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# INVESTIGATION OF HEART RATE VARIABILITY IN PATIENTS UNDER LOCAL ANAESTHESIA

K. Shafqat<sup>1</sup>, P. A. Kyriacou<sup>1</sup>, S. K. Pal<sup>2</sup>, S. Kumari<sup>2</sup>

<sup>1</sup>School of Engineering and Mathematical Sciences, City University, London, EC1V 0HB, UK

<sup>2</sup>St Andrew's Centre for Plastic Surgery & Burns, Broomfield Hospital, Chelmsford, CM1 7ET, Essex

[K.Shafqat@city.ac.uk](mailto:K.Shafqat@city.ac.uk), [P.Kyriacou@city.ac.uk](mailto:P.Kyriacou@city.ac.uk), [Sandip.Pal@meht.nhs.uk](mailto:Sandip.Pal@meht.nhs.uk), [KumariSanthi@aol.com](mailto:KumariSanthi@aol.com)

## ABSTRACT

Spectral analysis of Heart Rate Variability (HRV) is widely used for the assessment of cardiovascular autonomic control. Several studies have shown the effect of anaesthetic agents on HRV parameters. In this study a systematic approach of HRV analysis has been employed. The effect caused by the ectopic beats on the spectral measurements has been investigated and results are presented. A detrending method using Wavelet Packets has been developed which was able to remove slow varying trend from HRV signals without causing significant changes in the low frequency (LF) and high frequency (HF) component of the HRV signal. Using this methodology Electrocardiogram (ECG) signals from 14 patients undergoing local anaesthesia (brachial plexus block) were analysed with non-parametric (Welch's periodogram) method. The results show that the  $\frac{LF}{HF}$  ratio values calculated from the HRV signal decreases within an hour of the application of the brachial plexus block compared to the values at the start of the procedure. This change was noticed in approximately 80% of the patients.

## KEY WORDS

Heart rate variability, Spectral analysis, Local anaesthesia

## 1. Introduction

The Autonomic Nervous System (ANS) modulates the cardiac pacemaker and provides the beat to beat regulation of the cardiovascular system through complex interaction between the sympathetic and the parasympathetic nervous system input to the sinus node. Heart Rate Variability (HRV) is the study of this inter-beat variability of the ECG signal and therefore, can be used as a non-invasive technique to assess the autonomic influence on the heart [1]. Different methods have been used in HRV analysis but the two most commonly used are the time domain and frequency domain analysis. The use of spectral analysis in HRV studies was first proposed in the experiment carried out by Akselrod et al. [2, 3], Pomeranz et al. [4] and Pagani et al. [5]. Researchers took a keen interest in HRV research after the late 1980s when it was shown to be a strong and independent predictor of mortality after an acute myocardial infarction. In the frequency domain, three frequency bands can be

distinguished in the spectrum of short term (2 to 5 minutes) HRV signals [1]. These components are termed as:-

a) High-Frequency (HF) band which is related to the respiration frequency and is also termed as "Respiratory Sinus Arrhythmia (RSA) related band" (0.15 Hz to 0.4 Hz).

b) Low-Frequency (LF) band or "Mayer wave-like sinus arrhythmia related band", which occurs at about 0.1 Hz spontaneous vasomotor activity (0.04 Hz to 0.15 Hz)

c) Very Low-Frequency (VLF) band where the frequencies are less than 0.04 Hz

The HF region is mediated almost entirely by the vagal tone. The LF region is an indicator of sympathetic tone, but is also modulated by vagal activity. The physiological aspects behind the VLF band are not well established and therefore, in short term recording this band should be avoided [1]. Using this information the HRV indexes such as the ratio of  $\frac{LF}{HF}$  power or the fractional LF power have been used to describe sympathovagal balance [6].

HRV has also been used to study the effects of different drugs on the cardiovascular system. Spectral analysis techniques have been used to show the effect of anticholinesterase edrophonium on heart rate and blood pressure variability [7]. Changes in HRV due to atropine administration have also been studied using spectral analysis techniques [8]. Such studies have shown that with the first dose of atropine the HRV increased followed by a progressive decrease with higher doses. In the case of propranolol, there was a significant increase in heart rate variability, which progressively disappeared after the last dose [8]. Studies of heart rate variability in the field of anaesthesia for predicting the depth of anaesthesia were also undertaken over the last 12 years [9]. However, the outcome of such studies did not provide enough evidence so that such a technique could be used as a routine clinical tool.

The aim of this study is the investigation of the effect of local anaesthesia (induced with Bupivacaine + Lignocaine, in patients having brachial plexus block) on HRV.

## 2. Methods

### 2.1 Subject and Protocol

Research ethics committee approval was obtained prior to commencing the study on ASA 1 and 2 patients. Fourteen patients (7 males and 7 females) aged  $50.6 \pm 20.7$  years (mean weight  $67 \pm 15.3$  Kg, mean height  $1.6 \pm 0.2$  m) undergoing elective general surgery under local anaesthesia were recruited to the study. In all cases the axillary approach was used for the brachial plexus block. Combination of 30 ml Lignocaine and 29 ml of 0.5% Bupivacaine was used as anaesthetic agent. Patients were also given 1:200000 part adrenaline at the time of the block.

An AS/3 Anaesthesia Monitor (Datex-Engstrom) was used to collect lead II ECG signals from the patients. The monitoring started about 30 minutes before the start of the block and continued for approximately another 30 minutes postoperatively in the recovery ward. The ECG signal was digitised at 1 kHz sampling frequency using a 12-bit data acquisition card (National Instruments Corporation, Austin, Texas).

### 2.2 ECG R-wave detection

The algorithm for R wave detection implemented in this work is based on the algorithm presented by Sahambi et al. [10]. The wavelet used for the characterisation of the ECG signal is the first derivative of the Gaussian smoothing function. As the sampling frequency of 1 kHz is higher than the 250 Hz sampling frequency used by Sahambi et al. different wavelet scales ( $2^m$ ,  $m=4, 8, 12, 15, 20$ ) were used to get the desired filter response.

### 2.3 Ectopic Beat correction and HRV signal representation

For the representation of the HRV signal, the Heart Timing (HT) signal [11] was used. In the absence of ectopic beats the HT signal can be written, using the Integral Pulse Frequency Modulation (IPFM) model, as shown in equation 1.

$$ht(t_k) = kT - t_k = \int_0^{t_k} m(\tau) d\tau \quad \text{Eq. 1}$$

Where  $t_k$  is the  $k^{\text{th}}$  beat time and  $T$  is the mean heart rate. This signal was preferred as it provides an unbiased estimate of the modulating signal  $m(t)$ .

Dealing with ectopic beats is essential in the analysis as the time domain signal associated with the HRV can exhibit a sharp transient at the ectopic beat, making it unsuitable particularly in the Power Spectral Density (PSD) estimate of the HRV. It has been shown previously that the area of the low frequency (LF) component of the HRV spectrum in normal subjects can increase up to 89% if the analysis signal has 4% of ectopic beats. The same amount of ectopic beats also causes an increase of up to

402% in the area of the high frequency (HF) component of HRV [12].

The criterion for the detection of the ectopic beats using the HT signal depends on the beats location. This criterion is based on the fact that instantaneous Heart Rate (instHR) is bandlimited so it is possible to impose a threshold ( $U$ ) on the estimate of the derivative of instHR, as given in equation 2, to classify the beats as anomalies if the derivative passes the threshold.

$$\left| \hat{r}_k^i \right| = 2 \left| \frac{t_{k-1} - 2t_k + t_{k+1}}{(t_{k-1} - t_k)(t_{k-1} - t_{k+1})(t_k - t_{k+1})} \right| \quad \text{Eq. 2}$$

The threshold is defined as  $U = \min(4.3\sigma_{\hat{r}_k}, 0.5)$  where  $\sigma_{\hat{r}_k}$  is the standard deviation of the derivative of  $instHR_k$  [13].

In order to see the effectiveness of the HT signal for the representation of the HRV signal and to evaluate the performance of the ectopic beat handling algorithm, the modulating signal presented in equation 3 was used.

$$m(t) = 0.1 \cdot \cos(2\pi \cdot 0.1 \cdot t) + 0.1 \cdot \cos(2\pi \cdot 0.25 \cdot t) \quad \text{Eq. 3}$$

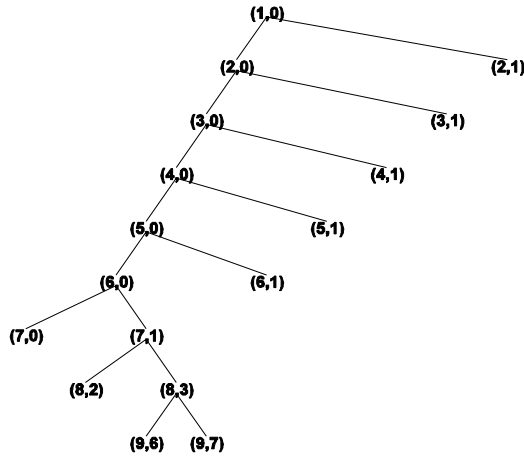
The HT signal was generated using this modulating signal in the IPFM model and this signal was resampled using cubic spline at a sampling rate of 4 Hz as recommended for HRV studies [14].

### 2.4 Detrending Method

Nonstationarities in the HRV signal can cause distortion in the time and frequency domain analysis. In particular these nonstationarities will distribute large amount of variance in the lowest frequency. In order to deal with this problem many researchers detrend the data prior to analysis. Detrending is usually based on first order [15, 16] or higher order [16] polynomial model or by using successive-difference filters. As mentioned before, for short term HRV analysis the VLF component does not reflect important information therefore, the cutoff frequency of the detrending filter should be such that it could remove most of the VLF component of the HRV signal.

The detrending algorithm was implemented using Wavelet Packets (WP). This algorithm is based on the technique mentioned by Wiklund et al. [17]. In this study WP were used so that better frequency separation could be achieved. The WP algorithm was implemented using Daubechies compactly supported orthonormal wavelet transform method with an order of 12. The basis used for the WP analysis is shown in figure 1. This basis was chosen according to the bandwidth of the resulting filters. Using the information regarding the cutoff frequencies of these filters, the frequencies from approximately

0 to 0.039 Hz were attenuated by making the coefficients of the WP decomposition coming from nodes (7,0) and (9,6), shown in figure 1, zero and reconstructing the signal from the remaining coefficients.



**Figure 1:** WP tree used for detrending. The pair of numbers in the bracket at each node represents particular part of the decomposition. The first number in the pairs shows the level of the decomposition  $j$  and the possible values for the second number ( $n$ ) are  $0, \dots, 2^{(j-1)} - 1$  except for  $j - 1$  which represent the original signal

This detrending method was evaluated using a simulated signal that consisted of three sine waves of equal amplitude at frequencies of 0.025 Hz, 0.045 Hz and 0.18 Hz. The frequencies were chosen so that each one represented the VLF, the LF and the HF part of the HRV signal respectively.

### 2.5 Spectrum analysis

In this study, Welch's periodogram method was used for spectral analysis. This non-parametric method of spectral analysis has been used in various HRV studies [18]. For spectral analysis five minutes of data (1200 points at

4 Hz) was used and the data was windowed using a Hamming window of 600 points with 68% overlap.

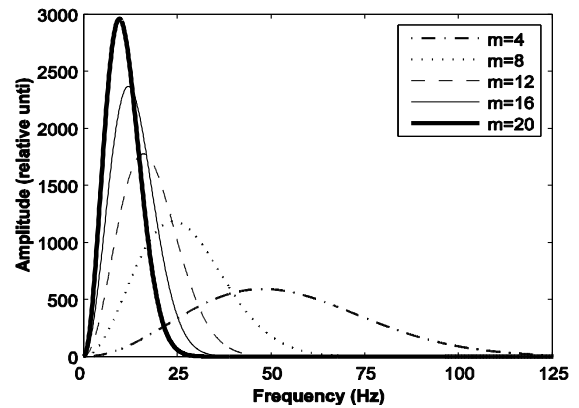
In order to see the effect of using five minutes of data with the Welch's periodogram method the performance of the Welch's periodogram, for the spectrum of simulated signal given in equation 3 was evaluated using the following parameters:

- Location of the peaks
- LF and HF peak height and LF/HF ratio
- Peak width at 3 dB below each peak
- Fraction of power within  $\pm 0.001$  Hz of each peak
- Power in the first sidelobe of each major peak ( $P_{sl}$ )

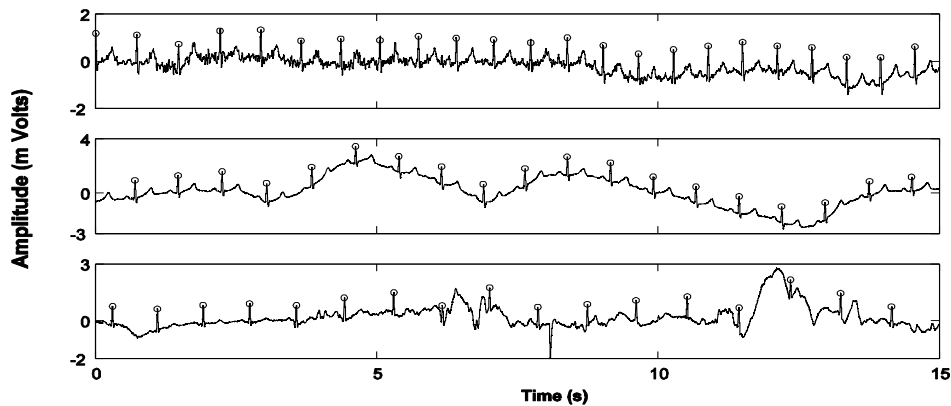
### 3. Results

The filter responses obtained for the Gaussian derivative wavelet at scale ( $2^m$ ,  $m=4, 8, 12, 16, 20$ ) are presented in figure 2. Using the acquired ECG signals, the peak detection algorithm achieved an accuracy of 99.96% and sensitivity of 99.7%. Typical examples of peak detection of ECG signals are shown in figure 3.

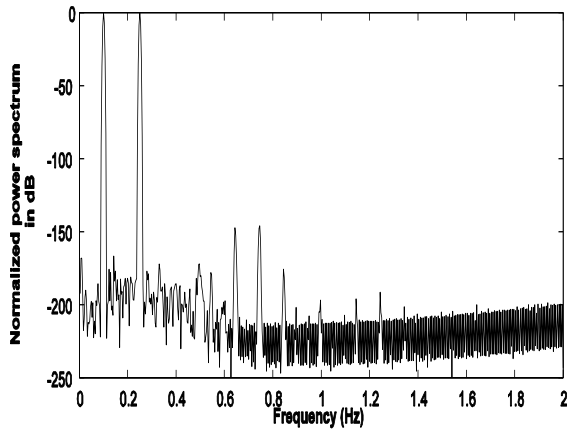
The power spectrum using Welch's periodogram method, of five minutes of the simulated signal given in equation 3 is presented in figure 4.



**Figure 2:** Fourier transform of wavelet at  $F_s = 1$  kHz at scale  $2^m$  with  $m=4, 8, 12, 16$  and  $20$



**Figure 3:** ECG peak detection in three signals acquired from different patients involved in the study



**Figure 4:** Normalised spectrum obtained for the modulating signal of Eq. 3 with 5 minutes of data using Welch’s periodogram method

Using the results shown in figure 4 the metrics defined in section 2.5 were calculated and the results are presented in table 1

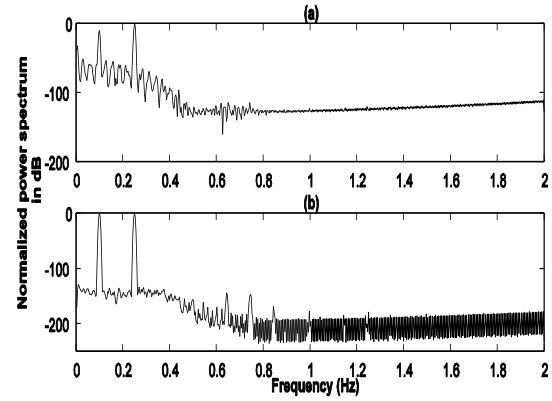
Table 1 shows that the peak locations are detected quite accurately with a difference of 0.0003 Hz and 0.0001 Hz in the LF and HF respectively. The bandwidth is approximately 0.003 Hz and the first sidelobes after the main peaks are at -167 dB and -175 dB.

Figure 4 shows that the biggest spurious peak occurs at around 0.75 Hz which is approximately 145 dB below. The spectral leakage is also minimum as power within 0.01 Hz of the main peaks represents 49.86% and 49.59% of the total power for LF and HF respectively, which is quite close to the theoretical value of 50%, expected for both components.

In order to see the effect of ectopic beats on the power spectrum ten signals were generated by randomly removing five beats from the same signal that was used to generate the spectrum shown in figure 4.

**Table 1:** The performance metrics for five minutes of simulated data. Total power ( $P_t$ ), location of the frequency peak ( $f_{peak}$ ), power at the frequency peak ( $P_{peak}$ ), 3 dB bandwidth ( $\Delta f_{3dB}$ ), fraction of power in a  $\pm 0.01$  Hz band centred on the peak ( $P_{peak}/P_t$ ) and amplitude of the first sidelobe ( $P_{sl}(dB)$ ) using figure 4

$P_t$		$f_{peak}$ (Hz)	$P_{peak}$ (dB)	$P_{peak}/P_t$	$\Delta f_{3dB}$	$P_{sl}$ (dB)
2.93	LF	0.1003	0.0	0.499	0.003	-167
	HF	0.2501	-0.086	0.496	0.003	-175



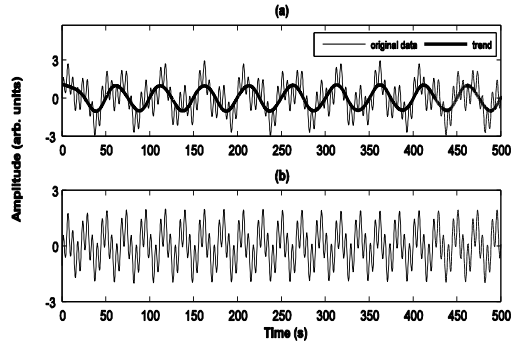
**Figure 5:** Normalised spectrum of the signal (a) by randomly removing five beats; (b) spectrum obtained after beat correction (compare with the spectrum shown in figure 3)

Figure 5(a) shows one of the power spectrums obtained after removing five random beats and figure 5(b) shows the spectrum obtained after performing beat correction. Figure 5(a) shows that the peak power of the lower frequency has been reduced. Instead of 0 dB the peak is at about -10 dB. Similarly the sidelobe peaks have increased. In the case of low frequency (0.1 Hz) the sidelobes are about 50 dB below and for high frequency (0.25 Hz) the sidelobes are approximately at -60 dB. The performance matrices used in table 1 were also calculated for each of the ten power spectrum results obtained after performing beat correction on the ten signals, in which five beats were removed randomly. The results are presented in table 2.

In general, the difference in LF peak location was 0.00031 Hz and for the HF peak the difference was 0.00011 Hz. The fraction of power in the  $\pm 0.01$  Hz band centred on the LF peak was 49.35% and for the band centred on the HF peak was 49.64%. There was a slight decrease in the power of the HF component. The peak value decreased from -0.0855 dB to -0.17 dB. The power in the LF component showed decrease in only two cases (first and last one, see table 2). The first sidelobe power  $P_{sl}(dB)$  (not shown in table 2) was approximately -135 dB for both LF and HF components. The 3 dB bandwidth ( $\Delta f_{3dB}$ ) for both the components remained unchanged at 0.003 Hz.

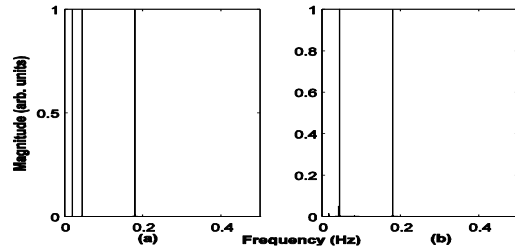
The result obtained by using the WP algorithm for detrending the simulated signal (see section 2.4) is presented in figure 6.

The magnitude spectrum of the original signal was compared before and after detrending. The spectrum of the signal before and after detrending is presented in figure 7.



**Figure 6:** Result of detrending simulated signal using WP algorithm part (a) shows the original signal and the trend removed part; (b) shows the detrended signal

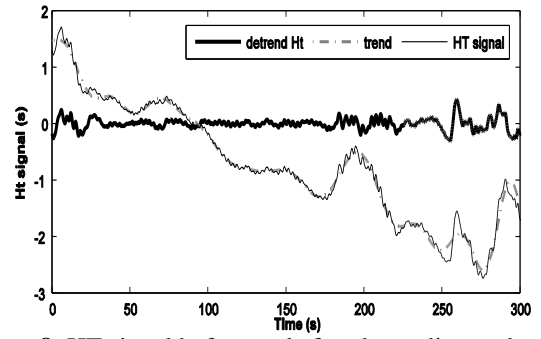
Figure 7 shows that the detrending method was able to attenuate the trend. Also, the magnitudes of the other two components corresponding to the LF and the HF part of



**Figure 7:** (a) Magnitude spectrum of the original signal; (b) the spectrum of the signal after removing trend using WP algorithm

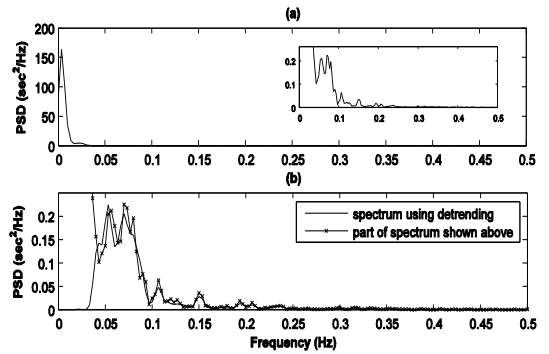
the HRV signal have not been altered significantly.

The result obtained by detrending a typical HT signal from a patient is presented in figure 8. The spectrum of the same HT signal after detrending with WP algorithm and after removing a liner trend is presented in figure 9.



**Figure 8:** HT signal before and after detrending and trend removed from the signal

Figure 9(a) shows that the spectrum is dominated by a large component at very low frequency, and because of this, interpretation of other smaller frequency components is difficult.



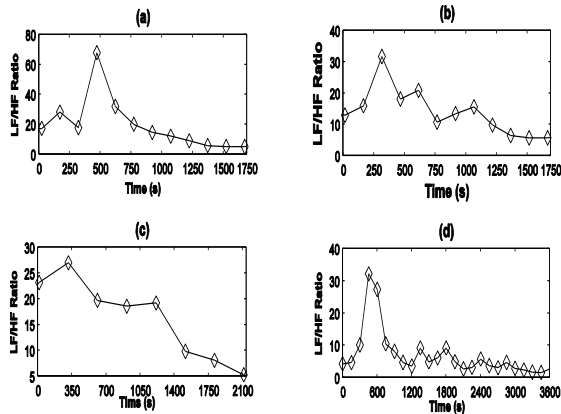
**Figure 9:** (a) Spectrum of HT signal after removing linear polynomial trend; (b) Spectrum of the HT signal after detrending using the WP method and part of the spectrum shown in part (a) (line with cross marks)

**Table 2:** Performance matrices after beat correction for the ten randomly generated signals. Location of the frequency peaks, power at the frequency peak ( $P_{peak}$ ), 3 dB bandwidth ( $\Delta f_{3dB}$ ), fraction of power in a 0.01 Hz band centred on the peak ( $P_{peak} / P_{total}$ ) and amplitude of the first sidelobe ( $P_{sl}(dB)$ )

Data no.	LF peak (Hz)	$P_{LF}(dB)$	$P_{LF} / P_{total}$	HF peak (Hz)	$P_{HF}(dB)$	$P_{HF} / P_{total}$
1	0.10027	-0.0338	0.494	0.2501	0.0	0.497
2	0.10029	0.0	0.499	0.2501	-0.0870	0.501
3	0.10029	0.0	0.495	0.2501	-0.1791	0.498
4	0.1003	0.0	0.498	0.2501	-0.1739	0.502
5	.10027	0.0	0.496	0.2501	-0.2602	0.489
6	0.10029	0.0	0.494	0.2501	-0.1089	0.497
7	0.10053	0.0	0.476	0.2501	-0.5394	0.479
8	0.10027	0.0	0.495	0.2501	0.0135	0.498
9	0.10029	0.0	0.494	0.2501	0.3384	0.506
10	0.10029	-0.1259	0.494	0.2501	0.0	0.497

On the right hand side of figure 9(a) a magnified version of the spectrum is presented, which is also shown in part (b) of the same figure (line with cross marks). Comparing this with the spectrum obtained after WP detrend removal (solid line in figure 9(b)) it is clear that the components in the LF and HF region of the signal are not affected by the trend removal technique. Furthermore, in this case it is relatively easier to distinguish the spectrum components.

From the patients data the power spectrum was again calculated using 1200 points, but in this case the signal was overlapped by 50% so that each time 600 new samples were used. The analysis of the signal from the patients revealed that in most cases the  $\frac{LF}{HF}$  ratio increases just after the application of the brachial plexus block and then decreases considerably. The timing of the drop in the ratio value differs from patient to patient, but in each case the drop occurs within an hour of the start of the block.



**Figure 10:** Ratio  $\frac{LF}{HF}$  changes after the brachial plexus block. In each case there is an increase right after the block and then the ratio values shows considerable decrease

Some examples of the changes in the ratio values after the application of the block are presented in figure 10. The drop in the  $\frac{LF}{HF}$  ratio value was expected as a result of the introduction of the local anaesthesia. The anaesthetic agents Lignocaine and Bupivacaine are known to have a decreasing effect on the heart rate.

#### 4. Conclusion

In this study various techniques of HRV analysis have been implemented and successfully evaluated. The HT signal which provide unbiased estimate of the modulating signal has been used. The effects caused by ectopic beats on the power spectrum of a five minute signal are shown in figure 5. Results shown in table 2 indicate that the ectopic beat correction has managed to reduce the distortion caused by the missing peaks in the spectrum of the signal.

The detrending algorithm developed using Wavelet Packets (WP) has successfully removed the slow moving trend from the signal without creating significant distortion or reduction in the power of the signal in the LF and HF region. Figures 6 and 7 demonstrate the effect of the detrending algorithm on a simulated signal that consists of three sine waves each having equal amplitude representing the VLF, LF and HF part of HRV signal. Figure 8 shows the detrending of a typical HT signal and figure 9 provides the evidence that this technique has removed the slow varying components successfully without significant changes in the power of the signal in the LF and HF region.

Finally the ECG data acquired from 14 patients undergoing local anaesthesia, using a combination of 30 ml Lignocaine and 29 ml of 0.5% Bupivacaine as the anaesthetic agent were analysed using Welch's periodogram. The results from the power spectral analysis indicate that the  $\frac{LF}{HF}$  ratio increases just after the block is applied and then decreases significantly (figure 10) .

This pilot clinical study suggests that during brachial plexus block, using a mixture of Lignocaine and Bupivacaine, there is a noticeable and almost consistent change in HRV. Such encouraging results suggest further and more rigorous clinical studies.

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#### References

- [1] Task Force of the Euro. Society of Cardiology the N. American Society of Pacing Electrophysiology, Heart Rate Variability Standards of Measurement, Physiological Interpretation, and Clinical Use, *European Heart Journal*,18, 1996, 354-381.
- [2] S. Akselrod, D. Gordon, F. A. Ubel, D. C. Shannon, A. C. Berger and R. J. Cohen, Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control, *Science*, 213, 1981, 220-222.
- [3] S. Akselrod, D. Gordon, J. B. Madwed, N. C. Snidman, D. C. Shannon and R. J. Cohen, Hemodynamic regulation: investigation by spectral analysis, *American Journal of Physiology*, 249, 1985, H867-H875.
- [4] B. Pomeranz, R. J. B. Macaulay, M. A. Caudill, I. Kutz, D. Adam, D. Gordon, K. M. Kilborn, A. C. Barger, D. C. Shannon, R. J. Cohen RJ and H. Benson, Assessment of autonomic function in humans by heart rate spectral analysis, *American Journal of Physiology*, 248, 1985, H151-H153.



- [5] M Pagani, F. Lombardi, S. Guzzetti, O. Rimoldi, R. Furlan, P. Pizzinelli, G. Sandrone, G. Malfatto, S. Dell'Orto, E. Piccaluga, M. Turiel, G. Baselli, S. Cerutti and A. Malliani, Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympathovagal interaction in man and conscious dog, *Circulation Research*, 59, 1986, 178-193.
- [6] J. J. Goldberger, Sympathovagal balance: How should we measure it?, *Am. J. Physiol.*, 276, 1999, H1273-H1280.
- [7] A. Deschanps, S. B. Backman, V. Novak, G. Plourde, P. Fiset and D. Chartrand, Effects of the Anticholinesterase Edrophonium on Spectral Analysis of Heart Rate and Blood Pressure Variability in Humans, *The journal of Pharmacology and experimental therapeutics*, 300(1), 2002, 112-117.
- [8] V. Pichot, J. M. Gaspoz, S. Molliex, A. Antoniadis, T. Busso, F. Roche, F. Costes, L. Quintin, J. R. Lacour and J. C. Barthélémy, Wavelet transform to quantify heart rate variability and to assess its instantaneous changes, *Journal of Applied Physiology*, 86, 1999, 1081-1091.
- [9] S. Z. Fan, Y. J. Cheng and C. C. Liu, Heart rate variability--a useful non-invasive tool in anesthesia, *Acta Anaesthesiol Sin*, 32(1), 1994, 51-56.
- [10] J. S. Sahambi, S. N. Tandon, and R. K. Bhatt, Using wavelet transforms for ECG characterization. An on-line digital signal processing system, *IEEE Eng Med Biol Mag*, 16(1), 1997, 77-83.
- [11] J. Mateo and P. Laguna, Improved heart rate variability signal analysis from the beat occurrence times according to the IPFM, *IEEE Trans. Biomed. Eng.*, 47(8), 2000, 985-996.
- [12] N. Strock, M. Ericson, L. Lindblad, and M. Jensen-Urstad, Automatic computerized analysis of heart rate variability with digital filtering of ectopic beats, *Clinical Physiology*, 21(1), 2001, 15-24.
- [13] J. Mateo and P. Laguna, Analysis of heart rate variability in the presence of ectopic beats using the heart timing signal, *IEEE Trans. Biomed. Eng.*, 50(3), 2003, 334-343.
- [14] D. Singh K. Vinod and S. C. Saxena, Sampling frequency of the RR interval time series for spectral analysis of heart rate variability, *Journal of medical Engineering & Technology*, 28(6), 2004, 263-272.
- [15] D. Litvack, T. Oberlandern, L. Carney, and J. Sau, Time and frequency domain methods for heart rate variability analysis: A methodological comparison, *Psychophysiol.*, 32, 1995, 492-504.
- [16] I. Mitov, A method for assessment and processing of biomedical signals containing trend and periodic components, *Med. Eng. Phys.*, 20, 1998, 660-668.
- [17] U. Wiklund, M. Akay, and U. Niklasson, Short-Term Analysis of Heart-Rate Variability by Adapted Wavelet Transforms, *IEEE Engineering in Medicine and Biology*, 16(5), 1997, 113-118.
- [18] D. Singh, K. Vinod, S. C. Saxena and K. K. Deepak, Effects of RR segment duration on HRV spectrum estimation, *Physiol. Meas.*, 25, 2004, 721-735.