened “homeostasis” or threatened equilibrium by stressors. The “stressors” are any threatening or disturbing forces from the physiology, the psychology and the environment (Chrousos & Gold, 1992; Johnson, Kamilaris, Chrousos, & Gold, 1992; Lusk & Lash, 2005). Many studies showed that the physiological, psychological and environmental stress in intensive care units (ICU) could affect patients’ health condition and cause substantial morbidity (Rotondi et al., 2002; Schelling et al., 2003; Schelling, Briegel, Roozendaal, Stoll, & Rothenha, 2001; Vermetten & Bremner, 2002a, 2002b). For example, a study in 2003 showed that exposure to high stress in the ICU had negative effects on health-related quality-of-life outcomes after cardiac surgery (Schelling et al., 2003). Other studies indicated stress resulted in long-standing changes in biological stress response and induced posttraumatic stress disorder (Schelling et al., 2003; Vermetten & Bremner, 2002a).

The most disturbing stressors for ICU patients are the pathological conditions leading to critical illness (infection, tissue injury, trauma/surgery, metabolic intoxication) and the illness-induced feelings of pain and discomfort (Bork, 1999). In addition almost patients in ICU are administered drugs that affect ANS, such as morphine, epinephrine/norepinephrine etc. which may secondarily activate SNS and promote inflammatory responses (Schelling et al., 2003). Moreover, ICU

patients undergo frequent injections and physical examinations; they are stressed by inserted intravenous and arterial lines (Porter, 1995), mechanical ventilation and pharmaceutical support (Rotondi et al., 2002; Thomas, 2003). A high proportion of patients experience emotional problems (such as fear of death, depression, distress and anxiety) and noisy environment (talking, alarms and equipment) which influence sleep quality (Bennun, 2001; Bork, 1999; Cornock, 1998; Jastremski, 2000; Novaes, Aronovich, Ferraz, & Knobel, 1997). All these stress from physiological, psychological and environmental factors may cause the increase in stress hormones (catecholamine and cortisol), decrease in lymphocyte function, pain tolerance, and the inhibition of healing (Bork, 1999; Desborough, 2000; Grumet, 1993; Morrison, Haas, Shaffner, Garrett, & Fackler, 2003; Pope, 1995). Therefore, the excessive stress should be reduced for ICU patients in order to control these negative effects and detrimental consequences.

### **2.1.2 Stress and Inflammatory Response**

Normally, when homeostasis is disturbed by stress, “stress response” is generated as a way to “remove or sequester the source of the disturbance” by removing stressors or repairing tissues, and to allow the host to adapt to the abnormal conditions and ultimately to restore functionality and homeostasis. Therefore, the “stress responses” are described as the counteracting forces that are put forth to neutralize the stressors’ effects and reestablish homeostasis (Chrousos & Gold, 1992; Johnson et al., 1992; Lusk & Lash, 2005). Excess physiological stress may cause uncontrolled stress responses that induce a series of significant deleterious impacts on organisms (Medzhitov, 2008).

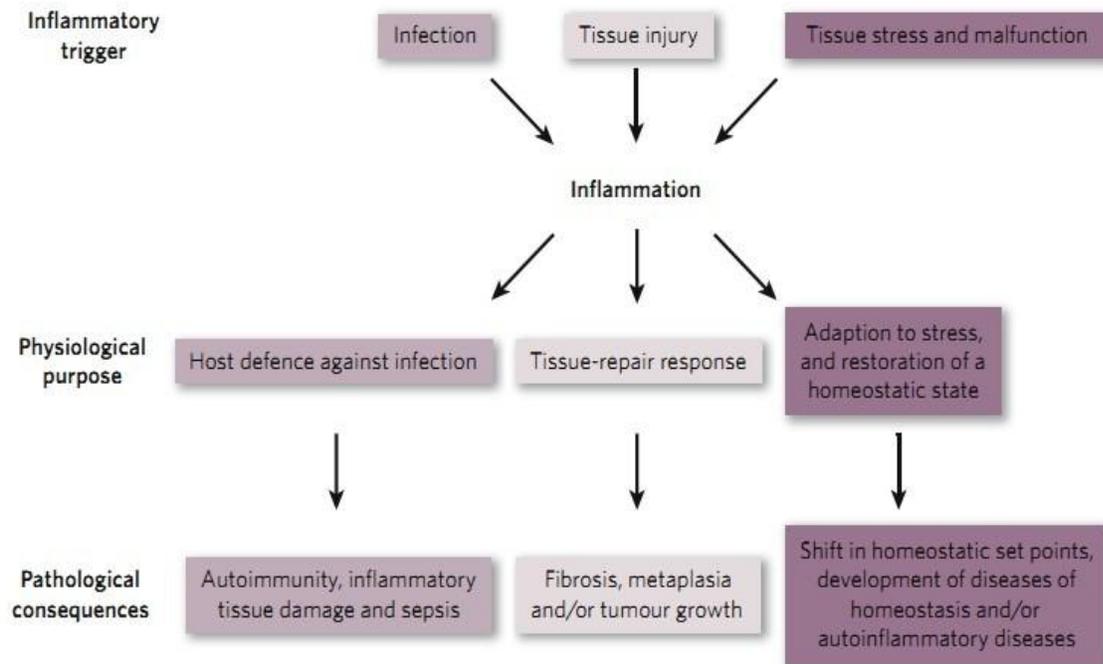
The inflammatory response is one of the stress responses. It underlies a wide variety of physiological and pathological processes and plays a crucial role in human physiology (Medzhitov, 2008). The classic inflammatory response ‘pathway’ consists of inducers, sensors, mediators and effectors (Medzhitov, 2008). Inducers (exogenous and endogenous) are the signals caused by stressful events, such as infection, tissue injury, trauma/surgery and metabolic intoxication, which initiate the inflammatory response. The inducers can activate specialized sensors (such as the Toll-like receptors on macrophage cells and mast cells), which then elicit the production of a variety of mediators, including cytokines, chemokines, vasoactive amines, eicosanoids and products of proteolytic cascades. These mediators have an important role in the activation, mediation and maintenance of the inflammatory response, by opening the functional states of effectors (endothelium and leukocytes), inducing the acute-phase response and initiating neuroendocrine and metabolic responses (Medzhitov, 2008). Cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), are important pro-inflammatory factors. They are produced by activated leucocytes (macrophages and monocytes), fibroblasts and endothelial cells early in the inflammatory response (Cohen, 2002; Medzhitov, 2008; Tracey, 2002); and interact with the surface receptors on a variety of target cells to stimulate a number of changes in these cells (for instance, inducing the formation of the exudates and influencing the protein synthesis) (Desborough, 2000; Sheeran & Hall, 1997).

Physiologically, the inflammatory response is a local protective response. It is beneficial to the body with different physiological purposes, by helping host defenses against infection, repairing tissues, adapting to stress and ultimately restoring homeostasis state (Figure 2-1) (Medzhitov, 2008). The classic inflammatory reaction includes swelling (tumor), redness (rubor), pain (dolor)

and heat (calor). These characters are advantageous for the organism by resulting in a localized hyperaemia to the damaged or infected area, bringing oxygen, clotting factors and glucose for repair, and making humoral and cellular components of the immune system to fight with the original inciting causes (i.e. stressor) (Medzhitov, 2008; Tracey, 2002).

The magnitude of inflammatory reaction must be regulated precisely (Serhan, 2007; Serhan & Savill, 2005). Insufficient inflammation results in poor control of the initial stressors, while excess inflammatory response may cause pathological consequences, morbidity and even mortality (Figure 2-1). Those negative effects include severe systemic acute inflammatory conditions if inflammation spreads into the bloodstream (such as systemic inflammatory response syndrome (SIRS), sepsis, septic shock and multiple organ dysfunction syndrome (MODS) (description provided in Appendix A) and chronic inflammatory conditions (observed in diabetes, cardiovascular diseases and atherosclerosis, for instance,) (Cohen, 2002; Medzhitov, 2008). The uncontrolled inflammatory response may become more dangerous than the original triggers/stimuli. Therefore, the inflammatory response is kept under tight control by a powerful inhibitory system that is activated immediately when the inflammatory response is triggered (Tracey, 2002).

**Figure 2-1:** Causes, physiological purposes and pathological outcomes of inflammation (Medzhitov, 2008)



Depending on the triggers, the inflammatory response has different physiological purposes and pathological consequences (Medzhitov, 2008).

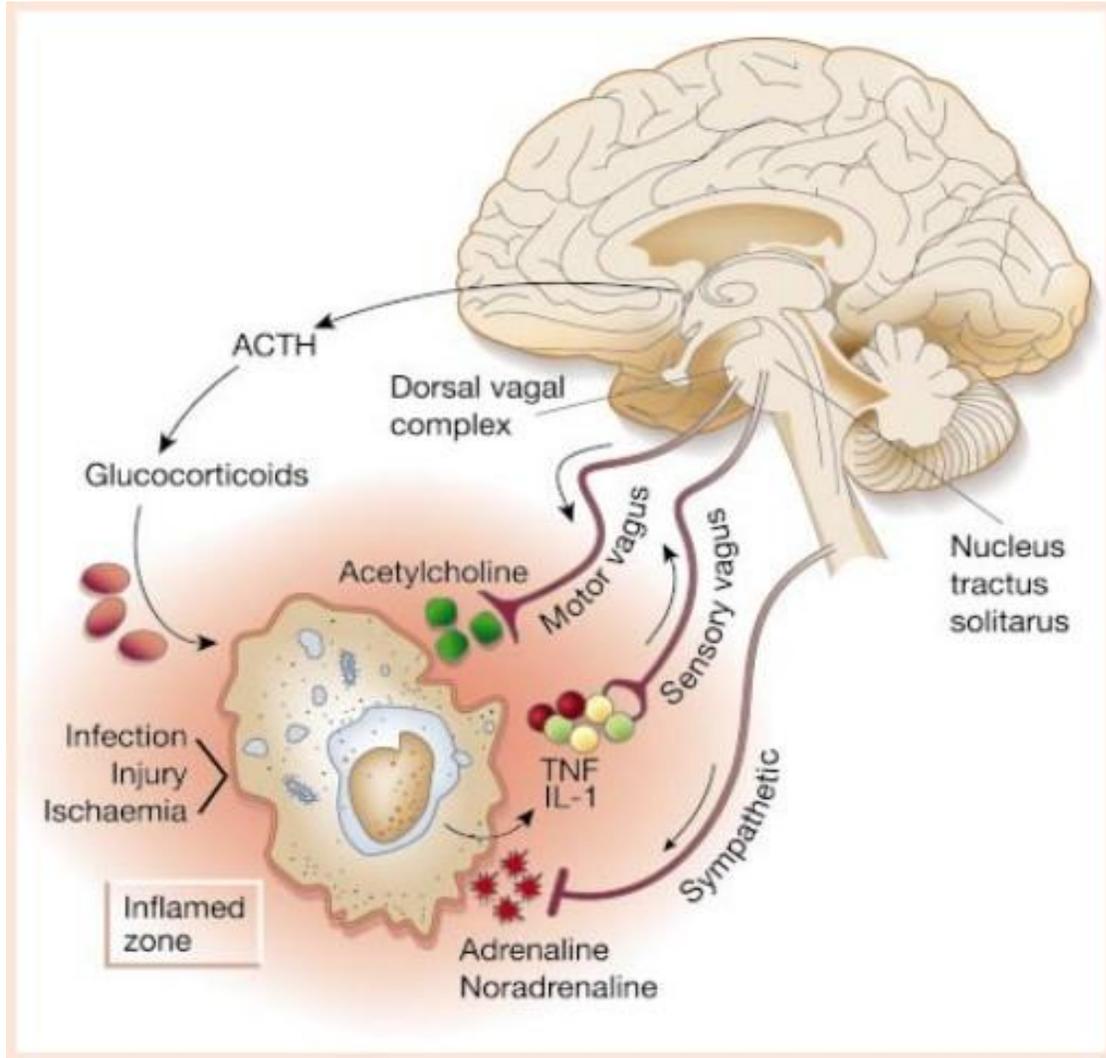
### **2.1.3 The Effect of Autonomic Nervous System in Stress Response**

The autonomic nervous system (ANS) plays an important role in regulating and controlling stress responses. The inflammatory response is characterized by the activation of ANS and increased secretion of several endocrine hormones (such as, catecholamines, cortisol, beta-endorphin, prolactin, growth hormone, insulin and glucagon, thyroid hormones and gonadotropins) that provide energy sources and maintain cardiovascular homeostasis (Desborough, 2000; Schrader, 1996). However, such powerful inflammatory response is nonspecific and potentially dangerous for the host to some extent. Homeostasis requires a rapid and efficient anti-inflammatory control that can limit the intensity, spread and duration of the response.

The three anti-inflammatory reflexes were described by Tracey in 2002 (Tracey, 2002) (Figure 2-2). They are “cholinergic anti-inflammatory pathway”, “humoral anti-inflammatory pathway” and sympathetic anti-inflammatory pathway. The pro-inflammatory cytokines (TNF-alpha, IL-6 and IL-1) which are produced by local macrophages induce the afferent neuronal impulses to travel along the sensory vagus nerve and synapse in the nucleus tractus solitarius. Then the activated efferent vagus nerves produce the acetylcholine (ACh) (the principle parasympathetic neurotransmitter) that interacts with the macrophage nicotinic receptors to effectively inhibit macrophage activation and reduce cytokine synthesis. This is the most important anti-inflammatory reflex by parasympathetic nervous system (PNS) to control the inflammatory response-“cholinergic anti-inflammatory pathway” (Tracey, 2007). In addition to this local, rapid and discrete anti-inflammatory reflex, a systemic humoral anti-inflammatory pathway is also

triggered. The information travels through the dorsal root of the spinal cord, up the spinal cord to the medulla and ultimately activates the hypothalamus. The “releasing hormones” from the hypothalamus stimulate the anterior pituitary to synthesize a number of “tropic hormones”, including the ACTH. ACTH has a critical role in stimulating two important substances: cortisol (from the adrenal cortex) and aldosterone (from the adrenal medulla) to maintain the anti-inflammation. This hypothalamus-pituitary-adrenal (HPA) axis is called “humoral anti-inflammatory pathway” (Tsigos & Chrousos, 2002). Moreover, the stress and pain caused by cytokine production can activate the sympathetic nervous system (SNS) and “flight-or-fight” responses, which then cause the release of epinephrine (E) from the adrenal medulla and norepinephrine (NE) from peripheral nerve endings. Such stress hormones/catecholamines (NE and E) elicit another anti-inflammatory effect at the macrophage level by suppressing pro-inflammatory cytokines release and stimulating anti-inflammatory cytokine release (such as beta-adrenergic-receptor-dependent interleukin-10) (Tracey, 2002). Therefore, both PNS and SNS are synergistic to suppress the inflammation at this level. Their combined anti-inflammatory action is to localize and shorten the duration the inflammatory reaction. Figure 2-2 shows the anti-inflammatory responses controlled by the three pathways.

**Figure 2-2:** Anti-inflammatory reflexes (Tracey, 2002)



Inflammatory products produced in damaged tissues activate afferent signals that are relayed to the nucleus tractus solitarius; subsequent activation of vagus efferent activity inhibits cytokine synthesis through the cholinergic anti-inflammatory pathway. Information can also be relayed to the hypothalamus and the dorsal vagal complex to stimulate the release of ACTH, thereby activating the humoral anti-inflammatory pathway. Activation of the sympathetic outflow by flight-or-fight responses or pain, or through direct signaling, can increase local concentrations of adrenaline and noradrenaline, which suppress inflammation further.

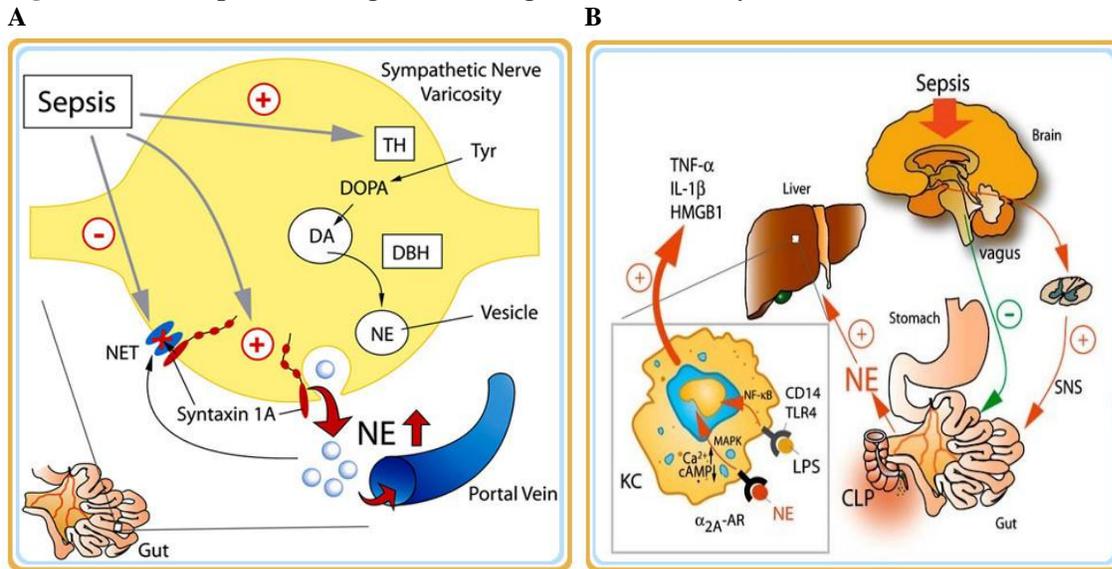
### ***2.1.3.1 “Paradoxical Pro-Inflammatory Effect” of SNS***

The synergistic “anti-inflammatory function” by both PNS and SNS in usual situations is nullified when the inflammation is uncontrolled. In patients with uncontrolled acute inflammation, the high circulating NE and E derived by over-activated SNS and gut elicits a pro-inflammatory effect through binding to the activated alpha2A-adrenoreceptor rather than the blunted beta-adrenoreceptor on Kupffer cells. This “paradoxical” pro-inflammatory effect of SNS plays an essential role in the development of SIRS, sepsis and MODS (Miksa et al., 2005; Rittirsch et al., 2008; Yang, Koo, Zhou, Chaudry, & Wang, 2000; Yang, Zhou, Chaudry, & Wang, 2001).

The adrenergic effect of SNS is mediated primarily by NE and partly by E through different receptors. Alpha2-adrenoceptors mediate the pro-inflammatory reaction, regulate the release of neurotransmitters and modulate physiological functions (such as pain and central blood pressure); while Beta2-adrenoreceptors mediate anti-inflammatory reaction (Miksa et al., 2005). In the situation of systemic inflammation, when the catecholamines’ concentration is high (under septic condition: 20 nano-molar), the anti-inflammatory effect of catecholamines is blunted by the down regulation of beta2-adrenoreceptors. This blockage meanwhile converts the catecholamines’ normal function into a pro-inflammatory action by binding to alpha2A-adrenoceptors on hepatic Kupffer cells (Bergmann & Sautner, 2002; Miksa et al., 2005; Tracey, 2002; Yang et al., 2001).

In sepsis for instance, the plasma levels of catecholamines are boosted, not only in the early stage but also in the late stage (Yang et al., 2000). The gut is the major source of the sustaining elevation of NE in sepsis with a 74% higher concentration of NE in the portal system compared to the systemic circulation (Yang et al., 2000). The process of how NE is produced in gut and systemically circulated is shown in Figure 2-3. The sepsis-induced elevated tyrosine hydroxylase (TH) in gut is eventually converted into NE after a series of biological events (Zhou, Hank Simms, & Wang, 2004). The norepinephrine transporter (NET), as major NE uptake, is decreased and also blocked by Syntaxin 1A, which attenuates NE uptake and causes a large amount of NE accumulation (Masson, Sagne, Hamon, & Mestikawy, 1999; Sung et al., 2003). Such massive spillover of NE is then released from the sympathetic nerve and enters into the circulation through the mediation of Syntaxin 1A (Miksa et al., 2005; Shukla et al., 2001) (Figure 2-3A). The high dose of circulated NE binds to the alpha2-receptors on Kupffer cells of the liver, thereby inducing a large amount of pro-inflammatory cytokines release (TNF-alpha, IL-1, IL-6) that could produce toxic effects on tissues and contribute to the development of systemic inflammation (Miksa et al., 2005) (Figure 2-3B).

**Figure 2-3:** The process of “paradoxical pro-inflammatory effect” of SNS (Miksa et al., 2005)



**A:** Altered activities of the sympathetic nerve varicosity in sepsis (Miksa et al., 2005). In sepsis, the increased TH induces an increase of NE production. The increased syntaxin 1A mediates the fusion of the presynaptic NE-vesicle with the varicosical membrane and promote NE release. The expression of NET, the major uptake and signal termination mechanism in the noradrenergic system, is decreased and also blocked by Syntaxin 1A, which attenuate the NE uptake and leads to an extraneuronal accumulation of NE. Thus, the high levels of NE enter into the circulation via the portal vein where they reach the liver and bind to Kupffer cells. NE=norepinephrine, NET=NE transporter protein, Tyr=tyrosine, TH=tyrosine hydroxylase, DA=dopamine, DBH=dopamine beta-hydroxylase, Syntaxin 1A=a member of soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) family.

**B:** Sympathetic excitotoxicity inducing systemic inflammatory response in sepsis (Miksa et al., 2005). Severe trauma and injury cause pain and stress, which activate the sympathetic nervous system. The increased levels of NE in the gut spill over into the portal circulation and reach Kupffer cells in the liver. NE and LPS along with Gram-negative bacteremia synergistically increase the release of pro-inflammatory cytokines (TNF-alpha, IL-1beta and HMGB1) which enter the circulation and eventually lead to severe sepsis, septic shock or MODS. SNS=sympathetic nervous system, CLP=cecal ligation and puncture, CD14=cluster of differentiation protein 14, TLR4=toll-like receptor4, LPS=lipopolysaccharides, alpha2A-AR=alpha2A-adrenoceptor, KC=Kupffer cells, cAMP=cyclic adenosine monophosphate, Ca<sup>2+</sup>=calcium, MAPK=mitogen-activated protein kinase, NF-kappa B=nuclear factor kappa B, TNF-alpha=tumor necrosis factor-alpha, IL-1beta=interleukin 1beta, HMGB1=high mobility group box protein-1.

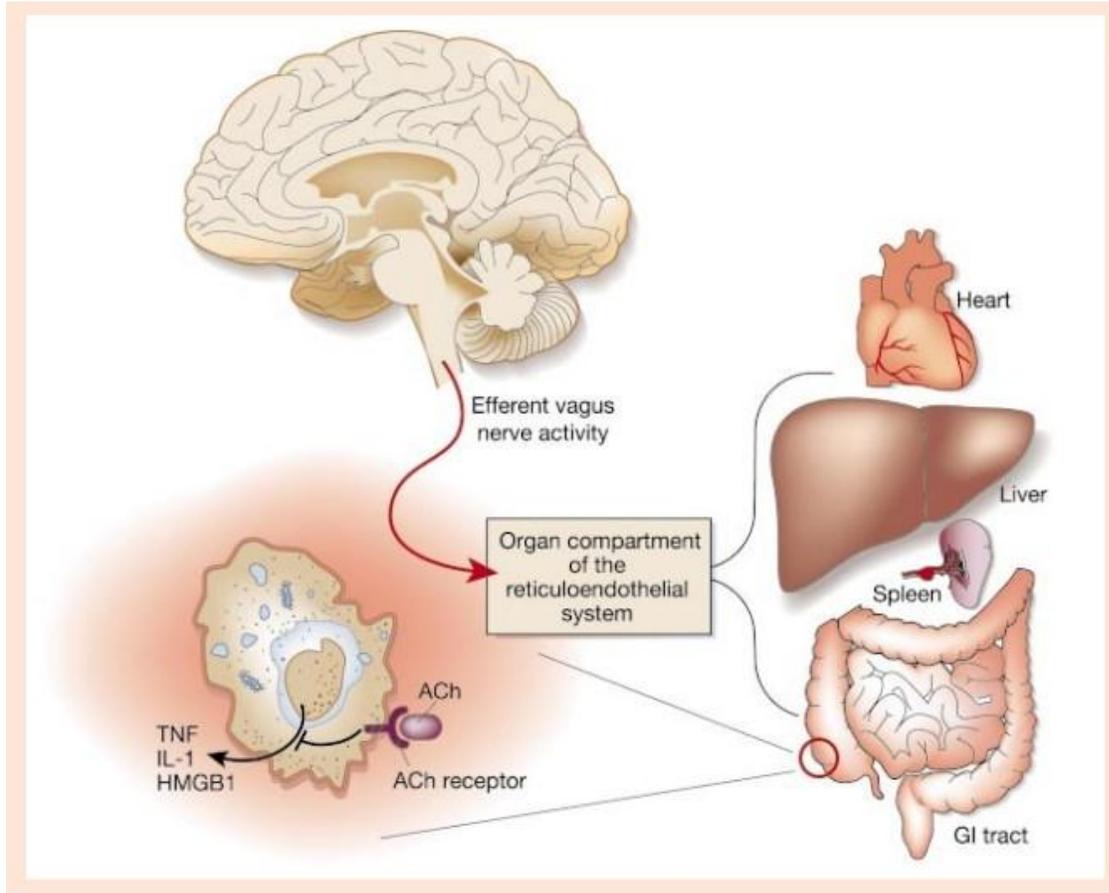
### ***2.1.3.2 The Blunted Parasympathetic Anti-Inflammatory Pathway***

As stated above, the PNS is the major anti-inflammatory force. Macrophages that are exposed to acetylcholine are effectively deactivated (Tracey, 2002). Given the vagus nerve innervating most of the vital organs, including liver, lung, spleen, kidneys and gut (Robertson, 2004), vagus nerve stimulation can inhibit the body systemic inflammatory response to endotoxin (Borovikova et al., 2000) (Figure 2-4). Several studies documented that the stimulation on the efferent vagus could activate the cholinergic anti-inflammatory pathway, thereby inhibiting the synthesis of TNF- $\alpha$  and reducing its concentration in serum during experimental endotoxemia (Bernik et al., 2002; Borovikova et al., 2000).

On account of such an important role of PNS in controlling inflammation, the blunting of this powerful effect may cause unopposed systemic spread of inflammation. In stressful situations such as sepsis, the parasympathetic anti-inflammatory function is blunted due to the high doses of circulatory catecholamines (Ellenby et al., 2001). This was exemplified by studies showing that the intravenously applied endotoxin was effective for inducing reversible heart rate “stiffness” and causing myocardial autonomic dysfunction (Paul J Godin et al., 1996; Werdan et al., 2009). Moreover, Schmidt et al. showed a decreased baroreflex sensitivity in ICU patients with high stress that indicated the suppressed parasympathetic function in ICU patients (H. B. Schmidt, Werdan, & Müller-Werdan, 2001).

Overall, the stress-related changes in ANS activity, in terms of the suppression of PNS and the overexcitation of SNS, significantly contribute to the development of systemic inflammation. In the next section, we will discuss the how to measure the ANS function in stressful situations.

**Figure 2-4:** The cholinergic anti-inflammatory pathway to body organs (Borovikova et al., 2000)



Efferent vagus nerve leads to acetylcholine (ACh) release in organs of the reticuloendothelial system, including the liver, heart, spleen and gastrointestinal tract. Acetylcholine interacts with the alpha-bungarotoxin-sensitive nicotinic receptors (ACh receptor) on tissue macrophages, which inhibit the release of TNF, IL-1, high-mobility group box-1 (HMGB-1) and other cytokines.

## **2.2 Autonomic Nervous System, Heart Rate Variability and Stressful Situations**

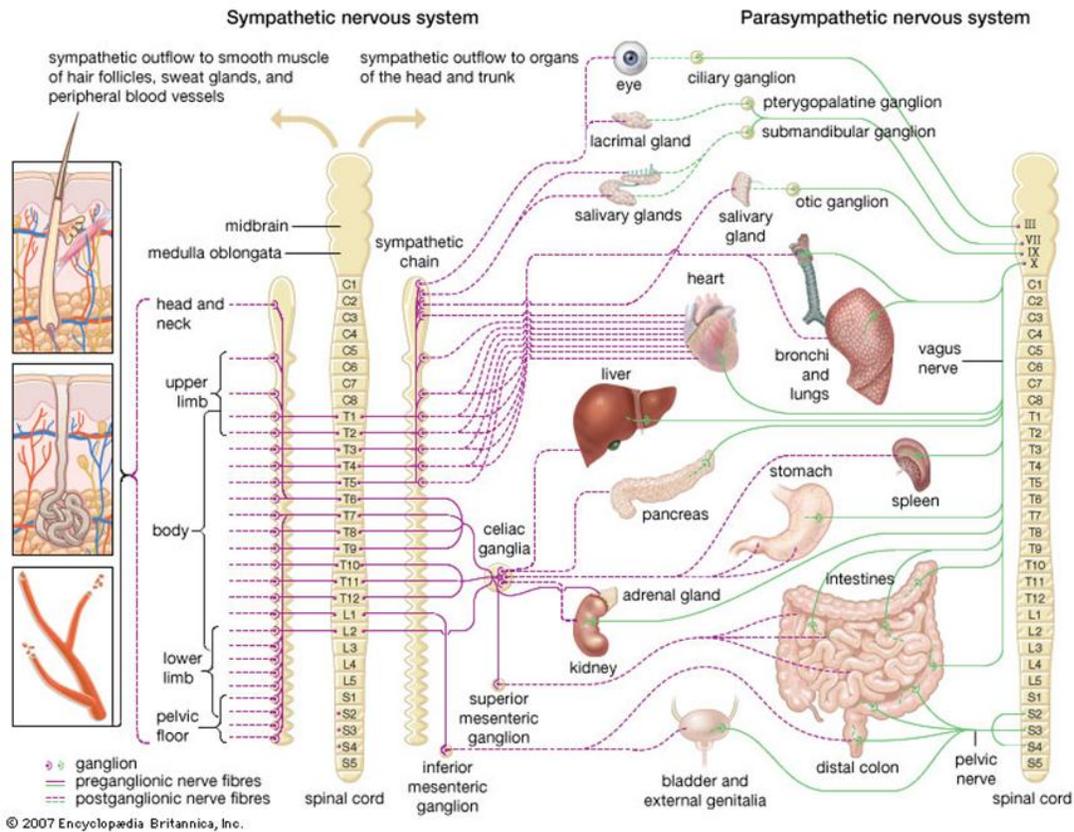
### **2.2.1 Autonomic Nervous System**

#### ***2.2.1.1 General Picture of ANS***

The ANS is a part of the peripheral nervous system that is not under voluntary control (Robertson, 2004). ANS controls visceral function, with the effects on heart rate, digestion, respiration rate, salivation, perspiration, urination and sexual arousal. The ANS is composed of two divisions: the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) which innervate most of the vital organs (Robertson, 2004) (Figure 2-5).

The SNS is responsible for the “fight or flight” response, while PNS promotes the relaxation response. These two divisions are considered as two parallel systems rather than antagonists; as they sometimes work synergistically and sometimes in opposition, to regulate the physiological functions of the body (Table 2-1) (Robertson, 2004). The SNS and PNS together maintain the metabolic equilibrium by making adjustments whenever something disturbs the balance.

**Figure 2-5:** Schematic diagram of the autonomic nervous system



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**Table 2-1:** Comparison of the sympathetic and parasympathetic function (Robertson, 2004)

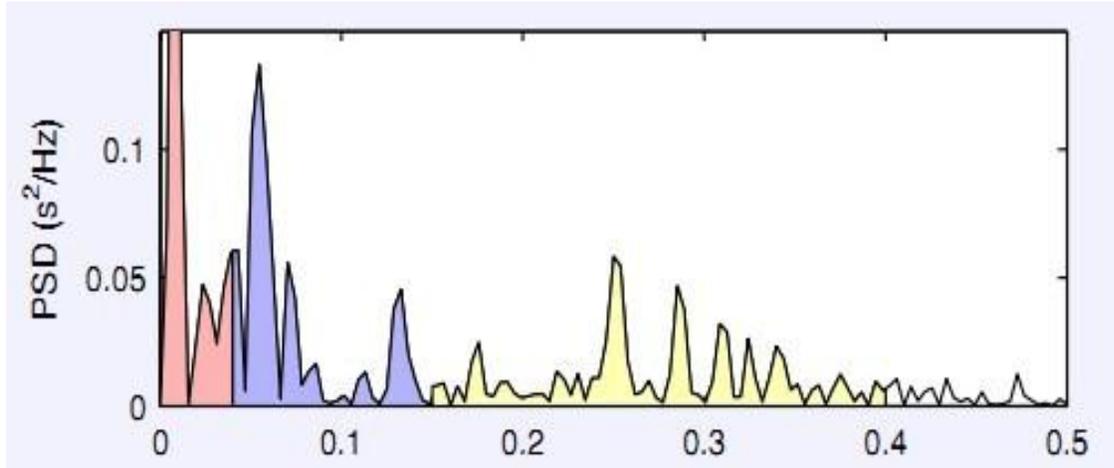
	SNS	PSNS
Heart	<p><u>1.Positive chronotropic effect:</u> increases heart rate</p> <p><u>2.Positive dromotropic effect:</u> increases conduction velocity in atrio-ventricular junction</p> <p><u>3.Positive inotropic effect:</u> increases cardiac (ventricle and atrium) contraction</p>	<p><u>1.Negative chronotropic effect:</u> decreases heart rate</p> <p><u>2.Negative dromotropic effect:</u> decreases conduction velocity in atrio-ventricular junction</p> <p><u>3.Negative inotropic effect:</u> decreases cardiac (atrium) contraction</p>
Blood vessel	Coronaries: Constricts ( $\alpha$ ); dilates ( $\beta$ )	little effect in most organs and dilation in several organs
Immune system	Increase cytokines synthesis and release	Inhibit inflammatory synthesis and release
Respiratory system	Dilate bronchiole	Contract bronchioles
Digestive system	Inhibits peristalsis inhibits urinate	Improve peristalsis Promotes urinate inhibits urinate
Urinary system	Contracts the pregnant uterus; relax non-pregnant uterus, inhibit reproductive function	Enhances reproductive function
Eyes	Dilates pupil	Contracts pupil
Skin	Contracts arrector muscle; increases sweating	NA
Metabolism	Promotes glycogenolysis; promotes adrenal medulla hormone secretion	Promotes glycogen synthesis; promotes insulin secretion

### ***2.2.1.2 Heart Rate Viability to Measure ANS Function***

In most physiological conditions, the activation of either sympathetic or parasympathetic system is accompanied by suppression of the other. Such equilibrium between the two subsystems of ANS is important for maintaining homeostasis. To explore the interaction of SNS and PNS, a noninvasive technique, HRV, defined as the fluctuations in the interval between normal heartbeats, to reflect the modulation of autonomic input to the sinus node is useful (Akselrod et al., 1981; ESC/NASPE Task Force, 1996; Pomeranz et al., 1985). Usually, increased variability of the heart rate reflects adequate autonomic modulation on heart rate and low HRV values reflect a lack of central modulation or a lack of responses of the sinus node (Stein & Kleiger, 1999).

HRV is mainly analyzed by three ways: frequency-domain, time-domain and non-linear methods (ESC/NASPE Task Force, 1996) (see Appendix B). The spectral analysis is a precise and commonly used procedure for assessing HRV, which uses power spectral density (PSD) and provides basic information on the power distribution across frequencies. Interest is focused on four components of PSD that involve ultra-low frequency power (ULF <0.003 Hz), very low frequency power (VLF: 0.003-0.04 Hz), low frequency power (LF: 0.04-0.15 Hz) and high frequency power (HF: 0.15-0.4 Hz) (ESC/NASPE Task Force, 1996). Figure 2-6 displays the PSD of one of my subjects.

**Figure 2-6:** Power spectral density



Red area: ULF and VLF components; blue area: LF component; yellow area: HF component; white area: very high and ultra-high frequency, which is highly influenced by respiration and other physiological factors

HF, an index of PNS, is solely mediated by the parasympathetic modulation. It is associated with the respiratory sinus arrhythmia (RSA) that is a vagally-mediated modulation of heart rate, with an increased heart rate (HR) during inspiration and a decreased HR during expiration. LF is mediated by a complex mixture of SNS and PNS modulation. The VLF band may represent the influence of the peripheral vasomotor and renin-angiotensin systems and the ULF is influenced by many poorly understood mechanisms. The ratio of LF to HF reflects the balance of the sympathetic and parasympathetic functions (Akselrod et al., 1981; Berntson et al., 1997; Pomeranz et al., 1985; Saul, 1990). The three parameters, HF, LF and LF/HF, are least influenced by the physiological factors and frequently investigated in studies (Delaney, Leong, Watkins, & Brodie, 2002; Hatayama, Kitamura, Tamura, Nagano, & Ohnuki, 2008; Takamoto et al., 2009). Thus in our study, we assess the HF, LF and LF/HF to estimate the different aspects of ANS.

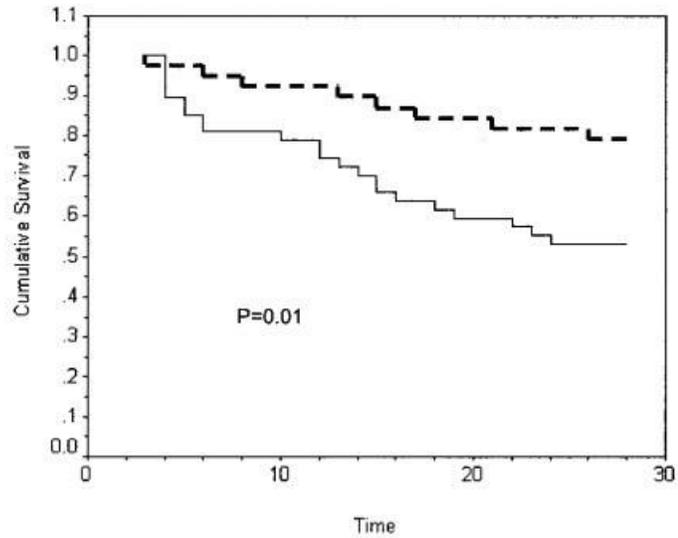
### **2.2.2 ANS Dysfunction and HRV Decreases in Acute Stressful Situations**

As described in the first section of this chapter (2.1) and the findings in different studies, the sympatho-vagal balance is impaired in critically ill patients, with the decreased parasympathetic and increased sympathetic activities, characterized by decreases in HRV (Paul J Godin et al., 1996; Jan et al., 2009; Miksa et al., 2005; H. Schmidt et al., 2005, 2008; H. B. Schmidt et al., 2001).

In a study conducted by Schmidt et al in 2005, they followed up 90 ICU patients diagnosed with multiple-organ dysfunction (an extreme stressful situation). Within 48 hours of the patient's admission to ICU, all patients showed significant decrease in HRV values: LF=129.3+/-405.1

msecs<sup>2</sup>; HF=112.3+/-267.3 msecs<sup>2</sup> and LF/HF=1.1+/-0.9, compared to the normal range values in the literature: LF=791+/-563 msecs<sup>2</sup>; HF=229+/-282 msecs<sup>2</sup> and LF/HF=4.61+/-2.33, respectively. Figure 2-7 showed the attenuation of autonomic function in patients with MODS correlated with decreased cumulative survival rate (H. Schmidt et al., 2005). The effect of endotoxemia on HRV was studied by Godin et al in a randomized clinical trial of healthy adult subjects (Paul J Godin et al., 1996). After endotoxin administration, averaged power spectra from each experimental group showed a statistically significant loss of spectral power at all frequencies ( $p < 0.001$ ) (Figure 8A-D); while the ratio of LF/HF significantly increased after endotoxin administration ( $p < 0.001$ ) (Figure 8E). Another study aimed at assessing the effect of the epinephrine on the HRV was conducted by Jan et al (Jan et al., 2009). In measuring HRV from baseline to +24 hours, the epinephrine+LPS group showed a significantly reduced HF compared with the placebo group, which confirmed the already known vagolytic effect by epinephrine. This effect may in turn limit the parasympathetic anti-inflammatory pathways in acute stressful situations, as mentioned in the first section of this chapter. Based on all these studies, we think ANS dysfunction with HRV decreases are fairly regarded as characters of acute stressful situation.

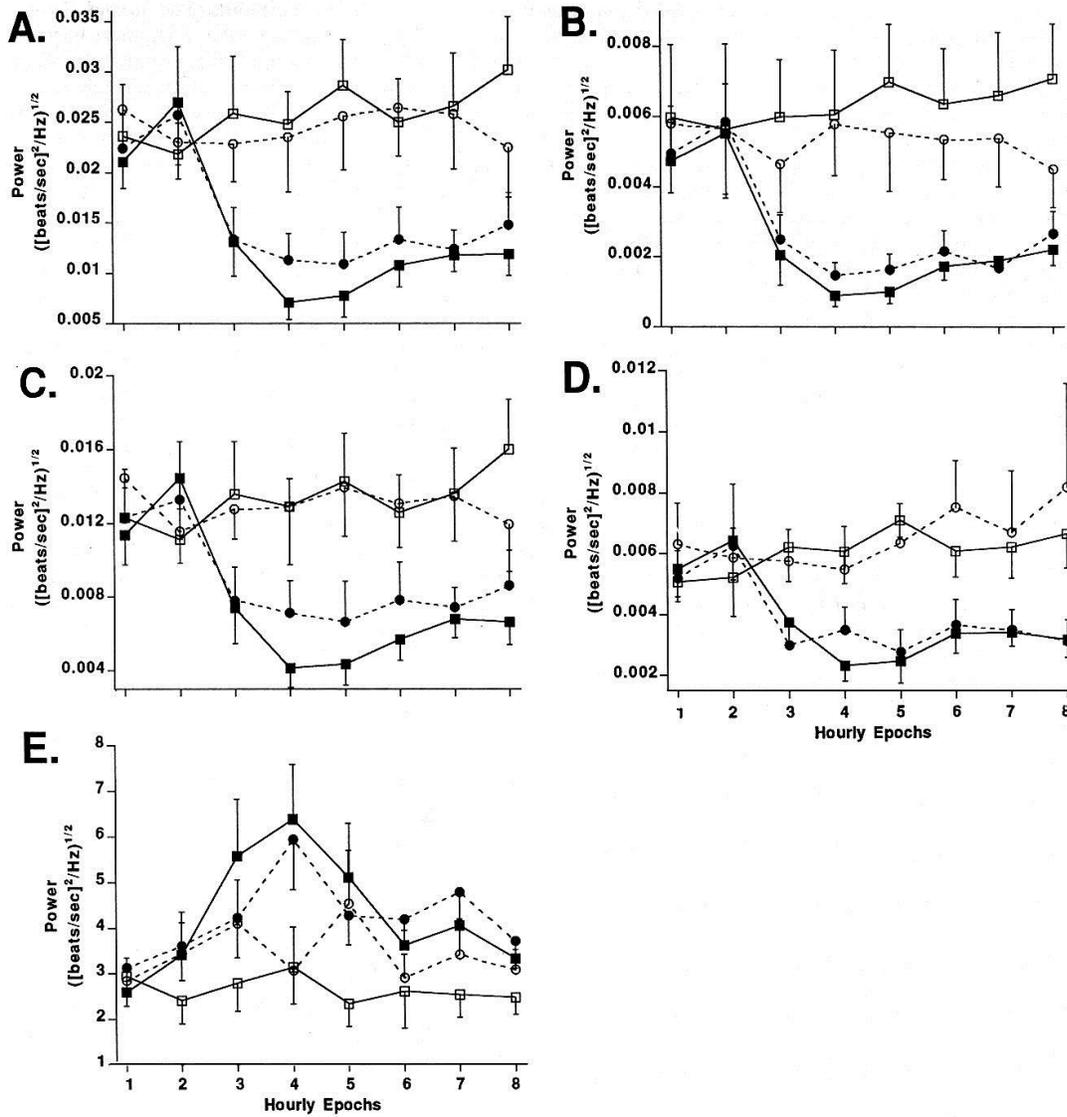
**Figure 2-7:** Kaplan-Meier survival curve (H. Schmidt et al., 2005)



Numbers at risk					
$\ln VLF > 3.9 \ln ms$	(dashed line)	38	34	31	30 (28 days)
$\ln VLF \leq 3.9 \ln ms$	(solid line)	47	37	27	25 (28 days)

Kaplan-Meier survival curve for 28-day mortality using the optimal cutoff point of  $\ln VLF$  (3.9  $\ln ms$ ) for the entire cohort of patients with MODS ( $n=85$ ). The dashed line indicates values above and the solid line below the cut point. The hazard ratio for 28-day mortality was 2.9 (95%CI: 1.3-6.6). \*Very low frequency power may represent physiologic influences like hormones, chemoreflexes, thermoregulation, and also parasympathetic modulation of the heart rate.

**Figure 2-8:** Comparison of HRV between endotoxin administration and placebo (Paul J Godin et al., 1996)



**A:** TP of 0 to 0.5 Hz ( $p < 0.001$ ) **B:** HF power of 0.2 to 0.35 Hz ( $p < 0.001$ ) **C:** LF power of 0.02 to 0.2 Hz ( $p < 0.001$ ) **D:** VLF of 0.01 to 0.04 Hz ( $p < 0.01$ ) **E:** LF/HF ( $p < 0.02$ )

Data are presented as mean plus minus standard error (SE); solid squares=endotoxin/placebo; open squares=saline/placebo; solid circles=endotoxin/ibuprofen; open circles=saline/ibuprofen; TP: total spectral power; HF: high frequency; LF: low frequency; VLF: very low frequency

### **2.2.3 Indicative Values of ANS and HRV on Disease Severity**

The “uncoupling theory” proposed by Godin et al suggested that during systemic inflammatory response, the organ responsiveness to autonomic signaling was diminished or disappeared, with decreased HRV; this process progressed with the disease severity (P J Godin & Buchman, 1996). This theory implies that the diminished autonomic modulation with the decreased parasympathetic activity and increased sympathetic activity leads to reduced variability of heart rate and indicates the severity of disease (Ellenby et al., 2001; Norris et al., 2006; Toweill, Sonnenthal, Kimberly, Lai, & Goldstein, 2000).

A prospective clinical study of 30 children demonstrated that sepsis severity inversely correlated with HRV (Toweill et al., 2000). Based on the results of their study, the authors concluded that HRV could be used as an indicator of sepsis severity. LF and HF components of HRV were documented to be considerably lower in patients with septic shock compared to the patients with sepsis (Table 2-2). Together, patients with sepsis and septic shock had lower HRV compared to the recovery states (Table 2-3).

**Table 2-2:** Cardiorespiratory analysis for pediatric patients with sepsis vs. septic shock (Toweill et al., 2000)

	Sepsis (n = 20) (mean ± SEM)	Septic Shock (n = 10) (mean ± SEM)	p Value
HR (bpm)	120 ± 5	154 ± 6	.0005
HR SD (bpm)	3 ± 1	1 ± 0	.06
MAP (mm Hg)	74 ± 4	66 ± 9	.45
HR LFP (bpm <sup>2</sup> )	3.37 ± 0.17	2.68 ± 0.24	.03
HR HFP (bpm <sup>2</sup> )	2.79 ± 0.23	2.18 ± 0.14	.04
log HR LFP/HFP	0.58 ± 0.12	0.49 ± 0.15	.64
MAP LFP (mm Hg <sup>2</sup> )	3.62 ± 0.17 <sup>a</sup>	3.38 ± 0.28 <sup>a</sup>	.48
HR α	1.00 ± 0.07	1.22 ± 0.06	.02

HR=heart rate; MAP=mean arterial blood pressure; LFP=low frequency power; bpm<sup>2</sup>=beats/min<sup>2</sup>; HFP=high-frequency power; α=detrended fluctuation analysis scaling exponent; <sup>a</sup> For MAP LFP: n=10 for patients with sepsis and n=7 for septic shock.

**Table 2-3:** Cardiorespiratory analysis for pediatric patients with sepsis vs. patients in recovery (Toweill et al., 2000)

	Sepsis/Septic Shock (n = 20) (mean ± SEM)	Recovery (n = 20) (mean ± SEM)	p Value
HR (bpm)	134 ± 6	113 ± 4	<.0001
HR SD (bpm)	2 ± 1	3 ± 1	<.0001
MAP (mm Hg)	74 ± 5	87 ± 4	.01
HR LFP (bpm <sup>2</sup> )	3.05 ± 0.19	3.61 ± 0.15	<.0001
HR HFP (bpm <sup>2</sup> )	2.50 ± 0.22	3.11 ± 0.15	<.0001
log HR LFP/HFP	0.56 ± 0.12	0.50 ± 0.14	.67
MAP LFP (mm Hg <sup>2</sup> ) <sup>a</sup>	3.33 ± 0.24	3.68 ± 0.19	.06
HR α	1.01 ± 0.08	0.98 ± 0.06	.66

HR=heart rate; MAP=mean arterial blood pressure; LFP=low frequency power; bpm<sup>2</sup>=beats/min<sup>2</sup>; HFP=high-frequency power; α=detrended fluctuation analysis scaling exponent; SEM= standard error of the mean; <sup>a</sup> for MAP LFP: n=9

#### **2.2.4 Predictive Values of ANS and HRV on Disease Prognosis**

In opposition to the “uncoupling” process during acute stress, recovery from diseases is characterized by the recuperation of heart rate fluctuation, or “recoupling” of inter-organ communication (Ellenby et al., 2001). “Recoupling” theory signifies the return of increased parasympathetic activity and effectively suppression of sympathetic activity. Such re-balance of ANS, reflected by the increased HRV, can predict the improvement of diseases.

In Ellenby et al’ prospective study, the authors evaluated the “uncoupling” and “recoupling” phenomenon in seven children with septic shock by observing changes in HRV. The investigators calculated two components of HRV spectrum, normalized low-frequency (norm.LF) and normalized high-frequency (norm.HF), every 6 hours in the duration of patients’ stay in PICU. They also compared the changes in mean HR and HR standard deviation during the first 24 hours of PICU hospitalization versus the reminder of PICU stay. The main finding of this study was that the norm.LF and LF/HF decreased while norm.HF increased during the recovery phase (Ellenby et al., 2001). Such results suggested the significant uncoupling between the sympathetic and cardiac systems during the acute phase of septic shock, and recoupling during disease recovery.

#### **2.2.5 Therapeutic Values of ANS and HRV**

By understanding the parasympathetic anti-inflammatory function as outlined in chapter 2.1, it becomes apparent that the cholinergic anti-inflammatory mechanisms can potentially be

exploited for the treatment of inflammatory diseases. In particular, the improvement of PNS function is considered beneficial to inhibit cytokines with possible utility for disease treatment (Ellenby et al., 2001; Kumar & Sharma, 2010; Wang et al., 2004).

In an article published in *Nature Medicine*, Wang H et al showed the neurotransmitter acetylcholine could inhibit the release of HMGB-1 (the high mobility group box 1 protein: a late mediator of lethal systemic inflammation in sepsis) from human macrophages and improve survival in experimental sepsis (Wang et al., 2004). The vagus nerve stimulation on cholinergic pathway was found to be one of the effective ways to improve parasympathetic activity thus to elicit the therapeutic effect for sepsis (Bernik et al., 2002; Kumar & Sharma, 2010). Moreover, complementary and alternative medicines, such as massage and acupuncture which have been studied for many years, may also be effective on improving ANS function and treating diseases. In the next section, we will discuss the effects of massage therapy on improving ANS function.

### **2.3 The Effect of Massage Therapy on Autonomic Nervous System**

Massage therapy, as a part of complementary and alternative medical therapy, is considered as a noninvasive intervention to stimulate the ANS (T. M. Field, 1998; Moyer, Rounds, & Hannum, 2004). In modern practice, massage is a tactile stimulation that works on superficial and deeper layers of skin, muscles and soft tissues by using various manipulations and techniques which include holding, causing movement and applying pressure to the body (T. M. Field, 1998; Moyer et al., 2004). Massage therapy is “a profession in which the practitioner applies manual techniques and may apply adjunctive therapies with the insertion of positively affecting the

health and well-being of the client”(American Massage Therapy Association, 1999). There are a variety of massage techniques, for instance Swedish massage, sports massage, trigger point massage, full body massage, deep tissue massage and peripheral massage (reflexology); different types of massage have different maneuvers and functions (T. Field, 2002). Peripheral massage therapy is a technique which via kneading or stroking of “points” stimulates the peripheral areas, such as feet and hands that are highly innervated by ANS (T. M. Field, 1998; Moyer et al., 2004).

Massage therapy has been shown to be beneficial for human psychological and physiological health (Huang, 2009; Moyer et al., 2004). Many articles emphasized that massage manipulation influenced the whole body by reducing stress and pain, relaxing muscles and nerves, improving circulation and promoting sleep (Donnelly, 2000; T. Field et al., 2002; T. M. Field, 1998; Ironson et al., 1996; Malkin, 1994; Moyer et al., 2004). One of the important mechanisms by which massage elicits its effects is the stimulation of PNS coupled with the suppression of SNS.

### **2.3.1 Massage Therapy: Stimulate PNS Activity and Suppress SNS Activity**

Massage therapy provides its benefits by shifting the ANS from a state of sympathetic dominance to a state of parasympathetic dominance (Moyer et al., 2004). The effects of massage therapy on stimulating PNS activity and suppressing SNS activity is mediated by the stimulation of dermal and/or sub dermal pressure receptors that are innervated by vagal afferent fibers, which ultimately project to structures involved in ANS regulation (Delaney et al., 2002; Diego & Field, 2009; T. Field, Diego, & Hernandez-Reif, 2010; T. M. Field, 1998; Hatayama et al., 2008; Takamoto et al., 2009).

Massage therapy was effective for relaxation and reducing pain as the decrease in SNS activity by massage “closes the gate” to pain stimulus (T. Field, Diego, & Hernandez-Reif, 2007), decreases the substance P which is considered to cause pain (Henry, 1980) and increases pain thresholds hence suppresses alert reaction (pain) (Drummond, Finch, Skipworth, & Blockey, 2001; Hernandez-reif, Field, Krasnegor, & Theakston, 2001). A single facial massage session showed through massage therapy, decreased SNS activity and increased PNS activity (increased HF) were observed associated with reduced anxiety and depression, reduced pain and improved mood status accompanying with calmness (Hatayama et al., 2008). Massage therapy was also demonstrated to be able to decrease stress hormones (average decreases 31% through massage therapy) (T. Field, Hernandez-Reif, Diego, Schanberg, & Kuhn, 2005).

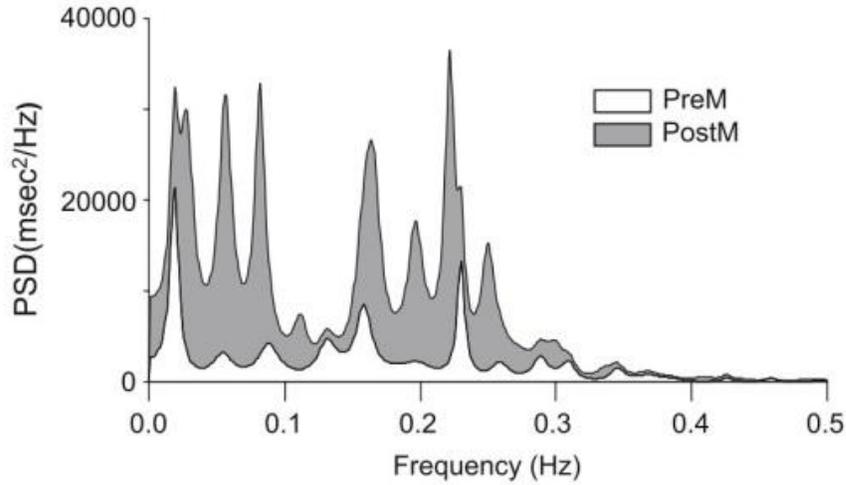
M Diego and T Field did extensive research and demonstrated that moderate-pressure massage could elicit a shift from sympathetic to parasympathetic activity, but light-pressure massage had no response or a sympathetic response. In their study of effects among vibrator stimulation, light and moderate massage, decreased heart rate, anxiety and stress were in all groups, but the moderate massage group expressed the greatest effect (Diego, Field, Sanders, & Hernandez-Reif, 2004). In their follow up study of 20 healthy people in 2009, HRV was used to measure the change of PNS activity and responses. HF was showed increased significantly through 15-min of moderate pressure massage, which strongly proved the mechanism under which moderate level of pressure elicits its positive effect on vagal activity and parasympathetic arousal (Diego & Field, 2009; Diego et al., 2004). Another study on short-term (5-minutes) myofascial trigger-point massage therapy demonstrated similar results with increased PNS activity characterized by

increased HF in 30 healthy subjects (16 female and 14 male aged 32 $\pm$ 1.5 years) (Delaney et al., 2002).

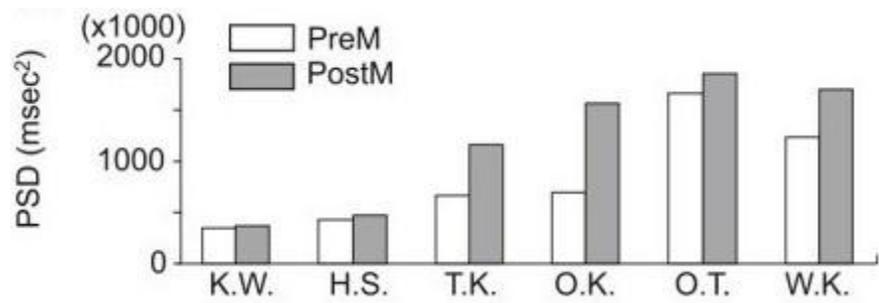
Moreover, one study conducted by K Takamoto in 2009 showed that one type of massotherapy (pressure stimulus applied over trigger points-TPs) increased PNS on healthy subjects. The results showed an overall increase in power spectrum density (PSD) with increases in both LF and HF components of HRV after 20 minutes of pressure application over the lower-limb (Figure 2-9A). Figure 2-9B showed the individual increase on PSD ranging from 5% to 125% after the massotherapy. They also showed the suppressed effects of pressure stimulus on sympathetic activity, by showing significant decreases in heart rate (from 67.3 bpm to 65.2 bpm), systolic blood pressure (119 mmHg to 102 mmHg) and diastolic blood pressure (from 68 mmHg to 54 mmHg) after massotherapy (Takamoto et al., 2009). These similar results were also observed in other massage studies (Ahles et al., 1999; Delaney et al., 2002; Diego et al., 2004; Kubsch, Neveau, & Vandertie, 2000).

**Figure 2-9:** HRV for pre-massotherapy and post-massotherapy

**A**



**B**



**A:** Power spectrum density (PSD) of HRV for pre-massotherapy and post-massotherapy. Low-frequency (LF) and high-frequency (HF) components increase after pressure application over the pressure stimulus applied over trigger points (TPs). LF component: 0.05–0.15 Hz; HF component: 0.15–0.50 Hz.

**B:** Individual PSD changes (ranging from 5% to 125%) through massotherapy. PSD for post-massotherapy (grey column) is higher than PSD for pre-massotherapy (white) in each subject (n = 6). Initials of each subject are under the horizontal scale.

However, as stated above, previous studies focused on the effect of immediate/short-duration (from 5-min to 20-min) or single session massage; and few articles aimed to assess the ANS conditions of ICU patients. Our present study first addresses the effects of long-duration (30-min) and multiple sessions of massage intervention on ANS function in young critical care patients, by using HRV measurements.

### **2.3.2 Patients in Pediatric Intensive Care Units (PICU)**

The PICU at BC Children's Hospital serves patients aged from newborn to 17 years old in British Columbia, Yukon and part of Northwest Territories, with over 1200 admissions per year. It has 22 beds with 16 intensive care unit and 6 transitional care unit beds. Types of patients admitted include 60% unplanned (e.g., traumas, infections) and 40% planned (e.g., surgeries, sleep apnea studies): 40 to 50% heart problems (e.g., congenital heart disease, open heart surgery, closed heart surgery, heart failure); 20% breathing difficulties (e.g., obstructions, infections, respiratory failure); 10 to 20% head problems (e.g., head injuries, seizures, infections, tumors) and other complex problems during high risk interventions such as orthopedic surgeries, kidney transplants or accidents, such as extended severe burn for instance. We decided to study patients in PICU, who experience significant more physical and psychological stress than most studied populations. We are interested to know whether in such a stressful environment, massage could have an effect to reduce the stress response by decreasing SNS activity and stimulating PNS activity.

## **CHAPTER 3: RATIONALE**

### **Our rationale was based on the following:**

- ANS is a tight control system for regulating inflammatory responses. ANS dysfunction (assessed by decreased HRV) is strongly associated with biological stress level. HRV as a sensitive measurement of ANS function can be a marker of disease severity and a predictor of prognosis.
- PNS activity is down regulated during severe acute stress situations. Parasympathetic down regulation may facilitate the development of an overwhelming inflammatory reaction with detrimental consequences.
- Massage could stimulate the parasympathetic pathway and down-regulate the overactive sympathetic pathway, and hence decrease the overwhelming biological stress, control the inflammatory response and potentially prevent further consequences.

### **Aims:**

In our study conducted in PICU, we wanted to:

- Describe the changes in HRV which reflect ANS function due to the massage intervention
- Assess the relationship between the HRV magnitude and the clinical severity of ICU patients measured by Pediatric Logistic Organ Dysfunction (PELOD) scores.

## **CHAPTER 4: OBJECTIVES AND HYPOTHESES**

### **4.1 Objectives**

In our study, we had two folds of objectives: the clinical research objectives and the pilot study objectives.

#### **4.1.1 Clinical Research Objectives**

##### ***4.1.1.1 Primary Objective***

The primary objective of this pilot clinical study was to assess the effect of massage on changing and rebalancing the ANS function (reflected by HRV changes) in an intensive care setting (PICU).

##### ***4.1.1.2 Secondary Objective***

The secondary objective of this pilot clinical study was to investigate associations between HRV magnitude (markers of ANS function) and clinical status/severity (measured with the clinical feature, diagnosis and PELOD Scores) of PICU patients.

### 4.1.2 Pilot Study Objectives

The pilot study objectives were:

- To determine the feasibility of conducting a study of massage effectiveness in the ICU (protocol compliance, missing data and recruitment);
- To determine the characteristics of each variable (HRV components, PELOD scores) with regard to the distribution and dispersion. This information would be useful for developing a future larger-scale study;
- To get preliminary data regarding correlations and variances, so as to better inform future sample size estimates.

### 4.2 Hypotheses

- In ICU patients both low frequency and high frequency components of HRV would decrease compared to normal values (provided in Appendix B), which reflected the impairment of ANS in acute stressful situations.
- In response to the massage therapy, we expected to see (a) the HF which reflects parasympathetic function would increase; (b) the function of the sympathetic nervous system would decrease; and (c) the ratio of LF/HF would be in or approach the normal range (pediatric standard:  $0.73 \pm 0.08 \sim 2.43 \pm 0.88$ , details provided in Appendix B) (Finley & Nugent, 1995; Kazuma, Otsuka, Wakamatsu, Shirase, & Matsuoka, 2002; Zhang, 2007). LF/HF above the normal range would decrease; LF/HF below the normal range would increase and LF/HF within the normal range would remain stable through

massage therapy. The fact that almost all patients received drugs that likely affected the ANS should not affect the hypothesis because we were assessing the ANS changes in the same patient (same condition) over time.

- We expected the group receiving higher frequency of massage intervention to have greater effect.
- We expected to observe a positive correlation between baseline HRV magnitude and clinical severity. We believed that decrease of heart rate fluctuation during inflammation was a marker of disease severity, equivalent to or even better than PELOD scores for some aspects because of its direct connection with several essential vital functions. HRV should also be a predictor of survival, although this association was not an objective of our study.

## **CHAPTER 5: METHODS**

### **5.1 Study Design**

This was a pilot prospective clinical trial in which eligible subjects hospitalized in the PICU were randomly allocated to one day of two foot and hand massage intensities: “light intensity”, defined as a single session; and “high intensity”, defined as six sessions offered within 24 hours. Massage was performed by Registered Massage Therapists (RTM) from the Massage Therapy program, Utopia Academy, Vancouver Campus.

HRV was recorded continuously as PICU routine through ECG monitoring and stored in a central station. ANS function was assessed through HRV analysis from the central station recording. Follow-up was for 72 hours, with 24 hours for the intervention and another 48 hours without intervention. During follow-up, the clinical status/severity was assessed by PELOD scores at the time of regular care. The clinical information was collected from medical records and charts. The follow-up stopped when patients recovered/discharged, transferred to other wards or died.

### **5.2 Study Population**

All PICU patients were eligible for our study during their stay in PICU.

### **5.2.1 Inclusion Criteria**

- Between 28 days to 18 years of age (pediatrics definition) (Marcdante, Kliegman, Behrman, & Jenson, 2010) and admitted in PICU
- Stability in subjects' condition
- Parents / guardians understood and signed the consent form; subjects assented to participate in the study.

### **5.2.2 Exclusion Criteria**

- For any reason massage therapy was not acceptable by patients or their parents.
- Subjects who took Precedex that affected all variability of heart rate.
- Subjects with arrhythmia or other cardiovascular diseases that affected the cardiac rhythm.
- Patient's parents/guardians did not speak English, and could not provide the interpreter.

We recruited 22 patients for this pilot study. This sample size was sufficient to assess the “pilot” aspects of the study and get preliminary data of HRV in the ICU.

### **5.3 Recruitment**

Patients were identified as eligible on their admission to PICU and families were approached for consent. The ICU registered nurses or research coordinator provided initial screening, informed the family about the study, invited them to participate and gave them the study information as soon as the patient was admitted to ICU. Parents/legal guardians who decided to participate in

this study would receive the informed consent (provided in Appendix C). If the subjects were between 14 to 18 years old and able to communicate, they also received a consent form; if the subjects were under 13 years old, their parents/legal guardians had to sign the consent form and subjects (7-13 years) received an assent form. Initially many subjects were unconscious and not able to give their consent/assent; those who regained consciousness during the course of the study were approached according to the protocol to assent to continue participating in the study. A copy of the informed consent form was given to parents/legal guardians for their reference.

## **5.4 Patients' Monitoring**

### **5.4.1 Clinical Monitoring**

All the recruited ICU patients had a 24-hours electrocardiogram and respiratory monitoring starting from their admission (as usual). The total urine elimination and fluid balance was measured twice a day. The severity of illness and organ dysfunction was assessed by using PELOD scores (Appendix D) every day. Follow up of relevant clinical and demographic information (shown below) was collected from patient's medical records and charts and talking with doctors and nurses until patients' discharge ("Clinical Information Collection Form" is provided in Appendix D).

- Personal information
- Clinical information regarding diagnosis, clinical feature, physiological variables, concomitant diseases, medication list and survival status

- PELOD scores (cardiovascular function, respiratory function, neurological function, hepatic function, renal function and hematological function).

The follow-up could be interrupted any time pending the following critical events: clinical deterioration or death, changing beds and being monitored by a system not connected to the central station, or leaving ICU for another unit.

#### **5.4.2 HRV Recording**

HRV were obtained from ICU 24-hours electrocardiograph recordings from the central station indirectly. ECG recording was obtained by using a program that enabled transferring ECG data waves from the central station to the external (study) computer. HRV data was obtained from the ECG recording and analyzed by using Acqknowledge software (version 4.1) (Appendix E).

ECG data were extracted from the central station retrospectively according to the starting time and end time of massage intervention which was recorded by the massage therapists in the “Massage Information Collection Form” (provided in Appendix F). ECG data were abstracted 5 minutes before massage (i.e. baseline), every 5 minutes during the 30-min massage session and 5 minutes after session for each massage session. Before each HRV analysis the data had to be cleaned manually. The Acqknowledge software identified each “R” peak on the ECG and marked on the recording. The researcher then manually identified and modified the incorrect tags due to artifacts and ectopic beats to ensure that only normal RR intervals were kept in the HRV analyses. At each assessment the following parameters were analyzed: (1) High frequency power (HF), purely representing vagal activity (2) Low frequency power (LF), reflecting a mix of

sympathetic and parasympathetic modulation (3) Ratio of LF to HF, a marker of sympathetic-parasympathetic balance (ESC/NASPE Task Force, 1996).

### **5.4.3 Video Camera Recording**

We used video cameras to record patients in bed during the day when the massage sessions were performed. This constant visual monitoring allowed us to compare the time period of heart rate change and time of the environmental disturbances in the patient's surroundings; this allowed for precise justification of changes in patient heart rate.

The videotape was used solely for the research purpose (i.e. to restore and record a chronology of the events that potentially influence patient's heart rate). The patient's confidentiality was protected.

### **5.5 Study Intervention**

Our study intervention was the foot and hand (F&H) massage as it was the most convenient intervention that could be conducted in ICU (lines and monitoring machines were on patient's head and body). The foot and hand (F&H) massage was administered by the Registered Massage Therapists (RTM) who came from the Massage Therapy program, Utopia Academy, Vancouver Campus. This program was fully accredited by both the College of Massage Therapists of BC and the Private Career Training Institution Agency. The whole process of a one massage session was around 30 minutes. The massage therapeutic session had a completed process with six steps:

introduction touch to head, right hand massage, left hand massage, right foot massage, left foot massage and closure (F&H massage protocol is provided in Appendix F).

The patients were randomized to two groups of different massage frequencies: low frequency (once per 24 hours) and high frequency (six times per 24 hours) for a single day intervention. The six sessions were given only in day time, normally from 9:00am to 7:00pm. The time interval between two sessions was around 1 to 1.5 hours. The allocation followed a randomization list created by the Clinical Research Support Unit.

The F&H massage was administered as soon as the patient's critical illness was stable and consent was obtained. The registered massage therapists administered the massage, recorded the time of the procedure and described the reaction of patients and environment in the ward in the "Massage Information Collection Form" which were kept in the ICU.

## **5.6 Study Outcomes**

### **5.6.1 Clinical Research Outcomes**

Our primary outcome for clinical research was the change of HRV (ANS function) during and after massage therapy compared to baseline. We assessed high-frequency (HF) component (as a maker of PNS function), low-frequency (LF) component (as the mix of SNS and PNS function) and LF/HF (as the balance of SNS and PNS) for the 5-minute baseline, every 5-minute fragment for 30 minutes' massage duration and 5-minutes after massage. The mean of six 5-minute

fragments was used for the During-massage value. For multiple massage sessions, the trend of variables (HF, LF and LF/HF) over 6 massage sessions was assessed, for (a) Baseline, (b) During-massage, and (c) After-massage.

The secondary outcome was to detect the correlation between HRV magnitude and clinical status/severity measured by PELOD score. The PELOD scoring system is commonly used to estimate disease severity in both clinical follow-up and research (Honna & Triratna, 2010; Leteurtre et al., 2003; Timsit et al., 2002). This system includes 12 items for six key organ dysfunctions: cardiovascular, respiratory, hematological, neurological, renal and hepatic and gives the priority to vital organs assessment (cardiovascular and neurological) of children. Total PELOD score is 71. The score for each item has four levels: 0, 1, 10 and 20. Higher score reflects more severe conditions. The PELOD score construct validity as measurement of disease severity is based on a best cut-off point of each continuous variable to predict severity. The score was developed and validated in two studies with different populations of patients (Leteurtre et al., 2003). PELOD scoring system showed high reliability with a k coefficient ranging from 0.73 to 1 (Leteurtre et al., 2003). It also has a good calibration (goodness of fit, Hosmer-Lemeshow value of 4.03,  $p=0.54$ ) (Leteurtre et al., 2003) and excellent discrimination properties (area under the receiver operating characteristic (ROC) curve (AUC) = 0.91 in Leteurtre's study in 2003 and 0.86 in Honna's study in 2010) (Honna & Triratna, 2010; Leteurtre et al., 2003).

## 5.6.2 Pilot Study Outcomes

To assess study feasibility, we examined the protocol compliance, discovered and solved problems during implementation. We assessed the study recruitment and evaluated the practicability of conducting a larger multicenter study.

## 5.7 HRV Analyses

Our analyses were based on answering the following two key questions. (1) Does massage have an effect on ANS (measured by HRV) of ICU patients? (2) Is the effect of multiple sessions of massage intervention greater than the effect of a single session? Log transformation of original data (HF and LF) was used to decrease the huge differences among values in the original dataset. These differences (from  $0.01\text{msec}^2/\text{Hz}$  to  $500\text{msec}^2/\text{Hz}$ ) among the original dataset were caused by the different health status of the patients.

In chapter 6.1.1, we assessed the effect of the first massage session by using Wilcoxon Signed-Rank Test and paired T-test. The 5 minutes before massage was the baseline; the mean of around 30 minutes massage duration (six 5-minutes fragments) and 5 minutes after massage were presented and compared with baseline in the analysis. We also used graphs to present the results.

In chapter 6.1.2, for the analyses of multiple massage interventions, we showed the changes of HF, LF and LF/HF over the course of 6 massage sessions, respectively. We also separately compared the HF, LF and LF/HF at baseline, during massage and after massage over multiple massage sessions.

## **5.8 HRV Magnitude & Clinical Severity Analysis**

We presented a table that showed the relationship among HRV values (order of magnitude), clinical diagnosis and clinical status/severity (measure by PELOD scores) of patients.

## **5.9 Feasibility Analysis**

Regarding the pilot aspects of this study, we assessed the feasibility of recruiting patients and collecting clinical information in PICU. The detected problems and the identified solutions generated the amendments on initial protocol.

## CHAPTER 6: RESULTS

We recruited 22 subjects. One dropped out from the study at the beginning. The data from one subject could not be used because his cardiac disease resulted in an ectopic rhythm on the ECG making the analysis impossible. The data from the other two subjects were lost because of information technology (IT) problems. Consequently, we included 18 subjects' HRV data for analysis. Among these 18 subjects, eight subjects received a single session of massage per 24 hours; and ten subjects received six sessions of massage per 24 hours.

In the high massage frequency group (n=10), among the ten who received six sessions, we obtained completed HRV data (six sessions' data) on seven subjects, while the data for three subjects were partly missed due to technical problems which will be further described in the last section of this chapter. In the low massage frequency group (n=8), we obtained completed data for all eight subjects. Table 6-1 describes the HRV data collection: We obtained the HRV data for the first massage session for 18 subjects (including eight in single-session group and ten in six-sessions group), the second session for ten subjects, the third session for eight subjects, the fourth and fifth session for eight subjects and the sixth session for seven subjects (Table 6-1).

Following our study objectives, the first two sections of this chapter described the HRV changes due to massage therapy. The third subsection presents the relationship between HRV magnitude and clinical status. The final section addressed study feasibility.

**Table 6-1: HRV data collection**

Subjects#	HRV data collection					
	The 1st massage	The 2 <sup>nd</sup> massage	The 3 <sup>rd</sup> massage	The 4 <sup>th</sup> massage	The 5 <sup>th</sup> massage	The 6 <sup>th</sup> massage
#1 (6 sessions group)	X	X	X		X	
#2 (6 sessions group)	X	X		X	X	
#3 (1 session group)	X					
#4 (1 session group)	X					
#5 (1 session group)	X					
#6 (1 session group)	X					
#7 (1 session group)	X					
#8 (6 sessions group)	X	X	X	X	X	X
#9 (1 session group)	X					
#10 (1 session group)	X					
#11 (6 sessions group)	X	X	X	X	X	X
#12 (1 session group)	X					
#13 (6 sessions group)	X	X	X	X	X	X
#14 (6 sessions group)	X	X	X	X	X	X
#15 (6 sessions group but just received twice)	X	X				
#16 (6 sessions group)	X	X	X	X	X	X
#17 (6 sessions group)	X	X	X	X	X	X
#18 (6 sessions group)	X	X	X	X	X	X
Total number of subjects	18	10	8	8	8	7

## **6.1 HRV Analysis of Massage Effects on ANS in PICU Patients**

The following questions were answered in the two subsections. (1) Does massage intervention have an effect on ANS? (2) Do multiple sessions of massage intervention have a greater effect?

### **6.1.1 The Effect of Single Massage Session**

The effect of the 1<sup>st</sup> massage session was analyzed among 18 subjects. Log transformation was applied for HF and LF original values in statistical analyses.

#### ***6.1.1.1 The Effect of Single Massage on HF***

Table 6-2 summarized the original data of HF and its changes during the 1<sup>st</sup> session of massage intervention. We could see the lower HF values in PICU patients compared to normal values (Appendix B) and the huge differences among HF data (due to different health conditions/stress levels).

The boxplot of logHF for baseline, during-massage and after-massage during the 1<sup>st</sup> massage session showed that logHF rose from baseline  $1.63 \pm 2.77$  to  $2.02 \pm 2.76$  during massage, and declined to  $1.69 \pm 2.62$  after massage (Figure 6-1). We found a significant increase of logHF during massage compared to baseline (Paired t-test:  $p=0.01$ ), with the similar result by using Wilcoxon Signed-Rank Test ( $p=0.02$ ). However, the increase in logHF after massage compared to baseline was not significant (Paired t-test:  $p=0.46$ ; Wilcoxon signed rank test:  $p=0.77$ ).

In the figure showing individual percentage change of HF, 13 subjects had increases in HF with two of them (#14 and #17) showing approximately 300% increases during massage, while five subjects showed decreases in HF during massage. We found that the HF values during massage were significantly increased by a mean of 75.06% (95% CI: 19.9%-130.23%, Student's t-test with  $p= 0.01$ ) compared to the baseline value (Figure 6-2A). For the 5 minutes after session, ten subjects had increases in HF with three of them (#14, #17 and #8) showing more than 150% increases of baseline; eight subjects showed decreases in HF. The average percentage increase on HF after massage was 33.52% to baseline, but it was not significant (95% CI: -10.59% to 77.64%, Student's t-test with  $p= 0.13$ ) (Figure 6-2B).

**Table 6-2: HF in the 1<sup>st</sup> massage session**

	Baseline	During-massage		After-massage	
	High-frequency power(msec <sup>2</sup> /hz)	High-frequency power(msec <sup>2</sup> /hz)	Percentage change of HF between During-massage and Baseline (%)	High-frequency power(msec <sup>2</sup> /hz)	Percentage change of HF between After-massage and Baseline (%)
Subject#1	2.62	1.82	-30.44%	1.99	-24.02%
Subject#2	42.04	25.19	-40.08%	37.43	-10.98%
Subject#3	9.17	4.94	-46.13%	4.01	-56.28%
Subject#4	46.42	66.26	42.74%	24.92	-46.31%
Subject#5	1.0489	0.9854	-6.05%	0.448	-57.29%
Subject#6	288.71	226.86	-21.42%	51.92	-82.02%
Subject#7	0.36	0.59	62.9%	0.52	45.28%
Subject#8	0.0734	0.1502	104.4%	0.2482	<b>237.75%</b>
Subject#9	22.37	38.88	73.83%	36.25	62.05%
Subject#10	8.35	14.26	70.92%	12.59	50.89%
Subject#11	8.21	19.99	143.54%	10.39	26.58%
Subject#12	334.64	577.64	72.61%	512.33	53.10%
Subject#13	0.1277	0.1523	19.29%	0.1099	-13.92%
Subject#14	66.03	309.16	<b>368.24%</b>	182.38	<b>176.22%</b>
Subject#15	0.2254	0.2663	18.15%	0.2432	7.91%
Subject#16	14.62	35.74	144.46%	9.86	-32.54%
Subject#17	0.0429	0.1713	<b>299.43%</b>	0.1117	<b>160.46%</b>
Subject#18	25.86	45.2	74.75%	53.42	106.52%
Mean +/- SD			75.06% +/- 110.93%		33.52% +/- 88.72%

*Baseline: the five minutes before massage;*

*During-massage: the massage duration;*

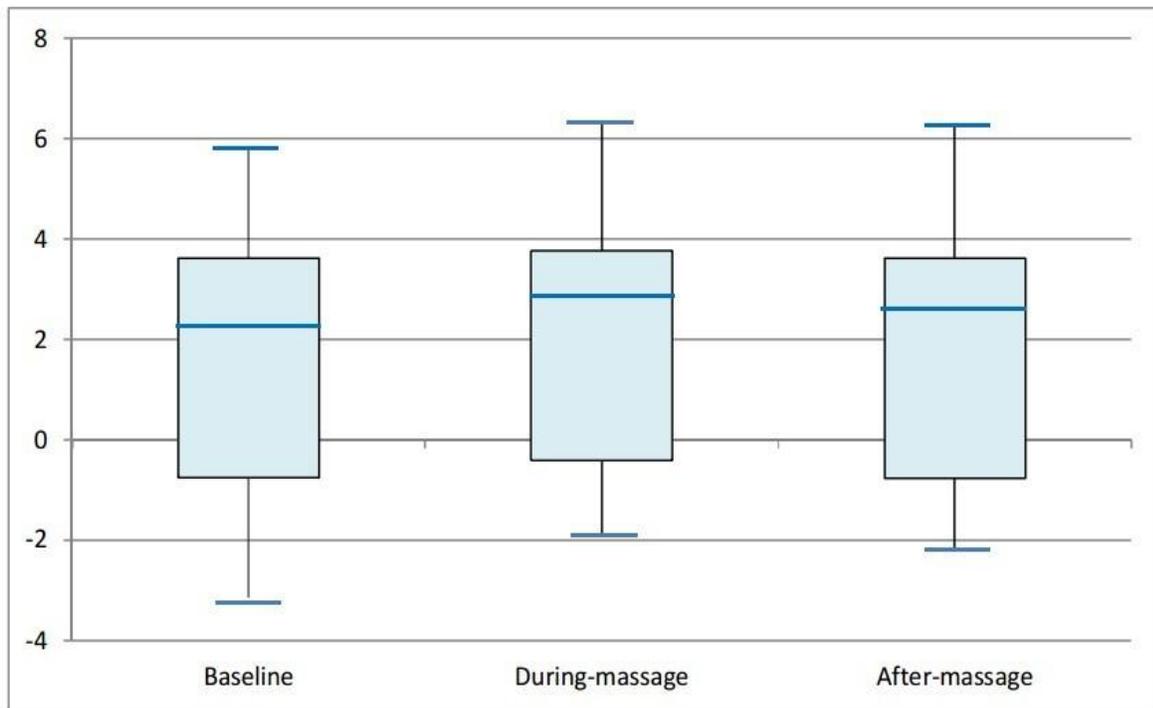
*After-massage: the five minutes after massage;*

*HF: high frequency component of HRV;*

*Percentage difference of HF change between During-massage and Baseline (%): [HF (During-massage)-HF (Baseline)] / HF (Baseline)\*100%;*

*Percentage difference of HF change between After-massage and Baseline (%): [HF (After-massage)-HF (Baseline)] / HF (Baseline)\*100%*

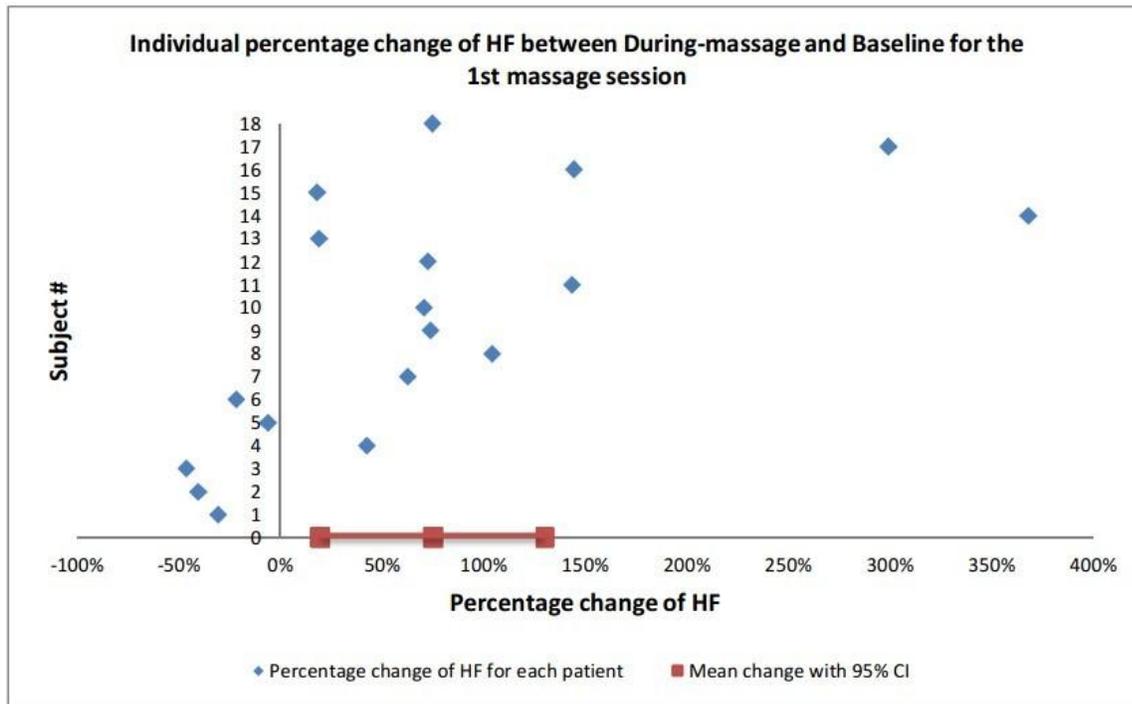
**Figure 6-1:** Change of logHF in the 1<sup>st</sup> massage session



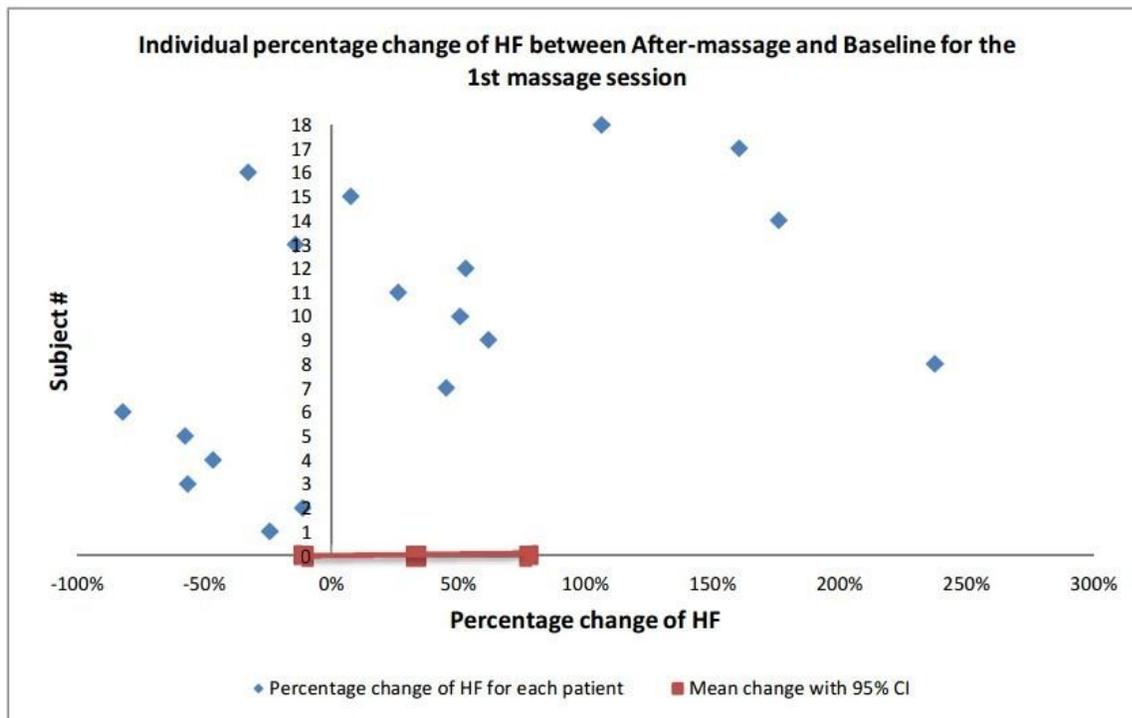
Boxplot for logHF changes in the 1<sup>st</sup> massage session

*Baseline (Min.: -3.15; Median: 2.17; Max.: 5.81), the mean of HF with SD at Baseline: 1.63+/-2.77; During (Min.: -1.9; Median: 2.83; Max.: 6.36), the mean of HF with SD during massage: 2.02+/-2.76; After (Min.: -2.21; Median: 2.31; Max.: 6.24), the mean of HF with SD after massage: 1.69+/-2.62*

**Figure 6-2:** Individual percentage change of HF for the 1<sup>st</sup> massage session  
**A**



**B**



**A:** Individual percentage change of HF between During-massage and Baseline. *The mean of percentage increase of HF during massage: 75.06% (95% CI: 19.90% to 130.23%)*

**B:** Individual percentage change of HF between After-massage and Baseline. *The mean of percentage increase of HF after massage: 33.52% (95% CI: -10.59% to 77.64%)*

### ***6.1.1.2 The Effect of Single Massage on LF***

Table 6-3 summarized the original data of LF and its changes during the 1<sup>st</sup> session of massage intervention. We saw the lower LF values compared to normal values (Appendix B) and the huge differences among LF data that reflected the different health condition.

The boxplot of logLF for baseline, during-massage and after-massage during the 1<sup>st</sup> massage session showed that the logLF rose from 2.44+/-2.64 (baseline) to 2.78+/-2.59 during massage, and fell to 2.59+/-2.4 after massage (Figure 6-3). The increase in logLF during massage compared to baseline values was statistically significant (p-value=0.01 for both paired t-test and Wilcoxon signed-rank test). However, there was no statistically significant difference of logLF between after-massage and baseline (Paired t-test for p=0.37 and Wilcoxon signed rank test for p=0.25).

In the graph showing individual change of LF, 13 subjects had increases in LF with two of them (#12 and #18) showing approximately 200% increases during massage, while 5 subjects showed decreases in LF during massage. We saw the LF significantly increased by a mean of 56.3% (95% CI: 20.31% to 92.29 %, Student's t-test with p= 0.004) compared to baseline (Figure 6-4A). For the 5 minutes after session, 12 subjects had increases in HF with one of them (#8) showing 230% increase of baseline; 6 subjects showed decreases in LF. The LF values after massage increase by a mean of 37.41 % compared to baseline, but it was not statistically significant (95% CI: -0.5% to 75.33%, Student's t-test with p= 0.053) (Figure 6-4B).

**Table 6-3: LF in the 1<sup>st</sup> massage session**

	Baseline	During-massage		After-massage	
	Low-frequency power(msec <sup>2</sup> /hz)	Low-frequency power(msec <sup>2</sup> /hz)	Percentage change of LF between During-massage and Baseline (%)	Low-frequency power(Msec <sup>2</sup> /hz)	Percentage change of LF between After-massage and Baseline (%)
Subject#1	7.96	14.5	82.17%	9.56	20.04%
Subject#2	179.96	144.81	-19.53%	234.95	30.56%
Subject#3	27.54	19.18	-30.37%	16.6	-39.72%
Subject#4	18.36	27.55	50.02%	18.44	0.4%
Subject#5	6.21	4.91	-20.98%	3.42	-44.86%
Subject#6	482.27	312.47	-35.21%	100.1	-79.24%
Subject#7	0.9367	1.9745	110.80%	1.6547	76.65%
Subject#8	0.0163	0.0361	121.19%	0.0542	<b>231.86%</b>
Subject#9	46.07	64.33	39.64%	86.21	87.13%
Subject#10	47.86	60.8	27.05%	37.19	-22.29%
Subject#11	24.02	43.08	79.31%	13.09	-45.49%
Subject#12	199.5	600.8	<b>201.16%</b>	363.06	81.99%
Subject#13	0.6026	0.9127	51.45%	1.4602	142.3%
Subject#14	167.85	236.21	40.72%	294.59	75.51%
Subject#15	2.4	1.76	-26.80%	2.18	-9.06%
Subject#16	27.39	62.4	127.80%	47.68	74.07%
Subject#17	0.2614	0.3287	25.73%	0.3472	32.82%
Subject#18	20.35	58.85	<b>189.26%</b>	32.71	60.75%
Mean +/- SD			56.30%+/- 72.37%		37.41%+/- 74.09%

*Baseline: the five minutes before massage;*

*During-massage: the massage duration;*

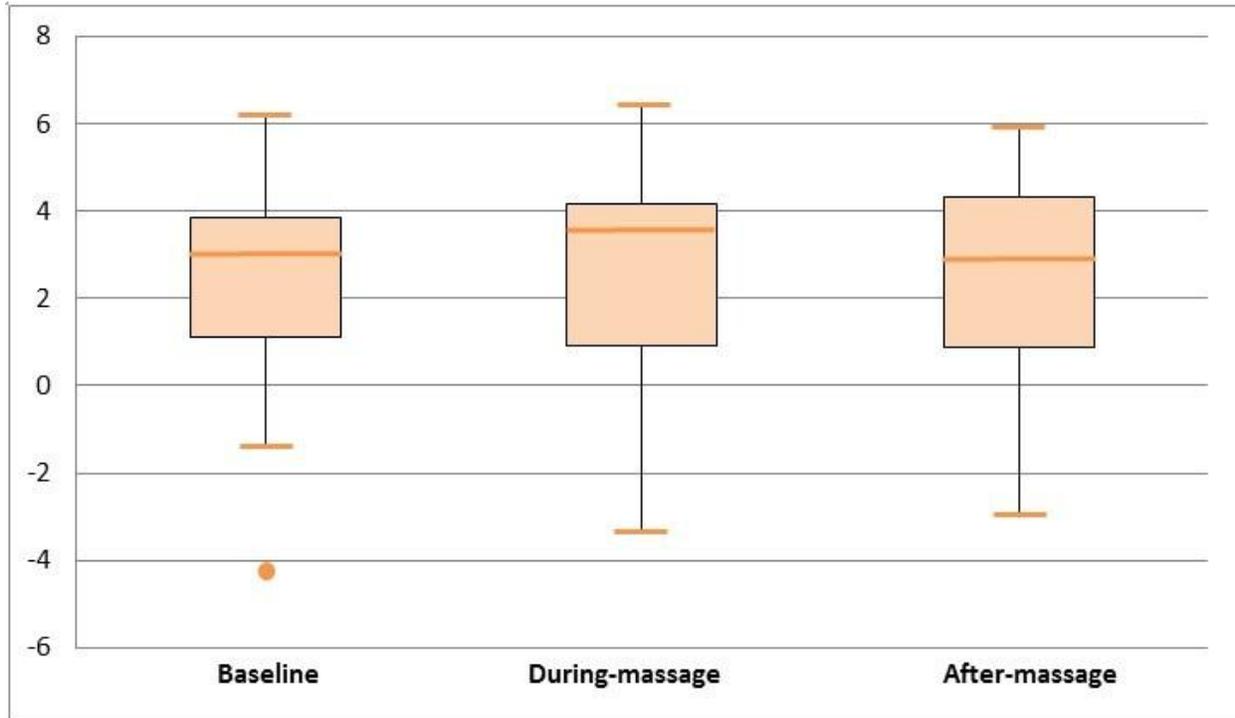
*After-massage: the five minutes after massage;*

*LF: low frequency component of HRV*

*Percentage difference of LF change between During-massage and Baseline (%): [LF (During-massage)-LF (Baseline)] / LF (Baseline) \* 100%;*

*Percentage difference of LF change between After-massage and Baseline (%): [LF (After-massage)-LF (Baseline)] / LF (Baseline) \* 100%*

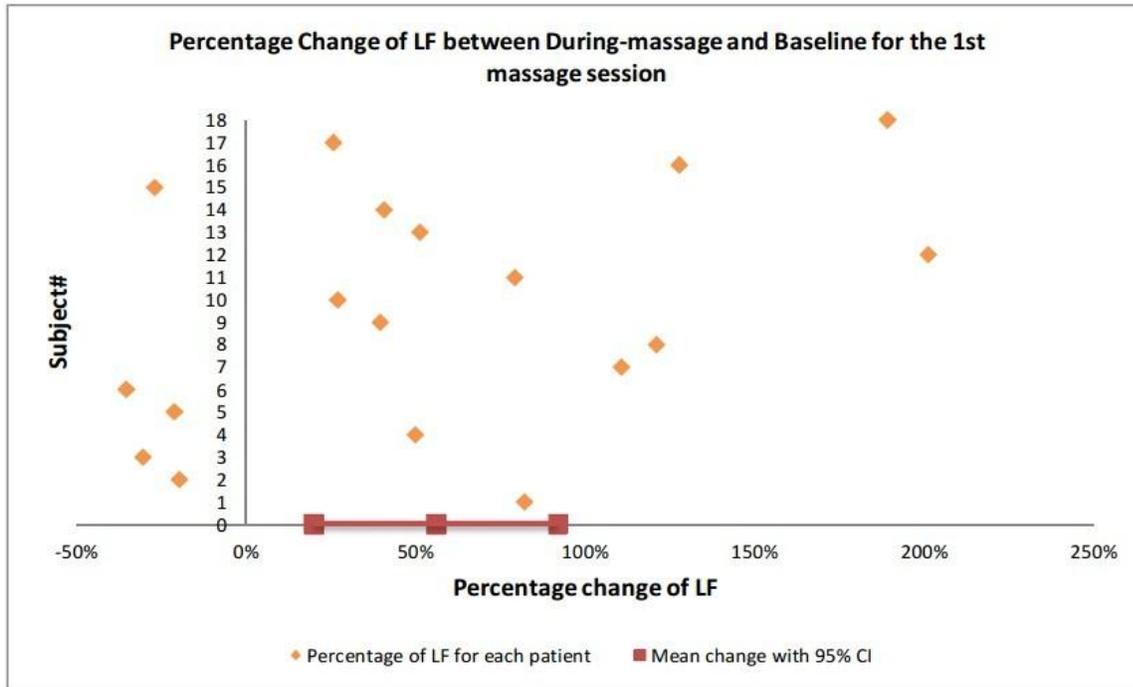
**Figure 6-3:** Change of logLF in the 1<sup>st</sup> massage session



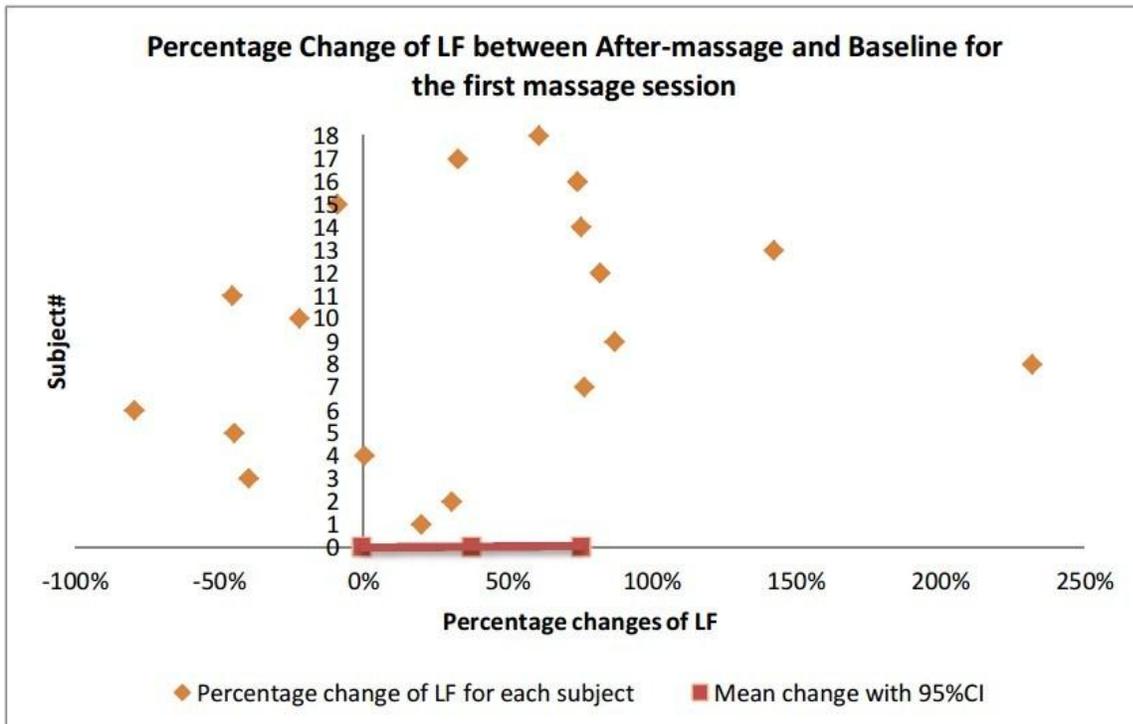
Boxplot of logLF changes in the 1<sup>st</sup> massage session

*Baseline (Min: -4.12; Median: 3.1; Max.: 6.18), the mean of LF with SD at baseline: 2.44+/-2.64;  
During (Min.: -3.32; Median: 3.54; Max.:6.4), the mean of LF with SD during massage: 2.78+/-2.59;  
After (Min.: -2.92; Median: 2.86; Max.:5.9), the mean of LF with SD after massage: 2.59+/-2.4*

**Figure 6-4:** Individual percentage change of LF for the 1<sup>st</sup> massage session  
**A**



**B**



**A:** Individual percentage change of LF between During-massage and Baseline. *The mean of percentage increase of HF during massage: 56.30% (95% CI: 20.31% to 92.29%)*

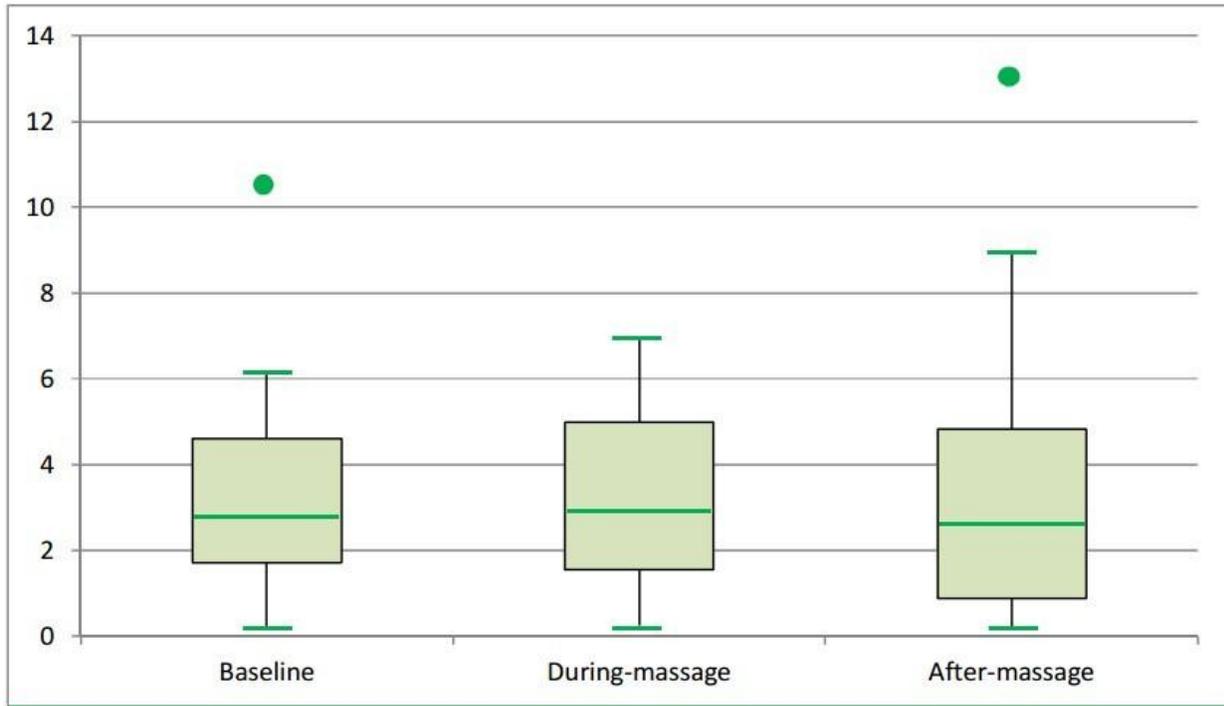
**B:** Individual percentage change of LF between After-massage and Baseline. *The mean of percentage increase of HF after massage: 37.41% (95% CI: -0.5% to 75.33%)*

### ***6.1.1.3 The Effect of Single Massage on LF/HF***

The comparison of LF/HF at baseline, during-massage and after-massage was presented in figure 6-5. There was no significant change on the average LF/HF during massage (mean=3.34+/-2.25) and after massage (mean=3.81+/-3.43), compared to the baseline (mean=3.28+/-2.26) ( $p>0.05$  for both Paired t-test and Wilcoxon signed rank test).

Table 6-4 summarized the LF/HF changes during the 1<sup>st</sup> session of massage intervention. (1) Six subjects had baseline LF/HF over the normal range (pediatric standard: 0.73+/-0.08~2.43+/-0.88, details provided in Appendix) (Finley & Nugent, 1995; Kazuma et al., 2002; Zhang, 2007). Among them, four subjects had decreases in LF/HF while two had increases in LF/HF during massage. (2) Three subjects with LF/HF below the normal range had increases in the ratio through massage. (3) The other nine subjects had LF/HF originally in the normal range. Following the massage, seven of them were in the normal range (do not affected by the massage therapy) and two of them had increases in LF/HF.

**Figure 6-5:** Change of LF/HF in the 1<sup>st</sup> massage session



*Baseline (Min: 0.22; Median: 2.76; Max.: 10.65);*

*During (Min.: 0.24; Median: 2.87; Max.:6.97);*

*After (Min.: 0.22; Median: 2.67; Max.: 13.29)*

**Table 6-4:** LF/HF changes in the 1<sup>st</sup> massage session

	Baseline	During-massage		After-massage	
	LF/HF	LF/HF	Difference of LF/HF between During-massage and Baseline	LF/HF	Difference of LF/HF between After-massage and Baseline
<b>Subjects with higher LF/HF</b>					
Subject#5	5.92	5.14	-0.78	7.64	1.72
Subject#10	5.73	4.52	-1.21	2.95	-2.78
Subject#15	10.65	6.56	-4.09	8.98	-1.68
Subject#17	6.1	2.8	-3.3	3.11	-2.99
Subject#2	4.28	6.78	2.5	6.28	2
Subject#13	4.72	6.4	1.68	13.29	8.57
<b>Subjects with lower LF/HF</b>					
Subject#4	0.4	0.9	0.5	0.74	0.34
Subject#8	0.22	0.24	0.02	0.22	0
Subject#10	0.6	1.04	0.44	0.71	0.11
<b>Subjects with normal LF/HF</b>					
Subject#6	1.67	1.5	-0.17	1.92	0.25
Subject#7	2.60	3.34	0.74	3.16	0.56
Subject#9	2.06	2.03	-0.02	2.38	0.32
Subject#11	2.93	2.94	0.01	1.26	-1.67
Subject#14	2.54	0.87	-1.68	1.62	-0.93
Subject#16	1.87	2.52	0.65	4.84	2.96
Subject#18	0.79	1.65	0.87	0.61	-0.17
Subject#1	3.04	6.97	3.93	4.8	1.76
Subject#3	3	3.87	0.87	4.14	1.14

*The normal range of LF/HF (pediatric standard: 0.73+/-0.08~2.43+/-0.88, details provided in Appendix) (Kazuma et al., 2002; Walton & Children, 1995; J. Zhang, 2007)*

*Baseline: the five minutes before massage;*

*During-massage: mean of massage duration;*

*After-massage: the five minutes after massage;*

## **6.1.2 The Effect of Multiple Sessions of Massage**

In this subsection, we were aiming to address the question: whether multiple massage intervention has a greater effect than a single session of massage. We obtained seven patients completed six sessions of massage.

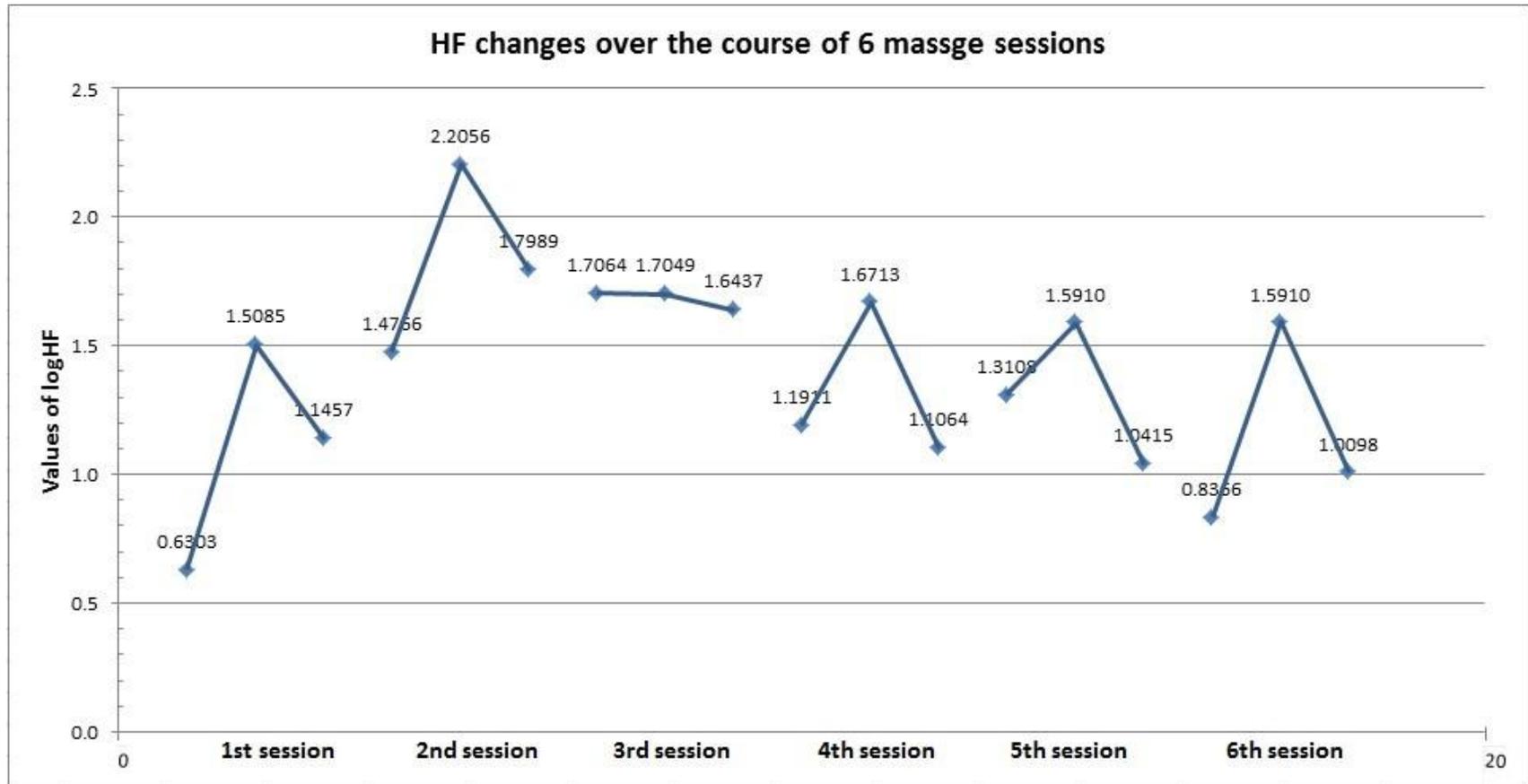
### ***6.1.2.1 HF Changes over the Six Sessions of Massage***

Figure 6-6 showed six sections of turning curves which represented the HF changes of seven patients over the course of six massage sessions. An impressive peak (1.5085) was observed in the first massage duration, followed by a fall (1.1457) after massage, which was still higher than the initial baseline (0.6303). Then we saw a sustained rise in the 2<sup>nd</sup> baseline (1.4766) compared to the 1<sup>st</sup> end point. The second line kept the similar trend to the first one and possesses the peak of HF (2.2056) during massage administration. In the third session, we saw that the HF basically leveled off through massage therapy. Then in the last three sessions of massage, HF values elevated during massage and descended after massage, but overall declined compared to the second and third sessions.

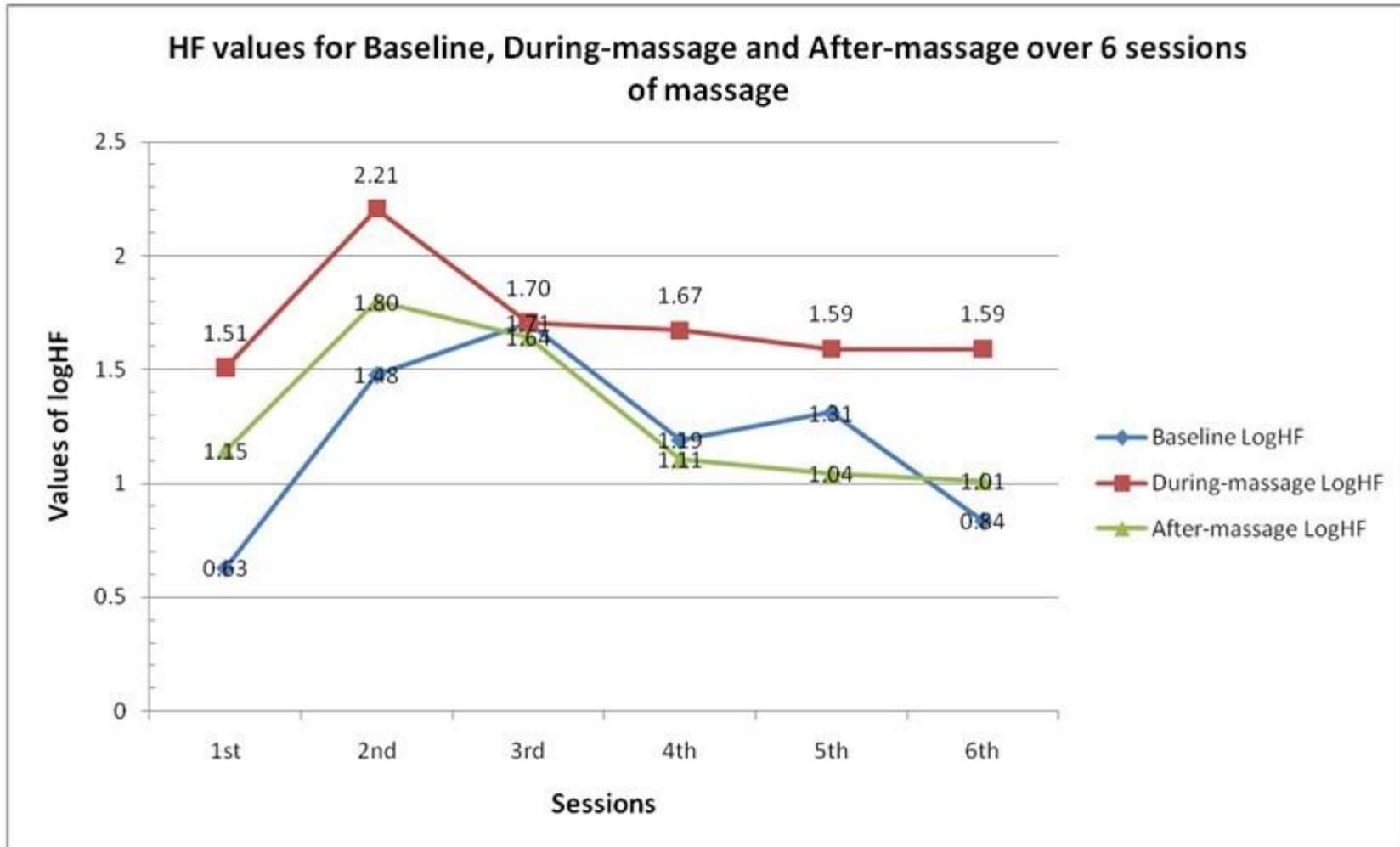
From another perspective, a description of HF changed among six baselines, during-massage periods and after-massage periods was presented in figure 6-7. We observed the highest red line of the three which represented the HF changes in massage procedure. The interlaced blue and green lines represented the baseline and after-massage, respectively. For baseline-HF (blue), we found HF peaked in the initial three sessions of massage (1.7064), descended from the fourth

session and stopped in the last session (0.8366) at a higher level than the initial baseline (first session: 0.6303). For HF during massage, we observed that HF peaked in the second session (2.2056), declined from the third session and then leveled off during the remaining sessions. HF in the last session of massage procedure was still higher than the starting point (1.5085) and initial baseline (0.6303). The change of HF for after-massage over six sessions was parallel with the baseline-HF, except the end point (1.0098) returning to near the starting point (1.1457). The final point of HF (1.0098) was higher compared to the initial baseline HF (0.6303).

**Figure 6-6:** HF changes over the course of six massage sessions



**Figure 6-7:** HF values for Baseline, During- and After-massage over six message sessions



### ***6.1.2.2 LF Changes over the Six Sessions of Massage***

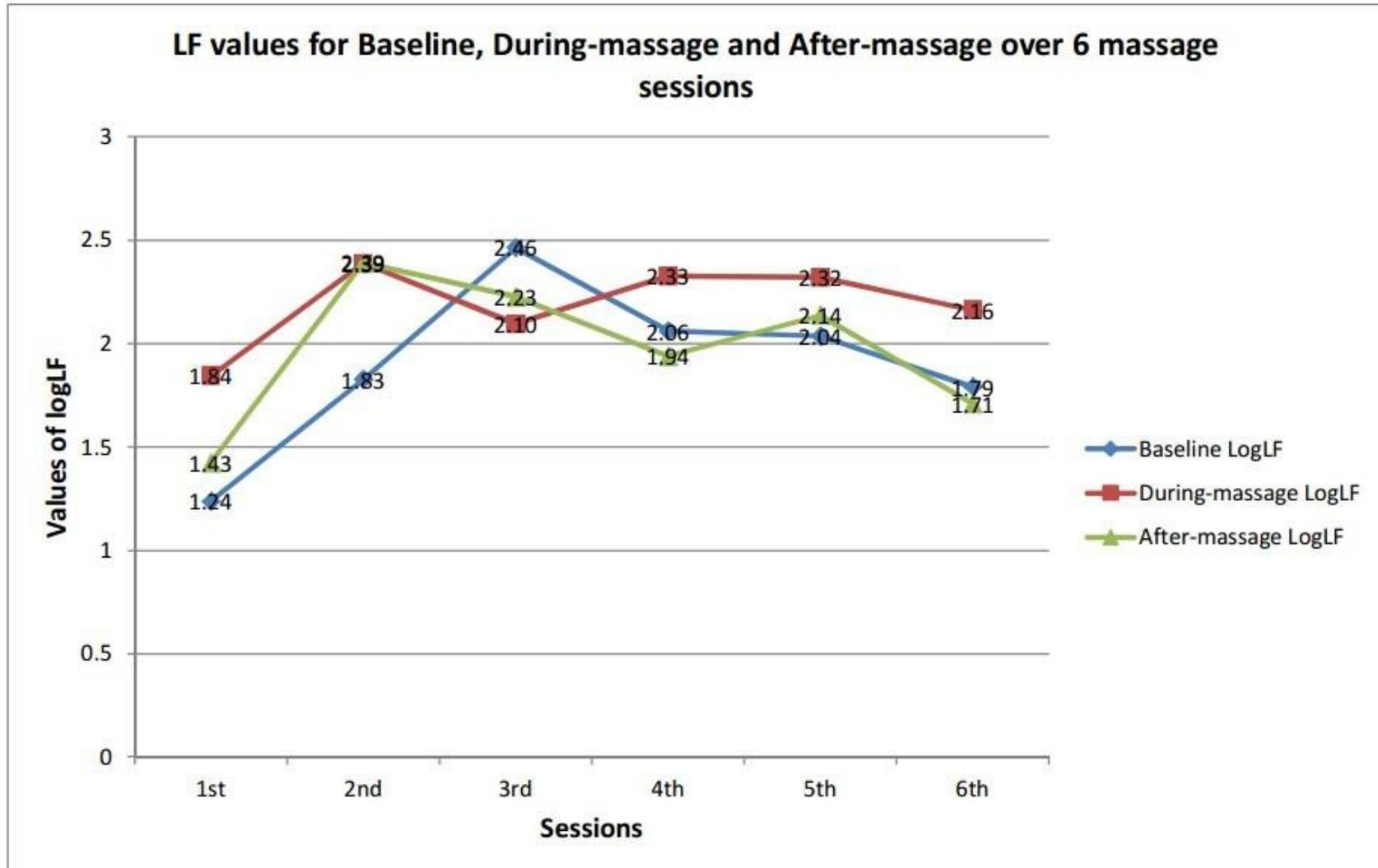
The six sections of turning curves in figure 6-8 represented the LF changes over the course of six massage sessions. LF rose up during massage and decreased after massage in the first massage session, with a peak at 1.844. In the 2<sup>nd</sup> massage session, an elevated baseline LF (1.8266) sustained the increase during massage (2.3883) and after massage (2.3914). However, the third line exhibited a total inverse trend from others. A decline during massage and subsequent ascent after massage were observed in the 3<sup>rd</sup> session. LF at baseline of the 4<sup>th</sup> session started from a high value (2.0614), then increased during massage and fell back to the 4<sup>th</sup> baseline after intervention. Both the remaining two sessions kept this trend. The LF after all sessions of massage was at a level 1.7087, which was increased compared to the initial baseline (1.2374)

Figures 6-9 described the change of LF among six baselines, six during-massage periods and six after-massage periods. Similar to HF, Baseline-LF (blue) continued rising to the peak of 2.46 in the initial three sessions of massage, then descended from the fourth session and ended at 1.79 in the last session. LF during massage (red) increased from 1.84 to 2.38 in the second session, and leveled off in the subsequent sessions. LF after massage (green) peaked in the second session (2.39) and fluctuated in the following sessions. The after-massage LF value for the 6<sup>th</sup> session (1.71) was higher compared to the initial baseline (1.24).

**Figure 6-8:** LF changes over the course of six massage sessions



**Figure 6-9:** LF values for Baseline, During- and After-massage over six massage sessions

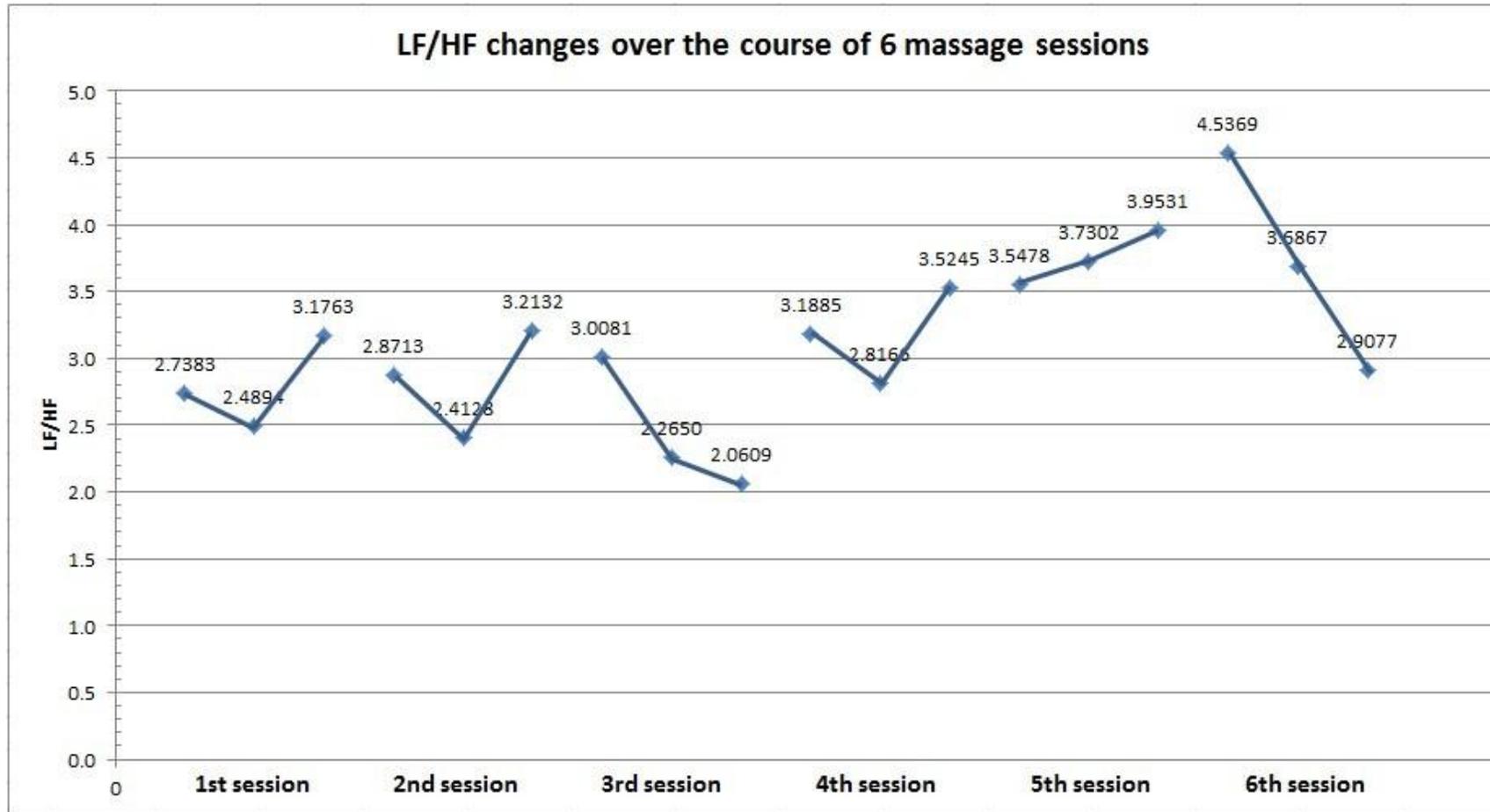


### ***6.1.2.3 LF/HF Changes over the Six Sessions of Massage***

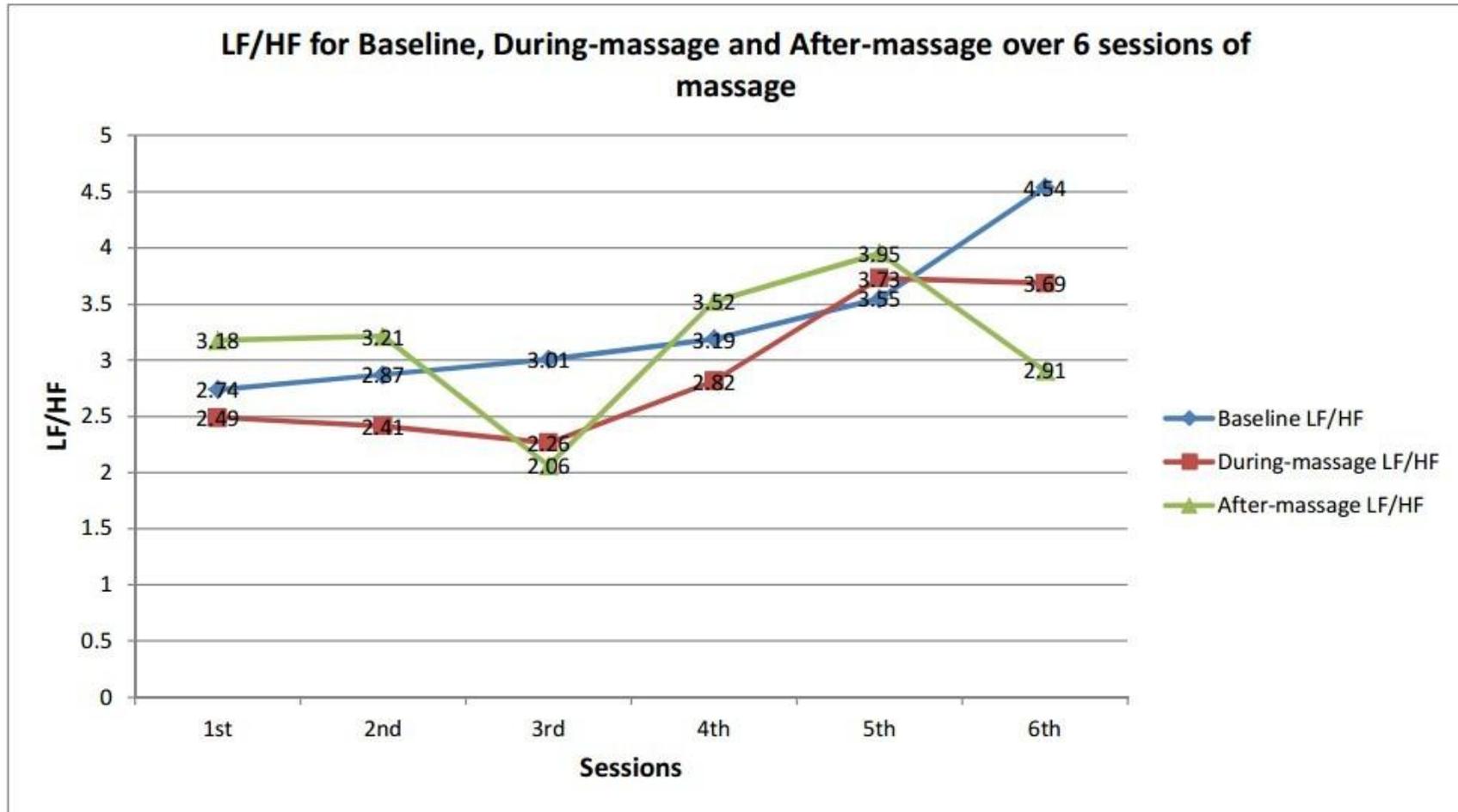
The average LF/HF for the first five sessions was all in the normal range at pediatric standard:  $0.73 \pm 0.08 \sim 2.43 \pm 0.88$  (details provided in Appendix) (Finley & Nugent, 1995; Kazuma et al., 2002; Zhang, 2007). The ratio declined slightly during massage and rose after massage in the first five sessions. The increased baseline (4.5369) in the 6<sup>th</sup> session was pushed down to the normal range through massage (Figure 6-10).

Figure 6-11 displayed two slightly increased lines: baseline-LF/HF (blue) and during-massage-LF/HF (red). Except the last baseline, other points were all in the normal range of LF/HF (1~3.5 for children under 18 years old) (Acharya U, Kannathal, Sing, Ping, & Chua, 2004; Massin, 1997). The green line, which represented LF/HF after-massage, fluctuated from 2.06 to 3.96 over six massage sessions.

**Figure 6-10:** LF/HF changes over the course of six massage sessions



**Figure 6-11:** LF/HF for Baseline, During- and After-massage over six message sessions



## 6.2 HRV Analysis and Clinical Status of PICU Patients

Table 6-5 summarized the relationship between patients' clinical status/severity (diagnosis and PELOD scores) and HRV magnitude (HF+LF). The subject number was listed by HRV magnitude (HF+LF), from high to low.

Among the 18 patients, four patients with high HF+LF (order of magnitude:  $10^2 \text{ ms}^2/\text{hz}$ ) had a PELOD score of 0; eight patients with moderate HF+LF ( $10^1 \text{ ms}^2/\text{hz}$ ) had a PELOD score of 0; three patients with low HF and LF ( $1-10 \text{ ms}^2/\text{hz}$ ) had a PELOD score of 1 or 10, except Subject#15; and three patients who had very low HF+LF values ( $10^{-1} \text{ ms}^2/\text{hz}$ ), which had high PELOD scores of more than 10.

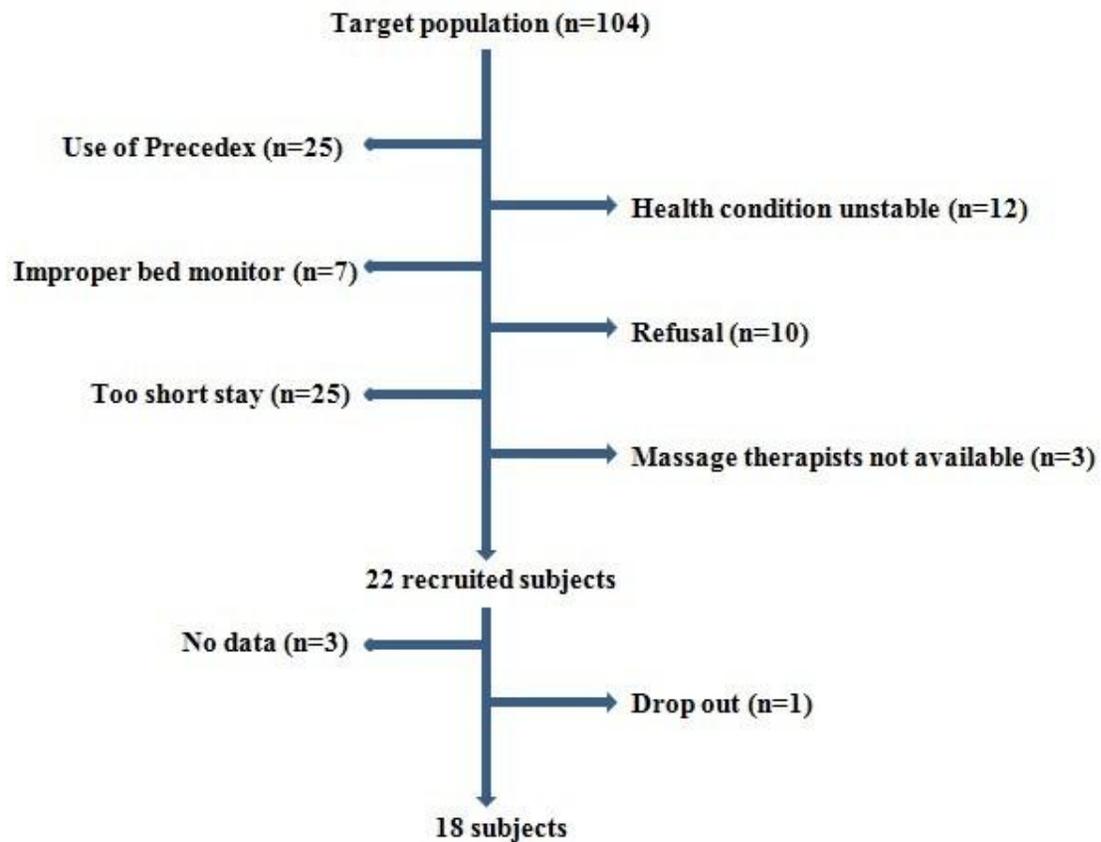
We observed that the high HRV values ( $>100 \text{ ms}^2/\text{hz}$ ) correspond to pneumonia, bronchiolitis and postoperative care; moderate HRV values ( $10-100 \text{ ms}^2/\text{hz}$ ) corresponded to burns, strider and pulmonary hypoplasia + upper GI bleeding; low HRV values ( $1-10 \text{ ms}^2/\text{hz}$ ) corresponded to trauma (car accident), sepsis shock (with presumed pyelonephritis and vesico-ureteral reflux); and very low HRV values ( $<1 \text{ ms}^2/\text{hz}$ ) were related to acute renal failure sepsis, septic shock, leukemia. An exception was the Subject#15 with febrile seizures (not so severe condition) who had low HRV values.

**Table 6-5:** HRV magnitude and PELOD scores

Subject #	Magnitude of HF+LF	Diagnosis	PELOD Scores
#6	100-1000	Pneumonia, respiratory distress,	0
#12	100-1000	Post-operation	0
#14	100-1000	Pneumonia, respiratory distress,	0
#2	100-1000	RSV bronchiolitis	0
#9	10-100	Stridor	0
#4	10-100	Difficulty breathing	0
#10	10-100	Vertical expandable prosthetic titanium rib (VEPTR) revision	0
#18	10-100	Respiratory infection, congenital heart disease	0
#16	10-100	Pulmonary hypoplasia + upper GI bleeding	0
#11	10-100	Respiratory disorder	0
#3	10-100	Burns	0
#1	10-100	RSV bronchiolitis	0
#5	1-10	Trauma	1
#15	1-10	Febrile seizures	0
#7	1-10	Sepsis shock, presumed pyelonephritis, vesico-ureteral reflux	10
#13	0.1-1	Acute renal failure, sepsis shock, respiratory distress	10
#17	0.1-1	Acute lymphoblastic leukemia	20
#8	0.01-0.1	Acute myeloid leukemia, E. coli sepsis	12

### 6.3 Study Feasibility

The recruitment was described as following. The different problems encountered were presented along with the corresponding solutions (Table 6-6), and protocol amendments (Table 6-7).



**Table 6-6: Problems and solutions**

	Problems	Solutions
Study population	1. Rare diseases/cases <i>Our primary inclusion criterion was to recruit patients with sepsis or septic shock. Such cases were very rare which created obstacle for recruiting patients.</i>	<i>Solution- we modified the primary research protocol in terms of inclusion criteria: we expanded the inclusion criteria to recruit all PICU patients. It was considered that PICU was a stressful environment for all subjects.</i>
	2. Using Precedex <i>Many PICU patients used Precedex, which affected HRV.</i>	<i>Solution- we stopped recruiting patients who were in use of Precedex.</i>
	3. Cardiac problems <i>We lost one subject because he had arrhythmia and this makes ECG with lots of ectopic rhythm waves which were not appropriate for HRV analysis.</i>	<i>Solution- we stopped recruiting patients with cardiac problems.</i>
Intervention	4. Two days' massage impossible <i>Patients sometimes stayed short in ICU, which made impossible to be administrated two days' massage following original protocol.</i>	<i>Solution- we switched two days massage intervention to one day</i>
	5. Massage refusal <i>Patients and their parents in ICU were depressed, which made them not open to other intervention.</i>	<i>Solution- we gave the detailed explanation about our research to patients and gave them enough time to make their decision.</i>
Data collection	6. Cytokines assessment difficulty <i>It was complex to arrange cytokines assessment in ICU</i>	<i>Solution- after we changed our study population, cytokines assessment was not necessary to be assessed; hence we decided to not collect cytokines.</i>
	7. Poor patients monitoring <i>Beds changes caused challenges to record ECG data in central station. Another problem was related to beds that were not connected to the central station.</i>	<i>Solution- we asked the massage therapists to record the bed number and the exact time with minute accuracy. We made more connection with nurses and checked the recording computer in central station to ensure the exact time of changing bed.</i>
	8. Data collection difficulty <i>The young patients moved and cried a lot; such emotion and behavior influenced ANS and induced lots of chaos in the data.</i>	<i>Solution- we tried our best to inform patients not to move a lot. We also had the permission to use video cameras to record the patients' movement, cry, sleep status and mode to explain the influence on results.</i>
	9. Technical problems <i>We lost several patients' data because of IT problems and central station capture box malfunction.</i>	<i>Solution- Technical experts provided assistance.</i>

**Table 6-7:** Protocol items amendments

Amendments	Date
1. We modified the research protocol regarding the inclusion criteria. We recruited all PICU patients rather than the ones with sepsis and sepsis shock.	June 28,2011
2. We stopped recruiting patients with cardiac problems or using Precedex.	June 28,2011
3. We switched from two day massage intervention to one day.	June 28,2011
4. We amended one study objective: assess inflammatory cytokines amount.	June 28,2011
5. We obtained permission to use cameras to record patients' reaction during the massage.	July 29, 2011

## **CHAPTER 7: DISCUSSION**

In our PICU study, we found that massage therapy could elicit ANS effects by increasing parasympathetic activity, reflecting an improved ANS modulation on heart rate and re-balancing the sympatho-vagal equilibrium both in single and multiple sessions on PICU patients. This study was conducted for one year (from April 2011 to April 2012) to include 22 patients, as the recruitment of patients was difficult. The ICU staff screened eligible patients every morning, and asked their consent/assent of participant in the study. We contacted a total of 104 eligible subjects; however due to problems of contaminant drug use (Precedex, n=25), unstable health condition (n=12), improper bed monitor (n=7), refusal (n=10), short stay (n=25) and RMTs not available (n=3), 22 ICU subjects were recruited in our study.

In this chapter, we will separate the discussion between the pilot aspect and the clinical aspects (HRV results interpretation and HRV & PELOD scores) of our results.

### **7.1 The Feasibility of the Pilot Study**

One important objective of pilot clinical study was to gain information on the feasibility and practicability of conducting future larger-scale studies. Our research project was the first one to address the effects of long-duration and repeated massage interventions on ANS among special population-PICU patients. Our primary protocol focused on sepsis and septic shock, with one objective of assessing the amount of inflammatory cytokines. We planned to administrate two days' massage intervention. However, we encountered several problems (table 6-9) in the

process of study implementation, in terms of recruitment, intervention and data collection. Solutions (table 6-9) were then found and amendments (table 6-10) were made to the primary protocol.

### **7.1.1 The Primary Research Protocol and Protocol Amendments**

We met several difficulties during the completion of the study regarding the study population, the intervention and data collection.

- *Regarding the study population: from sepsis to all PICU illness*

We initially planned to recruit subjects with sepsis or septic shock, because despite well-established modern medical treatment, sepsis is the most common cause of shock leading to multi-organ dysfunction and death. However, sepsis and septic shock are clinically rare diseases compared to other conditions in ICU in developed countries. Such rare disease incidence together with the short stay in ICU (average stay time: 24 hours) created challenges for patient recruitment; we spent three months recruiting two PICU patients diagnosed with acute bronchiolitis of the viral etiology (respiratory syncytial virus). Therefore, we expanded the inclusion criteria of primary research protocol to recruit all PICU patients who were also with high stress as our study population. The new population with high stress had suppressed PNS activity and over-activated SNS activity as well and the effect of massage was to improve the ANS dysfunction. Therefore this change of protocol did not change the study objective; it just facilitated the recruitment. The amendment was accepted on June 28, 2011.

- *Regarding study population: subjects using Precedex or with cardiac problem were excluded*

In our primary protocol, we did not exclude patients who were administered Precedex (Dexmedetomidine), a sedative medication, which affects HRV (eliminating most of the variability) thereby making it impossible to study ANS (Carollo, Nossaman, & Ramadhyani, 2008; Pandharipande, Ely, & Maze, 2006). After observing the important impact on study results, we then decided to stop recruiting patients who were taking Precedex and made the amendment on protocol on June 28, 2011. Moreover, we excluded one subject who had arrhythmia, as the ectopic rhythm strongly affected the HRV analysis. Hence, we also excluded subjects with cardiac problems (June 28, 2011).

- *Regarding study intervention: from two days' massage to one day*

We found that high mobility of patients produced challenges for administration of two days' massage intervention following the primary protocol. Due to the short-stay of patients in ICU, we switched two days' massage intervention to one day. This change affected the amount of intervention, but did not change the study objective. The protocol amendment was accepted on June 28, 2011.

- *Regarding data collection: cancelling pro-inflammatory cytokines measurement*

In the primary protocol, we planned to assess the inflammatory cytokines. However, we found it impossible to keep this objective due to the difficulty to arrange blood samples in a timely way to establish a valid correlation. Initially we intended to measure the cytokines from the laboratory sample leftovers, however the samples were not readily available and the cytokine tests performed were not compatible. Technically, it was not feasible to perform multiple cytokine tests from laboratory sample leftovers and biological data at each important time: 5

minutes before massage, during massage and 5 minutes after massage, as was planned. Additionally, because we changed the study population, it was not necessary to assess cytokines any more. The protocol amendment was accepted on June 28, 2011.

- *Regarding data collection: using video camera*

Another problem regarding data collection was related to the recording quality when young children moved or cried or because of environmental disturbances during massage. Heart rate can be strongly influenced by emotion, behavior (Kreibig, 2010) and environmental (e.g. physical) factors such as sudden loud sound, therapeutic manipulations and many others. It is also well known that the PICU is a very busy environment with considerable psychological and physical stress on patients. In our observations, ECG recording that we used for the HRV analysis had some disturbances and artifacts that could not be explained solely by massage. We then organized the massage interventions to be conducted during quiet time slots as much as possible. We also obtained ethics approval to use a camera to record the patients' movement, sleep status, emotional state (including crying) and environment to help interpreting results. The videotape was used solely for the research purpose. The patient's confidentiality was protected. The protocol amendment regarding using camera was obtained on July 29, 2011.

- *Regarding data collection: HRV data collection*

In ICU, one monitor is allocated to one bed; the ECG recording and other physiological data for each bed is transmitted to the central station through the device. Therefore, knowing the bed number is necessary to obtain ECG data. However, for optimal management, patients were transferred to other beds during their stay in ICU, which created some difficulties to follow the patient across several data sets (bed-related). Regarding this, we asked the massage therapists to record the bed changes and the exact time with minute accuracy when they administered

massage. We also established tight communication with the nurses and frequently checked the recording computer in central station to ensure the exact time of changing beds. Another issue was the transfer to beds that were not connected to the central station. Lastly, we experienced unexpected technical problems with the central station capturing box. In the initial stage of the study, the “capturing box” in central station only captured data on odds hours, while in the intermediate stage of the study, only data from even hours could be obtained. Due to this, we lost valuable data of several patients.

This pilot study, because of the difficulties encountered, raised some important questions. Overall, despite strong support by PICU team and high interest from parents to experience massage, it was not easy to recruit the study population. Moreover, assessing the change in biological parameters (such as pro-inflammatory cytokines) required drawing blood especially for the study purpose, as using blood sample leftover did not fit with the study protocol. Extra blood sample requirement would affect negatively the study with expected even lower recruitment rate; the extra sample would also create a new stress that defeated the purpose of conducting a massage intervention aimed at decreasing stress. Our experience showed that studying massage in the PICU should remain simple and pragmatic, with clinical objectives or non-invasive objectives, such as HRV recording.

## **7.2 HRV Interpretation**

This study contributed to a growing evidence of the role of the ANS in critical illness. In some sense it was not surprising given the active involvement of afferent and efferent from both branches of ANS in stressful situations (Ellenby et al., 2001; P J Godin & Buchman, 1996;

Norris et al., 2006). In our study, the long-duration (30-min) massage therapy elicited distinct ANS responses represented by three parameters of HRV analyses.

## **7.2.1 Significance and Interpretation of HRV Results**

### ***7.2.1.1 HRV for Single Session of Massage***

Vagal activity is the major contributor to the HF component (ESC/NASPE Task Force, 1996). The HF, as an index of pure parasympathetic regulation, is sensitive and unambiguous to reflect PNS responses. Patients who received a single session of 30-min massage therapy produced statistically significant increase in HF values compared to the baseline HF (5-min before massage), which signified increased vagal activity and PNS responses through massage therapy (Table 7-1). These results were in agreement with previous study findings that noted immediate effects of short-term (5-min) myofascial trigger-point massage therapy to the head, neck and shoulder areas on adults was effective in increasing cardiac parasympathetic activity (decreased heart rate, systolic and diastolic blood pressure) and improving relaxation (muscle tension and emotional state), characterized by increased HF ( $p < 0.01$ ) (Delaney et al., 2002). Studies on preterm babies also indicated that moderate pressure massage therapy could increase PNS activity characterized by increased HF, vagal activity and gastric motility (Diego et al., 2007; Diego, Field, & Hernandez-Reif, 2005).

Moreover, in our study, the original objective was to assess the SNS activity precisely rather than use HR and BP, whereas due to the PICU restriction, we could not use the BIOPAC, a device

with analysis software, to assess impedance and calculate the PEP (pre-ejection period) which is affected very specifically by SNS (Goedhart, Willemsen, Houtveen, Boomsma, & De Geus, 2008; Houtveen, Groot, & Geus, 2005; Schachinger, Weinbagher, Kiss, Ritz, & Langewitz, 2001). Thereby, we decided to assess the LF and LF/HF ratio to reflect the ANS modulation and the balance between SNS and PNS. The LF is complexly mediated by both parasympathetic and sympathetic in varying proportions (Akselrod et al., 1981; Pagani et al., 1986; Pomeranz et al., 1985). In particular it is not possible to state with certainty the extent to which LF may depend on sympathetic modulation. Thus it is much more difficult to interpret LF than HF. However, we know that both LF and HF vary in the same direction with total power when the spectral components are expressed in absolute units ( $\text{ms}^2/\text{hz}$ ) (ESC/NASPE Task Force, 1996), which means the increase of LF indicates the improvement of HRV, while decrease on LF indicates the decrease in HRV. Due to the fact that stress increases SNS and decreases PNS, which then decreases HRV (i.e. HF+LF; we did not focus on VLF as it is affected by many uncertain factors that was stated in chapter 2 and we did not assess it in this study), while stress reduction can increase HRV, we considered that the increase in LF indicated the improvement of HRV which was an indicator of stress decrease and ANS modulation improvement. The result of LF changes during single session of 30-min massage therapy revealed a statistically significant increase in LF during massage, compared to baseline LF (5-min before massage) (Table 7-1).

The ratio of LF to HF is said to reflect the balance of sympathetic and parasympathetic activities (ESC/NASPE Task Force, 1996). The massage intervention modified most of the HF and LF in the same direction, thereby keeping the ratio with little modification (Table 7-1). Overall, by analyzing individual LF/HF, we found for most subjects, LF/HF was brought towards the normal

range: LF/HF above normal range decreased; LF/HF below normal range increased; and LF/HF originally in the normal range kept stable through massage therapy (Table 6-4 in chapter 6). The massage intervention seemed to bring the dispersed LF/HF towards the normal range. The change in LF/HF ratio could be interpreted as a shift from a sympathetic predominance or parasympathetic predominance toward a sympatho-vagal balance.

- LF/HF above normal range was decreased by massage

The decrease in high LF/HF could be in principle achieved by several alterations: (1) increase in PNS and fix in SNS; (2) decrease in SNS and fix in PNS; or (3) increase in PNS and decrease in SNS

- LF/HF below normal range was increased by massage

On the other hand, the low LF/HF implied an originally parasympathetic predominance/sympathetic suppression; hence a boost in low LF/HF by massage indicated the enhancement on the originally low sympathetic system and re-balance of sympatho-vagal activities. As described in a previous chapter (chapter 2.1), an adequate activated sympathetic activity and controlled inflammation was beneficial for physiological function, as for some weak patients, they needed active sympathetic activity to “arouse body function”.

- LF/HF originally in the normal range kept stable

Subjects with LF/HF ratio in the normal range (pediatric standard:  $0.73 \pm 0.08 \sim 2.43 \pm 0.88$ ) (Finley & Nugent, 1995; Kazuma et al., 2002; Zhang, 2007) did not change during massage, that was to say, they kept the stability of SNS-PNS balance through the massage.

**Table 7-1:** Statistical analysis of HF, LF and LF/HF

		Baseline	During massage	After massage
logHF	Mean+/-SD	1.63+/- 2.77	2.02+/-2.76	1.69+/-2.62
	p-value for Wilcoxon signed rank test		0.02	0.77
Percentage change of HF	Percentage change with 95% CI		75.06% (19.9%~130.23%)	33.53% (-10.59%~77.64%)
	p-value for student's t-test		0.01	0.13
logLF	Mean+/-SD	2.44+/- 2.64	2.78+/-2.59	2.59+/-2.4
	p-value for Wilcoxon signed rank test		0.01	0.25
Percentage change of LF	Percentage change with 95% CI		56.3% (20.31%~92.29%)	37.41% (-0.5%~75.33%)
	p-value for student's t-test		0.004	0.053
LF/HF	Mean+/-SD	3.28+/- 2.26	3.34+/-2.25	3.81+/-3.43
	p-value for Wilcoxon signed rank test		>0.05	>0.05

### **Special cases analyses:**

Among the total 18 subjects, 12 subjects who gained increases in both HF and LF (also HF+LF) during massage intervention, which indicated that the massage elicited an effect on increasing their PNS activity and improving ANS modulation on heart rate. Except subject#13, the ratio LF/HF for other 11 subjects was all brought towards the normal range by massage. Subject#13 was a 6 year old girl with acute renal failure and sepsis shock (PELOD score of 10 and HRV<1). From the video camera, we saw that she was negative, restless and crying during massage therapy. Her LF increased more than HF which contributed to the raised ratio of LF to HF.

Among the 12 subjects who had increases in both HF and LF, two subjects showed approximately 300% increase in HF (Figure 6-2 and Table7-2), and two showed more than 190% increase in LF during massage (Figure 6-4 and Table 7-2). The subject (#17) who gained 299% HF during massage was a 5 year old girl who had acute lymphoblastic leukemia (ALL) and with a very low variability of heart rate ( $0.04\text{msec}^2/\text{hz}$ ) at baseline. From the video recording, we saw that she had fever all through the massage procedure, but reacted calmly and positively to the intervention (Table 7-2). The other subject (#14) is a 6.5 year old boy with pneumonia and PELOD score of 0; he gained 368% HF during massage. We collected his initial baseline HF for  $66\text{msec}^2/\text{hz}$ , which was much lower than the HF at any other time points in the subsequent sessions (2<sup>nd</sup> to 6<sup>th</sup>) that all showing above  $100\text{msec}^2/\text{hz}$ . It was possible that the baseline HF values were particularly low in relation to the negative emotion to the unfamiliar massage; as massage was probably stressful for him at the beginning. We watched video recording and found notes from “Massage Information Collection Form” that he was restless at the beginning of the intervention (did not want to be touched), but gradually calmed down during massage. This

information matched to our supposition. During massage HF increased sharply was due to the effect of massage and the occurring of a better mood (Table 7-2). In LF analysis, we found subject (#12) who was a 2 year old boy hospitalized in ICU for post-operation care gained 201% LF during massage. He had high variability of heart rate ( $199.5\text{msec}^2/\text{hz}$ ) at baseline. He was calm, rest and positive to massage. The other subject (#18) who gained 189% LF was a 10 months boy with moderate HRV ( $20.35\text{msec}^2/\text{hz}$ ). He was calm and positive to the intervention as well.

However, there were four subjects whose both of HF and LF (as well as HF+LF) decreased through massage intervention (Figure 6-2, 4 and Table 7-2). Such decreases in HF, LF and HF+LF variability indicated that massage was source of extra-stress. Among these four subjects, one subject was with trauma, one with burns, one with respiratory distress/pneumonia and the other one with RSV bronchiolitis. Three subjects had moderate or low baseline HRV (from  $2\sim 40\text{msec}^2/\text{hz}$ ); one had high baseline HRV ( $200\text{msec}^2/\text{hz}$ ). The LF/HF for the subjects with burns and RSV bronchiolitis were originally in and above normal range, but still rose during massage. We considered that the massage for these subjects could be a stress, for instance, if they were tired and wanted to sleep, or if they had pain and did not want to be touched. The pressure of massage intervention might be another reason, given the findings in a study by T Field and M Diego in 2009 showed the subjects who received light pressure massage exhibited a sympathetic nervous system response (arousal) characterized by decreased vagal activity and an increased LF/HF ratio (Diego & Field, 2009). Or it may be considered that massage as a stressor/stimulus, which induced a stress reaction to the body, would not only be distress but also eustress (positive reaction such as a sense of meaning, hope or vigor) (Selye, 1974, 1975). A

study on facial massage presented a reasonable concept that conceived the mix of psychologically relax and physically active through massage therapy might well be regarded as “the refreshment of the body” (Hatayama et al., 2008). We thus could also consider that massage was a positive eustress to well renovate body functions. Finally, we needed to take into account the effects of ANS drugs (catecholamines, morphine, etc.) used for PICU patients. Our objection is to assess the positive effects of massage on improving PNS on the basis of ANS drugs use; the effects of drugs on counteracting the effects of massage should be taken into account.

Additionally, two patients showed opposite changes on HF and LF during massage. One subject (a 2 year old girl) with febrile seizures and detected with influenza A-virus obtained increase in HF, decrease in LF and decrease in HF+LF. The other subject with RSV bronchiolitis gained increase in LF, decrease in HF and increase in HF+LF (Table 7-2). The subject with febrile seizures had good physical condition with PELOD score of 0, whereas her HRV was low in terms of HF at  $0.2\text{msec}^2/\text{hz}$  and LF at  $2\text{msec}^2/\text{hz}$ . It was because febrile seizure was a huge stress for the brain and induced nervous system injury (for example, lose consciousness) (Sillanpää & Shinnar, 2010) and contributed to the dysfunction of ANS and low HRV basis. Moreover, from the video camera, we observed that she had intravenous (IV) needles in two hands when receiving massage. It was probably that both the illness and massage on her hands aggravated stress for her and cause little increase HF ( $0.04\text{ms}^2/\text{hz}$ , 18%) and decrease in HF+LF variability (Table 7-2). The other subject was a 2 month boy with RSV bronchiolitis and PELOD score of 0. He was resting and a little coughing during massage intervention.

**Table 7-2: HRV results**

		Subject#									
		Big Increase in HF		Big increase in LF		Decrease in both HF and LF				HF and LF change conversely	
		#17	#14	#12	#18	#2	#3	#5	#6	#1 (decrease in HF and increase in LF)	#15 (increase in HF and decrease in HF)
Baseline	HF	0.0429	66.03	334.64	25.86	42.04	9.17	1.0489	288.71	2.62	0.2254
	LF	0.2614	167.85	199.5	20.35	179.96	27.54	6.21	482.27	7.96	2.4
	HF+LF	0.3	233.88	534.14	46.21	222	36.71	7.26	770.98	10.58	2.63
	LF/HF	6.1	2.54	0.6	0.79	4.28	3	5.92	1.67	3.04	10.65
During-massage	HF	0.1713	309.16	577.64	45.2	25.19	4.94	0.9854	226.86	1.82	0.2663
	LF	0.3287	236.21	600.8	58.58	144.81	19.18	4.91	312.47	14.5	1.76
	HF+LF	0.5	545.37	1178.44	104.05	170	24.12	5.89	539.33	16.33	2.02
	LF/HF	1.92	0.87	1.04	1.65	6.78	3.87	5.14	1.5	6.97	6.56
	Percent change of HF	<b>299.43%</b>	<b>368.24%</b>	72.61%	74.75%	<b>-40.08%</b>	<b>-46.13%</b>	<b>-6.05%</b>	<b>-21.42%</b>	<b>-30.44%</b>	18.15%
	Percent change of LF	25.73%	40.72%	<b>201.16%</b>	<b>189.26%</b>	<b>-19.53%</b>	<b>-30.37%</b>	<b>-20.98%</b>	<b>-35.21%</b>	82.17%	<b>-26.8%</b>
After-massage	HF	0.1117	182.38	512.33	53.42	37.43	4.01	0.448	51.92	1.99	0.2432
	LF	0.3472	294.59	363.06	32.71	234.95	16.6	3.42	100.1	9.56	2.18
	HF+LF	0.46	476.97	875.39	86.12	272.38	20.61	3.87	152.02	11.55	2.43
	LF/HF	3.1	1.62	0.71	0.61	6.28	4.14	7.64	1.92	4.8	8.98
	Percent change of HF	160.46%	176.22%	53.1%	106.52%	-10.98%	-56.28%	-57.29%	-82.02%	-24.02%	7.91%
	Percent change of LF	32.82%	75.51%	81.99%	60.75%	30.56%	-39.72%	-44.86%	-79.24%	20.04%	-9.06%

We found an interesting result that four severe ill patients with acute leukemia, acute renal failure or sepsis shock all obtained positive effects from massage. It was probably that massage was more effective on increasing very low HRV. This might be because the patients with very severe condition concentrated on their critical illness and the huge pain from illness; the soft touch of massage on their body was a comfort and relaxation even though there was a certain pressure from massage. Moreover, it was shown that leukemia could cause ANS dysfunction (measured by HRV) (Nevruz & Yokusoglu, 2007) because of paraneoplastic neuropathy (Corbo & Balmaceda, 2001), anemia and chemotherapy-induced side effect (Nevruz & Yokusoglu, 2007). Massage therapy was reported to be able to decrease the risk of cardiovascular complications (Wesa & Cassileth, 2009), improve paraneoplastic autonomic neuropathy and decrease SNS thereby decreasing the risk of disseminated intravascular coagulopathy (DIC) (von Känel & Dimsdale, 2000) and relieve the side effect of chemotherapy (Wesa & Cassileth, 2009). Based on this, future studies are suggested to provide insight on leukemia and ANS research.

#### ***7.2.1.2 HRV for Multiple Sessions of Massage***

The HF changes over the course of six massage sessions were summarized in tables 7-3. The effects observed on improving HRV peaked in the initial sessions of massage procedure. We found the first three sessions of massage induced an impressive and persistent increase in HF. The effects remained or decreased slightly in the subsequent sessions, but were still higher than the effect of the first session and initial baseline. Such results showed that multiple sessions of massage extended effects on improving PNS function. Our results were consistent with one previous study that supported repeated massage intervention (three 15-min per day for 5 days) to

elicit increased PNS function (consistent short-term increases in vagal activity and gastric motility on both the first and the last days of the 5-day study that associated with weight gain,  $p < 0.05$ ) in preterm infants (Diego et al., 2007). We considered that the level off of HF from the fourth session to the last session was difficult to interpret. Hypotheses included the saturation of skin and muscle receptors, thereby making massage be not transmitted to the brain; the boredom and tiredness of patients receiving long time reproducible manipulation, thereby the initial positive massage was transformed to a neutral one; a possible confounding effect of nycthemeral rhythms of plasma hormone and cytokines, cannot be excluded which may affect the massage impact at different times of the day. Taking account both the physiological and psychological factors that influenced massage's effect, we raised a number of important considerations regarding the duration of massage session (30 minutes) and the optimal number of sessions (probably three sessions). Finally, these results deserved cautions interpretation due to the small sample size ( $n=7$ ) involved in the multiple sessions group.

Demonstrating similarities to HF, LF for patients who completed 6 sessions of massage increased during the initial two sessions and remained stable in the subsequent sessions (Table 7-3 and chapter 6.1.2). Together with the increased HF, the increases in LF showed that massage improved HRV in ICU patients, which had been found to be associated with better health conditions. Our results were consistent with the study conducted by J Delaney in 2002 showing that both experimental and control groups had increased LF after a short-term myofascial trigger point massage therapy (Delaney et al., 2002) and another study in 2009 showing that both LF and HF components were increased by pressure stimulus on trigger points (massotherapy) in leg muscles (Takamoto et al., 2009). Also, Some ICU studies showed that low variability was

associated with deterioration, while increased variability of heart rate (HF and LF) was associated with recuperation (Ellenby et al., 2001; Norris et al., 2006), as stated in chapter 2.

**Table 7-3: HF and LF changes over six massage sessions**

		1 <sup>st</sup> massage	2 <sup>nd</sup> massage	3 <sup>rd</sup> massage	4 <sup>th</sup> massage	5 <sup>th</sup> massage	6 <sup>th</sup> massage
HF	logHF at baseline (mean+/-SD)	0.63+/-3.11	1.48+/-2.66	1.71+/-3.08	1.19+/-3.37	1.31+/-3.08	0.84+/-3.34
	logHF during massage (mean+/-SD)	1.51+/-3.25	2.21+/-3.17	1.7+/-2.98	1.67+/-3.37	1.59+/-3.38	1.59+/-3.21
	logHF after massage (mean+/-SD)	1.15+/-3.06	1.8+/-3.01	1.64+/-3.48	1.11+/-3.08	1.04+/-2.88	1.01+/-3.06
LF	logLF at baseline (mean+/- SD)	1.24+/-3.28	1.83+/-3.02	2.46+/-3.27	2.06+/-3.38	2.04+/-2.52	1.79+/-3.44
	logLF during massage (mean+/-SD)	1.84+/-3.32	2.39+/-3.34	2.1+/-3.11	2.33+/-3.26	2.32+/-2.88	2.16+/-3.16
	logLF after massage (mean+/-SD)	1.43+/-3.41	2.39+/-3.2	2.23+/-3.28	1.94+/-2.87	2.14+/-2.69	1.71+/-3.01

## **7.2.2 The Statistical Significance, Practical Significance and Clinical Significance**

Our pilot study' aims were to estimate the feasibility and potential impacts of conducting a massage study in ICU. Statistical significance is defined as a statistical assessment of whether observations reflect a pattern rather than just chance (Pagano & Gauvreau, 2000). It only demonstrates the theoretical meaning in statistics, limited by small sample size. Statistical significance does not imply clinical or practical relevance (Pagano & Gauvreau, 2000). The practical relevance, which is defined as whether the detected statistical significance is valuable in practical sense (Pagano & Gauvreau, 2000), can be investigated in future larger-scale confirmatory studies.

Clinical relevance is related to the importance of the effect with regard to possible clinical impacts. HRV, as a surrogate, is associated with health improvement or deterioration, but the relationship between changes in HRV and changes in health outcomes are not clear. In our study, the massage elicited the effect on increasing HF and LF during its administration; and the six sessions further increased and sustained this positive HRV changes. We could thus consider this increase of HF as a positive sign in favor of better health; and the multiple sessions had a greater effect than the single session. However, our intervention was too short to be able to assess a change in clinical outcomes, such as disease improvement, days of recovery, duration of stay in the PICU and etc. We could not then make a precise link between “increase of HF” and “improvement of patients”. The HRV is just an index of physiology, rather than the clinical endpoints. But its variation is in the good direction that is associated with improved clinical outcomes. Given that (1) most patients in the ICU had eventually a good prognosis; and (2) patients had a large range of medical conditions and stress related factors, future designs of

massage in PICU will need to consider more clinical issues and aspects (see section 7.5 “Future direction”).

### **7.3 HRV Magnitude and Clinical Status/Severity**

We found a higher order of magnitude of HRV (HF+LF) corresponding to lower PELOD scores (see table 6-5) which indicated better clinical conditions. This finding was supported by studies showing the predictive values of HRV in ICU patients (Ellenby et al., 2001; P J Godin & Buchman, 1996). One subject with febrile seizures had a different reaction: she did not have a severe condition (PELOD score=0), but had very low HRV ( $2\text{msec}^2/\text{hz}$ ) (described in chapter 7.2.1). We considered this particular profile might be due to the huge stress for the brain related to febrile seizures; and such stress induced nervous system injury, for example, lose consciousness (Sillanpää & Shinnar, 2010) that contributed to the dysfunction of ANS and low modulation of ANS on heart rate.

### **7.4 Unique Aspects and Limitations of the Study**

#### **7.4.1 Unique Aspects of Study**

Our study comprised a number of unique aspects with regard to: (1) the type of intervention (F&H massage therapy); (2) the intervention duration (long-term 30 minutes) and (3) the intervention frequency (6 sessions per 24 hours). Rare studies applied F&H massage techniques in research although other local massage or whole body massage were tried (Delaney et al., 2002;

Diego et al., 2005; T. Field et al., 2002; Hayes & Cox, 1999; Ironson et al., 1996). F&H massage is the most convenient to be administrated to ICU patients as some lines and therapeutic machines could remain on their heads and bodies. Previous studies just administrated single massage intervention (at most 30 min) or multiple sessions (at most 3 sessions) with short duration per session (15 min) (Diego et al., 2007, 2004; Takamoto et al., 2009). We designed such intervention duration and frequency with the aim of measuring the change of massage effect in a certain long time, thus improving understanding of massage and ANS study and development of future research plan.

Our second clinical objective to assess the relationship between HRV magnitude and PELOD scores (clinical status) was unprecedented. The finding of a positive relationship between them provided an interesting research perspective that may deserve further studies.

At last, as a pilot study, the problems we encountered in the study process and the corresponding solutions offered valuable information to further develop research in this area. All of the knowledge could be directly applicable for the development of ANS, HRV and massage studies.

#### **7.4.2 Limitations**

- Despite strict standard rules for the practice of massage sessions, we recognized that the therapist who administered the massage intervention may influence the massage effect, either due to the difference in technical skills or to personal interaction with the child. This concern was one key challenge of all manual interventions. This problem was

particularly important for all patients' reported outcomes (PRO). However, our primary outcome, ANS functioning and the balance between sympathetic and parasympathetic, was objectively assessed and less likely influenced by subjects' performance as stated above.

- Besides, due to several problems, we obtained only 7 patients with a completed total six sessions of massage. Such small sample size was certainly a limitation for the analysis and interpretation of the results for multiple sessions.
- We did not correlate the changes in HRV with the changes in clinical outcomes (see section 7.2.2).

## **7.5 Future Directions**

From demonstrating the effects of massage on ANS in this pilot study, future studies should enlarge the sample size. A broad recruitment for all eligible PICU patients will be carried out to assess the massage effect on reducing stress by increasing the PNS and decreasing the SNS activities. We will conduct a randomized control trial (RCT). The recruited ICU subjects will be randomly allocated to two groups: (a) three sessions of massage per day (b) no massage, through ANS profiles (health conditions). We will explore long-term effect of massage and assess clinical outcomes, such as disease improvement, days of recovery and duration of stay in ICU, on the subjects. We will also take into account the emotional influences on ANS, conducting a comprehensive analysis in future study.

## CHAPTER 8: CONCLUSION

This study provided the first evaluation of the effectiveness of long-duration and multiple sessions of massage on the ANS in PICU patients. The findings also estimated the feasibility of conducting a larger study.

We showed significant increases in both HF and LF (HF+LF) values which reflected the increased PNS activity and decreased SNS activity by 30-minute single session of massage therapy. With regard to the multiple sessions, the optimal number of massage sessions seemed to be three times per 24 hours. HF gradually increased during the initial three sessions. LF was also shown to increase during the initial two or three sessions. The LF/HF decreased in the high ratio, increased in the low ratio and remained stable when the ratio was originally in the normal range (pediatric standard:  $0.73 \pm 0.08 \sim 2.43 \pm 0.88$ ) (Finley & Nugent, 1995; Kazuma et al., 2002; Zhang, 2007). However, we also observed several subjects whose HF and/or LF were not improved by massage therapy. We then considered the massage therapy might be a stress for some patients in some certain conditions, such as when they felt painful, wanted to sleep, did not want to be touched or resisted massage.

At last, this pilot study exhibited a series of problems encountered in the study process, which provided valuable first-hand information for future studies. In the future, a RCT for three sessions' massage versus no massage will be conducted, with exploring long-term effects of massage and clinical outcomes on patients. All the knowledge offered in this study can be

directly applicable for the development of ANS and HRV studies, and offer further insight on effects of longer-duration and multiple sessions of massage therapy on critical ill patients.

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## APPENDICES

### Appendix A: Diagnosis and Pathophysiology of SIRS, Sepsis, Septic Shock and MODS

SIRS<sup>a</sup>: The presence of at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count:

- Core<sup>b</sup> temperature of >38.5 C or <36 C.
- Tachycardia, defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli; or otherwise unexplained persistent elevation over a 0.5- to 4-hr time period OR for children <1yr old: bradycardia, defined as a mean heart rate <10<sup>th</sup> percentile for age in the absence of external vagal stimulus, β-blocker drugs, or congenital heart disease; or otherwise unexplained persistent depression over a 0.5-hr time period.
- Mean respiratory rate >2 SD above normal for age or mechanical ventilation for and acute process not related to underlying neuromuscular disease or the receipt of general anesthesia.
- Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leucopenia) or >10% immature neutrophils.

Infection: A suspected or proven (by positive culture, tissue stain, or polymerase chain reaction test) infection caused by any pathogen OR a clinical syndrome associated with a high probability of infection. Evidence of infection includes positive findings on clinical exam, imaging, or laboratory tests (e.g. white blood cells in a normally sterile body fluid, perforated viscus, chest radiograph consistent with pneumonia, petechial or purpuric rash, or purpura fulminans).

Sepsis: SIRS in the presence of, or as a result of suspected or proven infection.

Severe sepsis: Sepsis plus one of the following: cardiovascular organ dysfunction OR acute respiratory distress syndrome OR two or more other organ dysfunctions. Organ dysfunctions are defined in Table 1.

#### Septic shock

- Age-specific ranges for physiological and laboratory variables are defined in Table 1
- Core temperature must be measured by rectal, bladder, oral, or central catheter probe.

**Table 1**-Age-specific vital signs and laboratory variables (lower values for heart rate, leukocyte count, and systolic blood pressure are for the 5<sup>th</sup> and upper values for heart rate, respiration rate, or leukocyte count for the 95<sup>th</sup> percentile)

Age Group	Heart Rate (beats/min)		Respiratory Rate, Breath/min <sup>a</sup>	Leukocyte Count Leukocytes 10 <sup>3</sup> /mm <sup>3</sup>	Systolic Blood Pressure, mm Hg
	Tachycardia	Bradycardia			
					X

0 days to 1 week	>180	<100	>50	>34	<65
1 week to 1- mon	>180	<100	>40	>19.5 or <5	<75
1-mon to 1 year	>180	<90	>34	>17.5 or <5	<100
2-5 years	>140	N/A	>22	>15.5 or <5	<94
6-12 years	>130	N/A	>18	>13.5 or <4.5	<105
13 to <18 years	>110	N/A	>14	>11 or <4.5	<117

N/A, not applicable

**Table 2-Organ dysfunction criteria**

Cardiovascular dysfunction: Despite administration of isotonic intravenous fluid bolus  $\geq$  40ml/kg in 1 hour

- Decrease in BP (hypotension)  $<5^{\text{th}}$  percentile for age or systolic BP  $<2$  SD below normal for age<sup>a</sup>, or
- Need for vasoactive drug to maintain BP in normal range (dopamine  $>5$   $\mu\text{g}/\text{kg}/\text{min}$  or dobutamine, epinephrine or norepinephrine at any dose), or
- Two of the following
- Unexplained metabolic acidosis: base deficit  $>5.0\text{mEq}/\text{L}$
- Increased arterial lactate  $>2$  times upper limit of normal
- Oliguria: urine output  $<0.5$  mL/kg/hr
- Prolonged capillary refill:  $>5$ secs
- Core to peripheral temperature gap  $>3$  °C

Respiratory<sup>b</sup>

- $\text{PaO}_2/\text{FIO}_2 <300$  in absence of cyanotic heart disease or preexisting lung disease, or
- $\text{PaCO}_2 >65$ torr or 20 mm Hg over baseline  $\text{PaCO}_2$ , or
- Proven need<sup>c</sup> or  $>50\%$   $\text{FIO}_2$  to maintain saturation  $\geq 92\%$ , or
- Need for nonelective invasive or noninvasive mechanical ventilation<sup>d</sup>

Neurologic

- Glasgow Coma Score  $\leq 11(57)$ , or
- Acute change in mental status with a decrease in Glasgow Coma Score  $\geq 3$  points from abnormal baseline

Hematologic

- Platelet count  $<80,000/\text{mm}^3$  or a decline of 50% in platelet count from highest value recorded over the past 3 days (for chronic hematology/oncology patients), or
- International normalized ratio  $>2$

Renal

- Serum creatinine  $\geq 2$  times upper limit of normal for age or 2-fold increase in baseline creatinine

Hepatic

- Total bilirubin  $\geq 4$  mg/dl (not applicable for newborn), or
- ALT 2 times upper limit of normal for age

*BP, blood pressure; ALT, alanine transaminase; <sup>a</sup> see Table 1; <sup>b</sup> acute respiratory distress syndrome must include a  $PaO_2/FIO_2$  ratio  $\leq 200$  mm Hg, bilateral infiltrates, acute onset, and no evidence of left heart failure. Acute lung injury is defined identically except the  $PaO_2/FIO_2$  ratio must be  $\leq 300$  mm Hg; <sup>c</sup> proven need assumes oxygen requirement was tested by decreasing flow with subsequent increase in flow if required; <sup>d</sup> in postoperative patients, this requirement can be met if the patient has developed an acute inflammatory or infectious process in the lungs that prevents him or her from being extubated*

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**Table 3**-Criteria for diagnosis of septic shock

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1. Suspected infection manifested by
    - Hypothermia (temperature,  $\leq 36.0$  °C) or
    - Fever (temperature,  $\geq 38.0$  °C)
  2. Clinical signs of altered perfusion (one of the following)
    - Depressed mental status
    - Capillary refill  $>2$ secs
    - Flash capillary refill
    - Decreased pulses
    - Bounding pulses
    - Mottled, cool extremities
    - Decreased urine output ( $<1$  mL/kg/hr)
  3. Hypotension
    - Infants (birth -11 months): Systolic BP,  $\leq 65$  mm Hg
    - Children (12 mos-12yrs): Systolic BP,  $\leq 75$  mm Hg
    - Adolescents (13yrs-21yrs): Systolic BP,  $\leq 85$  mm Hg
- 

**Table 4**-Criteria for diagnosis of multiple organ system failure

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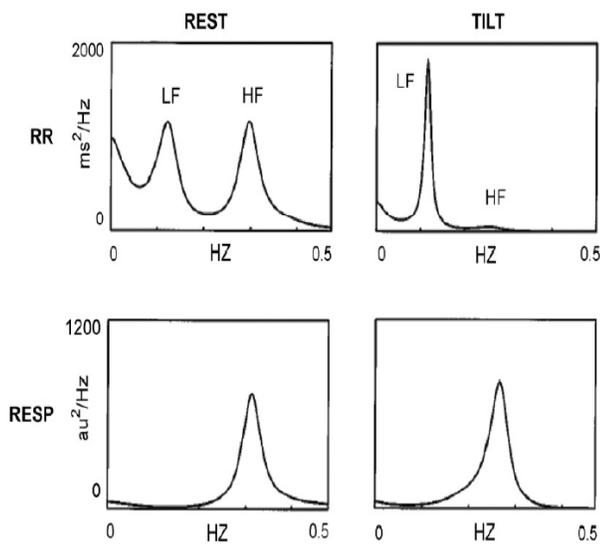
1. Cardiovascular
  - Systolic blood pressure, mm Hg
    - $\leq 65$  in infants
    - $\leq 75$  in children or
    - $\leq 85$  in adolescents
  - Heart rate (beats/min)
    - $<50$  or  $>220$  in infants
    - $<40$  or  $>200$  in children

- Continuous infusion of inotropic agents
  - Serum pH <7.20 (with a normal PaCO<sub>2</sub>)
2. Respiratory
- Respiratory rate (breaths/min)
    - >90 in infants or
    - >70 in children
  - PaO<sub>2</sub>/FIO<sub>2</sub> <200 (in the absence of congenital heart disease)
  - Mechanical ventilation (>24hrs in a postoperative patient)
  - PaCO<sub>2</sub> >65 torr
  - PaO<sub>2</sub> <40 torr (in the absence of congenital heart disease)
3. Neurologic
- Glasgow Coma Scale score of <5
  - Fixed and dilated pupils
4. Hematologic
- Hemoglobin, <5 g/dL
  - White blood cell count, <3000/ mm<sup>3</sup>
  - Platelet count, <20,000/ mm<sup>3</sup>
  - Prothrombin time, >20 sec or Activated partial thromboplastin time, >60 sec
5. Renal
- Blood urea nitrogen >100 mg/dL
  - Creatinine, >2.0 mg/dL (in the absence of preexisting renal disease)
  - Dialysis
6. Gastrointestinal
- Blood transfusion >2- mL/kg in 24 hrs because of hemorrhage
7. Hepatic
- Total bilirubin >5 mg/dL and aspartate or lactate dehydrogenase greater than twice normal (without evidence of hemolysis)
-

## Appendix B: Heart Rate Variability Measurement and Normal Values

### Heart Rate Variability to assess ANS function

The neural regulation of circulatory function is mainly affected through the interaction of the sympathetic and vagal outflows. In most physiological conditions, the activation of either sympathetic or vagal outflow is accompanied by the inhibition of the other suggesting the concept of sympathovagal balance. This interaction can be explored by assessing cardiovascular rhythm with appropriate spectral methodologies. Spectral analysis of cardiovascular signal variability and in particular of RR period (heart rate variability, HRV) is considered precise and convenient procedure to investigate the status of autonomic nervous system, and/or target function impairment. In the frequency domain, the oscillatory pattern that characterizes the



spectral profile of heart rate variability consists of two major components, at low (LF, 0.04–0.15 Hz) and high (HF, synchronous with respiratory rate) frequency respectively, related to vasomotor and respiratory activity. In Figure 3 below (from Neuroscience and Biobehavioral Reviews (Montano et al, 2009), the respiratory rhythm of heart period variability, defined as HF spectral component, is a marker of vagal modulation (Akselrod et al, 1981; Pagani et al, 1986; Montano et al, 1994); the rhythm defined as LF, present in RR and systolic arterial pressure (SAP) variabilities and corresponding to vasomotor waves is a marker of sympathetic modulation; in physiological conditions, a reciprocal relation exists between the relative amplitude of these two

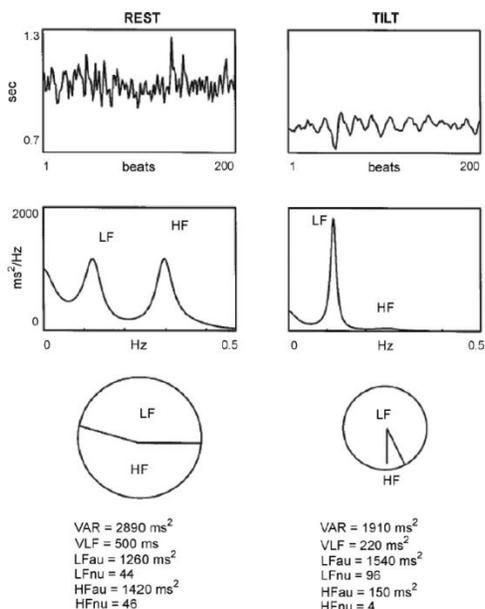
rhythms that is similar to that characterizing the sympathovagal balance (Malliani 2000). Figure 3 also shows that at supine rest, there is evident synchronization between high frequency (HF) components of RR and respiration rate variability. Stress stimulation (standing up) is associated with an increase in LF and decrease in HF component of HRV.

The dynamic assessment of the autonomic changes may provide important diagnostic, therapeutic and prognostic information, not only in relation to cardiovascular, but also non-cardiovascular diseases. There are two methods of measuring heart rate variability in patients: time domain measures and frequency domain methods. Each method requires different recording techniques and statistical analytical approach. The time domain analysis, initially based on simple statistics, such as standard deviation (SD) of RR interval variation, does not provide information on the time structure or periodicity of data. Conversely, with the frequency domain analysis, the signal series can be represented by the sum of sinusoidal components of different amplitude, frequency and phase values. In this study we will focus on frequency domain measurements of HRV.

The spectral profile of human HRV contains three components, with frequencies at rest centered at 0.00 Hz (VLF=very low frequency), 0.10 Hz (LF=low frequency), and around the respiratory

rate (HF=high frequency), respectively. The amplitude of LF and HF components is assessed by the area (i.e. power) of each component. Square units are used for its absolute value

This following figure is adopted from Neuroscience and Biobehavioral Reviews (Montano et al, 2009). It shows spectral analysis of HRV in young subject at rest and during 90 ° tilt.



The RR interval time series (tachograms) are illustrated in the top panels. The middle panels contain the autospectra which indicate the presence of two major components (LF=low frequency; HF=high frequency). During tilt, the LF component becomes largely predominant. In this example the total power (variance) is markedly reduced during tilt and consequently LF and HF powers are both decreased when expressed in absolute units (LFau, Hfau). The use of normalized units (nu) indicates the altered relation between LF and HF during tilt. Pie charts show the relative distribution together with the absolute power of the two components represented by the area. VAR=variance; VLF=very low frequency.

#### Time domain measures

The simplest method to perform is the time domain measure. With this method, either heart rate at any point in time or the intervals between successive normal complexes are determined. Normal-to-normal intervals are detected on the continuous electrocardiographic (ECG) record. Normal-to-normal (NN) intervals are intervals between adjacent QRS complexes resulting from sinus node depolarizations. Also, the instantaneous heart rate can be determined by this method. Time-domain variables can be calculated include the mean NN interval, the mean heart rate, the difference between the longest and the shortest NN interval, the difference between night and day heart rate, and others. The variations in instantaneous heart rate secondary to respiration, tilt, Valsalva manoeuvre, can be also used.

#### Statistical measures

Statistical time-domain measures can be calculated from the series of instantaneous heart rate or cycle intervals recorded over 24 hours. There are two classes of measures:

- those derived from direct measurements of NN intervals or instantaneous heart rate;
- those derived from the differences between NN intervals.

These variables may be calculated from the analysis of smaller segments of ECG or derived from the total electrocardiographic recording (24 hours).

The variables that will be calculated:

*SDNN* [ms] is the standard deviation of the Normal-to-Normal (NN) interval. In many cases SDNN is calculated over 24-hours period. SDNN reflects all the cyclic components responsible for variability in the period of recording. In practice it is inappropriate to compare the SDNN measures obtained from recordings of durations because its value depends on the length of recording period. Thus the durations of ECG recordings used to determine SDNN should be standardized.

*SDANN* [ms] is the standard deviation of the average NN interval calculated over short periods (usually 5 min). It estimates the changes in heart rate due to cycles longer than 5 min and the *SDNN* index, the mean of the 5-min standard deviation of the NN interval calculated over 24h, which measures the HR variability due to cycles shorter than 5 min.

Other commonly used measurements of short-term variation that estimate high-frequency variations rate (and also highly correlated): *RMSSD* [ms] (the square root of the mean squared differences of successive NN intervals), *NN50* (the number of interval differences of successive NN intervals > 50ms, and *pNN50* [%] the proportion derived by dividing *NN50* by the total number of NN intervals.

### Frequency domain methods

Spectral components: Short-term recordings: three main spectral components are distinguished in a spectrum calculated from short-term recordings of 2 to 5 minutes: very low frequency (VLF), low frequency (LF) and high frequency (HF) components. The distribution of the power and the central frequency of LF and HF may vary relatively to the changes in autonomic modulations of the heart period. VLF, on the other hand, is much less defined. The existence of the specific physiological process which is responsible for these heart period changes is not clear. LF and HF could be measured in absolute values of power ( $ms^2$ ) or in normalized units (n.u.). Long-term recordings may also be used to analyze the sequence of NN intervals in the 24h period. In these recordings, an ultra-low frequency component (ULF) is included in addition to VLF, LF and HF.

### Normal Values of HRV (Kazuma et al., 2002)

**Table 2.** Effect of Age on Heart Rate Variability

Age	Lower Classes (n = 18) 6.3 ± 0.5 Years	Third and Fourth Grades (n = 14) 8.7 ± 0.5 Years	Higher Classes (n = 38) 10.9 ± 0.9 Years	P < 0.001
TF ( $ms^2$ )				
24 h	6372 ± 3999	7974 ± 3155	9774 ± 4192	0.01
Wake time	4664 ± 3575	6622 ± 2817	8524 ± 4470	0.004
Sleep time	8329 ± 5484	9930 ± 3633	11510 ± 4695	0.06
LF ( $ms^2$ )				
24 h	820 ± 438	1062 ± 397	1360 ± 624	0.003
Wake time	608 ± 342	871 ± 360	1142 ± 623	0.001 <sup>k</sup>
Sleep time	1151 ± 660	1338 ± 526	1665 ± 763	0.03
HF ( $ms^2$ )				
24 h	863 ± 880	777 ± 514	737 ± 465	0.81 <sup>k</sup>
Wake time	398 ± 377	469 ± 275	541 ± 332	0.08 <sup>k</sup>
Sleep time	1610 ± 1768	1176 ± 900	1028 ± 965	0.46 <sup>k</sup>
LF/HF				
24 h	1.37 ± 0.64	1.71 ± 0.86	2.34 ± 1.10	0.002
Wake time	2.15 ± 1.09	2.08 ± 0.75	2.43 ± 0.88	0.36
Sleep time	1.08 ± 0.54	1.65 ± 1.07	2.37 ± 1.34	0.002 <sup>k</sup>
NNA (ms)				
24 h	661 ± 90	707 ± 73	756 ± 79	0.0004

<sup>k</sup>: Kruskal-Wallis test.

## Appendix C: Consent Form



### Subject Information and Consent Form

#### Massage and Autonomic Nervous System in Patients in Pediatric Intensive Care Units: A Pilot Prospective Study

Principal Investigators: Dr. Jean Paul Collet, MD, PhD  
Professor, Department of Pediatrics, UBC  
Associate Director, Partnership Development, CFRI  
(604) 875 3130

Study Team: Dr. Tex Kissoon,  
Dr. Mark Ansermino,  
Dr. Pascal Lavoie,  
Dr. Peter Skippen,  
Rosella Jefferson.

For questions regarding the consent, please call the study coordinator:  
(604) 875 2000 ext 5322

In this form “You” refers to You/Your child/Your ward

## 1. INTRODUCTION

You are invited to take part in this research study because you are an Intensive Care Unit (ICU) patient aged between 28 days and 18 years old in stable condition with systemic inflammatory response syndrome (SIRS).

## 2. YOUR PARTICIPATION IS VOLUNTARY

Your participation is entirely voluntary, it is up to you to decide whether or not to participate in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

If you decide that you can participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you do not have to provide any reason for your decision not to participate nor will you lose the benefit of any medical care to which you are entitled or are presently receiving.

Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

## 3. WHO IS CONDUCTING THE STUDY?

The study is being conducted by the staff of Pediatric Intensive Care Department (Dr. Niranjana Kisson) and Department of Pediatrics at BC Children’s Hospital (Dr. Jean-Paul Collet). The investigators will not be paid for conducting this study.

## 4. BACKGROUND

### *4.1 What is Systemic Inflammatory Response Syndrome (SIRS)?*

Systemic Inflammatory Response Syndrome (SIRS) is an enormous inflammatory response of the human body to the infection or massive tissue damage (for instance, large burn injury). SIRS is a life-threatening condition, and if not treated, progresses to sepsis, shock, and disturbance in organs function.

### *4.2 How does SIRS begin?*

In response to a microbial invasion or injury, the immune system produces special cells (from white blood cells) and substances (like cytokines) that help to fight the infection or heal the wound. When the infection or injury is small (for instance when you cut the finger), it is easy for the body to localize the source of the infection or damaged cells and quickly heal it with help of immune cells, cytokines and other specific substances. The finger cut is usually easily healed. But sometimes, when your defense system (immune system) is weak or the infection/injury is

massive, the body cannot properly fight the infection and systemic (spread) inflammation can occur.

#### *4.3 What are cytokines?*

Cytokines are chemical substances that help body cells to communicate with each other. Many cytokines and their functions are known, but perhaps more are undiscovered yet. Some cytokines such as TNF, IL-1 and others promote the inflammation, by stimulation of the production of other inflammatory cytokines and substances. Other cytokines (like IL-10) work as anti-inflammatory agents. Cytokines are produced by immune cells in the presence of infection.

#### *4.4 What happens when the bacteria enters the body?*

Our immune system is finely tuned to fight the infection. It works very well when the infection is mild and isolated on the site (like a sore throat). When the bacteria enter the body, the immune system recognizes the bacteria as an “enemy” and activates cells and cytokines to fight the infection. The cytokines help cells to communicate with each other. As long as cytokines production remains confined to the site of infection, the inflammation response is beneficial for the organism. Unfortunately, the production of the cytokines and other cells are not always beneficial. Not only bacteria can cause the systemic inflammation in the body, but different bacterial and non-bacterial toxic substances can cause it as well.

#### *4.5 What happens when the infection or toxins spreads throughout the organism?*

During sepsis the inflammation occurs in many places in the body at the same time. The immune system produces an *enormous* amount of immune cells and other related substances of inflammation rapidly to fight the infection or to eliminate the toxins. Although this overwhelming production of cytokines and other inflammatory substances is defensive against infection, it could be harmful, sometimes even fatal for the body.

#### *4.6 What regulates the immune system?*

The regulation of the immune system and immune response in sepsis is very complicated and some aspects are unknown yet. It was suggested that the immune system is closely connected with the autonomic nervous system (ANS). This nervous system is called “autonomic” because it does not greatly depend on the consciousness. The main responsibility of the autonomic nervous system is to coordinate the function of the body organs. For instance, you do not think how to contract your heart muscle in order to pump your blood - the autonomic nervous system will coordinate this for you. The autonomic nervous system responsible for many functions in the body and the coordination of the immune response could be one of them. During SIRS, the autonomic nervous system does not work properly. There is also some evidence that the stimulation of the autonomic nervous system (ANS) can reduce the overwhelming inflammation.

#### *4.7 Can massage help in the situation of systemic inflammation?*

Patients in ICU are under huge stress as shown by increased heart rate (HR), high blood pressure (BP), increased hormones of stress, poor sleep quality and emotional distress. Many patients also lose control of the inflammatory response. Recent studies documented evidence showing that massage could stimulate the autonomic nervous system and generate anti-stress response. We therefore propose to conduct a small study in ICU with children who have SIRS, to assess

whether foot and hand massage, apart from relaxing effects, may also decrease inflammatory reaction. The study is designed to establish massage as complementary therapy in ICU.

#### 5. WHAT IS THE PURPOSE OF THE STUDY?

The primary objective of this study is to assess the effect of foot and hand (F&H) massage on the autonomic nervous system. Secondary objectives are to document the effect of massage on (a) decreasing the level of inflammation (b) decreasing the use of medications that help to control blood pressure and (c) decreasing stress. The study may also generate useful information for a large study in the future.

#### 6. WHO CAN PARTICIPATE IN THE STUDY?

If you are:

Greater than 28 days, - less or equal 18 years of age;

Diagnosed with systemic inflammatory response syndrome (SIRS);

Your condition is stable. Stable condition is defined as no change in use of inotropic agents (drugs that change the force of heart contractions) in the last 6 hours

You understand the study and can sign the informed consent.

#### 7. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You should not participate if:

You had severe immunodeficiency before the onset of sepsis (inherited or acquired immunodeficiency disease and/or condition);

You have been taking immunosuppressant medications before the onset of sepsis;

You have another serious chronic condition that may affect the outcomes independently of sepsis;

You do not speak English, and cannot provide the interpreter.

#### 8. WHAT DOES THE STUDY INVOLVE?

This study of 22 subjects who will be randomized to 2 groups of different massage frequencies for two consecutive days. Massage will be hand and foot massage administered at three frequency levels: once per 24 hours, and 6 times per 24 hours over a period of two days. Each massage session will last for 30 min. It will be conducted by Registered Massage Therapists to assure continuity of care. Relevant data including diagnosis, heart rate, heart rate variability, blood pressure, and drugs administered will be abstracted from the medical charts.

ECG (Electrocardiograph) recordings will be acquired from the central ICU station. Blood test for inflammatory response will also be performed using the blood leftovers from the ICU routine tests. No extra blood will be collected for this test.

#### 9. STUDY DURATION

We will keep a very small amount of blood from one of your first laboratory tests before you sign the informed consent because we need to assess the cytokine level at the beginning of your stay in ICU. If you do not want to participate in the study, this sample will be destroyed. Once you sign the informed consent, we start the follow-up (to assess the cytokines level and monitoring blood pressure/heart beat). If your condition is stable we will perform the massage for 2 days. The study follow-up will stop when you are discharged or transferred from BC Children's hospital Pediatric Intensive Care Unit.

**10. WHAT ARE MY RESPONSIBILITIES?**

If you decide to participate, you will be randomly allocated to receive either once per 24 hours, or 6 times per 24 hours hand and foot massage over a period of two days. Each massage session will last for 30 min.

If you feel uncomfortable or want to interrupt for any reason, the massage therapy will stop. Your situation (sleep, feeling tired) will be respected and the massage therapy will be adjusted according to your situation.

**11. WHAT ARE THE POSSIBLE HARMS AND SIDE EFFECTS OF PARTICIPATING?**

There are no possible harms or side effects of participating. The study procedure will not involve additional skin puncture, or any other invasive procedures. Massage is a safe intervention and the massage therapist and nurses are well-trained and experienced.

**12. WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?**

You do not need to pay for the massage therapy. There may or may not be direct medical benefit to you for taking part in this research study. We hope the information collected from the study will benefit ICU children in the future.

**13. WHAT IF NEW INFORMATION BECOMES AVAILABLE THAT MAY AFFECT MY DECISION TO PARTICIPATE?**

If new information arises during the research study that may affect your decision to remain in the study, you will be advised of this information.

**14. WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?**

Your participation in this research is entirely voluntary. You may withdraw from this study at any time. If you decide to join the study and decide to withdraw at a later time, there will be no penalty or loss of benefits to which you are entitled and your future medical care will not be affected. You can withdraw without providing any explanation or reasons, but if you decide to stop participating in the study, please contact a study coordinator at (604) 875 2000 ext. 5322

**15. WHAT HAPPENS IF SOMETHING GOES WRONG?**

Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else. In the event you become injured or unexpectedly ill as a result of participating in this study, necessary medical treatment will be available at no additional cost to you.

**16. CAN I BE ASKED TO LEAVE THE STUDY?**

Study doctors and investigators may decide to discontinue the study at any time, or withdraw you from the study at any time without your consent if they feel that it is in your best interests.

**17. AFTER THE STUDY IS FINISHED**

The results of the study may be published in a peer-reviewed medical or scientific journal. Publication will not include personal information or any information that may enable identifying a person.

**18. WHAT WILL THE STUDY COST ME?**

You will not be paid to take part in this study. The study will provide all study interventions at no cost to you as long as you receive treatment in this study.

#### 19. WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records identifying you may be inspected in the presence of the investigator or his designate by representatives of the UBC Research Ethics Boards for the purpose of monitoring the research. However, no records which identify you by any personal identifier will be allowed to leave the hospital.

You do not waive any of your legal rights by signing this consent form.

All information about you will have the name removed and be stored in a locked filing cabinet with regulated access. Electronic data will be regulated according to the strictest of safety procedures. All data about you will be stored for 5 years and will be destroyed after that.

Statistical analysis will be done by researchers directly involved in this study in a way that will not identify you. For scientific communication of the results of the study, the data and statistics will be published on an anonymous basis.

Your right to privacy are legally protected and guaranteed by federal and provincial laws that require safeguards to insure that your privacy is respected. You also have the right to access the information about your health that has been provided to the investigator and, if need be, an opportunity to correct any errors in this information.

#### 20. WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If you have any questions or desire further information about this study before or during participation, you can contact the study coordinator at: (604) 875 2000 ext.5322.

#### 21. WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?

If you have any concerns about your rights as a research subject you may contact the Research Subject Information Line at the UBC Office of Research Services at the University of British Columbia at 604 822-8598; toll free number: 1-877-822-8598 or email to: [RSIL@ors.ubc.ca](mailto:RSIL@ors.ubc.ca)

#### Check List:

*I have read and understood the subject information and consent form.*

*I have had sufficient time to consider the information provided and to ask for advice if necessary.*

*I have had the opportunity to ask questions and have had satisfactory responses to my questions.*

*I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.*

*I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.*

*I understand that I am not waiving any of my legal rights as a result of signing this consent form.  
I understand that this study will provide any personal benefits.  
I have read this form and I freely consent to participate in this study.  
I have been told that I will receive a dated and signed copy of this form.*

**I CONSENT BY SIGNING BELOW TO PARTICIPATE IN THIS STUDY**

Printed name of subject (child) \_\_\_\_\_

Printed name of subject's legally acceptable representative: parent/legal guardian \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

Printed name of principal investigator/designated representative \_\_\_\_\_  
Signature \_\_\_\_\_ Date \_\_\_\_\_

Printed name of translator (if applicable) \_\_\_\_\_ Signature \_\_\_\_\_ Date \_\_\_\_\_

Signed and dated

***SUBJECT'S ASSENT TO PARTICIPATE IN RESEARCH***  
*(Children 14-18 years)*

I have had the opportunity to read this consent form, to ask questions about my participation in this research, and to discuss my participation with my parent(s)/legal guardian(s). All my questions have been answered. I understand that I may withdraw from this research at any time, and that this will not interfere with the availability to me of other health care. I have received a copy of this consent form. I assent to participate in this study.

First Name (Printed) \_\_\_\_\_ Last Name (Printed) \_\_\_\_\_  
Signature \_\_\_\_\_ Date: Month \_\_\_\_\_ Day \_\_\_\_\_ Year \_\_\_\_\_

## Appendix D: Clinical Information Collection Form

### Clinical Information Collection Form

Subject study number: CYTR519 - \_\_\_\_\_

#### 1. Individual Information

Name	
Gender	
Age	
Weight	
Date of Birth	
Admission to PICU (date and time)	
Discharged from PICU(date, time and place)	

#### 2. Clinical Feature

Fever (temperature)		
Infection	Site of infection	
	Pathogenic organism	
Others		

#### 3. Diagnosis

Systemic Inflammatory Response Syndrome	
Sepsis and/or septic shock	
Post-operative care	
Others	

#### 4. Pediatric Logistic Organ Dysfunction Score (PELOD)

					DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10
PELOD Score	0	1	10	20	Maximum score	At randomization / / / 2011	/ / / 2011	/ / / 2011	/ / / 2011	/ / / 2011	/ / / 2011	/ / / 2011	/ / / 2011	/ / / 2011
Respiratory System: PaO <sub>2</sub> mmHg (kPa)/FiO <sub>2</sub> PaCO <sub>2</sub> mmHg (kPa) Mechanical ventilation	> 70 (9.3) and ≤ 90 (11.7) and no ventilation	Ventilation	≤ 70 (9.3) or > 90 (11.7)		10									
Cardiovascular System: Heart rate (rate/min) < 12 yr ≥ 12 yr Systolic BP (mmHg) 1 month 1 month - 1 yr 1 yr. - 12 yr. ≥ 12 yr	≤ 195 ≤ 150 and > 65 > 75 > 85 > 95		> 195 > 150 or 35 – 65 35 – 75 45 – 85 55 – 95	< 35 < 35 < 45 < 55	20									
Neurological System: Glasgow Pupillary reaction	12 – 15 and both reactive	7 – 11	4 – 6 or both fixed	3	20									
Hepatic System: ALT SGOT (UI/L) PT or INR	< 950 and > 60% (10-13s) or < 1.4	≥ 950 or ≤ 60% (10-13s) or ≥ 1.4			1									
Renal System: Creat: μmol/L (mg/dL) < 7 days (7 days – 1 yr.) (1 yr. – 12 Yr.) ≥ 12 yr.	< 140 (<1.59) < 55 (<0.62) < 100 (<1.13) < 140 (<1.59)		≥ 140 (≥1.59) ≥ 55 (≥0.62) ≥ 100 (≥1.13) ≥ 140 (≥1.59)		10									
Hematological System: White blood cell (10 <sup>9</sup> /L) Platelet count (10 <sup>9</sup> /L)	> 4.5 and ≥ 35	1.5 – 4.4 or < 35	< 1.5		10									
TOTAL					71									

### 5. Laboratory Test

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
WBC ( $\times 10^9/L$ )						
Neutrophil Count ( $\times 10^9/L$ )						
Hgb (G/L)						
PLT ( $\times 10^9/L$ )						
K (mmol/L)						
Urea (mmol/L)						
Creatinine ( $\mu\text{mol/L}$ )						
Total Bilirubin ( $\mu\text{mol/L}$ )						
Gluc (mmol/L)						
Serum Osm (mosm/L)						
Urine osmolality (mosm/L)						
Urine Sodium (mm/L)						
AST (U/L)						
ALT (U/L)						
PT (secs)						
PTT (secs)						
INR						

6. Treatment (Day 1, 2, 3, etc.)

Name of drugs	Day 1			
	Time	Dose	Effects	Effect on ANS

6\* Medication List

Subject #	Medication list
P1	Precedex, Chloral hydrate, Tylenol, Ventolin, etc.
P2	Tylenol, Ventolin, Cefotaxime, etc.
P3	Morphine, Midazolam, Fentanyl, Ketamine, Tylenol, etc.
P4	Morphine, Midazolam, Atropine, Ketamine, etc.
P5	Morphine, Acetaminophen, ibuprofen, Cefotaxime, Lasix, etc.
P6	Midazolam, Acetaminophen, Cefotaxime, etc.
P7	Cefotaxime, Ondansetron, Tylenol, Ibuprofen, etc.
P8	Morphine, Midazolam, Esmolol, Labetalol, etc.
P9	Epinephrine, Ventolin, Tylenol, etc.
P10	Morphine, Acetaminophen, Tylenol, Ventolin, Budesonide, etc.
P11	Cefotaxime, Vitamin D, Prevacid, Flovent, etc.
P12	Precedex, morphine, Ondansetron, Midazolam, Tylenol, etc.
P13	Amlodipine, Omeprazole, Ondansetron, Gravol, Erythropoietin alpha, Vancomycin, Lorazepam, Methadone, Vitamin D, etc.
P14	Vancomycin, Tylenol, Ventolin, etc.
P15	Morphine, Midazolam, Tylenol, Vancomycin, Acyclovir, etc.
P16	Tylenol, Omeprazole, Vitamin D, etc.
P17	Epinephrine, Tylenol, Advil, Vancomycin, Cefotaxime, etc.
P18	Tylenol, Cefotaxime, etc.

## 7. Mortality

Alive or dead when discharged	
Cause of death	

## 8. Notes

## **Appendix E: BIOPAC and Acqknowledge Software**

<http://www.biopac.com/>

**Appendix F: Massage Information Collection Form**

PID: CYTR519-

GROUP: 1 massage session

DATE: Day \_\_\_\_\_ Month \_\_\_\_\_ 2011 BED #: \_\_\_\_\_

RMT's NAME \_\_\_\_\_

1<sup>st</sup> DAY: SESSION # 1

Start Time: HH \_\_\_\_\_ MIN \_\_\_\_\_ PM or AM (circle)

End Time: HH \_\_\_\_\_ MIN \_\_\_\_\_ PM or AM (circle)

Session Duration (in minutes) \_\_\_\_\_ MINUTES

Area of the intervention (PLEASE CHECK THE SEQUENTIAL ORDER): PLEASE USE THE TIME SHOWN ON THE BEDSIDE MONITOR (upper line, in the middle)

Extremity/Sequence	#1	#2	#3	#4
Left hand	Start time ___:___	Start time ___:___	Start time ___:___	Start time ___:___
Yes___ No___	End time ___:___	End time ___:___	End time ___:___	End time ___:___
Right hand	Start time ___:___	Start time ___:___	Start time ___:___	Start time ___:___
Yes___ No___	End time ___:___	End time ___:___	End time ___:___	End time ___:___
Left foot	Start time ___:___	Start time ___:___	Start time ___:___	Start time ___:___
Yes___ No___	End time ___:___	End time ___:___	End time ___:___	End time ___:___
Right foot	Start time ___:___	Start time ___:___	Start time ___:___	Start time ___:___
Yes___ No___	End time ___:___	End time ___:___	End time ___:___	End time ___:___

Comments regarding the massage session:

Child:

When starting message	Asleep or awake	
	Mood/emotion: exciting, calm, unhappy, other	
	Crying, restless	
During message	Asleep or awake	
	Reaction to the touch: positive, negative, neutral, other	

period	Mood/emotion: exiting, calm, unhappy, other	
	Crying, restless	
After massage	Asleep or awake	
	Mood/emotion: exiting, calm, unhappy, other	
	Crying, restless	
Other comments		

Patients:

Present/absent during the session	
Positive/negative attitude	
Other	

Other comments:

## Appendix G: Massage Protocol

### MASSAGE THERAPY PROTOCOL--Foot & hand treatment

#### Introduction of therapist's touch to child:

**Introductory touch to head** – rest dominant hand on vertex/top of head for a moment; transition to frontal area/forehead and occiput (anterior/posterior aspects of head) – simultaneous, gentle compression sustained briefly, and released – *5 seconds*

- **Right hand massage**

Introductory touch to right shoulder – similar to head (anterior and posterior aspects of shoulder capsule, firm compression) – *5 seconds*

Firm compressions with mobilizations to the radio-ulnar area (one hand of therapist grasps radial aspect of hand/wrist, other hand of therapist grasps ulnar aspect of hand/wrist; firm compression into the wrist and palm of hand) – *10 seconds*

While holding/stabilizing the dorsal aspect of child's right hand, therapist's dominant hand mobilizes palmar surface with superior/inferior rhythmic movement, from the proximal to distal aspect of the hand – *30 seconds*

#### **Passive range of motion of the thumb**

Stabilizing at radius and carpals, move first metacarpal with gentle oscillations in flexion and extension

Stabilize first metacarpal, move proximal phalange ... in flexion and extension

Stabilize proximal phalange, move distal phalange... in flexion and extension

...work back to 1<sup>st</sup> carpal/MC (MC = metacarpal) joint

-- *60 seconds*

End with compression of saddle joint – *10 seconds*

#### **Metacarpals**

Stabilize third metacarpal, mobilize second in palmar/dorsal direction (oscillate rhythmically); stabilize 2<sup>nd</sup> MC, mobilize 3<sup>rd</sup> MC in palmar/dorsal direction, continue to ulnar aspect of hand, and return (one at a time) to radial aspect of hand with the same movements – *60 seconds*

#### **Each digit**

Stabilize 2<sup>nd</sup> MC, mobilize proximal phalange in flexion & extension (oscillate rhythmically 4x back and forth – each flexion/extension sequence = 1 time) ... continue full length of digit, then return to base of finger – (*60 seconds each digit*)

Firm compression of full palm, with focus on base of digit just mobilized

Continue with digits 3-5

*4 minutes*

Firm compressions with mobilizations to the radio-ulnar area (one hand of therapist grasps radial aspect of hand/wrist, other hand of therapist grasps ulnar aspect of hand/wrist; firm compression into the wrist and palm of hand) – *10 seconds*

“Leave-taking” touch to right shoulder – (anterior and posterior aspects of shoulder capsule, firm compression) – *5 seconds*

- **Left hand massage**

Introductory touch to left shoulder – as previous/above, for right shoulder (anterior and posterior aspects of shoulder capsule, firm compression) – *5 seconds*

**Continue with all steps as described for right hand, above, but apply to left hand, up to and including:**

“Leave-taking” touch to left shoulder – (anterior and posterior aspects of shoulder capsule, firm compression) – *5 seconds*

- **Right foot massage**

Introductory touch to right hip – (anterior and posterior aspects of hip, firm compression; if cannot access, bilateral touch – both hips, *gently* compressing toward the midline) – **5 seconds**

Palmar surface of non-dominant hand supports heel of right foot; dominant hand gently strokes proximal-to-distal from superior to the malleoli (as accessible), along the dorsal surface of the foot, to toes, covering medial, anterior, and lateral aspects – **10 seconds**

While holding/stabilizing the dorsal aspect of child's malleoli with fingertips, therapist's thumbs knead the "ball" of the foot – **30 seconds**

Continue petrissage/alternating thumb kneading from the hallux into the medial arch of the foot, to the heel. Therapist may switch to fingertips to ensure child's foot is not overly dorsi-flexed. Include all surfaces of the heel. With continuous contact, return to ball of foot with alternating thumb/fingertip kneading, along medial arch – **60 seconds**

NB: **Babinski's reflex** is elicited in children <2 years old when the sole of the foot is firmly stroked. Alternating thumb(fingertip) kneading must be into the tissue (*into* the foot) and not resemble stroking, to avoid eliciting this reflexive response.

**Passive range of motion of the hallux**

Stabilizing at navicular & medial cuneiform, move first metatarsal with gentle oscillations in flexion and extension

Stabilize first metatarsal (MT), move proximal phalange ... in flexion and extension

Stabilize proximal phalange, move distal phalange... in flexion and extension

...work back to 1<sup>st</sup> tarsal/MT joint

-- **30 seconds**

End with firm compression into transverse tarsal joint – **10 seconds**

**Metatarsals**

Stabilize third MT, mobilize second in plantar/dorsal direction (oscillate rhythmically); stabilize 2<sup>nd</sup> MT, mobilize 3<sup>rd</sup> MT in plantar/dorsal direction, continue to lateral aspect of foot, and return (one at a time) to medial aspect of foot with the same movements – **60 seconds**

**Each toe**

Stabilize 2<sup>nd</sup> MT, mobilize proximal phalange in flexion & extension (oscillate rhythmically 4x back and forth – each flexion/extension sequence = 1 time) ... continue full length of toe, then return to base of toe – **(20 seconds each toe)**

Firm compression of full plantar aspect of foot, with focus on base of toe just mobilized, followed by deep kneading of toe from its base to its tip, in pincer-grip of therapist's thumb and medial surface of first finger. Make small movements to travel full length of toe (while appreciating that an infant's toes are tiny, intention is specific, deep pressure into full length of the toe) – **(30 seconds each toe)**

Continue with toes 3-5

While holding/stabilizing the dorsal aspect of child's malleoli with fingertips, therapist's thumbs knead the "ball" of the foot – **30 seconds**

"Leave-taking" touch to right hip – (anterior and posterior aspects of hip, firm compression; if cannot access, bilateral touch – both hips, *gently* compressing toward the midline) **5 seconds**

- **Left foot massage**

NB: if "leave-taking" contact at hip was bilateral, continue to left foot. If contact at hip can be unilateral, proceed as follows:

Introductory touch to left hip – (anterior and posterior aspects of hip, firm compression) **5 seconds**

**Continue with all steps as described for right foot massage, above, but apply to left foot, up to and including:**

"Leave-taking" touch to left hip – (anterior and posterior aspects of hip, firm compression; if cannot access, bilateral touch – both hips, *gently* compressing toward the midline) **5 seconds**

- **CLOSURE:**

**“Leave-taking” of therapist’s touch to child:** rest dominant hand on vertex/top of head for a moment; transition to frontal area/forehead and occiput (anterior/posterior aspects of head) – simultaneous, gentle compression sustained briefly, and released – *5 seconds*

- **NOTES:**

Times are given for each “move”, to ensure standardized application of treatments. A baby has smaller hands and feet than a 5-year old child; times may vary as a result. The intention is that treatments are 30 minutes in duration. For a baby or infant, treatment may be shortened to 20 minutes (no less).

Due to the small size of a baby or infant’s feet and hands, it may be challenging for therapists to stabilize and mobilize individual fingers and toes (and metacarpals and metatarsals, as described in the sequences). The therapists’ intention will be to do so; firm/deep pressure is directed into the tissue.

- **GLOSSARY:**

**Firm compression:** compress to tissue resistance

**Mobilisation:** gentle oscillatory to-and-fro movement, in the plane/s specified. Slow, rhythmic motion that impacts proprioceptive sensors.