31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Transfer Function Analysis of Baroreflex Function in a Rabbit Model of Endotoxic Shock

Collin H.H. Tang, Gregory S.H. Chan, Paul M. Middleton, Grant Cave, Martyn Harvey, Andrey V. Savkin, and Nigel H. Lovell

Abstract— **Sepsis is characterized by profound cardiovascular abnormalities which may result from the derangements in the arterial baroreflex system and other autonomic regulatory functions. In this study, a mechanically ventilated and anesthetized rabbit model of endotoxic shock was utilized to mimic the behaviors of the cardiovascular system in a sepsis patient. 13 adult New Zealand white rabbits were studied, with 8 of them injected with endotoxin and the remaining given saline solution as sham fluid. Measurements of heart period (RRi) and systolic blood pressure (SBP) were obtained pre- and post-intervention after a 90 minute period, which allowed spectral and cross-spectral analysis of heart rate and blood pressure variabilities to be performed. A significant increase of blood lactate level ($p < 0.01$) in post-intervention stage signified the onset of distributive shock. Based on this model, the novel findings were that in the low frequency (LF) and mid frequency (MF) bands, there was a decrease in coherence ($p < 0.01$ and $p < 0.05$ respectively) and loss of phase delay ($p < 0.05$) between SBP and RRi, along with a depression in transfer function gain in the LF band ($p < 0.05$), which might indicate an impairment of baroreflex control of heart rate following the administration of endotoxin. None of the above variables changed significantly in the control group. Moreover, endotoxin also led to a decrease in RRi variability in the ventilatory frequency (VF) band, suggesting a suppression of cardiac vagal modulation. These results highlight the potential value of frequency spectrum analysis combined with transfer function analysis of cardiovascular variability in the assessment of autonomic and baroreflex-related changes associated with endotoxic shock.**

I. INTRODUCTION

Sepsis is characterized by profound cardiovascular abnormalities with seriously compromised hemodynamic, metabolic, and immunologic regulatory functions. This is often associated with autonomic cardiovascular dysfunction resulting in refractory systemic hypotension, leading to abnormal blood flow distribution to several vital organs, which may contribute along with other manifestations of sepsis to multiple organ dysfunction and death [1], [2]. Severe infection is believed to be the main cause of distributive shock in sepsis with a relatively insufficient intravascular blood volume secondary to extensive blood vessel dilation. It also results in fluid retention in the interstitial space [3]. This is accompanied by excessive secretion of cytokines and uncontrolled spillover of circulating catecholamines, which further undermines autonomic nervous system (ANS) control of the heart and vessels through the arterial baroreflex [4]-[6]. The baroreflex is important in short-term arterial blood pressure regulation; impairment of this mechanism is often of clinical relevance and assessment of the degree and type of impairment may provide useful diagnostic approaches to acute illness [7], [8]. Marked increases in heart rate, elevated blood lactate level, refractory hypotension and myocardial depression occur frequently in the late phase of sepsis syndrome, potentially demonstrating interference with the arterial baroreflex [5], [9].

Endotoxin or lipopolysaccharide (LPS), a major component of the gram-negative bacterial cell wall, is known to initiate many of the adverse effects of sepsis. To evaluate novel diagnostic approaches to sepsis syndrome, it is necessary to use a model that removes many of the confounding elements that make research in critically ill human patients challenging, to which end animal models of sepsis were evaluated. One approach to experimentally simulate the effect of sepsis and septic shock is via intravascular infusion of endotoxin or live bacteria in animals [10], [11]. Shen et al. evaluated the baroreflex gain in LPS-induced endotoxemia in rats, and highlighted the usefulness of arterial baroreflex function evaluation in determining the survival time in endotoxic shock [7]. Whilst that study used a conventional phenylephrine infusion method to assess baroreflex function, an alternative method is to utilize transfer function analysis of heart rate and blood pressure variability [8], [12]. In this study, the transfer function method was applied to assess alterations in baroreflex function in mechanically ventilated and anesthetized rabbits after Escherichia coli endotoxin administration.

II. METHODS

The protocol used in this study was approved by the Ruakura Animal Ethics Committee of the Ruakura Small Animals Research Facility, Hamilton, New Zealand. 13 adult New Zealand white rabbits (6 males and 7 females) between 90 and 110 days of age were studied. Animals

Manuscript received April 22, 2009. This work was supported in part by the Australian Research Council.

C.H.H. Tang, G.S.H. Chan, and A.V. Savkin are with School of Electrical Engineering and Telecommunications, University of New South Wales, Sydney, NSW 2052, Australia hhtang@student.unsw.edu.au, gregchan@unsw.edu.au, a.savkin@unsw.edu.au

P.M. Middleton is with Ambulance Service of New South Wales, Balmain Road (Locked Bag 105), Rozelle, NSW 2039, Australia pmmidleton@ambulance.nsw.gov.au

G. Cave is with Department of Surgery and Anaesthesia, School of Medicine and Health Sciences, University of Otago, Wellington, New Zealand and Department of Emergency Medicine, Hutt Hospital, Lower Hutt, New Zealand grantcave@gmail.com

M. Harvery is with Department of Emergency Medicine, Waikato Hospital, Hamilton, New Zealand harveym@waikatodhb.govt.nz

N.H. Lovell is with the Graduate School of Biomedical Engineering, University of New South Wales, Sydney, NSW 2052, Australia N.Lovell@unsw.edu.au

were initially anesthetized with 50 mg/kg ketamine and 4 mg/kg xylazine. Following intravenous cannulation of the marginal ear vein, animals were continuously sedated with ketamine at 10 mg $kg^{-1}hr^{-1}$ and xylazine at 2 mg $kg^{-1}hr^{-1}$. Tracheostomy was performed prior to the administration of pressure controlled mechanical ventilation (performed with 100% oxygen) at approximately 40 breaths per minute via a Nuffield series 200 pediatric ventilator (Penlon Ltd, Abington, England), with the inspiratory to expiratory ratio set at 1:4. Animals were paralyzed with 0.1 mg/kg vecuronium immediately on placement of the tracheostomy tube and on evidence of respiratory effort thereafter. The femoral artery was clamped proximally and ligated distally, incised, and a 1.2 mm Millar intravascular pressure transducer advanced past the relaxed clamp to the distal aorta.

Invasive femoral arterial blood pressure (ABP) and electrocardiogram (ECG) signals were recorded continuously throughout the experiment by a PowerLab data acquisition system (ADInstruments, Sydney, Australia) at a sampling rate of 1000 Hz. A stabilization period of thirty minutes was allowed following completion of surgical procedures. Animals were randomized into two treatment groups: i.e., control ($n = 5$) and endotoxic ($n = 8$) groups. Endotoxic shock was induced by single bolus injection of one mg/kg purified Escherichia coli 055:135 LPS (Sigma-Aldrich Inc, St Louis, USA) diluted to 5 ml in 0.9% saline solution and infused over one minute via the ear vein. Control animals received 5 ml 0.9% saline solution as sham fluid. After the intervention, all rabbits were continuously monitored for a period of 90 minutes. Along with the process, blood sample was drawn from the central venous catheter for the evaluation of lactate concentration (ISTAT1 i-STAT corporation, NJ, USA), immediately before the infusion of endotoxin (pre-intervention) and after the 90-minute monitoring period (post-intervention).

A. Signal Processing

Four minute segments of artifact-free ECG and ABP signals were selected from both pre- and post-intervention stages. ECG R-waves were detected using the modified Pan and Tompkin's QRS detector [13], whilst systolic peaks of the ABP signals were identified using the algorithm proposed by Treo et al. [14]. The identified beats were manually inspected, and any ectopic beats were replaced by linear interpolation of the beats immediately preceding and following the replaced beats. Short-term beat-to-beat heart period (RRi) and systolic blood pressure (SBP) time series were generated by interpolating the identified features to evenly spaced samples at 10 Hz using a previously proposed algorithm [15], [16].

B. Frequency Spectrum and Transfer Function Analysis

A power spectrum analysis technique based on the Welch algorithm of averaging periodograms was used to compute the auto-spectrum and the cross-spectrum of SBP and RRi [17]. Each of the extracted 4-minute time series of SBP and RRi was divided into nine equal segments with 50% overlap (50 seconds for each segment), before being Hanning windowed and fast Fourier transformed to its frequency, f representation. Periodograms were then averaged to produce the autospectra, $S_{\mathrm{xx}}(f)$ and $S_{\mathrm{yy}}(f)$ respectively. The power spectra of SBP and RRi were each defined as $P_{\mathrm{xx}}(f) = |S_{\mathrm{xx}}(f)|^2$ and $P_{\mathrm{yy}}(f) = |S_{\mathrm{yy}}(f)|^2$. The cross spectrum $S_{\mathrm{xy}}(f)$ is computed as the product of $S^*_{\mathrm{xx}}(f)$ and $S_{\mathrm{yy}}(f)$ (where asterisk denotes the complex conjugate). The transfer function $H(f)$ was evaluated as follows, with SBP as the input and RRi as the output for the assessment of baroreflex gain [12]:

$$H(f) = \frac{S_{\mathrm{xy}}(f)}{S_{\mathrm{xx}}(f)} \tag{1}$$

Signal coherence describes the linear coupling between SBP and RRi in the frequency domain. It has a value between zero (i.e., SBP and RRi are totally uncorrelated) and one (perfect linear relationship between SBP and RRi). It may be estimated as:

$$\gamma^2(f) = \frac{|S_{\mathrm{xy}}(f)|^2}{S_{\mathrm{xx}}(f)S_{\mathrm{yy}}(f)} \tag{2}$$

The complex transfer function in (1) is composed of two major components: i.e., the magnitude $|H(f)|$ and the phase $\theta(f)$ functions, which can be obtained from the following equations:

$$|H(f)| = \sqrt{\mathrm{real}[H(f)]^2 + \mathrm{imag}[H(f)]^2} \tag{3}$$

$$\theta(f) = \arctan\left(\frac{\mathrm{imag}[H(f)]}{\mathrm{real}[H(f)]}\right) \tag{4}$$

The transfer function gain, γ^2 and $\theta(f)$ within a given frequency band were obtained by averaging the corresponding values at frequencies where $\gamma^2 > 0.5$, or reporting only the values with maximum γ^2, if $\gamma^2 < 0.5$. This method allows an estimation of the baroreflex gain and phase shift of the output signal RRi with respect to the input signal SBP [12]. A negative phase shift (SBP precedes RRi) and a relatively high coherence (> 0.5) indicates a likely involvement of the arterial baroreflex in mediating the changes in RRi. Three different frequency bands were identified: the low frequency band (LF, 0.04 – 0.25 Hz), the mid frequency band (MF, 0.25 – 0.50 Hz), and the ventilatory frequency band (VF, 0.50 – 0.75 Hz). For ABP variability in rabbits, the MF band has been attributed to sympathetic nerve activity on peripheral vasculature, whilst the LF band was believed to include non-autonomic mechanisms, including myogenic vasomotion and tubuloglomerular feedback [18]. Since the rabbits were mechanically ventilated at approximately 0.67 Hz, a VF band has been defined to describe the respiratory change. For RRi variability in rabbits, previous studies involving autonomic blockade suggested that there was considerable overlap of sympathetic and vagal activities in the LF and MF bands, although sympathetic influence tended to be more concentrated in the LF band, while fluctuation in the VF band seemed to be purely vagal-mediated [11], [19].

C. Statistical Analysis

To analyze within-animal differences between the pre-intervention and post-intervention stages, a paired Student's t-test was applied. Prior to the test of means, the normality and homoscedacity of the samples were checked using the Lilliefors and Bartlett tests respectively. All of the non-normal data were logarithmically transformed. With $p < 0.05$ defined as statistically significant, 95% confidence intervals were calculated. For non-normal data, the geometric means and confidence intervals were reported.

III. RESULTS

Table I illustrates the central hemodynamic parameters of the endotoxic rabbits at pre-intervention and post-intervention stages. In the endotoxic group, SBP decreased significantly in the post-intervention stage whilst heart rate increased markedly. Blood lactate level was also found to increase significantly in post-intervention stage ($p < 0.01$). As shown in Table II, MF and VF powers of RRi decreased significantly in the post-intervention stage ($p < 0.01$ and $p < 0.05$ respectively). No significant changes were observed in the spectral powers of SBP variability. The control group did not show any significant change in any of the variables presented in Table I or Table II.

In the endotoxic group, a significant decrease in transfer function gain was observed in the LF band as shown in Table III ($p < 0.05$), whilst the coherence in the LF and MF bands also decreased ($p < 0.01$ and $p < 0.05$ respectively). Furthermore, the phase delays in LF, MF and VF bands were found to be significantly different between the two stages ($p < 0.05$). In the LF and MF bands, SBP tended to lead RRi in pre-intervention, then became roughly in-phase in post-intervention, as coherence decreased from close to 0.6 to less than 0.5. Conversely, in the VF band, there was an increase in phase lead from SBP to RRi. No significant changes were observed in the control group for any of the variables presented in Table III, except for a similar but smaller increase in phase lead in the VF band.

TABLE I
ASSESSMENT OF CENTRAL HEMODYNAMIC OF THE ENDOTOXIC RABBITS AT PRE- AND POST-INTERVENTION STAGES

	Pre-Intervention		Post-Intervention	
Physiology Index	**Mean**	**95% CI**	**Mean**	**95% CI**
Endotoxic Group				
SBP (mmHg)	84	(76,92)	70†	(63,77)
MAP (mmHg)	66	(60,73)	59	(53,64)
DBP (mmHg)	57	(51,63)	53	(48,57)
HR (bpm)	177	(163,191)	206†	(193,219)
Lact. (mmol/L)	3	(1,5)	5‡	(4,7)
Control Group				
SBP (mmHg)	78	(62,93)	73	(66,81)
MAP (mmHg)	61	(49,74)	60	(53,68)
DBP (mmHg)	53	(43,64)	54	(46,62)
HR (bpm)	178	(153,202)	192	(167,216)
Lact. (mmol/L)	4	(1,7)	4	(2,6)

$\dagger p < 0.05$ and $\ddagger p < 0.01$ for Pre- vs. Post-intervention.
Abbreviations: *SBP* systolic blood pressure *MAP* mean arterial pressure *DBP* diastolic blood pressure *HR* heart rate *Lact* blood lactate level.

TABLE II
COMPARISON OF PARAMETERS OBTAINED FROM FREQUENCY SPECTRUM OF SYSTOLIC BLOOD PRESSURE AND HEART PERIOD VARIABILITY IN PRE- AND POST-INTERVENTION PHASES

	Pre-Intervention		Post-Intervention	
Spectral Index	**Mean**	**95% CI**	**Mean**	**95% CI**
Endotoxic Group				
SBP LF ($mmHg^2$)	0.05	(0.02,0.11)	0.13	(0.06,0.26)
SBP MF ($mmHg^2$)	0.02	(0.01,0.03)	0.01	(0.01,0.02)
SBP VF ($mmHg^2$)	1.67	(1.19,2.36)	2.29	(1.39,3.78)
RRi LF (ms^2)	0.94	(0.27,3.25)	0.51	(0.22,1.20)
RRi MF (ms^2)	0.58	(0.25,1.36)	0.30‡	(0.12,0.71)
RRi VF (ms^2)	4.48	(2.35,8.54)	2.03†	(1.22,3.38)
Control Group				
SBP LF ($mmHg^2$)	0.04	(0.02,0.07)	0.16	(0.08,0.34)
SBP MF ($mmHg^2$)	0.01	(0.00,0.04)	0.04	(0.02,0.08)
SBP VF ($mmHg^2$)	1.79	(0.79,4.07)	2.19	(0.90,5.36)
RRi LF (ms^2)	0.56	(0.16,1.89)	0.30	(0.11,0.83)
RRi MF (ms^2)	0.53	(0.13,2.14)	0.29	(0.14,0.60)
RRi VF (ms^2)	4.30	(2.20,8.40)	1.88	(0.78,4.53)

$\dagger p < 0.05$ and $\ddagger p < 0.01$ for Pre- vs Post-intervention.
Abbreviations: *CI* confidence intervals *RRi* heart period *SBP* systolic blood pressure *LF* low frequency power *MF* mid frequency power *VF* ventilatory frequency power

IV. DISCUSSIONS

In this study, the occurrence of endotoxic shock was confirmed with a significant increase of blood lactate level, which signified the onset of distributive shock in the late phase of experiment. Based on this model, several novel findings on the use of cross-spectral transfer function analysis for assessing baroreflex-related cardiac control have been presented in this paper. A decrease in coherence and loss of phase delay between SBP and RRi, along with a depression in transfer function gain in the LF band showed that the baroreflex mechanism has been impaired by endotoxin intoxication. The impairment of baroreflex was believed to be partly responsible for the autonomic disturbances observed in human septic shock [5], [19], [20]. Prior to endotoxin infusion, SBP was leading RRi in both the LF and MF bands with a coherence of close to 0.6, suggesting a potentially baroreflex-mediated relationship. However, subsequent to endotoxic shock, the coherence dropped to below 0.5, along with a loss of phase delay from SBP to RRi (LF SBP led LF RRi with 3.6 heart-beats initially, and deteriorated to 0.5 heart-beats subsequently). These findings altogether tended to suggest a derangement of heart rate control by the arterial baroreflex as a result of endotoxic shock.

A significant increase in phase delay from SBP to RRi in the ventilator frequency band was noted in the post-intervention stage. This change could not be solely attributed to the effect of endotoxin, as it also occurred in the control group. A possible explanation for this might be the effect of anesthesia, which could lead to an increase in the delay of the baroreflex-mediated heart rate response [21]. However, despite the potential confounding effects of anesthesia, it was highly unlikely that anesthesia alone could explain the other changes that were observed only in the endotoxic group, but not in the control group.

TABLE III

COMPARISON OF PARAMETERS OBTAINED FROM TRANSFER FUNCTION ANALYSIS OF SYSTOLIC BLOOD PRESSURE AND HEART PERIOD VARIABILITY IN PRE- AND POST-INTERVENTION PHASES

	Pre-Intervention		Post-Intervention	
Transfer Index	**Mean**	**95% CI**	**Mean**	**95% CI**
Endotoxic Group				
Coh. LF	0.59	(0.52,0.66)	0.47‡	(0.41,0.52)
Coh. MF	0.57	(0.48,0.65)	0.46†	(0.36,0.55)
Coh. VF	0.83	(0.76,0.89)	0.81	(0.78,0.85)
Mag. LF	4.72	(2.90,6.55)	2.42†	(1.24,3.60)
Mag. MF	5.63	(2.02,9.25)	5.87	(3.16,8.57)
Mag. VF	3.15	(0.45,5.85)	2.11	(0.80,3.41)
Pha. LF	-63	(-89,-38)	-8†	(-29,12)
Pha. MF	-18	(-47,12)	12†	(-19,43)
Pha. VF	5	(-31,41)	-60†	(-93,-27)
Control Group				
Coh. LF	0.56	(0.44,0.68)	0.50	(0.39,0.61)
Coh. MF	0.52	(0.43,0.61)	0.57	(0.40,0.73)
Coh. VF	0.80	(0.64,0.96)	0.85	(0.81,0.89)
Mag. LF	4.13	(0.94,7.31)	1.32	(0.73,1.91)
Mag. MF	7.26	(2.17,12.34)	2.06	(1.46,2.65)
Mag. VF	3.39	(0.20,6.58)	1.20	(0.41,1.99)
Pha. LF	-25	(-79,29)	20	(-29,68)
Pha. MF	0	(-41,41)	-35	(-93,23)
Pha. VF	2	(-33,38)	-30†	(-54,-5)

$\dagger p < 0.05$ and $\ddagger p < 0.01$ for Pre- vs Post-intervention.
Abbreviations: *CI* confidence intervals *Coh* coherence *Mag* transfer function magnitude (ms/mmHg) *Pha* transfer function phase (degree) *LF* low frequency *MF* mid frequency *VF* ventilatory frequency.

Results of spectral analysis on SBP and RRi variabilities in this study are in agreement with previous researches demonstrating that heart rate variability is significantly depressed in endotoxic shock, which may result from the poor responsiveness of the heart to central autonomic control [11]. The decrease was most significant in the MF and VF bands, suggesting that the impairment in cardiac autonomic control occurred mostly in the vagal branch.

V. CONCLUSIONS

Frequency spectrum and transfer function analysis was utilized in this study to investigate the changes in SBP and RRi variabilities, and their relationship in a mechanically ventilated and anesthetized rabbit model of endotoxic shock. RRi variability was found to be significantly suppressed in endotoxic shock rabbits, whilst profound reduction was also observed in the coherences, transfer function gains and phase delays between SBP and RRi in the relevant frequency bands, which might indicate impairment in the baroreflex control of heart rate. In general, the results have highlighted the potential value of frequency spectrum and transfer function analysis of cardiovascular variability in assessing autonomic and baroreflex-related changes in endotoxic and potentially septic shock.

VI. ACKNOWLEDGMENTS

The authors gratefully acknowledge the staffs in Ruakura Small Animals Research Facility, Hamilton, New Zealand for their assistance in the data collection.

REFERENCES

[1] D. Di Giantomasso, C.N. May, and R. Bellomo, "Vital organ blood flow during hyperdynamic sepsis," *Chest*, vol. 124, no. 3, pp. 1053-1059, 2003.

[2] J. Brierley and M.J. Peters, "Distinct hemodynamic patterns of septic shock at presentation to pediatric intensive care," *Pediatrics*, vol. 122, no. 4, pp. 752-759, 2008.

[3] L.B. Hinshaw, "Sepsis/septic shock: participation of the microcirculation: an abbreviated review," *Crit. Care Med.*, vol. 24, no. 6, pp. 1072-1078, 1996.

[4] G. Bernardin, A.D. Strosberg, A. Bernard, M. Mattei, and S. Marullo, "Beta-adrenergic receptor-dependent and-independent stimulation of adenylate cyclase is impaired during severe sepsis in humans," *Intensive Care Med.*, vol. 24, no. 12, pp. 1315-1322, 1998.

[5] D. Annane, F. Trabold, T. Sharshar, I. Jarrin, A.S. Blanc, J.C. Raphael, and P. Gajdos, "Inappropriate sympathetic activation at onset of septic shock: a spectral analysis approach," *Am. J. Respir. Crit. Care Med.*, vol. 160, no. 2, pp. 458-465, 1999.

[6] C. Martin, C. Boisson, M. Haccoun, L. Thomachot, and J.L. Mege, "Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock, and severe trauma," *Crit. Care Med.*, vol. 25, no. 11, pp. 1813-1819,1997.

[7] F.M. Shen, Y.F. Guan, H.H. Xie, and D.F. Su, "Arterial baroreflex function determines the survival time in lipopolysaccharide-induced shock in rats," *Shock*, vol. 21, no. 6, pp. 556-560, 2004.

[8] G.D. Pinna, R. Maestri, S. Capomolla, O. Febo, E. Robbi, F. Cobelli, and M.T. La Rovere, "Applicability and clinical relevance of the transfer function method in the assessment of baroreflex sensitivity in heart failure patients," *J. Am. Coll. Cardiol.*, vol. 46, no. 7, pp.1314-1321.

[9] E.J. Bridges and S. Dukes, "Cardiovascular aspects of septic shock: pathophysiology, monitoring, and treatment," *Crit. Care Nurse*, vol. 25, no. 2, pp. 14, 2005.

[10] L.F. Poli-de-Figueiredo, A.G. Garrido, N. Nakagawa, and P. Sannomiya, "Experimental models of sepsis and their clinical relevance," *Shock*, vol. 30 Supplement 1, no. 7, pp. 53-59, 2008.

[11] B. Goldstein, M.H. Kempski, D. Stair, R.B. Tipton, D. DeKing, D.J. DeLong, and *et al.*, "Autonomic modulation of heart rate variability during endotoxin shock in rabbits," *Crit. Care Med.*, vol. 23, no. 10, pp. 1694-1702, 1995.

[12] H.W. Robbe, L.J. Mulder, H. Rüddel, W.A. Langewitz, J.B. Veldman, and G. Mulder, "Assessment of baroreceptor reflex sensitivity by means of spectral analysis," *Hypertension*, vol. 10, no. 5, pp. 538-543, 1987.

[13] J. Pan and W.J. Tompkins, "A real-time QRS detection algorithm," *IEEE Trans. Biomed. Eng.*, vol. 32, no. 3, pp. 230-236, 1985.

[14] E.F. Treo, M.C. Herrera, and M.E. Valentinuzzi, "Algorithm for identifying and separating beats from arterial pulse records," *Biomed. Eng. Online*, vol. 4, pp. 48, 2005.

[15] R.D. Berger, S. Akselrod, D. Gordon, and R.J. Cohen, "An efficient algorithm for spectral analysis of heart rate variability," *IEEE Trans. Biomed. Eng.*, vol. 33, no. 9, pp. 900-904, 1986.

[16] C.H.H. Tang, A.V. Savkin, P.M. Middleton, "Non-invasive sepsis patient classification using least squares support vector machine," in *Proc. 2nd International Conference on Bio-inspired Systems and Signal Processing*, Porto-Portugal, 2009.

[17] P. Welch, "The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms," *IEEE Trans. Audio Electroaccoust.*, vol. 15, no. 2, pp. 70-73, 1967.

[18] B.J. Janssen, S.C. Malpas, S.L. Burke, and G.A. Head, "Frequency-dependent modulation of renal blood flow by renal nerve activity in conscious rabbits," *Am. J. Physiol.*, vol. 273, no. 2 Pt 2, pp. R597-R608, 1997.

[19] V.A. Moguilevski, L. Shiel, J. Oliver, B.P. McGrath, "Power spectral analysis of heart-rate variability reflects the level of cardiac autonomic activity in rabbits," *J Auton Nerv Syst*, vol. 58, no.1-2, pp. 18-24, 1996.

[20] M. Piepoli, C.S. Garrard, D.A. Kontoyannis, and L. Bernardi, "Autonomic control of the heart and peripheral vessels in human septic shock," *Intensive Care Med.*, vol. 21, no. 2, pp. 112-119, 1995.

[21] C. Keyl, A. Schneider, M. Dambacher, U. Wegenhorst, M. Ingenlath, M. Gruber, L. Bernardi, "Dynamic cardiocirculatory control during propofol anesthesia in mechanically ventilated patients," *Anesth Analg*, vol. 91, no. 5, pp. 1188-95, 2000.