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Multi-parametric Analysis of Heart Rate Variability with Novel Entropy Based Measures in Ex-preterm Babies

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Contents

List of Figures

List of Tables

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Sommario

Introduzione

La morte infantile improvvisa (Sudden Infants Death Syndrome, SIDS) è una delle principali cause di mortalità infantile nei paesi sviluppati. La prima definizione è stata data da Bergman nel 1970 [1], il quale sosteneva che la SIDS non dipendesse da un'unica causa che conduce il bambino alla morte, ma da un'interazione di fattori di rischio con probabilità variabili, la cui concomitanza può portare a tale sindrome. A causa del drammatico impatto sui genitori, questa sindrome è stata a lungo studiata, ma ad oggi i meccanismi fisiologici alla sua base non son ancora stati chiariti.

Al momento la spiegazione più supportata in letteratura è la recente ipotesi del *triplo rischio* (*triple risk hypothesis*), ideata da Filiano e Kinney [3]. Tale ipotesi individua rischi intrinseci ed estrinseci per il neonato e afferma che un bambino è a rischio SIDS quando esposto a tutti e tre i seguenti fattori: vulnerabilità del neonato (ad esempio a causa della prematurità), essere in un periodo di sviluppo critico (il primo anno di vita, specialmente tra il secondo e il quarto mese) e l'azione di fattori esogeni di stress (ad esempio la posizione prona durante il sonno).

Alla luce di questi aspetti, il presente studio si propone di analizzare una popolazione di bambini ex-prematuri valutando l'impatto della posizione del sonno sui meccanismi di controllo che agiscono sul ritmo cardiaco.

Prematuro è definito ogni bambino nato prima della 37esima settimana di gestazione, la prematurità può poi essere suddivisa in estrema, precoce e tardiva. Si parla di estrema prematurità nel caso di bambini nati prima di 26 settimane di gestazione, precoce tra le 26 e le 34 settimane ed infine tardiva per i nati tra le 34 e 37 settimane. La condizione di prematurità espone precocemente il neonato all'ambiente esterno prima che il suo Sistema Nervoso Autonomo (Autonomic Nervous System, ANS) sia completamente sviluppato e in grado di affrontare efficacemente la regolazione di alcuni meccanismi fisiologici, come la respirazione e la termoregolazione, comportando perciò una maggior vulnerabilità nei soggetti nati prematuri.

Per quanto riguarda un possibile fattore esterno di rischio, la posizione del neonato

durante il sonno è stata intensamente studiata fino a giungere nel 1994 ad una campagna proposta dal National Institute of Child Health and Development (NICHD) negli USA, nota come "Back to Sleep" Campaign. L'obiettivo era di raccomandare che i neonati fossero posizionati in posizione supina a dormire, con il presupposto che la posizione prona potesse contribuire all'origine di episodi di SIDS. Una possibile spiegazione è che i bambini dormano più profondamente in tale posizione, risvegliandosi meno frequentemente. La capacità di svegliarsi durante il sonno è un importante meccanismo di sopravvivenza che, se compromesso, potrebbe portare alla SIDS. L'ipotesi che la posizione prona sia una condizione ulteriore di rischio per il neonato è stata avvalorata dal fatto che, a seguito della campagna del NICHD, il tasso di SIDS negli Stati Uniti è diminuito di più del 50% in 10 anni.

Grandi fattori di rischio per la SIDS, quali, per esempio, essere in un periodo di sviluppo critico, come il primo anno di vita, e la prematurità sono condizioni entrambe caratterizzate da un ANS ancora immaturo nel bambino. Questa considerazione suggerisce che l'ANS giochi un ruolo centrale nei meccanismi che possono innescare la SIDS, ci si propone perciò in questo studio di analizzarne il comportamento.

L'ANS è uno dei sistemi che concorrono al mantenimento dell'omeostasi del nostro organismo; esso, grazie alle informazioni inviate da chemocettori e barocettori localizzati nel sistema circolatorio, agisce regolando il battito cardiaco (Heart Rate, HR) e la pressione sanguigna (BP). L'ANS è suddiviso in due rami, simpatico e parasimpatico, che in genere agiscono in modo anatagonistico, la loro azione sul cuore è infatti descritta come bilancia simpato-vagale. Questi rami sono sempre attivi e agiscono contemporaneamente sul cuore, ma con differenti costanti di tempo. La stimolazione del ramo simpatico è di solito guidata dal neurotrasmettitore noradrenalina, i cui effetti includono l'aumento di battito cardiaco, di pressione sistolica (SAP), di contrattilità cardiaca, di velocità di conduzione, di frequenza respiratoria, di vasocostrizione e di molte altre funzioni fisiologiche. L'azione del ramo parasimpatico è invece mediata dall'acetilcolina ed è caratterizzata, ad esempio, da diminuzione di HR e volume sistolico, da costrizione coronarica e diminuzione della SAP.

È possibile accedere indirettamente all'ANS osservando una delle variabili da esso controllate, l'HR.

Heart Rate Variability (HRV) è lo studio delle oscillazioni ritmiche nella frequenza cardiaca che sono attribuibili a modulazioni simpatiche e parasimpatiche della funzione cardiovascolare da parte dell'ANS.

In questo studio sono state effettuate misure tradizionali di HRV nel dominio del tempo e della frequenza, ed inoltre sono stati estratti indici da analisi non-lineari con la proposta di nuove misure basate sul calcolo di Entropia.

Materiali e Metodi

Questa tesi sviluppa e approfondisce un precedente progetto del 2013 [27] svoltosi presso il Columbia University Medical Center.

Il dataset analizzato è un follow up composto da 24 bambini, tra i 37 partecipanti al primo studio, la cui età gestazionale è in media di 27.9 ± 1.6 settimane, mentre l'età post menstruale è di 49.16 ± 3.21 settimane. La durata media delle acquisizioni è di 60 minuti, nei quali i bambini sono stati posizionati a dormire per 30 minuti circa in posizione supina e poi per altri 30 minuti circa in posizione prona. Di questi 30 minuti, la prima metà è di baseline, mentre nella seconda metà è stata eseguita una serie di tilt.

La popolazione del primo studio invece comprendeva tutti i 37 neonati prematuri, la cui età post-mestruale al momento dello studio era invece di 35,7 settimane. Lo studio per ogni bambino aveva una durata di 6 ore: durante le prime 3 i bambini erano in posizione supina, e nelle 3 successive erano in posizione prona. Due sequenze di tilt sono state eseguite in ciascuna posizione all'inizio delle 3 ore.

Gli stati del sonno sono stati codificati a mano su apposite cartelle da clinici esperti ogni 30 secondi nel primo studio e ogni minuto nel secondo. La misurazione dell'ECG è stata eseguita con un cardiofrequenzimetro standard (Hewlett Packard 3680) con frequenza di campionamento a 500 Hz.

La fase di pre-processing ha permesso di ottenere le serie temporali di intervalli RR importando il segnale ECG in un sistema di analisi, chiamato GMark (proprietà della Columbia), in grado di selezionare automaticamente i picchi R nel tracciato, mediante un algoritmo di pattern recognition. Questo programma permette inoltre di eseguire una facile ispezione visiva del segnale, al fine di correggere eventuali riconoscimenti mancanti o identificati in modo scorretto.

Per ogni bambino, ogni minuto è stato codificato in: Sonno Attivo (Active Sleep, AS), Sonno Quieto (Quiet Sleep, QS), Allattamento (Feeding), Sveglio (Awake) o Indeterminato.

Dato che i neonati spendono più tempo in AS, sono stati trovati più segnali classificati in questo stato. Sono state quindi analizzate le Baseline in AS (Baseline Active, BA) in posizione supina (Baseline Supine Active, BSA) e in posizione prona (Baseline Prone Active, BPA).

Il dataset finale per l'analisi è composto da 10 dei 24 pazienti iniziali, di cui ogni BA è stata poi suddivisa in segmenti da 3 minuti di lunghezza, usati per le successive analisi.

Tre tipologie di metodi sono state considerate per questo studio: calcolo di indici nel dominio del tempo, della frequenza e indici derivati da approcci non lineari.

Dal momento che la prematurità è una condizione collocata a metà tra la vita fetale e neonatale si è deciso, coerentemente con il primo studio, di utilizzare indici nel dominio del

tempo che provengono sia da analisi effettuate su adulti, sia analisi nell'ambito fetale. Sono stati utilizzati i parametri proposti da Malik et. al [17] per il dominio del tempo: la media dell'intervallo RR, la deviazione standard dell'intervallo NN (SDNN) e la radice quadrata della media dei quadrati delle differenze di intervalli successivi NN (RMSSD). I parametri dell'analisi HR fetale sono invece [34]: indice di variabilità a lungo termine (LTV), indice di variabilità a breve termine (STV), indice di intervallo (II) e il successivo sviluppo di Arduini (IIa), indice differenziale (DI), radice quadrata media delle differenze dall' intervallo medio (RMSM) ed indice di irregolarità a lungo termine (LTI) .

Per l'analisi in frequenza i segmenti da 3 minuti sono stati ricampionati con spline cubiche a 10 Hz, la densità spettrale di potenza (Power Spectral Density, PSD) è stata poi stimata attraverso il metodo non-parametrico di Welch con finestre di 1 minuto e overlap del 50%. È stata successivamente calcolata la potenza nelle bande di bassa frequenza (low frequency, LF) e alta frequenza (high frequency, HF), che sono state spesso associate nell'adulto rispettivamente ai sistemi simpatico e parasimpatico [17]. Poiché la regolazione cardiovascolare del neonato differisce da quella dell'adulto, vi è quindi la necessità di modificare le bande di potenza proposte per gli adulti. In questa tesi si è deciso di seguire il lavoro svolto nel primo studio impostando perciò la banda HF tra 0,15-1,40 Hz e la banda LF, invece, tra 0,03-0,15 Hz [27].

È stata inoltre verificata l'occorrenza del *cardiac aliasing*, poiché nel neonato può alterare significativamente la PSD del segnale [26]. Il cardiac aliasing è una distorsione della PSD che si verifica quando l'HR è "troppo lento" per campionare la respirazione, ossia quando la media della frequenza respiratoria supera la metà dell'HR.

Il primo fra i metodi non lineari implementati è la Phase-Rectified Signal Averaging (PRSA). Questa metodologia fu definita originariamente da Bauer et al. [32]. La PRSA serve per quantificare le quasi-periodicità del segnale spesso nascoste dalla natura non-stazionaria del segnale stesso e dal rumore. E' possibile studiare le periodicità del segnale di ritmo cardiaco per accelerazioni e decelerazioni del segnale a differenti scale temporali.

Per quanto riguarda altri metodi non lineari sono state implementate diverse misure di Entropia. L'Entropia è una misura di regolarità e complessità di un segnale, in termini di quantità di generazione di informazione. Si associano a maggiori valori di Entropia più casualità, meno ordine e minore complessità. La stima del valore di Entropia si basa sul calcolo del numero template di lunghezza m che combaciano (*match*) entro una tolleranza r per $m+1$ campioni.

Le misure di Entropia incluse in questo studio sono: Approximate Entropy, Sample

Entropy, Quadratic Sample Entropy, Multiscale Sample Entropy e Permutation Entropy.

L'Approximate Entropy (ApEn) fu proposta da Pincus nel 1994 per calcolare l'Entropia approssimata su segnali con rumore e di lunghezza finita [36], dimostrando delle potenzialità applicative in ambito fisiologico neonatale [37, 35].

Per risolvere alcuni limiti nel calcolo della ApEn, è stata introdotta la Sample Entropy (SampEn). La SampEn elimina i self-matches presenti nel calcolo dell'ApEn, presenta una migliore indipendenza dalla lunghezza del segnale ed inoltre ha una consistenza relativa che la ApEn invece non presenta.

Per ridurre la dipendenza da parametro r è stato introdotta un'evoluzione della SampEn, ossia la Quadratic Sample Entropy (QSE).

Si è proposto in letteratura di convertire la probabilità condizionale stimata ad una densità di probabilità normalizzando il numero dei *matches* per il volume della regione di *matching* [41]. Questo si traduce nel passaggio di aggiungere $ln(2r)$ al valore di SampEn. Questa procedura ha mostrato buone potenzialità nel migliorare la distinzione tra una popolazione sana da una popolazione affetta da fibrillazione atriale [41].

Nel 2014 è stata proposto da E.M. Cirugeda-Roldán et al. [42] un metodo per il calcolo della QSE particolarmente adatto per segnali di breve durata. In questo metodo viene innanzitutto introdotto un contatore, come proposto in [30, 41], che consente di avere un numero minimo di *matches M*. M viene poi fatto opportunamente variare e viene scelto l'M che meglio distingue le due condizioni (Supino-Prono) mediante il calcolo di curve ROC. Il vantaggio di questo approccio è che la tolleranza r viene automaticamente impostata per avere un numero minimo di matches M, che ha garantito una stima più consistente del valore di Entropia anche per segnali molto brevi, con soli 50 samples [42].

Un diverso approccio è quello proposto da Costa et al. [43] ed è la Multiscale Sample Entropy (MSE). Questa misura consiste nel calcolo della SampEn su cosiddette serie temporali *coarse-grained* ("a grana grossa"), ottenute mediando le serie temporali su finestre di dimensione crescente. Queste serie permettono di calcolare l'Entropia su scale temporali maggiori, da qui il nome *multiscale*. La MSE viene rappresentata in grafici con curve dei valori SampEn in funzione della scala temporale.

L'ultimo indice di Entropia calcolato è la Permutaion Entropy (PermEn). Questa misura, differentemente dalle precedenti, è basata su un approccio ordinale (o simbolico) e per il suo calcolo è stato seguito l'approccio presente in [44, 46]. Il procedimento prevede di estrarre finestre di campioni consecutivi del segnale aventi lunghezza D, e di catalogarle secondo la loro ampiezza relativa. Si calcola la distribuzione di probabilità delle possibili D! combinazioni di campioni e da essa si estrae la misura di Entropia.

Risultati ed analisi statistica

La prima parte del lavoro di tesi si è focalizzata sulla valutazione dell'andamento del battito cardiaco in funzione dell'età post-mestruale (PMA) tra primo e secondo studio nei 10 bambini, al fine di valutare se questi segnali potessero essere rappresentativi della popolazione generale. L'HR mostra una decrescita all'aumentare della PMA, in un periodo che va dalla 33esima (primo studio) alla 53esima (follow up) settimana, sia nella posizione Supina sia in quella Prona.

In seguito sono stati calcolati per ogni Baseline in sonno Active (Baseline Active, BA) i parametri precedentemente descritti al fine di confrontare gli indici per le due posizioni.

È stata poi eseguita l'analisi di significatività statistica usando il test dei ranghi con segno di Wilcoxon (*Wilcoxon Signed-Rank Test*). È un test non-parametrico per popolazioni con campioni accoppiati, il livello di significatività impostato è $\alpha = 5\%$.

Nell'analisi del tempo i parametri che mostrano significatività, ovvero sono in grado di distinguere Supino e Prono, sono: il valor medio dell'RR (Mean), RMSSD, STV e DI. Tutti i parametri mostrano valori maggiori nella posizione Supina rispetto la Prona.

È stata poi studiata la correlazione tra questi parametri, provando che tutti e quattro sono correlati. Questo aspetto, per gli ultimi tre indici, è ragionevole poiché stimano tutti la variabilità nel breve termine.

Per quanto riguarda l'analisi nel dominio delle frequenze, entrambi i parametri valutati, la potenza nelle bande HF e LF, non permettono una consistente distinzione tra le due posizioni. Il test statistico è infatti non significativo.

Nell'analisi non-lineare, utilizzando il metodo della PRSA, il numero limitato di samples a disposizione in ciascuna finestra di 3 minuti, non ha permesso di ottenere un numero adeguato di curve PRSA da mediare. Non è stato possibile perciò eliminare sufficientemente il rumore nelle curve di periodicità, motivo per cui i risultati non sono significativi.

Per quanto riguarda le misure di Entropia di ApEn e di SampEn, implementate sulle finestre di 3 minuti del dataset, si evince dall'analisi statistica che non si ha una significativa distinzione tra Supino e Prono.

Successivamente è stato calcolato per $m = \{1,2,3\}$ il numero minimo di matches migliore M_{ottimo} che consente di calcolare il valore di QSE che permette di distinguere le due popolazioni Prono e Supino con più significatività. Il parametro M_{ottimo} è risultato essere uguale a 4'000, 8'000 e 16'000 rispettivamente per la dimensione *embedded* $m = \{1,2,3\}$. La dimensione embedded più significativa è $m = 2$ ($p<0.01$) mentre per $m = 1$ e $m = 3$ $p<0.05$.

Questo procedimento ha mostrato dei miglioramenti significativi quando paragonato alla SampEn. Si sono studiate infatti le curve di SampEn e QSE in funzione sia del minimo numero di matches M, sia della lunghezza delle serie temporali (N) per le posizioni Prono e Supino. Si è visto che le le curve di QSE Prono vs. Supino sono più separabili rispetto alle curve di SampEn per un maggior range di M. Studiando poi l'andamento di SampEn e QSE in funzione di N, si può osservare che la QSE assicura un calcolo di Entropia più consistente per più ampi valori di N rispetto a SampEn.

Lo stesso parametro di QSE è stato successivamente calcolato per ulteriore verifica anche sui segnali del primo studio, mostrando anche in questo caso, alta significatività e dando valori di M_{ottimo} pari a 18'000, 44'000 e 20'000, sempre per $m = \{1,2,3\}$.

Per quanto riguarda il metodo della MSE nessun risultato significativo è stato ottenuto, a causa del limitato numero di samples che inficia il processo di media utilizzato per calcolare le serie coarse-grained.

Anche per la PermEn non si sono ottenuti risultati significativi.

È stata poi calcolata la cross-correlazione lineare tra tutti i parametri significativi di questo studio, trovando un'elevata correlazione (>0.9) tra la Media di RR e la QSE.
In seguito è stato svolto un confronto tra il primo studio e il follow up per gli indici calcolati, al fine valutarne l'andamento di crescita o decrescita.

Per quanto riguarda i parametri del dominio del tempo si è riscontrata una crescita significativa di tutti i parametri, per ciascun paziente, dal primo studio al follow up. L'analisi statistica è significativa per tutti gli indici tranne che per l'indice di IIArduini. Anche per quanto riguarda l'analisi delle frequenze, entrambi i parametri di potenza nelle bande HF e LF mostrano una crescita dal primo al secondo studio. Analoga discussione può essere fatta per i parametri di Entropia: la ApEn e la SampEn crescono, fatta eccezione per la dimensione $m = 3$. Anche la QSE presenta valori maggiori per il follow up in ciascuna embedded dimension.

Discussione

Per quanto riguarda l'analisi nel dominio del tempo, sono stati utilizzati parametri provenienti sia dall'analisi fetale, sia da analisi effettuate sugli adulti. Questa scelta si basa sul fatto che la prematurità dei bambini costituenti il dataset è una condizione transitoria tra questi due stadi di maturazione.

I parametri che sono risultati significativi sono: la Media di RR, RMSSD, STV e DI, che generalmente mostrano valori maggiori nella posizione Supina rispetto a quella Prona.

Questi quattro parametri sono tutti rappresentativi di variazioni a breve termine, ad eccezione della Media.

Nel primo studio i parametri più significativi risultavano essere quelli legati a variazioni a lungo termine, questo aspetto suggerisce forse una prevalenza del ramo simpatico, mentre nel follow up la maggior significatività di quelli a breve termine può essere un effetto della maturazione del ramo parasimpatico.

Dalla letteratura è noto che il ramo simpatico è il primo a svilupparsi durante la gravidanza, mentre il parasimpatico si sviluppa successivamente e continua a maturare durante i primi mesi di vita [22].

Questo ramo dell'ANS controlla la decrescita del HR ed ha un controllo definito come beat-to-beat, la cui alta velocità di risposta si manfiesta con un rapido controllo del HR e quindi nei parametri a breve termine.

Come descritto in letteratura [15], anche in questo studio la Media del tacogramma (l'inverso del HR) è generalmente maggiore nella posizione Supina rispetto quella Prona.

Per quanto riguarda il RMSSD, questo parametro cresce dal Supino al Prono, implicando che nella posizione Supina c'è un maggior grado di dispersione attorno alla media, ovvero una maggior variabilità. La minor varianza nella posizione Prona potrebbe essere legata ad una predominanza di controllo da parte del sistema simpatico, evidenziando un possibile squilibrio nel controllo dell'ANS.

Dal confronto tra i due studi è stato possibile osservare un aumento di tutti i parametri, ad eccezione del IIArduini, per tutti i pazienti, dal primo studio al follow up, associato probabilmente ad una maturazione dell'ANS in concomitanza con il crescere della PMA.

Nel dominio delle frequenze è stato scelto il metodo non parametrico di Welch per stimare la densità spettrale dei segnali (PSD). Gli indici estratti dalla PSD per l'analisi sono la potenza nelle bande HF e LF. Essendo varie le proposte in letteratura su quale sia la scelta di tali bande, in questa tesi si è deciso di settare gli intervalli analogamente al precedente studio.

Questa analisi non ha portato a conclusioni significative, non riuscendo a distinguere le popolazioni Supina e Prona.

Dal confronto tra i due studi i parametri mostrano una crescita dal primo studio al follow up, sia nel confronto tra le popolazioni Supine che tra quelle Prone. Questo aspetto potrebbe essere legato ad una regolazione fisiologica più stabile.

Essendo i valori nel follow up superiori rispetto al primo studio, i risultati possono essere interpretati come l'effetto di uno sviluppo dell'ANS nei bambini più maturi, come visto anche nella precedente analisi.

I risutati ottenuti con questo metodo possono essere parzialmente spiegati considerando due aspetti: il primo è che in generale ci si aspetta che il respiro nei neonati sia molto debole e irregolare, rendendo difficile l'individuazione del picco in HF, inoltre è stata provata in alcuni soggetti l'occorrenza del *cardiac aliasing*, altro fenomeno che può inficiare l'analisi spettrale. Questo fenomeno porta in soggetti che mostrano una certa regolarità nella respirazione (sotto forma di picco nella banda HF dello spettro del respiro) a nascondere questa regolarità nello spettro del HR. Inoltre esso rende più difficoltosa la scelta delle bande per il calcolo delle potenze. Senza un'opportuna analisi del segnale di respiro non è possibile verificarne l'occorrenza e questo può inficiare l'analisi spettrale per i soggetti in cui si è presente.

Riguardo l'analisi dell'Entropia, sia la ApEn sia la SampEn sono state implementate ma non sono stati trovati risultati significativi. Questo aspetto potrebbe essere legato all'insufficiente numero di campioni per spezzone, infatti nel primo studio si sono ottenuti risultati significativi considerando finestre da 1500 campioni, ossia circa 12 minuti.

Perciò è stata introdotto il metodo basato sulla QSE con il minimo contatore di matches. Questo metodo è meno sensibile alla lunghezza del segnale, rivelando una migliore consistenza quando confrontato con la SampEn. La novità introdotta con questo algoritmo consiste in un metodo innovativo e ripetibile di stima del numero di matches minimo ottimale, che consente di calcolare il valore di Entropia associato che meglio distingue le due condizioni analizzate. In più, questa misura non necessita della selezione manuale dalla tolleranza r, che viene automaticamente impostata. La QSE permette una migliore separazione tra le popolazioni Supina e Prona in modo statisticamente significativo. In particolare il valore di Entropia di QSE nella posizione Supina è stato trovato maggiore rispetto alla posizione Prona. Bassi valori di Entropia sono solitamente associati ad una maggior predicibilità e minor complessità del segnale HR. Ciò suggerisce una diversa regolazione del HR nei neonati in questa posizione e può essere un indice del maggior rischio di SIDS.

Dal confronto tra i due studi si può osservare che tutti i parametri di Entropia calcolati crescono, per ciascun paziente, sia nella posizione Supina che in quella Prona. Valori più alti di Entropia corrispondono generalmente a maggior casualità ed impredicibilità, valori più bassi invece ad un maggior riconoscimento di pattern. La crescita dei valori di Entropia, con la crescita della PMA, può essere interpretato come un segno della maturazione dell'ANS in questi bambini, difatti, diventando più efficiente il loro sistema di controllo, essi mostrano una maggiore HRV.

I risultati ottenuti in questa tesi sono promettenti e sarebbe opportuno estendere l'analisi ad una popolazione più ampia, per avere conferma di quanto riscontrato.

A causa della necessità di finestre di almeno 3 minuti, in questo studio è stato analizzato solo il sonno Active per le Baselines, dovendo ignorare per mancanza di dati il sonno Quiet e le fasi di tilt. Un ulteriore sviluppo di questo lavoro sarebbe quello di acquisire registrazioni più lunghe, attraverso nuovi protocolli sperimentali, per avere la possibilità di confrontare lo stato Active e Quiet e le Baselines Quiet in posizione Supina e Prona, implementando in queste nuove analisi il nuovo metodo basato sulla QSE.

Essendo la posizione Prona uno dei principali fattori di rischio di sviluppo di SIDS, il metodo della QSE applicato ai bambini nati a termine e pre-termine potrebbe consentire di individuare una possibile condizione patologica latente che potrebbe portare alla SIDS.

Il segnale della respirazione è stato utilizzato in questo lavoro per valutare l'impatto della debole e irregolare respirazione neonatale e il *cardiac aliasing* sull'analisi spettrale. Un'analisi più approfonddita e più estesa è inoltre già atto per confermare i risultati ottenuti. Altri segnali, come la saturazione di ossigeno nel sangue e la pressione sanguigna, possono essere integrati con l'analisi dell'HR, per costruire un classificatore multi-parametrico che valuti eventuali cambiamenti nella condizione fisiologica dei bambini.

L'obiettivo finale è quello di ottenere uno strumento clinico utile per aiutare l'equipe medica nella diagnosi di una condizione rischiosa per il bambino, che potrebbe evolvere in un episodio di SIDS.

Summary

Introduction

Sudden Infants Death Syndrome (SIDS) is one of the leading causes of infant mortality in developed countries. The first definition was given by Bergman in 1970 [1], who argued that SIDS did not depend on a "single characteristic that ordains an infant for death," but on an interaction of risk factors with variable probabilities. Because of its dramatic impact on parents, this syndrome has been studied for long, but still nowadays the underlying physiologic mechanisms have not been cleared yet. At the moment, the most supported explanation in literature is the triple risk hypothesis, proposed by of Filiano and Kinney [5]. It takes into account intrinsic and extrinsic risks for the newborn and states that an infant is more likely to die of SIDS if he/she possesses all three factors: to be a vulnerable infant (for example due the prematurity), to be in a critical developmental period (like the first year of life, especially between the second and the fourth month of life) and the action of an exogenous stressors (for example the prone sleep position).
In light of this issue, the present study proposes to analyze a population of ex-preterm babies, evaluating the impact of sleep position on the control mechanisms that act on the heart rate (HR).

Any infant born before the 37^{th} weeks of gestation is defined as premature, then they can be further divided into extremely, early and late preterm birth with those infants being born before 26 weeks of gestation being regarded as extremely preterm, between 26 and 34 weeks of gestation regarded as early preterm and those born between 34 and 37 weeks regarded as late preterm. Prematurity sets the newborn to be exposed to the outer environment before his autonomic system is fully developed and able to effectively face life challenging situations, like breathing and thermoregulation; a preterm baby is understandably more vulnerable than a full term baby.

The sleep position as an external risk factor was investigated by numerous studies that lead in 1994 to a campaign proposed by the National Institute of Child Health and Development (NICHD) of the USA, named "Back to Sleep", in order to recommend that the babies were put supine while sleeping, with the assumption that the prone position could

increase the incidence of SIDS. A possible explanation is that infants sleep more deeply in that position and arouse less easily. The ability to arouse from sleep is an important survival mechanism, which if impaired may lead to SIDS. Prone position proved to be one major risk for SIDS by the fact that following the NICHD campaign, the rate of incidence of SIDS in the US decreased of more than 50% in 10 years.

Major risk factors for SIDS include, for instance, being in a critical developmental period and prematurity. These conditions are both characterized by a still growing Autonomic Nervous System (ANS) in the infant, this points at the fact that ANS plays a central role in SIDS, thus its behavior is investigated in this study.

The ANS is one of the systems that aim to the maintenance of the homeostasis of our organism, through the information gathered with chemoreceptors and baroreceptors located in the circulatory system it can induce variations in the heart rate (HR) and blood pressure (BP). ANS is divided in two branches, sympathetic and parasympathetic, that generally act antagonistically. Their action on the heart is described as sympatho-vagal balance. They are always active and the heart receives simultaneously signals from both systems, but with different time delays. Stimulation of the sympathetic nerves is usually driven by norepinephrine, and its effects include the increase of HR, systolic arterial pressure (SAP), cardiac contractility, conduction velocity, respiratory frequency, vasoconstriction and many others. The parasympathetic action is instead mediated by acetylcholine and it is characterized by the decreasing of the HR and of the systolic volume, coronary constriction, decrease of SAP and others.

It is possible to access indirectly the ANS by observing one of the variables that it controls, the HR. Heart Rate Variability (HRV) is an non-invasive methodology to study rhythmical oscillations embedded in heart period and blood pressure time-series, which represent the sympathetic and parasympathetic modulations of the cardiovascular function.

In this study have been computed measures coming from traditional HRV, in the time and frequency domain, and also other indices extracted from non-linear analysis and new entropy-based measures.

Materials and Methods

This thesis follows and develops a 2013 previous project [27] made at the Columbia University Medical Center.

The dataset of this research is a follow up composed of 24 babies (out of 37 of the first study), whose Post-Menstrual Age (PMA) at the time of the study was on average 49.16 ± 3.21 weeks and whose gestational age (GA) is on average 27.9 ± 1.6 weeks. The

mean duration of the acquisitions is 60 minutes, the babies were put to sleep for 30 minutes firstly in supine position and for other 30 minutes in prone position. For each position a 10-15 minutes long baseline was recorded in the first half of the 30 minutes, and then a tilt sequence was performed.

The population of the first study instead is composed of 37 premature infants, whose GA is on average 28,7 weeks, while PMA at the time of the study is 35,7. Each study was 6 hours long, during the first 3 hours babies were in the supine position, and for the remaining 3 hours they were turned into prone. Two tilt sequences for each position were performed, at the beginning of the three hours.

For both the first and the follow up studies sleep states were coded by expert clinicians every minute and for both the focus was on the ECG while only in the follow up also a brief analysis of the respiration was done. Measurement of the ECG is obtained from a standard HR monitor (Hewlett Packard 3680) with a 500 Hz sampling frequency.

The pre-processing phases allowed to obtain RR interval time series by importing the ECG tracks in a waveform marking and analyzing software, named Gmark (Columbia's property), capable of automatically recognizing the R peaks thanks to a patter recognition algorithm. This program let a fast visual inspection of the signal, in order to correct the missing or wrongly identified peaks.

For each baby the sleep state of every single minute is coded in: Active Sleep (AS), Quiet Sleep (QS), Feeding, Awake or Indeterminate. The most numerous signals were the one classified Baseline in AS (BA) in Supine position (Baseline Supine Active, BSA) and in Prone position (Baseline Prone Acrtive, BPA). This can be explained by the fact that newborns spend more time in AS during sleep than in QS. These consideration resulted in a dataset comprising 10 patients out of the original 24. Every BA was then divided in segments of 3 minutes length for the successive analysis.

Three types of methods were considered for this study: indices computed in the time and frequency domain analysis and indices derived form non-linear approaches.

Since prematurity is a condition in between fetal and newborn life in the time domain analysis parameter both from the fetal and the adult fields has been used, accordingly with the first study. It has be used parameters proposed by Malik et al. [17] for the time domain: the mean of the NN interval, the standard deviation of the NN interval (SDNN) and the square root of the mean squared differences of successive NN intervals (RMSSD). The typical parameters of the fetal HR analysis are instead long term variability index (LTV), short term variability index (STV), interval index (II) and its evolution the interval index of Arduini (IIa), differential index (DI), root mean square of differences from the mean interval (RMSM) and the long term irregularity index (LTI).

For the frequency analysis the 3 minutes segments are resampled with cubic spline at 10 Hz, than the power spectra density (PSD) is estimated with the non parametric Welch method, with 1 minute windows and 50% overlap. The two measures used to investigate the sympatho-vagal balance in adults, are Low Frequency (LF) and High Frequency (HF) power bands [17]. Since the cardiovascular regulation of the infant differs from the one of the adult, these bands need to be adjusted properly, so it is decided to follow the choice of the first study, setting the HF band to 0.15–1.40 Hz and the LF band to 0.03–0.15 Hz [27]. It is then investigated the occurrence of *cardiac alisaing*, that is a distortion of the PSD occurring when HR is "too low to sample" respiration (i.e. when the mean respiration rate exceeds half of the mean heart rate). This phenomenon may occur in newborns, altering the PSD estimate [26].

Phase-Rectified Signal Averaging (PRSA) is the first non-linear methodology implemented in this study. This methodology was originally defined by Bauer et al. [32]. PRSA is useful to quantify quasi-periodicities of the signal often hidden by the non-stationarity and noise. It is possible to study the periodicities of the HR for accelerations and decelerations at different time scales.

Regarding other non-linear methodologies, Entropy measures are implemented. Entropy is a measure of regularity and complexity of a signal, in terms of the rate at which information is generated. Entropy is based in the computation of the number of patterns of length m that match within a tolerance r for $m+1$ samples. The following measures of entropy are included in this study: Approximate Entropy, Sample Entropy, Quadratic Sample Entropy, Multiscale Sample Entropy and Permutation Entropy.

Approximate Entropy (ApEn) was proposed by Pincus in 1994 to provide applications on real, noisy and finite signals [36]. ApEn has demonstrated it capabilities in the clinical field, in applications on the HR both for diagnosis and predictions [37, 35]. It discriminated groups of subjects, in instances where classical statistical methods, such mean and standard deviation, did not show distinction [36]. To overcome the ApEn limits, Sample Entropy (SampEn) was then proposed. The advantages of the SampEn are that eliminates self-matches, it is a simpler parameter to calculate, has less dependence on record length and has relative consistency where ApEn has not.

To reduce the dependence on the tolerance r, in literature it was proposed an evolution of SampEn, that is the Quadratic Sample Entropy (QSE). It was suggested to convert the condition probability estimated into the density by normalizing the number of matches for the volume of the matching region [41]. This is done by adding $ln(2r)$ to the value of SampEn. This measure has proven its potential by improving the distinction between a healthy population and one affected by atrial fibrillation [41]. To improve the robustness of QSE for short signals a minimum numerator count is then introduced by Lake and Moorman.

In 2014 E.M. Cirugeda-Roldán et al. [42] proposed a method that combines a numerator counter with a way to compute the minimum number of matches. The advantage of this approach is that tolerance *r* is automatically set to achieve a minimum number of pre-defined matches M. Then the best M ($M_{optimum}$) is chosen as the one that has the greatest Area Under the Curve (AUC) of the ROC curves built to classify the two conditions (Healthy-Sick, Supine-Prone).

A different approach is the one developed by Costa et al. [43] is the Multiscale Sample Entropy (MSE). It is based on the computation of coarse-grained time series, averaging windows of the signal of increasing width. These time series allows to compute entropy on greater times scales by displaying the values of entropy in function of the time-scale itself.

The last index of Entropy computed is Permutation Entropy (PermEn). This Entropy measure differs from the others because it is based on an ordinal or symbolical approach [44, 46]. The method consists in classifying different windows of same length D based on their relative amplitude, computing the probability distribution of all the D! possible combination of the samples and lastly extracting from it the value of entropy.

Results and Statistical analysis

The first step of the work is to make an analysis of variation of the HR in function of the post-menstrual age (PMA) of the 10 babies, to assess if these recordings could exemplify a general population. The HR shows a decrease with the increase of the PMA from the 33rd week (first study) to the 53rd week (follow up), both for the Supine and Prone position.
Then for each Baseline in Active sleep (Baseline Active, BA) are computed the previously described parameters in order to compare their values in the Supine and Prone sleep position. They will be addressed as Baseline Supine Active (BSA) and Baseline Prone Active (BPA). The statistical analysis is done with a non-parametric test, the Wilcoxon Signed-Rank for paired samples, with a level of significance of 5%.

In the time domain analysis the parameters that show significance are: the Mean of the RR, RMSSD, STV and DI. All these parameters show higher values for the Supine than the Prone position. It is then studied the linear correlation among these parameters, finding that all four are highly correlated (>0.9). This is reasonable for RMSSD, STV and DI because they are all short term variability indices.

Regarding the frequency domain analysis, both the HF and LF power does not have a consistent difference for the Supine or Prone position, the statistical test show no significance. It has been proven for some subjects the occurrence of cardiac aliasing.

In the non-linear analysis, the PRSA methodology, due to the limited number of samples of each 3 minutes window, did not allow to obtain a sufficient number of PRSA curve to be averaged. It was not possible then to remove enough noise to compute the indices on the periodicity curves.

The ApEn and SampEn measures are implemented are not found significant in the distinction of the sleep position.

It is then computed for $m = \{1,2,3\}$ the optimal minimum number of matches ($M_{optimum}$) that allows the computation of the QSE that best separates the Supine and Prone positions. The optimum number of matches resulted for m=1 is 4'000, for m=2 8'000 and for m=3 16'000. The most significant embedded dimension is m=2 ($p<0.01$) while the other have $p<0.05$. This procedure leads to significative improvements when compared to the simple SampEn. Afterwards the curves of SampEn and QSE in function of both the number of matches M and the signal length N are studied, comparing the supine and prone curves. The QSE curves Supine vs. Prone are more distant with respect to the one of SampEn for a larger range of M, suggesting a greater consistence of QSE. The trend of the QSE and SampEn as a function of the length of the signal (N), instead, shows that the QSE is more robust for small N, while the curves of SampEn are more noisy as the Supine and Prone curves are mainly overlapped for short lengths. The same parameter is then tested on the signals of the first study, showing also in this case, high significance and giving a $M_{optimum}$ for the embedded dimension m = 1 of 18'000, for m = 2 of 44'000 and for m = 3 of 20'000.

It is then performed a comparison between the Baseline Supine Active of the first study and the follow-up study and between the Baselines Prone Active, in order to see their evolution in two months.

For the time domain, the value of the parameters, for each patient, generally increases from the first to the follow up study. The statistical analysis is significant for all the parameters except for the IIa.

For the frequency domain too, both the HF and LF power indices increase from the first to the follow up study, showing significance with the statistical test ($p<0.05$).

The same happens for the entropy measures: the ApEn and the SampEn show a significant increase, with exception for the m = 3. The QSE is still significant, showing an increase from the BSA of the first study to the BSA of the follow up, and from the BPA of the first study to the BSA of the follow up.

Discussion

For the time domain analysis, both parameters from the fetal and newborn world are tested. This choice is taken because of the condition of prematurity of the babies recorded in this

thesis, that is a dynamic condition of transition between these two stated of maturation.

The parameters that showed significance are: Mean of the RR, RMSSD, STV and DI. They generally are higher in the Supine than in the Prone position. They all are short term variability parameters, for exception of the Mean of RR.

It is known from the literature that the sympathetic system is the first to develop during pregnancy, while the parasympathetic develops later and continue to develop during the first months of life [22]

In the first study the higher significant parameters were the long term ones, this may be related to a prevalence of the sympathetic branch, while in the follow up a maturation of the parasympathetic branch is observed.

The parasympathetic branch of the ANS is the one that let the HR decrease and that has a beat to beat control; its higher velocity of response is manifested in a more rapid control of the HR and so on in the short term variability parameters.

In this work as in many others in literature [15], the mean of RR (the inverse of the HR) is generally higher in the Supine than in the Prone position, so generally a higher heart rate is found while Prone.

Regarding the RMSSD, it is resulted to generally increase from the Prone to the Supine position; this means that in the Supine position there is a higher degree of dispersion around the mean (higher variability). The smaller variability in the Prone position may be related to an increased sympathetic drive on the heart, that could bring to an instability in the ANS control.

From the comparison between the two studies it is then seen that each parameter shows an increase, for all the patients, from the first study to the follow up study. This increase is may associated to the maturation of the ANS related to the increase of the PMA.

The frequency domain analysis computed in this thesis uses the traditional Welch method to estimate the power spectral density (PSD) of the signals. Parameters drawn from the PSD are studied because of their utility in estimating the behavior of sympatho-vagal balance.

The parameters selected for the analysis are the HF and LF power bands.

In literature different bands are proposed for newborns, in this work they are set following the previous choice done in the first study.

This analysis did not lead to any useful conclusion in the comparison between the BSA and BPA.

When compared between the two studies, these parameters showed an increase, both for the Supine and Prone comparison. This is may related to the more stable regulation, for instance respiration is more regular in the follow up subjects. Since the total power

is equivalent to the variance of the signal, it is found that follow up has generally higher variability. These results confirm the already seen maturation of the ANS of these older babies.

These results can be partially explained considering two aspects: the first is that in general a weak and irregular breathing in newborns is expected, making difficult the identification of the HF peak. Secondly, it has been proven, at least in some subjects, the occurrence of cardiac aliasing. Its presence is particularly crucial because it affects infants that showed regularity in breathing. This type of aliasing is intrinsic to the system measured and it cannot be removed by filtering. When it occurs it is likely to have a distortion of the PSD, but without a spectral analysis of respiration, it would not possible to verify wether or not a distortion is happening, thus impairing spectral analysis of HR.

Regarding the entropy analysis, both the ApEn and the SampEn are computed, but the problem of the shortness of the recordings and the different number of samples in every 3 minutes windows, has influenced the statistical analysis. In fact in the first study significant results have been found considering windows of 1500 samples, more or less 12 minutes, unavailable for this study.

So the QSE parameter with the minimum count of matches is implemented, being not influenced by the length of the signal; because of this feature it is a very useful tool to be used in this work based on recordings with a limited number of samples. The novelty of this algorithm consists in the up-to-date analytical and automatic method to evaluate the $M_{optimum}$. Moreover, this parameter is not dependent on manual selection of the tolerance r, which is automatically computed for each M and then set to the one linked to the $M_{optimum}$.

The QSE parameter allow the curve of Supine and Prone to be more separated than the SampEn, so that the two populations can be distinguishable with the statistical test. Particularly the QSE for the Supine position is generally higher than the Prone. Low entropy values usually quantify more predictable and less random HR dynamics, suggesting significantly different HR regulation in the infant, thus may be linked to an higher risk for SIDS in the Prone position.

From the comparison between the first and the follow up study, it can be noticed that all the parameters' values increase, for each patient, both in the Supine and Prone comparison. The increase of the entropy values, with the increase of the PMA, may be linked to a maturation of the ANS in these babies. Their control system becomes more efficient, showing an higher HRV.

The results achieved in this thesis are promising and it would be opportune to extend the

analysis on a wider population, to obtain stronger results.

Because of the need of at least 3 minutes windows, in this study it has been possible to analyze only the Active Sleep Baseline, thus ignoring the Quiet sleep. It is then suggested to acquire longer recordings, through new experimental protocols, to have the chance to compare Active and Quiet sleep state and Supine and Prone Quiet Baselines, implementing the newly developed method of the QSE.

Being the Prone position one of the major risk factors of developing SIDS, this measure in full-term and preterm babies could allow to assess a possible pathological that could lead to SIDS.

The respiratory signal has been used in this work to draw some conclusions but a deeper and more extended analysis is already taking place to confirm the results obtained.

Other signals such as pulse-oximetry and blood pressure, could be integrated to the HR analysis, to build a multi parametric classifier to assess changes in the physiological condition of the babies.

The final aim should be to obtain a useful clinical tool to help the medical equip in diagnosing a risky condition for the baby that could lead to SIDS.

Chapter 1

Introduction

Sudden Infant Death Syndrome (SIDS) is one of the main causes of neonatal death and up to now there is no clear knowledge regarding the motivations of death.

At the moment, the triple risk hypothesis is one of the most supported explanation. It takes into account intrinsic and extrinsic risks which will be presented focusing on the role of position during sleep and the prematurity.

Being Autonomic Nervous System (ANS) and the cardiovascular system involved in this pathology, a description of the two systems is provided. Afterward a definition of Heart Rate Variability (HRV) is presented, since it is a useful and non-invasive way to access the ANS, used to understand the possible influence between ANS and SIDS.

Prematurity is a risky condition that presents the baby to the external environment before his/her systems are fully developed. Particularly, it has been studied that the Autonomic Nervous System (ANS) helps maintain the homeostasis of the organism, through the regulation of key parameters such as the Heart Rate (HR) and the Blood Pressure (BP). Thus, such a vulnerable baby is at high risk of encourring in different pathologies, such as the Sudden Infant Death Syndrome (SIDS). The present study proposes to analyze the control mechanisms on HR through a non invasive measure, the Heart Rate Variability (HRV) analysis, evaluating the impact of the sleep position.

1.1 Sudden Infant Death Syndrome

Sudden Infant Death Syndrome (SIDS) is one of the leading causes of infant mortality in the developed countries and accounts for nearly 30% of deaths during the post neonatal period, nonetheless the physiologic mechanisms that underlie this syndrome are still uncertain [2].

The first definition of SIDS was given by Bergman in 1970, who argued that SIDS did not depend on a "single characteristic that ordains an infant for death", but on an interaction of risk factors with variable probabilities [5]. Later, Wedgwoog, Raring, Rognum and Saugstad developed the first "triple risk hypothesis" which was succeeded by the most famous triple risk hypothesis of Filiano and Kinney [5]. The difference in this final version was the emphasis on prenatal origin.

The National Institute of Child Health and Development SIDS strategic plan 2000 quoting Kinney's work, stated unequivocally that "SIDS is a developmental disorder. Its origins are during fetal development." and later in 2001 "Knowledge acquired during the past decade supports the general hypothesis that infants who die from SIDS have abnormalities at birth that render them vulnerable to potentially life-threatening challenges during infancy [3, 5, 6]."

Filiano and Kinney's hypothesis was based on the concurrence of three factors: 1) a vulnerable infant, 2) a critical developmental period for the homeostatic control with a peak at 2 - 4 postnatal months and 3) an exogenous stressor [7], as summarized in Figure 1.1.1.

An infant is likely to die of SIDS if he/she possesses all three factors: the infant's vulnerability lies latent until he/she enters the crucial period and is subject to an exogenous stressor [5].

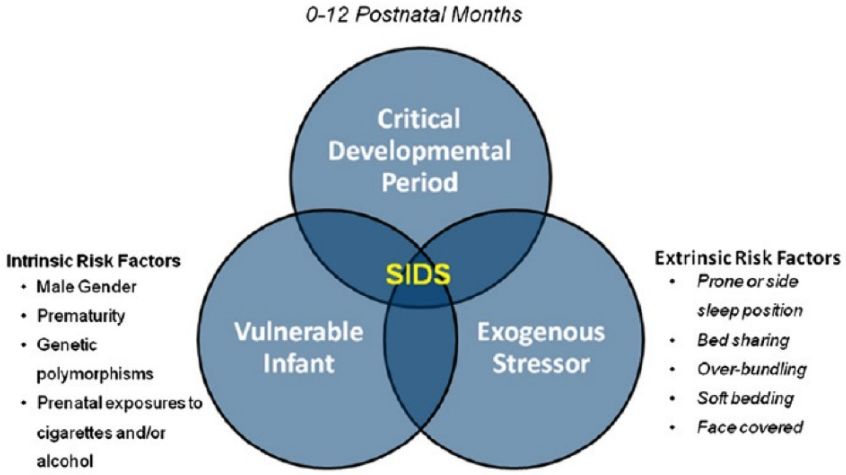


Figure 1.1.1: The Triple Risk Model.

Few infants die of SIDS in the first few weeks of life while the critical age for SIDS is between the second and the fourth month of life, after which a steady decline is observed [8]. Infants who later on died of SIDS showed an increased prevalence of short gestation, neonatal problems, resuscitation at delivery using intubation or cardiopulmonary resuscitation techniques and admission to a neonatal intensive care unit [8].

Two kinds of risk can be defined: intrinsic and extrinsic. An intrinsic risk is defined as a genetic or endogenous factor that affects susceptibility, including African American race, male gender, prematurity, prenatal maternal smoking or alcohol intake. An extrinsic risk is defined as a physical stressor around the time of death, that may increase the risk of SIDS for an already vulnerable infant. These factors include being placed or found in a prone sleep position, sleeping on an adult mattress, bed-sharing and high room temperature.

In the following sections the two most relevant risk factors for this research are presented: prematurity as intrinsic risk and sleep position as extrinsic.

1.1.1 Intrinsic risk: prematurity

Preterm birth occurs approximately 13'000'000 annually, and rates are increasing. Only in the United States ≈ 500'000 infants are born preterm each year [10].

Any infant born before the 37^{th} weeks of gestation is defined as premature, then they can be further divided into extremely, early and late preterm birth with those infants being born before 26 weeks of gestation being regarded as extremely preterm, between 26 and 34 weeks of gestation regarded as early preterm and those born between 34 and 37 weeks regarded as late preterms [11].

Preterm infants have recently been classified by age, as illustrated in Figure 1.1.2 [27].

- Gestational age (GA) (or menstrual age) is the time elapsed between the first day of the last normal menstrual period and the day of delivery. Gestational age is conventionally expressed as completed weeks. Therefore, a 25-week, 5-day fetus is considered a 25-week old fetus.
- Chronological age (or postnatal age) is the time elapsed after birth. It is usually described in days, weeks, months and/or years.
- Post-menstrual age (PMA) is the time elapsed between the first day of the last menstrual period and birth (gestational age) plus the time elapsed after birth (chronological age).
- Corrected age (CA) (or adjusted age) is a term most appropriately used to describe children up to 3 years of age who were born preterm. It represents the age of the child from the expected date of delivery and is calculated by subtracting the number of weeks born before 40 weeks of gestation from the chronological age.

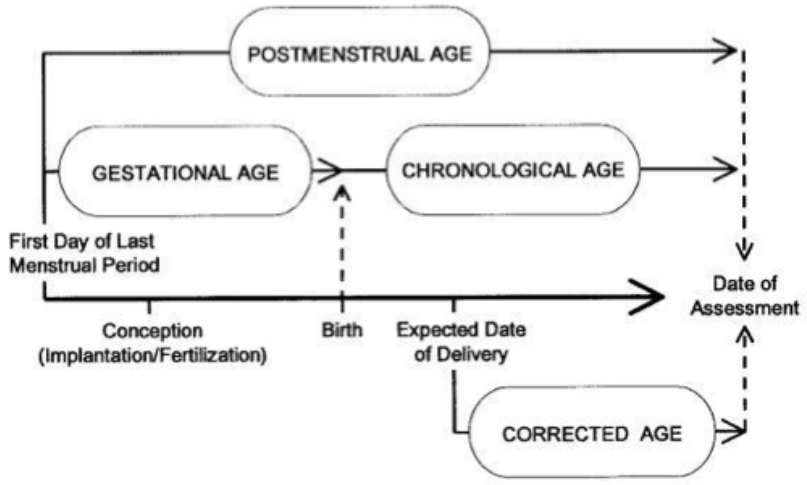


Figure 1.1.2: Nomenclature for prematures' age (Committee on Fetus and Newborn, 2004).

Prematurity sets the newborn to be exposed to the outer environment before his autonomic system is fully developed and able to effectively face life challenging situations like breathing and thermoregulation. A preterm baby is understandably more vulnerable than a full term baby.

1.1.2 Extrinsic risk: sleep position

Prone position is thought to be one of the major risks for developing SIDS for a newborn. The incidence of SIDS is highly reduced since the "Back to Sleep" campaign in 1994 was initiated by the National Institute of Child Health and Development in the United States. In June 1992 the American Academy of Pediatrics (AAP) Task Force on Infant Positioning and SIDS published a recommendation that healthy full-term infants be placed laterally or supine to sleep [9]. The SIDS rate in the United states declined by >50 % in the 10 years after the initiation of the campaign [7] .

A possible explanation for the increased risk of SIDS in the prone position is that infants sleep more deeply in that position and arouse less easily [14]. Results of the study of Ravindra Y. Bath et al [14] suggest that in prone position the infant sleeps significantly longer and he/she spends less time in AS and longer in QS. In supine position the infant had more awakenings and arousals but more obstructive apneas. It has been hypothesized that the ability to arouse from sleep is an important survival mechanism, which may be impaired in SIDS [15].

In healthy full term infants, HR decreases rapidly between 1 and 3 months and falls more slowly between 3 and 6 months. In Rita Tuladhar et al. study [15], maturational changes have been demonstrated, with the HR gradually decreasing with increasing postnatal age in both supine and prone positions and in both AS and QS. An exception is found in prone position in AS in which the HR did not follow the same patterns.

1.2 Cardiovascular system

A preterm infant is collocated between a fetus and a full-term newborn so the development of his cardiovascular system stands in the middle of these two conditions. Fetal circulation is characterized by 1) the placenta as the organ of respiration, 2) high pulmonary vascular resistance (VR), 3) low systemic VR and 4) the fact that the fetal ventricles pump in parallel with a dominant right ventricle. In addition, the fetus exists in a remarkably hypoxic environment compensated for by a relatively high cardiac output and a hemoglobin with a high affinity for oxygen.

The fetus receives all the necessary nutrition through the blood vessels in the umbilical cord and oxygen from the mother through the placenta. Waste products and carbon dioxide from the fetus are sent back through the umbilical cord and placenta to the mother's circulation to be eliminated.

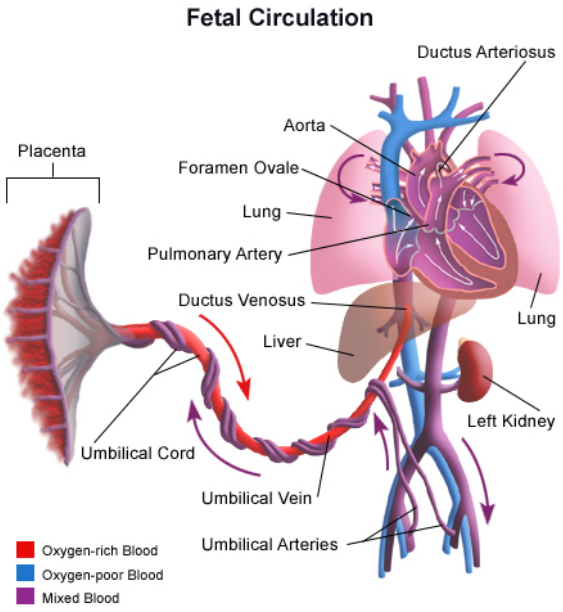


Figure 1.2.1: The Cardiovascular system.

The fetal circulatory system uses two right to left shunts, which are small passages that connect regions of the heart that are, in the adult, separated. The purpose of these shunts is to bypass certain body parts – in particular, the lungs and liver – that are not fully developed while the fetus is still in the womb. The shunt that bypasses the lungs is called the *foramen ovale*. It moves blood from the right atrium of the heart to the left atrium. The *ductus arteriosus* moves blood from the pulmonary artery to the aorta.

At birth, the umbilical cord is clamped and the baby no longer receives oxygen and nutrients from the mother. With the first breaths of life, a number of changes occur in the infant's lungs and circulatory system very rapidly: the lungs begin to expand, the *ductus arteriosus* and the *foramen ovale* both close. In infants born before the term, circulatory shunts do not always close immediately after birth, adding to the immaturity of the cardiovascular system and placing these infants at a higher risk of circulatory complications [11].

1.3 Autonomic Nervous System

Homeostasis was defined by Claude Bernard in 19th century like "the fixity of the 'milieu intérieur' (the environment within), which is the condition of free and independent life." The ANS is an essential regulator of the homeostasis and a main actor in the control of circulatory and respiratory systems [16].

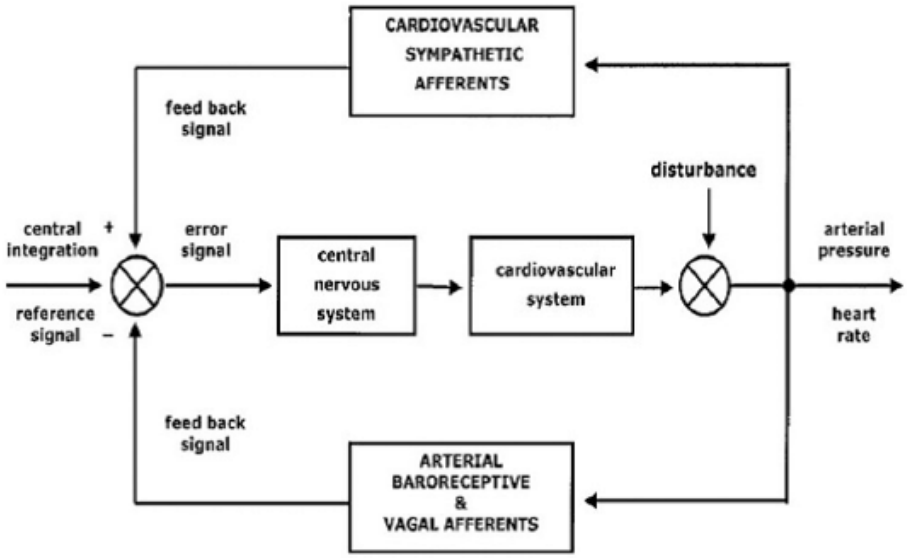


Figure 1.3.1: Autonomic Nervous System regulation of cardiovascular system.

To reach a condition of dynamic equilibrium our body uses different strategies. In the case of the cardiocirculatory system the baroreflex is used, which is one of the main regulatory mechanisms. Baroreflex means literally 'reflex reaction to arterial pressure changes', it starts from arterial stretch receptors in high-pressure areas called baroreceptors, located mainly in the carotid sinuses and in the aortic arch.

Baroreceptors function as sensors in the homeostatic maintenance of mean arterial pressure (MAP), by constantly monitoring pressure in the aortic arch and carotid sinuses. High levels of blood pressure (BP) result in an increase baroreceptors firing frequency. They induce the baroreflex response that ultimately decreases the HR, lowers cardiac contractility, causes vasoconstriction. This is a quick response with a protective stabilizing function.

Chemoreceptors are found in both the peripheral and central nervous systems. They mostly have their effects on the regulation of respiration, but they are also important in the control of the circulation. They respond to changes of oxygen and carbon dioxide quantity in blood.

The chemoreceptor and baroreceptor reflexes have important developmental implications in the infant and child; for instance the baroreflex is present but is still incompletely developed at term. In preterm infants, postural changes elicit no change in HR, but there is an increase in peripheral VR, illustrating an incomplete and attenuated baroreceptor response [2]. This can be related to the fact the the fetus spends approximately two months upside-down suspended in the amniotic liquid, a very different gravity condition than a born person and an overall high VR. The baroreflex then develops in the first months after birth as a function of a changed environment. The chemoreceptor response instead seems to be well

developed in-uterus.

The ANS is composed of two branches: the parasympathetic and sympathetic innervations.

Stimulation of the sympathetic nerves is usually driven by norepinephrine, effects include the increase of HR, SAP, cardiac contractility, conduction velocity, respiratory frequency, vasoconstriction and many others. The sympathetic stimulation decays very slowly when the stimulus is interrupted. Furthermore, the norepinephrine released even during intense stimulation, is enough to change the HR only by a small increment. This is due to the fact that the neurotransmitters of the two branches of autonomic innervations, acetylcholine and norepinephrine, are released at different rates during stimulation and also sympathetic activity depends on the intracellular accumulation of second messengers.

On the other hand the parasympathetic action is mediated by acetylcholine and it is characterized by the decreasing of the HR and of the systolic volume, coronary constriction, decrease of SAP and others. The effect of this system is ephemeral because the acetylcholine released is hydrolyzed right away; the latency is very short since the acetylcholine can activate the specific K+ channels without the help of any secondary messenger. When vagus nerve is stimulated the HR reaches a steady state value within 1 or 2 cardiac cycles and when stimulation is interrupted HR goes quickly back to its basal level. These two aspects are very important because they allow the parasympathetic system to have a beat to beat control.

The term *vagal tone* is often used to describe the predominancy of the parasympathetic action leading to a "relaxation" of the cardiovascular-respiratory system.

Sympathetic and parasympathetic effects are often antagonists, their action on the heart is described as sympatho-vagal balance; they are always active and the heart receives simultaneously signals from both the systems. The activity changes follow the 'push – pull' logic: when sympathetic activity increases, the parasympathetic decreases and vice versa [27].

1.4 Heart Rate Variability

Heart Rate Variability (HRV) has become the conventionally accepted term to describe variations of the frequency at which the heart is beating; this is commonly referred as HR and is usually measured in beats per minute (bpm) or R-R intervals (RR), defined as the period of time between two R peaks in two consecutive QRS complexes.

RR can be measured in seconds or milliseconds and can be seen as the inverse of HR, as $RR = {}^{60}/_{HR}$. Since HR is one of the most accessible biological, complex signal to analyze, the last two decades have witnessed a raising interest towards this signal thus a

significant relationship between the ANS functioning and cardiovascular mortality has been assessed [17].

In a human neonate, neural control of cardiovascular function has been assessed often by measuring alteration in HR in response to postural changes. HR is a commonly used indicator of the health of a newborn and reflects the infant's ability to transition from intra to extra-uterine life. Although HR may be lower initially amongst preterm infants, once hemodynamic stability is achieved, preterm infants display a high HR (higher than the average adult); this suggests a predominancy of the sympathetic system over an immature parasympathetic system [11].

The meaning of variability in biological signals is studied by Goldberger in 1997, who proposed that health is characterized by "organized variability" and disease is defined by decomplexification, increased regularity and reduction in variability [18].

The analysis of the HRV has been widely used as a non-invasive and reliable tool to evaluate cardiovascular autonomic control [19]. HRV is based on the assessment of rhythmical oscillations embedded in heart period and blood pressure time series, which represent the sympathetic and parasympathetic modulations of the ANS on the cardiovascular function.

1.4.1 HRV and sleep states

Newborn autonomic control of the respiratory and cardiovascular systems undergoes considerable maturation during the first 6 months of life; this maturation is linked to an increasing parasympathetic dominance and it allows full term babies to adapt their respiratory and hemodynamic responses to the internal and the external environment. Since these systems are in a critical stage of development, presenting rapid re-arrangements, it is easy to find that infants are at increased risk for cardio-respiratory disturbances and hypoxemia [12, 13]. Since sleep has a marked influence on cardio-respiratory control, these instabilities are even more evident during sleep itself; this is of particular importance since preterm infants spend even more time sleeping than full term infants that can spend up to 70% of the 24 hours sleeping [12].

Sleep states can be divided in Quiet Sleep (QS) and Active Sleep (AS), precursors of well-known non-REM and REM sleep stages respectively (REM = Rapid Eye Movement). Infants spend approximately equal amount in both states, while adults have a lower percentage of AS.

QS is characterized by the absence of eye movements and regular HR and respiration. In contrast, AS is characterized by eye movements, and irregular HR and respiration [12]. The proportion of QS increases with age, while the amount of AS decreases. Cardiorespiratory disturbances occur predominantly in AS or REM sleep, so the predominance of AS in early infancy may increase the risk of cardio-respiratory disturbances during this period [12].

Relevant insight on sleep related pathologies such as SIDS can be then assessed through studying how responses of cardiovascular and respiratory control might change in infants and preterm infants during sleep. AS has a more irregular breathing pattern in the infant, with short apneas occurring more frequently than in QS.

A preterm infant has an even more increased risk than full-term infants, he/she may present more potentially life threatening events, like apneas, that are more difficult to overcome with an immature autonomic control system.

1.4.2 HRV and tilt

In the study of J. E. Mazursky et al. is evaluated the developmental change in autonomic cardiovascular reflexes in preterm infants with increasing postnatal age in supine position and after 45 degree head-up tilt [23]. An advantage of the head up tilt is that it allows enhanced isolation of the sympathetic contribution to HRV [22].

Postural changes have been performed in infants to assess the integrity of the cardiovascular response to baroreceptor activation. Head up tilting produces a significant HR response and the magnitude of the response is proportional to the degree of tilting. Tilt to the upright position is accompanied by cardiac sympathetic excitation and vagal withdrawal to the sinus node pacemaker.

In Yallurou et al. it is shown that at postconceptional age range 35-39 weeks the tilt produces a small but significantly increase in HR compared with the resting value, while there is no difference at the postconceptional age range 28-32 weeks.

1.5 Respiration in newborns

Respiration in babies is largely different from adults. The respiration rate can be around 30-60 breaths per minute in newborns, widely higher than adults, that is around 15-20 breaths per minute.

Respiratory rate and variability decreases during the first 6 months of postnatal life. An increased respiratory rate in younger infants may be necessary due to the increased demand

for carbon dioxide clearance (due to an increased metabolic rate), while permitting the infant to maintain ventilation with minimal effort.

Two of the principal disorders in newborns, especially in preterm, are apneas, which usually occur during sleep, and tachyapneas.

Apneas are respiratory events that consist of breathing that slows down or stops for any cause, they can be classified as obstructive, central and mixed. Obstructive is when, despite chest and abdominal wall movements, there is no nasal airflow, central when there is no nasal airflow and an absence of chest and abdominal wall movements and mixed when there is a combination of central and obstructive apneas. There are several reasons why preterm babies may have apnea: their ANS is still immature, the muscles that keep the airway open are weak and they have been exposed to drugs. These babies will have short episodes (5 - 10 seconds) of either shallow breathing or stopped breathing, these episodes are followed by periods of normal breathing. So the respiratory pattern in preterm babies is highly irregular.

Transient tachypnea means rapid breathing. As the baby grows in the womb, the lungs make a particular fluid, that fills the developing baby's lungs and helps them grow. When the baby is born at term, chemicals released during labor tell the lungs to stop making this fluid. The baby's lungs start removing or reabsorbing it. The first few breaths a baby takes after delivery fill the lungs with air and help to clear most of the remaining lung fluid. Leftover fluid in the lungs causes the baby to breathe rapidly. It is harder for the small air sacs of the lungs to stay open.

Chapter 2

Materials and Methods

This thesis follows and develops a previous project [27], referred as first study , made at the Columbia University Medical Center. This work analyzes the follow up of the first study, which is firstly presented.

Successively the dataset of the follow up will be presented along with its features: the acquisition system, the experimental protocol and the signals recorded.

It is described the pre-processing of the ECG signal used to obtain the RR time series.

Then are presented the methods used, spacing among different domains, such as the time domain, the frequency domain, the Phase Rectified Signal Averaging (PRSA) domain and Entropy domain.

Among the Entropy measures the method proposed involving the QSE is explained in detail, because of its original contribution.

2.1 The first study

This thesis is the follow up of a previous project developed in 2012-2013 at the Columbia University Medical Center [27].

In the first study, the dataset consists of 37 preterm healthy babies, whose average gestational age (GA) is 28.7 weeks and the post-menstrual age (PMA) 35.7 weeks. It is a 6 hours long study and the signal analyzed is the ECG, for 35 of the 37 babies the sleep state is coded.

As illustrated in Figure 2.1.1, each study starts with feeding and is followed by two sequential 3-h periods of observation separated by another feeding period. Infants are assigned to the supine position for the first epoch and then the position is inverted.

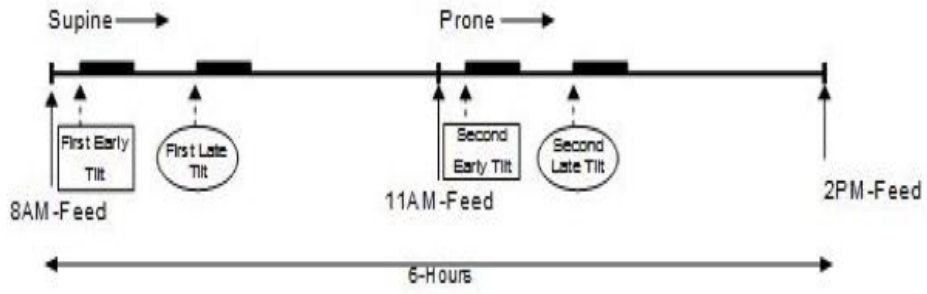


Figure 2.1.1: First study experimental protocol.

In the supine position infants are placed flat on the back with the head turned to the right, no further manipulations throughout the inter-feeding period are performed.

Two tilt sequences, formed of four alternating head-up tilts in a fixed sequence are performed; each takes around 4 minutes (249 sec) in total and is divided in the following way [27]:

- Horizontal to Tilted: starting from horizontal position the head-up angular change will occur over the first 5 second period (0-5 sec)
- Tilted: tilt position will be maintained for next 2 minutes (5-120 sec)
- Tilted to Horizontal: change back to horizontal position will occur over next 5 seconds (120-125 sec)
- Horizontal: horizontal position will be maintained for next 2 minutes (125-240 sec) after which next tilt sequence will be initiated.

PATIENTS NUMBER	PATIENTS CHARACTERISTICS	SIGNALS ACQUIRED
37	Preterm babies	ECG, EEG, Respiration, Pulse-oximetry
	GA 28.7 weeks	
	PMA 35.7 weeks	

Table 2.1.1: First study dataset.

2.2 The follow-up

The project of this thesis is collocated after the one previously described, using signals from the same babies of the first study but acquired 2 months later. With this dataset there is the possibility to analyze the HR during the critical period for the SIDS and to compare the results to the age of first study in order to have a longitudinal vision of the maturation of the babies and particularly of the development of their cardiovascular and autonomic control system.

2.2.1 Dataset

The dataset is composed of signals from 24 out of the 37 babies of the first study; here the main informations regarding this dataset are summarized.

PATIENTS NUMBER	PATIENTS CHARACTERISTICS	SIGNALS ACQUIRED
24	Preterm babies	ECG, EEG, Respiration, Pulse-oximetry
	GA 27.9 ± 1.6 weeks	
	PMA 49.16 ± 3.21 weeks	

Table 2.2.1: Follow up dataset.

Each baby is put to sleep after feeding and the sleep states are coded starting from the "coding time" which sometime corresponds to the start of acquisition, other times later, usually when the baby fall asleep.

The protocol mean duration of the acquisitions is 60 minutes and for each acquisition different signals are available (2.2.1), but the focus is on the ECG and respiration.

2.2.2 Experimental protocol

The experimental protocol comprehends four phases:

• Baseline Supine (BS): the baby is put supine on the bed for the first 10 minutes.

• Tilt Supine (TS): the baby is tilted in the supine position for 2 minutes for 4 times, with 2 minutes of resting in horizontal supine position between the tilts (approximately 16 minutes total).

• Baseline Prone (BP): the baby is put for 10 minutes in the prone position.

• Tilt Prone (TP): 4 prone tilts following the same procedures of the supine tilt.

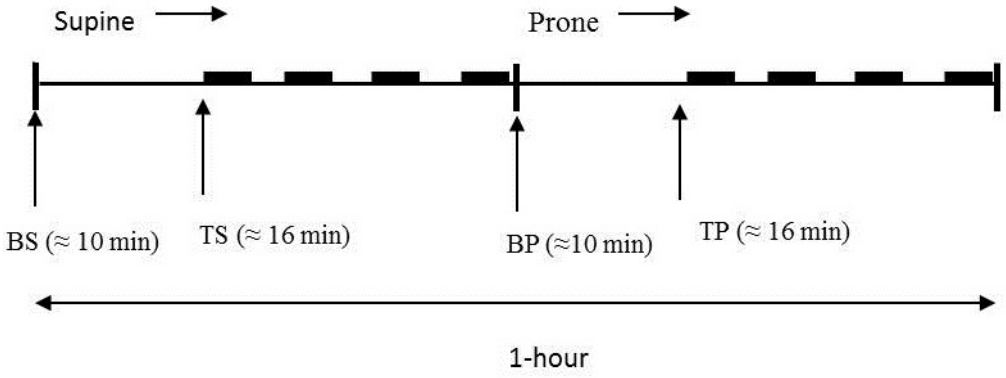


Figure 2.2.1: Follow up experimental protocol.

For two patients the protocol was inverted, thus the baby was firstly put to sleep in prone and then turned in supine position. Other 2 patients spent all the hours of study awake, so they were not taken in consideration in the study.

During the tilts, systolic, diastolic and mean BP measurements were made every minute and all other measurements (HR, EEG) were continue. Tilts were not initiated within 30 sec of periods of fussing or sustained body movements.

2.2.3 Acquisition system

Measurement of cardiorespiratory data are obtained for ECG from a standard HR monitor (Hewlett Packard 3680) at 500 Hz sampling frequency, after opportune filtering. The impedance pneumogram is digitized at a rate of 50 samples per second and a pattern recognition algorithm is than used to detect peaks and troughs in order to mark the beginning and the end of each breath in the filtered impedance tracing.

Pulseoximetry-derived instantaneous pulse rate, SpO2 and perfusion index are measured using the Masimo Set Radical oximeter (Masimo Corporatio, Irvine, CA, USA).

Dense Array EEG leads are connected to a Geodesics System 200 collection system (Electrical Geodesics, Eugene, OR).

EEG data are acquired with 128-electrode Geodesic Sensor Nets with a vertex reference. During recording, the infant's eyes are observed for movement and blinks. Minimal EEG artifact from eye movement of the sleeping infants is found.

Sleep state coding began generally some minutes after the beginning of the acquisition and terminated with the end of the study. Codes were assigned by direct observation each minute using a scoring system developed and validated in the Infant Physiology Laboratory at Children's Hospital of New York. States were coded every minute and Active Sleep (AS) was assigned when at least one rapid eye movement was observed. In this state, small body movements and movements of extremities and of the trunk occurred. Quiet Sleep (QS) was defined when the baby was sleeping and no rapid eye movements were occurring. During QS, movements were limited to startles and non-nutritive sucking. Sometimes small body movements were observed but no rapid eye movements occurred in the same minute, thus the sleep state was defined as indeterminate. Codes were also assigned for wakefulness (Awake), crying (Fussy/Crying), and feeding (Feeding) [27].

2.3 Pre processing

The first step to obtain the RR interval series is to import the ECG tracks in a waveform marking and analyzing system, named Gmark (Columbia's property). Thanks to this software, it is easy to visualize at the same time the peak positions and the tachogram extracted from them. This allows a fast visual inspection of the signal, in order to correct the peaks automatically detected by the program. Each signal is slid to identify if there is any missed peak or any wrongly identified peak as observed in figure.

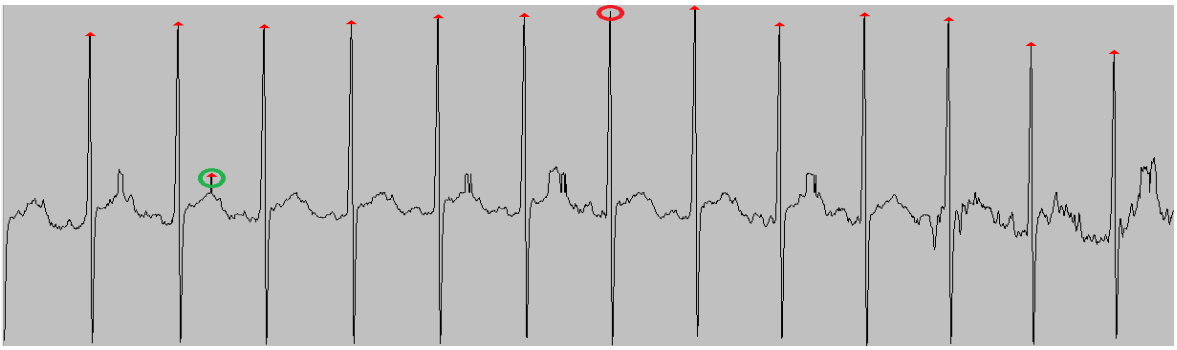


Figure 2.3.1: Example of missing peak (red circle) and wrongly identified peak (green circle) in a segment of ECG of patient ID 4.

These files (.mrk format) are then imported in MATLAB with an adequate code. When the recording is noisy to a level that affects the capability of peaks identification, two strategies are followed, distinguishing the case of one single peak missing and two peaks missing. In case of one peak missing it is used the mean of the three previous and the three following intervals RR to identify and add the peak. In case of two missing peaks the interval is simply subdivided in three equal parts and the space is filled with the new peaks. In case of more than two peaks the decision is not to proceed with the addition of peaks, in order to not distort excessively the signal.

After the pre processing, the correction of the missing peaks and the visual inspection of all recordings for quality check, the data available for each patient is the one shown in Table 2.3.1, divided for each protocol phase BS, TS, BP and TP and by sleep state.

ID	Sleep State	BS	TS	BP	TP
4	Active	4m		4m	3,8m
	Quiet		8m		
7	Active	10m		6m	4m, 2m, 3m
	Quiet		20m		
8	Active	3m	1m		
	Quiet				
...	...	...	...	...	...

Table 2.3.1: Example for three patients of the number of minutes available for the preliminary analysis.

Each protocol phase is considered acceptable for the study only if it has at least 3 minutes in a row of the same sleep state both for the Prone and the Supine sleep position. It was found that this condition was satisfied by 10 patients for the AS Baselines, 8 for the AS Tilt, 1 for the QS Baseline and 5 for the QS Tilt, here summarized in the Table 2.3.2.

	Baselines	Tilt sequences
Active Sleep	10	8
Quiet Sleep	1	5

Table 2.3.2: Number of patients divided for each protocol phase and sleep state.

The choice is to focus on the Active sleep Baselines (BA), since they are the most numerous, being the Active Sleep the most common during infants' sleep. BA allows a statistical analysis and a comparison with the results obtained in the first study.

This resulted in a dataset comprising 10 patients out of the original 24. Every BA is then divided in segments of 3 minutes length, suitable for the subsequent analysis. In Table 2.3.3 the number of segments for each patient and for each BA are shown.

Patient ID	Baseline Active	
	Supine	Prone
4	1	1
7	3	2
12	3	3
17	2	1
20	1	1
21	1	2
22	1	1
31	2	1
32	3	1
37	2	1

Table 2.3.3: Number of segments of Active Sleep Baselines available for the Supine vs. Prone comparison.

2.4 Time domain analysis

To investigate the HRV, time domain parameters are the simplest measures to perform; in this research the work done before in the first study is taken as example, leading to implement the parameters of the HRV Task Force 1996 [17].

The aim of the Task Force is to give guidelines to standardize nomenclature and definitions of terms and to specify standard methods of measurements. The parameters suggested are the MEAN of the RR interval, the Standard Deviation of the NN interval (SDNN) and the Square Root of the Mean Squared Differences of Successive NN intervals (RMSSD).

As in the first study other measures of fetal HRV (FHR) are added to the previous parameters described, since prematurity is a condition that lays between fetal and newborn life.

The parameters chosen are then: Long Term Variability index (LTV), Short Term Variability index (STV), Interval Index (II) and its evolution the Interval Index of Arduini (IIa), Differential Index (DI), Root Mean Square of differences from the Mean interval (RMSM) and the Long Term Irregularity index (LTI).

For each patient and for each segment of 3 minutes of the Supine and Prone Active Baseline (BSA and BPA) these parameters are calculated.

2.4.1 Task Force parameters

The simplest variable to calculate is the SDNN index. The variance is mathematically equal to the total power of spectral analysis, so SDNN reflects the cyclic components which influence the variability in the period of recording. This index depends on the length of the ECG signal, in fact the total variance of HRV increases with the length of recordings. Because of this dependence on the length, SDNN cannot be used to compare recordings of different duration, but it is necessary to standardize the signal length.

The Task Force suggests using short-term 5 minutes recordings but for this study parameters are implemented on a 3 minutes period. The choice does not influence the consistence of the results because for newborns HR is significantly higher than adults, hence the number of beats in a 3 minutes newborn's segment is quite the same of a 5 minutes adult's window.

RMSSD is the most commonly used measure derived from interval differences. This measurement of short-term variation can estimate high frequency variations in HR.

2.4.2 FHR parameters

Short Term Variability (STV) is defined as the beat to beat changes in the RR time series, it quantifies variability on a very short time scale. It is computed as the average of the beat-to-beat interval differences over cycles, expressed in milliseconds and it is useful to describe very rapid oscillations [24]. STV is also implemented by adopting the definition provided by Arduini et al (1993): for a 1 minute segment of the RR interval signal, STV is defined as [25]

$$STV = mean[|T_{24}(i+1) – T_{24}(i)|] = \frac{\sum_{i=1}^{23} |T_{24}(i+1) - T_{24}(i)|}{23}, i = 1,\ldots 23 \qquad (2.4.1)$$

The parameter is calculated on HR series gained by averaging the values over 2.5 seconds windows. Thus, every minute of signal contained 24 windows of 2.5 sec. This signal is called T_{24}.

Long Term Variability (LTV) is defined as the difference between maximal and minimal instantaneous HRs over a period of 3 minutes and is expressed in beats per minute [27]. When this parameter is measured for the amplitude and the number of cycles per minute, accelerations and decelerations are not included in the process [24]. LTV has two

shortcomings: first, as STV increases LTV increases linearly too and secondly LTV parameters do not present an increase linearly correlated to the raise of HR [28].

So in 1971 a new parameter for long time irregularity (LTI), which showed less correlation with STV, was introduced by Haan et al . LTI is usually computed from a short segment of the RR interval signal. Given 3 minutes of RR interval signal, the index is defined as the interquartile range $\left[\frac{1}{4};\frac{3}{4}\right]$ of the distribution of the modulus m(j), where

$$m_{24}(j) = \sqrt{T_{24}^2(j+1) + T_{24}^2(j)} \tag{2.4.2}$$

The Interval Index (II) is an adimensional parameter which measures the variability over short period. The original definition by Yeh et al. in 1973 is

$$II = \frac{(std[T(i)])}{(mean[T(i)])} \tag{2.4.3}$$

where T(i) corresponds to 30 seconds of the RR signal. The following modification by Arduini et al. in 1993 brings to a new definition for II in which the difference between two successive heart beat is considered

$$IIa = \frac{std[|T_{24}(i+1) - T_{24}(i)|]}{STV} \tag{2.4.4}$$

RMSM is another parameter used for fetal time analysis, it is defined by Siimes et al. in 1990 [29]

$$RMSM = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(RR_i - RR)^2} \tag{2.4.5}$$

where RRi is the ith interval, RR the mean R-R interval and N the number of intervals in the sample [29].

The last time domain parameter considered in this research is the Differential Index (DI) that measures the relatively long term fluctuations in the RR intervals. It was defined by Yeh et al. in 1973 as

$$DI = \sqrt{\sum_{i=1}^{n-1}(d_1 - \bar{d})} \tag{2.4.6}$$

where $d_1 = \frac{t_{n-1} - t_n}{t_{n-1+t_n}} K$ with K constant and $\bar{d} = \frac{1}{n-1}(\sum_{i-1}^{n-1} d_i)$ [27].

Summary tables

TIME DOMAIN	
LENGTH OF SEGMENTS: 3 MINUTES	
ACTIVE SLEEP BASELINES	PARAMETERS
Supine vs. Prone	MEAN
	SDNN
	RMSSD
	LTV
	STV
	II
	DI
	RMSM
	II - Arduini
	LTI

Table 2.4.1: Time domain summary.

2.5 Frequency domain analysis

The frequency analysis consists in estimating the Power Spectral Density (PSD) of a signal S(t). It is then estimated the power of two frequency bands: Low Frequency (LF) and High Frequency (HF), traditionally used to investigate the sympatho-vagal balance in adults.

HF power is influenced by parasympathetic activity alone and it is mainly modulated by ventilatory cycles (respiratory sinus arhytmia). LF power, in contrast, is influenced by both sympathetic and parasympathetic activity and related to the baroreflex system. The Very Low Frequency component (VLF, lower than 0,03 Hz) represents mechanism of very long term regulation such as the thermoregulation and peripheral vasomotor tonicity [16].

So HF and LF powers can be useful to describe the sympatho-vagal balance in action in the subject and to assess if there is any significant difference between the Supine and Prone conditions.

As the cardiovascular regulation of the infant differs from the one of the adult there is also the need to adjust properly the definition of LF and HF band, as in [27].

The high frequency component HF is then set to 0,15–1,40 Hz in newborns. The low frequency component LF is instead set to 0,03–0,15 Hz.

2.5.1 Parametric and Non-Parametric PSD estimate

The PSD represents how the power of each frequency component is distributed along the frequencies within the Nyquist frequency of the signal (sample frequency / 2), that is the maximum frequency to compute the power.

There are different methods to compute the PSD, mainly divided in Parametric and Non-Parametric methods.

Parametric methods consider the signal as if it is an Auto-Regressive (AR) or Moving-Average (MA) stochastic signal, and they are based on the computation of the parameters of the filter transfer function. They have the advantage to provide a smoother PSD and offer an easier calculation of the power for a selected band. On other hand these kind of methods need a verification of the suitability of the chosen model and of its complexity.

Non-Parametric methods have the advantage to be simple to implement and to have high processing speed. They are mostly based on the Fourier Transform, most often on the Fast Fourier Transform (FFT), which is fast and efficient, even though the spectrum obtained might be noisier. A limitation of Non-Parametric methods is that the computation uses windows, resulting in distortion of the PSD estimate due to window effects, thus a strict periodicity of the windowed data is desirable.

2.5.2 The Welch method

In this study a Non-Parametric method is used to compute the PSD, in particular the Welch method, which is an improvement of the standard periodogram spectrum estimating method. Particular attention is given to the choice of the window since it defines the resolution. A window of 1 minute was chosen since it allows to have a resolution of 0.01667 Hz.

The following is done for every 3 minutes segments available. The HR is an unevenly spaced signal, as it samples a beat when it happens, and if this kind of signal undergoes a Fourier analysis there is the problem to know the sample frequency, because the Nyquist frequency (the greatest frequency represented in the periodogram) is not known apriori. To face this problem the first step is to resample the data, in order to have a Nyquist frequency equal to the half of the sample frequency. The choice is to interpolate the data with cubic splines and resample it at 10 Hz as in [27] and [31]. The data is then de-trended by subtracting the mean and it is cropped into overlapping segments with a 50% overlap. Modified periodograms of the overlapping segments are computed through DFT, as each segment is windowed with a Hamming window of the same length of the segment. The resulting periodograms are averaged to produce the final PSD estimate.

2.5.3 Power of LF and HF bands

To compute the power P of an arbitrary frequency band within frequencies f_a and f_b of a spectral density $P_{xx}(f)$ the following formula is used:

$$P = \frac{f_{samp}}{L} \sum_{f=fa}^{fb} P_{xx}(f) \tag{2.5.1}$$

because it is a discrete PSD. f_{samp} is the sample frequency (10 Hz) and L the length of the signal. In this way P_{LF} and P_{HF} are computed, and also $P_{LF\%}$ as percentage of the total power $P_{LF} + P_{HF}$.

Then in order to make the statistical analysis one value for P_{LF} and P_{HF} is obtained for each BSA and BPA for every patient by averaging all the powers.

2.5.4 Cardiac Aliasing

In the spectral analysis the occurrence of the phenomenon of cardiac aliasing has to be investigated.

The Shannon's Theorem states that a signal, whose maximum frequency is $\hat{f}$, needs to be sampled at least at a frequency $2 \times \hat{f}$. This law could be re-stated as: the mean HR needs to be at least double of the mean respiration rate in the same segment analyzed, if one wants to see the respiration component through the spectral analysis of the HR (the HF component related to the vagal contribute) :

$$HeartRate \geq 2 \times RepirationRate \tag{2.5.2}$$

This law is generally respected in the adult, since:

$$70bpm/15bpm = 4.7 \gg 2 \tag{2.5.3}$$

where 70 bpm and 15 bpm are average values.

In the infant this could be not always verified, as this ratio, for instance, could measure:

$$90bpm/60bpm = 1.5 < 2 \tag{2.5.4}$$

So even if a higher but regular respiration rate could be observed directly in the infant, it is not always visible in the spectral analysis as a peak in HF in the HR spectrum. Cardiac aliasing can distort the PSD of the HR and could give an overall misleading interpretation of the sympatho-vagal balance in the infant.

To assess whether or not the occurrence of cardiac aliasing could happen, the respiration signal is analyzed following these steps:

- Respiration signal is filtered with an order n = 60, lowpass, FIR filter with a cutoff frequency of 5 Hz.
- Respiration is then down-sampled from 50 Hz to 10 Hz to be cross-analyzed with the RR.
- The PSD of respiration is computed for this signal with the Welch method, as in 2.5.2.
- The frequency range of the HF component is memorized.
- The cross-spectrum HR-Respiration is computed.
- It is investigated through visual inspection of the spectra whether or not the same HF components are found in both spectra.
- The mean HR in the period is measured and the Shannon's law for cardiac aliasing is verified: HR > 2 × Respiration Rate.

The aim is to assess if the law is verified when the HF component is found in the respiration, but not in the HR spectrum, while is not verified when the component can be found in both.

Summary tables

PSD Estimate, Welch Method	
Length of segments	3 minutes
Window Overlap	50 %
Resampling HR	10Hz, cubic spline
Resampling Respiration	Low-pass FIR 5Hz cutoff, order = 60 down-sampling 50Hz > 10Hz, cubic spline

(a) Frequency domain summary.

Active Sleep Baseline	Parameters
Supine vs Prone	P_{LF}
	P_{HF}

(b) Parameters computed for each Active Baseline.

Table 2.5.1: Frequency analysis summary table.

2.6 Phase-Rectified Signal Averaging

PRSA is a technique introduced by A. Bauer et al. in 2005 to detect and quantify quasi-periodic oscillations masked by the non-stationary nature of composite signals and noise. Non-stationarities are a major problem in the analysis of signals recorded over a long period of time. Many biological signals are influenced by internal and external perturbations causing interruptions of the periodic behavior, these interruptions 'reset' the regulatory processes resulting in phase de-synchronization of the oscillations and so the signal becomes quasi-periodic. The detection of quasi-periodicities in long-term records of human HR is of high clinical relevance because they reflect regulatory processes [32].

This technique is useful because of its capability to separate processes occurring during increasing and decreasing parts of the signals. The aim of PRSA is to compress the signal into a much shorter sequence, keeping all relevant quasi-periodicities but eliminating non-stationarities, artifacts and noise [32].

2.6.1 The method

The method consists of three steps, as represented in Figure 2.6.1.

In the first step anchor points are selected, following three different criteria:

- The anchor point corresponds to increases in the signal

$$x_i > x_{i-1} \tag{2.6.1}$$

- The anchor point corresponds to decreases in the signal

$$x_i < x_{i-1} \tag{2.6.2}$$

• The anchor pint is defined by comparing averages of T values of the time series

$$\frac{1}{T}\sum_{j=1}^{T-1} x_{i+j} > \frac{1}{T}\sum_{j=1}^{T} x_{i-j} \tag{2.6.3}$$

or

$$\frac{1}{T}\sum_{j=1}^{T-1} x_{i+j} < \frac{1}{T}\sum_{j=1}^{T} x_{i-j} \tag{2.6.4}$$

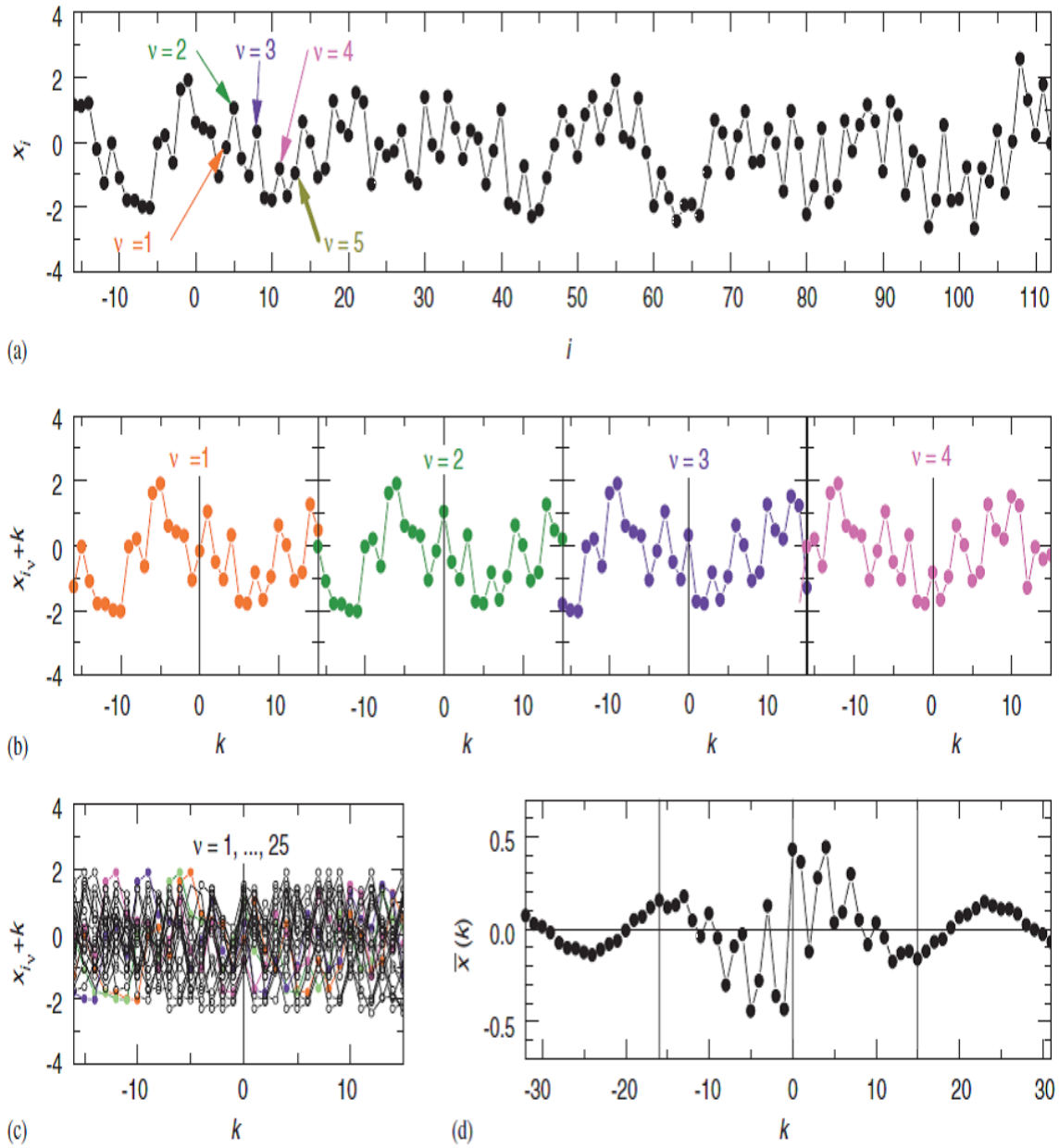


Figure 2.6.1: The PRSA technique:

(a) Anchor points are selected from the original time series (X_i); here increase events are selected, corresponding to $T = 1$. (b) Windows (surroundings) of length $2L$ with $L = 16$ are defined around each anchor point; here are shown the first four anchor points. (c) The surroundings of many anchor points (all located in the centre) are shown on top of each other. (d) The PRSA curve x(k) resulting from averaging over all surroundings is shown versus the offset k from the anchor points; the parameter L is increased to $L = 32$ in order to improve the visibility of the slow period. The original signal consists of 1/f noise (generated using the Fourier filtering method) with two additional quasi-periodicities with characteristic frequencies f = 0.05/dt and f = 0.3/dt; phase jumps are inserted after an average number of four periods [32].

The parameter T sets an upper frequency limit for the periodicities that can be detected by PRSA (low pass filter). A range of T values from 3 with step 3 until 60 is chosen (which on average corresponds to 2 to 40 seconds), knowing that the sympathetic and the parasympathetic branches have different time responses to external factors [33]. Quasi-periodic sinusoidal oscillations in the noisy series will cause anchor points predominantly in the phase of the steepest ascent or descent, this way the phase information of the oscillations is obtained from the signal itself and the signal can be phase-rectified using the anchor points [32].

In the second step a window of length $2L+1$, surrounding each anchor point, where $L = 75$ is defined. This parameter must be larger than the period of the slowest oscillation that has to be detected.

In the third step the PRSA series is obtained by averaging over all windows aligned at the anchor point. In this average non-periodic components that are not phase synchronized with the anchor points cancel out and only events that have fixed phase relationship with the anchor points survive the procedure [32].

The PRSA increase and decrease series ($T = 3{:}3{:}60$) is obtained for each patient, both for the Supine and Prone Active Baseline. Once the PRSA curve is ready it can be note that the central peak contains contributions of all quasi-periodicities in the original signal and that the decay of the oscillations towards the extremes conveys information about their coherence time [32].

2.6.2 Parameters

According to the first study, Δx, Δy and Slope are calculated from the PRSA series (increase and decrease, T varying) for each patient Supine/Prone Baseline. Δx is defined as the difference between the first maximum and the first minimum along the x axis. Δy is defined as the difference between the maximum and the minimum along the y axis. Slope is defined as the ratio between Δy and Δx. These parameters describe to which extent the heart is capable of increasing or decreasing its beating rate, taking this information from the central amplitude on the y axis. At the same time they take into account the velocity with which the heart manages to reach the required beating rate in response to external factors, taking this information from the x axis [33]. PRSA parameters should be less influenced by noise and thus be more robust and trustable than the time domain HRV parameters [33].

Summary tables

PRSA method settings	
T - anchor point search window width	from 3 to 60 points, with step 3, (3:3:60)
L - averaging window width	75 points for each side
Parameters computed on curves	Δx, Δy, slope

(a) The PRSA method summary.

Dataset	PRSA curves parameters	
	Acceleration	Deceleration
Active Sleep Baselines, Supine vs. Prone	Δx, Δy, slope	Δx, Δy, slope

(b) Parameters obtained form the PRSA curves.

Table 2.6.1: PRSA method and parameters summary.

2.7 Entropy domain analysis

HR arises from a complex combination of both deterministic and stochastic physiological processes [30]. It is often impossible to obtain sufficient insight on biological systems by noninvasive techniques, the knowledge on their behavior is limited by their intrinsic complexity, which is the result of mechanism contributing to the physiological performance. The development of the chaos theory supplied the framework to study nonlinear dynamics through a new approach [34]. Entropy is a concept that addresses system randomness and predictability, with greater entropy associated to more randomness and less system order [35]. Since the birth of this theory, many different entropies have been developed in order to address new problems.

One of the first entropies is the K-S entropy, developed by Kolmogorov and expanded by Sinai, which allowed to classify deterministic systems by rates of information generation [35]. The problem is that KS entropy is not developed for statistical applications and that it could be applied only with no noise and infinite signals, otherwise an ideal signal [35].

2.7.1 Approximate Entropy

To face shortcomings of KS entropy, Approximate entropy (ApEn) was introduced by Pincus [36] to provide applications on real, noisy and finite signals.

ApEn has four advantages in comparison to KS entropy: 1) it is less affected by noise of magnitude below *r* (tolerance), 2) it is robust to occasional artifacts, 3) it needs a reasonable number of data points and 4) it is finite for both stochastic and deterministic processes. So, ApEn has the capability to differentiate models in which both kinds of components are present. To calculate ApEn the parameters *N*, *m* and *r* must be fixed for each calculation. *N* is the length of the time series, *m* is the length of sequences to be compared and *r* is the tolerance for accepting matches. It is convenient to set the tolerance as *r* × SD, the standard deviation of the data set, allowing measurements on data sets with different amplitudes to be compared.

For a time series of *N* points, {u(j): $1 \leq j \leq N$} forms the $N - m + 1$ vectors $x_m(i)$ for {i| $1 \leq i \leq N - m + 1$}, where $x_m(i)$ = {u(i + k): $0 \leq k \leq m - 1$} is the vector of *m* data points from u(i) to u (i + *m* - 1). The distance between two such vectors is defined to be d[x(i), x(j)] = max {|u(i + k) - u(j + k)| : $0 \leq k \leq m - 1$}, the maximum difference of their corresponding scalar components. Let B_i be the number of vectors $x_m(j)$ within *r* of $x_m(i)$ and let A_i be the number of vectors $x_{m+1}(j)$ within *r* of $x_{m+1}(i)$. Define the function $C_i^m(r) = B_i$ / ($N - m + 1$). In calculating $C_i^m(r)$, the vector $x_m(i)$ is called the template and an instance where a vector $x_m(j)$ is within *r* of it is called a template match. $C_i^m(r)$ is the probability that any vector $x_m(j)$ is within *r* of $x_m(i)$. The function $\Phi^m(r) = (N - m + 1)^{-1} \sum_{i=1}^{N-m+1} lnC_i^m(r)$ is the average of the natural logarithms of the functions $C_i^m(r)$ and the parameter ApEn (*m, r*) $= lim_{N\to\infty}[\Phi^m(r) - \Phi^{m+1}(r)]$.

Given *N* data points, this parameter is estimated by defining the statistic ApEn (*m, r, N*) = $[\Phi^m(r) - \Phi^{m+1}(r)]$ [38].

ApEn measures the (logarithmic) likelihood that runs of patterns that are close for *m* observations remain close on next incremental comparisons. Greater likelihood of regularity, produces smaller ApEn values, and conversely [35]. ApEn is usually calculated via a short computer code. The value of *N* for ApEn computations is typically between 100 and 5000 while the choice of *m* and *r* is made to ensure that the conditional probabilities defined are reasonably estimated from the *N* input data points. To avoid a significant contribution from noise in the calculation, it is necessary to choose *r* larger than most of the noise, often *r* should be at least three times the estimated mean noise amplitude [35].

ApEn has demonstrated to be useful in the clinical field, for example in applications to HR both for diagnosis as described in [38], it discriminated groups of subjects, in instances where classical statistical methods, such mean and standard deviation, did not show distinction [36]. The decrease of the ApEn value is associated with sickness and

aging, following the hypothesis that compromised physiology in many systems is linked to a more regular, patterned sinus rhythm HR tracing, while physiology is associated to greater irregularity (randomness and complexity) [35].

Although its advantages, ApEn, as Pincus himself noticed, is affected by a bias effect and depends strongly on the record length [34]. The disadvantage of ApEn is that there is the risk to have a number of matches equal to zero, thus a logarithm of zero is obtained. This constraint is overcome by allowing each template to match itself (self-matching). So the ApEn is defined under all circumstances, because there will always be at least one self matches [38]. The limitation is that this solution could bring to an overestimate of entropy, because self-matching are counted, but they do not correspond to generation of new information. In this work ApEn is evaluated for every segment of 3 minutes of the patients, both for the BSA and BPA. Different values of m, from 1 to 3 are used and the r parameter is set, as suggested in literature as 20% of the standard deviation of the corresponding segment of signal. N is different from a segment to another because of the choice of the 3 minutes approach.

2.7.2 Sample Entropy

Sample entropy (SampEn) has been introduced by Richman and Moorman in order to overcome the limitations of ApEn.

SampEn can be conceived as the conditional probability (CP) that two short templates that match within an arbitrary tolerance will continue to match at the next point. A central idea for this work is that SampEn, like any probability, is estimated more accurately when more events are counted. A data record consists of a series of N consecutive inter-beat (RR) intervals, x_1, x_2, . . ., x_n. For a length $m < n$ and starting point i, the template $x_m(i)$ is the vector containing the m consecutive intervals x_i, x_{i+1}, . . ., x_{i+m-1}. For a matching tolerance $r > 0$, an instance where all the components of $x_m(i)$ are within a distance r of any other $x_m(j)$ in the record, is called a match (or template match). Let B_i denote the number of matches of length m with template $x_m(i)$ and A_i denote the number of matches of length m + 1 with template x_{m+1}(i). Let A = $\sum A_i$ and B = $\sum B_i$ denote, respectively, the total number of matches of length $m + 1$ and m. The ratio p = A/B is then the conditional probability (CP) that subsequent points of a set of closely matching m intervals also remain close and match. SampEn (m, r) is the negative natural logarithm of this probability, as follows [39]:

$$SampEn = -ln(p) = -ln(\frac{A}{B}) = ln(B) - ln(A) \tag{2.7.1}$$

SampEn (m, r, N) calculates the negative logarithm of a probability associated with the time series as a whole; it will be defined except when B = 0, in which case no regularity has been detected, or when A = 0, which corresponds to a conditional probability of 0 and an infinite value of sample entropy [38].

The advantages of the SampEn, in addition to eliminating self-matches, are that it is a simpler parameter to calculate and that it is largely independent of record length and has relative consistency where ApEn has not. That is, if ApEn of one data set is higher than that of another, it should, but does not, remain higher for all conditions tested. The disadvantages are that SampEn strongly depends on the size of the tolerance r and because of this it is not possible to compare entropy estimates made using different values of r. Smaller values of r lead to higher and less confident entropy estimates because of falling numbers of matches of length m and m+1. The selection of the parameters m and r is critically important: if m is too large or r is too small the number of template matches will be too small for confident evaluations; on the other hand, if m is too small and r is too large, all templates will match each other [39].

Furthermore both ApEn and SampEn cannot reveal the underlying dynamics of the generating system, with these parameters it can only be understood if one signal is more or less regular than another one [34]. It is here evaluated the SampleEn following the same method decided for the ApEn, using m varying from 1 to 3 and r equal to the 20% of the standard deviation of the 3 minutes segment.

Orthogonal Range Search algorithm

The time necessary to compute SampEn is proportional to the square of the length N of the signal. Since there is the need to compute SampEn several times for all the patients and different parameters, for example different embedded dimensions m and different r, a suitable computation time would be better; this aim is reached following the Orthogonal Range Search algorithm proposed by Yu-Hsiang Pan et al. 2011 [40] that provides a time of computing linearly proportional to N.

The problem of the match counting can be traduced to an equivalent range-count problem, so that the values of A and B in the SampEn equation can be computed by solving an orthogonal range counting problem. The first step is to build the m-dimensional space of N-m+1 points. For each point, his neighbors within a box of radius r (the tolerance) in an m-D space are found. Then the number of neighbors for all the N-m+1 points, n_n(for m) and

n_d(for m+1), is counted and B and A can be computed as: $B = \sum_i n_n^i$ and $A = \sum_i n_d^i$. For the range count the earliest and the simplest method to implement is the k-d tree, as suggested in [40].

2.7.3 Quadratic Sample Entropy

One of the largest problem in implementing Entropy estimation is the choice of the r tolerance value. An important new insight is presented by Lake in 2006 who suggested to convert the measured conditional probability to a density by normalizing it for $2 \times r$, that translates into adding ln($2r$) to the Sample Entropy estimate. It is suggested that, with this approach, Entropy estimation with different r can be compared each other directly. Furthermore r can be optimally varied for each data record. This measure is called Quadratic Sample Entropy (QSE) and it is defined as follows [39]:

$$QSE = -ln(p/(2r)) = -ln(p) + ln(2r) = SampEn + ln(2r) \tag{2.7.2}$$

As presented in [41], QSE can help to diagnose patient with atrial fibrillation (AF) separating them from healthy patients (Normal Sinus Rithm, NSR), as QSE more consistently let the curves to be separated than SampEn, as below in Figure 2.7.1.

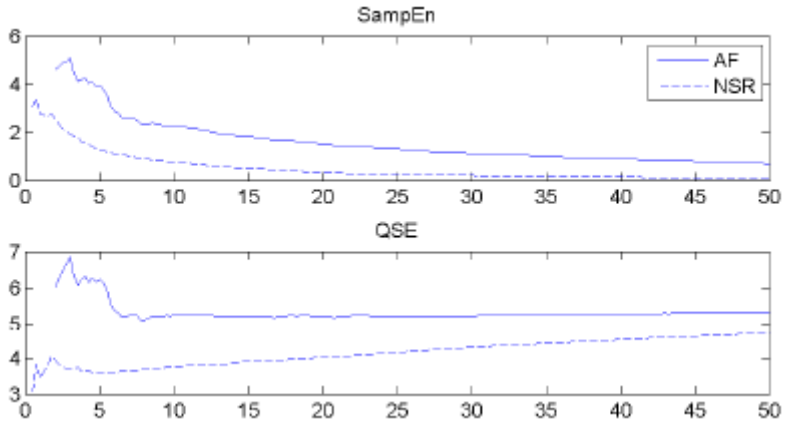


Figure 2.7.1: SampEn and QSE as a function of tolerance r for 2 signals. SampEn starts to diverge while QSE converges for small r.

In this dataset, that is characterized by relatively short of signals, both because the recording for each baby is 1 hour long, comprises Tilt and Baselines, both because in this analysis the cleaned segments of 3 minutes, in Active Sleep, of the Baseline Supine and Prone, are considered. Another improvement suggested in [41] to increase the statistical

significance and the reliability of QSE parameter is to imposed a minimum numerator counter.

The minimum numerator count method looks to make r as small as possible but varies r as needed until a pre-specified number of matches A are observed. Other additional restrictions, such as minimum denominator count on B, can be also used to control accuracy. The minimum numerator count method provides stable estimates of QSE for lengths as short as $n = 8$ [41].

As it can be seen in Figure 2.7.2 this method provides even more consistency especially for low record lengths, $N \lesssim 25$; it is clear then how this can be useful for this research.

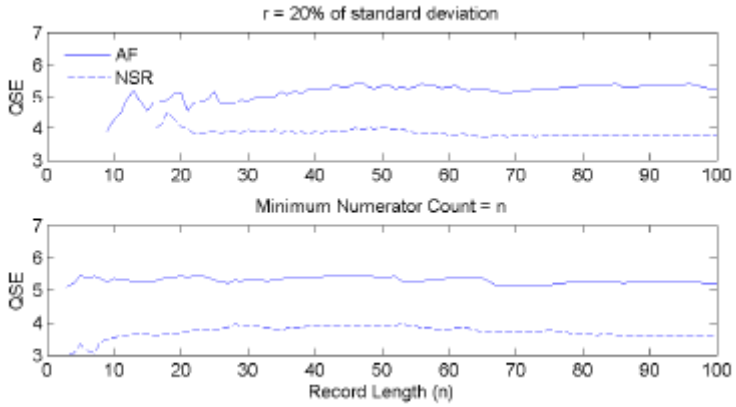


Figure 2.7.2: QSE as a function of first n points for AF and NSR. The minimum numerator count method provides stable estimates of QSE for lengths as short as n~8. Standard methods degrade starting for n~50 and degenerate starting at n~15 [41].

An automatic method for selecting the best minimum number of matches for the numerator is then proposed in [42], this new procedure is based on the computation the QSE with increasing number of minimum matches, and then selecting the best one (i.e. the one that most separates two conditions) through ROC curves.

The method

The method implemented consists of three main steps. The maximum number of matches for a each segment is computed as $M_{max} = (N_x - m)(N_x - m + 1)/2$ where N_x is the number of samples of the time-series, m embedded dimension. Among these the smallest M_{max} is chosen and it is set as M_{max} for all the series. Since the 3 minutes long segment has around $N = 300$, this translated in $M_{max} \approx$ 300^2 = 90'000 maximum number of matches (for the numerator).

It is than computed the QSE ensuring that a minimum number of matches for the numerator is assured, this is done for every minimum number of matches from 1 to the maximum with a step of 2'000 (M = 1:2000:M_{max}). The step of 2'000 is empirically chosen

to assess reasonable computation time and enough resolution for the r value. For each M, in order to compute a minimum number of matches M in the numerator, r is initialized to zero and increased of successive steps of: $r_{t+1} = r_t + 0.015 * std(sig)$. It is obtained one value of QSE for each M, going from 1 to M_{max} and it is used the following method, as in [42], to assess which is the best to distinguish the two populations, Supine and Prone Active Baselines. For each M the ROC curve is built based on the QSE value for classifying the two population, assigning target 0 to the Supine population and target 1 to the Prone population. It is then chosen the M that has the maximum area under the curve (AUC).

The M value found is called optimum, $M_{optimum}$; this whole steps where performed for $m = 1$, $m = 2$ and $m = 3$. This way the r ($M_{optimum}$) assures a minimum number of matches regardless of the signal length.

Gaussian noise

A problem for the interpretation of this numerical quantity could arise from the lack of a measure unit. An improvement could be made by converting the QSE value to the standard deviation of Gaussian white noise with the equivalent entropy.

This is possible thanks to the knowledge that for Gaussian noise with standard deviation σ, the value of the quadratic entropy rate is $\frac{1}{2}ln(4\pi) + ln(\sigma)$ or $1.266 + ln(\sigma)$. This conversion leads the Entropy measure to have units equal to the physiologic measurement, and may provide a better practical use.

2.7.4 Multiscale Entropy

To achieve an analysis at different scales it has been introduced by Costa et al the Multiscale Entropy (MSE) method, which is strongly related to different signal structures [34]. Compared to traditional complexity measures, MSE has the advantage of being applicable to both physiologic and physical signals of finite length [43].

Given a one-dimensional discrete time series, {x (1), ... x (i), ... ,x (N)}, the consecutive coarse-grained time series, {y^(τ)}, corresponding to the scale factor, τ, are constructed. First, the original times series are divided into non overlapping windows of length τ, second, the data points inside each window are averaged, as in the Figure 2.7.3. In general, each element of a coarse-grained time series is calculated according to the equation

$$y_j^\tau = \frac{1}{\tau} \sum_{i=(j-1)_\tau+1}^{j\tau} x_i, 1 \leq j \leq N/\tau \tag{2.7.3}$$

For scale one, the time series {y1} is simply the original time series. The length of each coarse-grained time series is equal to the length of the original time series divided by the scale factor, τ. Finally, an entropy measure (SE) for each coarse-grained time series, plotted as a function of the scale factor τ, is calculated.

The MSE curves are used to compare the relative complexity of normalized time series (same variance for scale one) based on the following guidelines: 1) if for the majority of the scales the entropy values are higher for one time series than for another, the former is considered more complex than the latter, 2) a monotonic decrease of the entropy values indicates the original signal contains information only in the smallest scale [43].

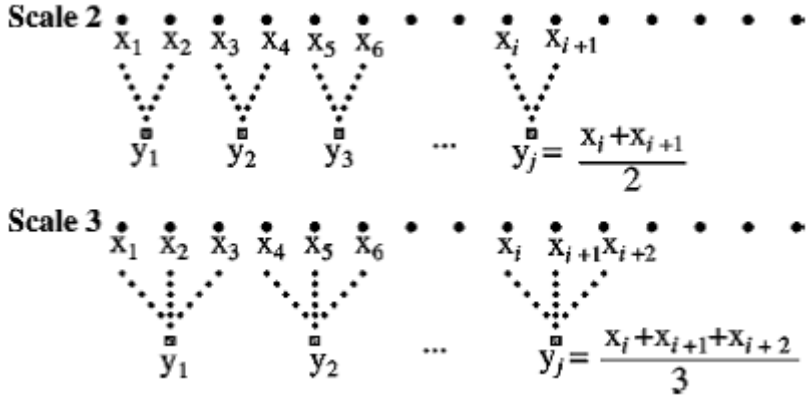


Figure 2.7.3: MSE: Schematic illustration of the coarse-graining procedure, from [43].

This approach gives more details about the time scales at which irregularities occur and it allows extracting the information about the signal structure, for example if the irregularity belongs to the first scale factor only (white noise) or it persists for all scale factors (1/f noise) [34].

In literature the values of the scale (L) were varied from 1 to 20 in HR analysis, in this work it is decided not to use L values higher than 8 because signals are too short, with approximately 300 samples in 3 minutes. $L = 20$ (averaging on 20 samples) is a too small number of samples (coarse grain series of length 300/20 = 15) to have a valid entropy estimation, since often the value of entropy for such high scales is infinite because of the lack of m+1 matches found, causing the denominator of the natural logarithm to be zero.

The tolerance (r) obtained from the QSE algorithm is used and the MSE value, for each scale L from 1 to 8, is calculated for each segment of 3 minutes Supine and Prone.

2.7.5 Permutation Entropy

As done previously in the first study, Permutation Entropy (PermEn) parameter is implemented [44]. PermEn is a complexity measure for time series operating on an ordinal level. Its main features are 1) its robustness with respect to some noise corrupting the data and 2) its easy computation [44]. PermEn measures the entropy of sequences of ordinal patterns derived from *m*-dimensional delay embedded vectors, the idea behind permutation entropy is that patterns may not have the same probability of occurrence, and thus, that this probability may unveil relevant knowledge about the underlying system. An extreme situation is represented by the forbidden patterns, that is, patterns that do not appear at all in the analyzed time series [45]. By assessing the presence, or absence, of some permutation patterns of the elements of a time series, it is possible to derive information about the dynamics of the underlying system. Even if all patterns eventually appear, the probability with which each one is present can unveil relevant information about this dynamics [45].
Its definition is presented in [44, 46] as follow.

The scalar time series $x(t)_{(t=1)}^{T}$ is embedded into an m-dimensional space Xt = [x(t), x(t+*L*),..., x(t + (*m* - 1)*L*)], where *m* is called the embedded dimension and *L* the embedded delay time. In general, there are just *m*! possible order patterns, which is the number of permutations of the m coordinates in X_t. Let p(π) denote the relative frequency of order pattern π,

$$p(\pi) = \frac{\#t|1 \leq t \leq T-(m-1)L}{T-(m-1)L} \tag{2.7.4}$$

Then, for fixed embedded dimensions $m \geq 2$, and fixed delay *L*, permutation entropy is defined as

$$H(m,L) = -\sum\nolimits_{\pi} p(\pi) log_2 p(\pi) \tag{2.7.5}$$

where the sum runs over all *m*! patterns π.

For convenience H(*m,L*) is normalized by its maximum value $log_2 m!$

$$0 \leq \frac{H(m,L)}{log_2 m!} \leq 1 \tag{2.7.6}$$

The choice of *m* and *L* parameters is important, PermEn is calculated for different embedded dimensions, in according with the literature with *m* at least 3 and maximum 6, limited by the length of the time series and the delay equal to 1 for simplicity [44].

Summary tables

DATASET ACTIVE SLEEP BASELINES	ENTROPY MEASURES	INPUT PARAMETERS
Supine vs. Prone	Approximate Entropy (AppEn)	(m, r)
	Sample Entropy (SampEn)	(m, r)
	Quadratic Sample Entropy (QSE) with minimum matching counter	m, $M_{min} : M_{step} : M_{max}$ r_{step}
	Multi Scale Entropy (MSE)	m, r, L_{min},$L_{max}(scale)$
	Permutation Entropy (PermEn)	m, L (delay)

Table 2.7.1: Entropy Summary.

Chapter 3

Results and Statistical analysis

In this chapter the results and the statistical analysis will be presented.

After a brief discussion about the evolution of the parameters with the PMA of the babies, the non parametric statistical test used is described: the Wilcoxon Signed-Rank test.

For each parameter the tables relative to the values obtained for a patient taken as example and the mean of the whole population will be reported.

The statistical test is done for every parameter using as population the difference between the Supine and Prone position.

In the last section of the chapter the statistical analysis on the comparison between this study (follow up) and the first study will be presented, to show the variation of the same parameter from the first study to the second one, recorded 2 months later.

3.1 Evolution of parameters with PMA

From the literature is known that the HR should decrease with the maturation of the baby, thus with his PMA.

In the first part of the chapter a short analysis of this evolution is presented , showing the trend of the HR, to assess if these recordings can exemplify a general population.

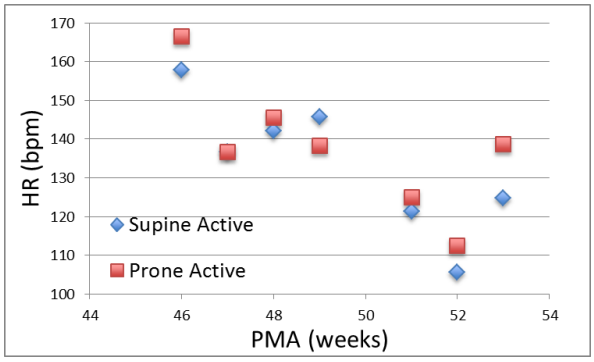
It is important to underline that the 10 babies of this study have not the same age, they have an average PMA of 49,3 weeks; hereinafter in Table 3.1.1 the number of babies, for each PMA value, and the HR value, both for the Prone and Supine position are reported.

PMA (WEEKS)	46	47	48	49	51	52	53
# Babies	1	2	1	2	2	1	1
HR ±std, Supine	157,91	136,71 ± 7,71	142,06	145,79 ± 1,47	121,26 ± 5,93	105,51	124,82
HR ± std, Prone	166,46	136,61 ± 4,46	145,50	138,34 ± 6,84	124,87 ± 5,25	112,46	138,63

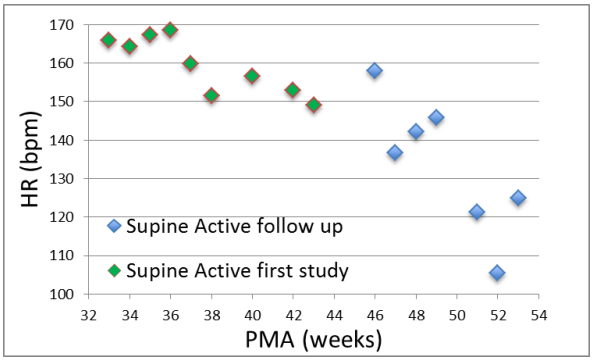
Table 3.1.1: Age distribution of the population according to PMA (# of babies for each PMA in weeks) and the HR for the Supine and Prone position, Active sleep.

With these data, the following Figure (3.1.1) is presented to demonstrate the decrease of the HR value in bpm, with the increase of the PMA. In the left image data from the follow up dataset are reported, showing the trend of the 10 babies in the Supine and Prone position, while in the right image there is the trend of the babies taken also from the first study dataset, only for the BSA.

It can be seen that the HR values from the 33rd to the 53rd week go down from an average of 165 bpm down to lower values, approximately around 120 bpm.



(a) Supine and Prone follow up.



(b) Supine first study and follow up.

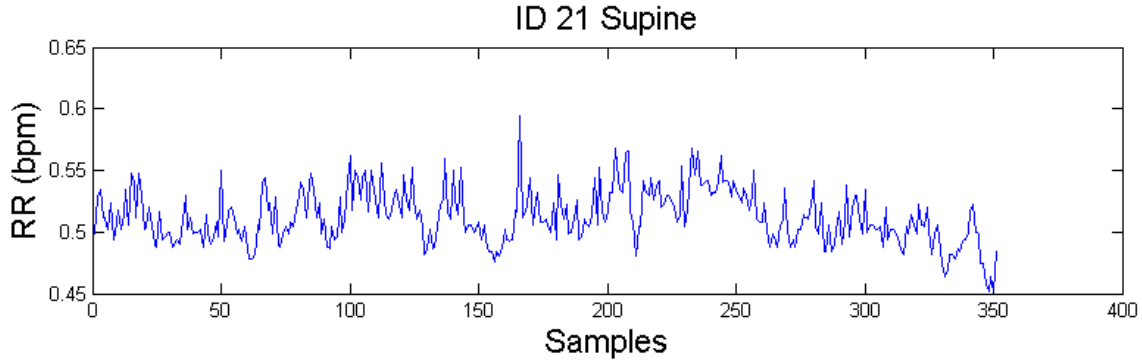
Figure 3.1.1: HR (beats per minute) as a function of PMA, expressed in weeks. In Figure a the average HR of the follow up babies in Active sleep is portrayed, both in Prone (red) and Supine (blue) position. In Figure b, instead, the average HR of both the first study (green) and follow up (blue) babies in the Supine position are portrayed.

3.2 Presentation of the results

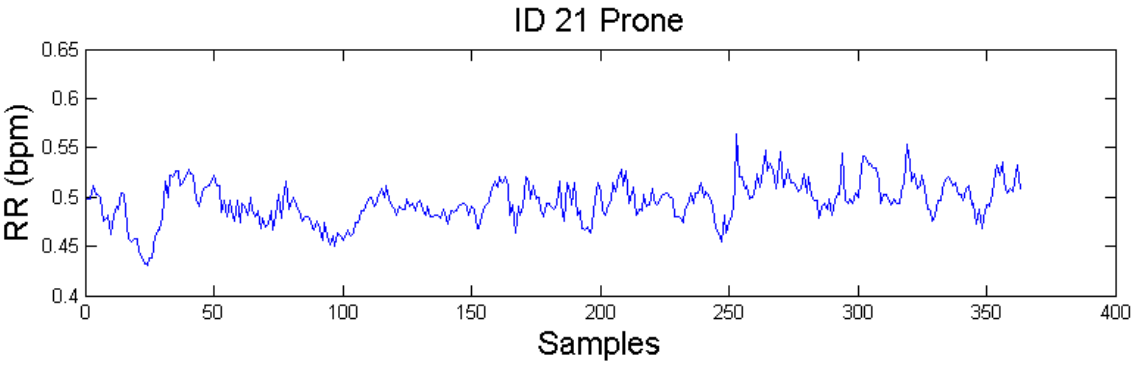
The results obtained will be presented by field, starting with the time domain, proceeding with the frequency domain and finally the Entropy measures.

For each section, results for a patient taken as example will be portrayed (usually patient ID 21), followed by the mean of the whole population (10 babies).

The patient ID 21 is generally a good example to validate the results achieved, in Figure 3.2.1 the tachogram for this patient is shown. In the upper image there is the Baseline Supine Active (BSA) of 3 minutes, in the lower one there is the Baseline Prone Active (BPA) just for one of the two segments of 3 minutes. It is already evident from the tachogram that in the Supine position the MEAN of the RR has higher values than in the Prone, as validated with the following results.



(a) Supine.



(b) Prone.

Figure 3.2.1: Tachogram for patient ID 21 of the 3 minutes BSA at the top and the BPA at the bottom.

For each field the statistical analysis too will be reported, showing the P-value obtained for each parameter, using a non parametric test as described in the following section. Among the large set of parameters chosen and described in Materials and Methods, only the most relevant results will be presented.

For the PRSA, MSE and PermEn no significant results has been obtained.

There will be a paricular focus on the QSE. This methodology is applied both to the follow-up and to the first study, so that a comparison and a further verification is possible, since in the first one it was not implemented.

The last part of the chapter is dedicated to the comparison between the two studies.

Table 3.2.1 summarizes the presentation of the results, describing the Baselines analyzed, the parameters and the number of patients available.

ACTIVE BASELINES	RESULTS AND STATISTICAL ANALYSIS	N° OF PATIENTS
Supine vs. Prone	Time Domain	10
	Frequency Domain	10
	ApEn and SampEn	10
	QSE	10 follow up
		29 first study
Supine vs. Supine Prone vs. Prone	Comparison follow up vs first study	9

Table 3.2.1: Summary of the presentation of the results.

3.3 PRSA, MSE and PermEn

The methodologies of the PRSA and MSE have been applied, even knowing they best performed on long signal, since they both involved averaging procedures. The results obtained from them were not significant and the cause is mainly linked to the shortness of signals of this study.

Regarding the PRSA, the limited number of samples in each 3 minutes segment does not allow to have a sufficient set of curves to be averaged. This way, the averaging procedure brings to noisy PRSA curve that impaired the computation of meaningful indices on the curves.

The MSE is computed to perform an analysis at different time scales, using an averaging process on large number of samples to obtain the coarse-grained time series. For large scales the averaging procedure causes to coarse-grained series with very few samples, which compromised the Entropy estimation for such scales.

The PermEn is another method used, but it is not found significant for this study.

3.4 The Wilcoxon signed-rank test

In order to perform a paired difference t-test, the samples need to be normally distributed. In this study only 10 Baselines can be compared and a simple test of normality shows that they

are not normally distributed.

So, methods of statistical analysis to be used in non-normal populations are investigated: non-parametric tests. Among the possible tests, the decision is to use the Wilcoxon test for paired samples, the Wilcoxon Signed-Rank test. It is a test based on paired differences and like the paired t-test it allows to avoid problems linked to the correlation between samples coming from the same subject, that could hide the differences between two populations. The populations tested are the indices measured on the Supine and the Prone Active Baselines. To have significance the null-hypothesis, that data comes from a distribution with null median at 5% of significance, has to be rejected, giving a P-value smaller than 0,05.

3.5 Time domain

3.5.1 Results

The time analysis described in Materials and Methods has been applied and the parameters for each patient have been calculated, both for the BSA and BPA, for each 3 minutes window. Then the values obtained in the segments (when more than one) are averaged to get a single measure. In Table 3.5.1 it is showed an example of the values obtained for the patient ID 21 of the dataset, who had one segment of 3 minutes in the Supine Baseline and 2 in the Prone.

In Table 3.5.2 the mean of the 10 patients and the relative standard deviation are reported, for each time domain parameter, both for the Supine and Prone position.

ID 21	SUPINE	PRONE
N° 3 MINUTES WINDOWS	1	2
MEAN	0,5125	0,4952 ± 0,00005
SDNN	0,0219	0,0346 ± 0,0138
RMSSD	0,0200	0,0136 ± 0,0001
LTV	31,7332	44,6758 ± 11,5239
STV	15,1714	10,4661 ± 0,0738
II	0,0427	0,0699 ± 0,0279
DI	0,0200	0,0137 ± 0,0001
RMSM	0,0218	0,0346 ± 0,0138
IIa	1,6339	0,8783 ± 0,3265
STI	0,0401	0,0570 ± 0,0217

Table 3.5.1: Time domain parameters for patient ID 21, 1 segment for the Supine Baseline, the mean of the 2 segments for the Prone Baseline, Active sleep.

N° OF PATIENTS: 10		
PARAMETERS	SUPINE	PRONE
MEAN	0,4548 ± 0,0570	0,4409 ± 0,0469
SDNN	0,0373 ± 0,0117	0,0357 ± 0,0154
RMSSD	0,0174 ± 0,0099	0,0142 ± 0,0085
LTV	53,0236 ± 10,5789	56,5646 ± 21,6363
STV	12,8231 ± 7,7307	10,2905 ± 7,0366
II	0,0817 ± 0,0198	0,0799 ± 0,0326
DI	0,0174 ± 0,0099	0,0142 ± 0,0085
RMSM	0,0373 ± 0,0117	0,0356 ± 0,0154
IIa	0,9258 ± 0,2899	0,8740 ± 0,0753
LTI	0,0736 ± 0,0239	0,0663 ± 0,0332

Table 3.5.2: Mean of the time domain parameters with the standard deviation of the 10 patients, for the Supine and Prone Baseline, Active sleep.

In order to understand if the parameters evaluated were correlated, the cross-correlation between them was computed. The result of this analysis is evident from the table here reported (Table 3.5.3), where the significant correlations are presented. It shows an high correlation between RMSSD and DI (>0.99), in fact their calculation is similar but they come from different fields, fetal and adult. Regarding the high correlation between RMSSD (and DI) and STV it can be explained with the fact that they both estimates short term variability in the HR.

SUPINE	MEAN	RMSSD	STV	DI
MEAN	1	0,9159	0,9217	0,9159
RMSSD	0,9159	1	0,9975	0,9999
STV	0,9217	0,9975	1	0,9975
DI	0,9159	0,9999	0,9975	1

(a) Supine table.

PRONE	MEAN	RMSSD	STV	DI
MEAN	1	0,8079	0,8324	0,8080
RMSSD	0,8079	1	0,9761	0,9999
STV	0,8324	0,9761	1	0,9762
DI	0,8080	0,9999	0,9762	1

(b) Prone table.

Table 3.5.3: Correlation among time domain parameters in the Supine and Prone position, only the high correlated parameters are reported.

Moreover it could be seen in Figure 3.5.1 the demonstration of linear correlation between (for example) STV and DI, noticing that, plotting the values, they are approximately on a line.

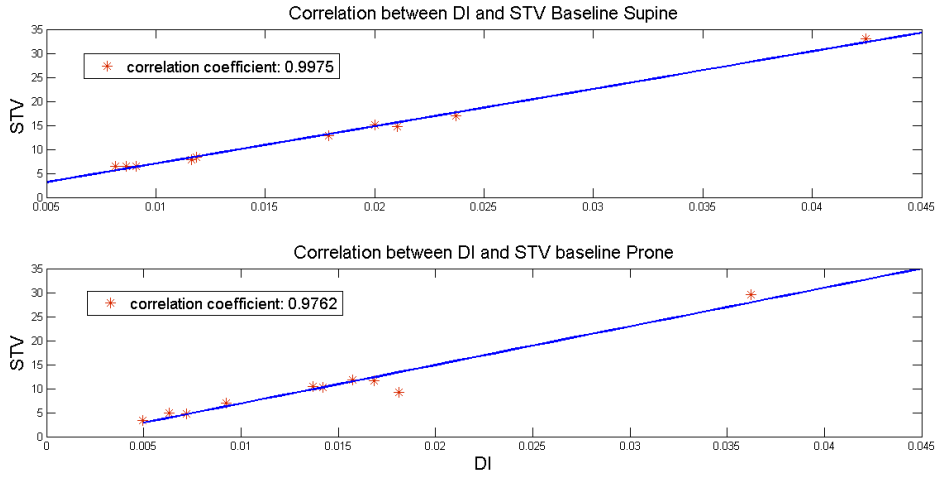


Figure 3.5.1: Example of correlation between STV and DI in the Supine Baseline (top) and the Prone Baseline (bottom), Active sleep.

The values for Supine and Prone position are then presented for three of the time parameters, chosen for their relevance. In Figure 3.5.2 the MEAN of RR is shown; in the plot at the top there is the value for Supine (blue) and Prone (red) for every patient. Notice that the number of the patient on the x-axis is not representing the ID of the patient, but it is an ordinal numeration. In the plot at the bottom of Figure 3.5.2 there is the difference between Supine and Prone mean values of each patient. It can be clearly seen that the Supine values are significantly higher than the Prone, so that the value of the difference is often above zero.

The use of the paired differences between the two populations let the results and the statistical test to not be influenced by the mean, that could mask the real trend. This way only the increase or the decrease of each parameter within the same subject is tested and a significance can be found even when the mean values of the populations are similar.

Other examples are reported in Figure 3.5.3 and 3.5.4, where there is the value for each patient of the RMSSM and STV in the Supine and Prone position and the difference between Supine and Prone. Also for these parameters the value for the Supine is higher than the Prone.

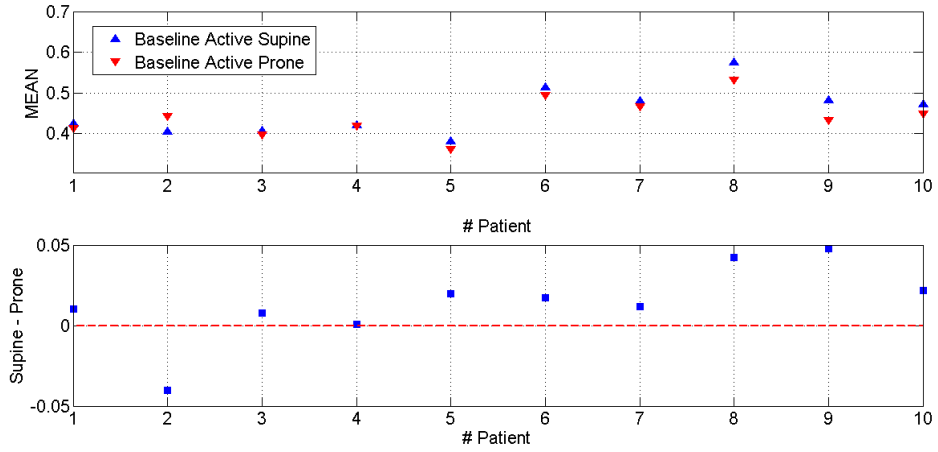


Figure 3.5.2: Mean of RR value for each patient for the Supine (blue) and Prone (red) Baseline is reported in the top figure. The difference between Supine and Prone is showed in the bottom figure, where the zero value is highlighted with the red dotted line.

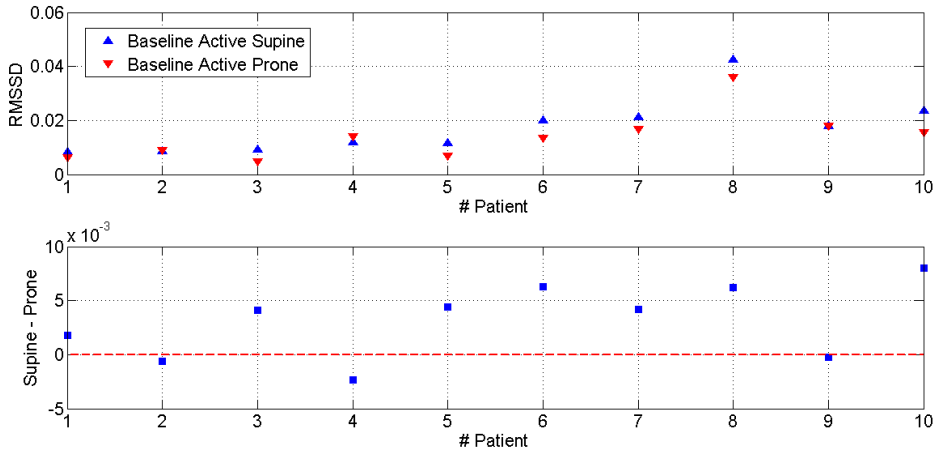


Figure 3.5.3: RMSSD value for each patient for the Supine (blue) and Prone (red) Baseline is reported in the top figure. The difference between Supine and Prone is showed in the bottom figure, where the zero value is highlighted with the red dotted line.

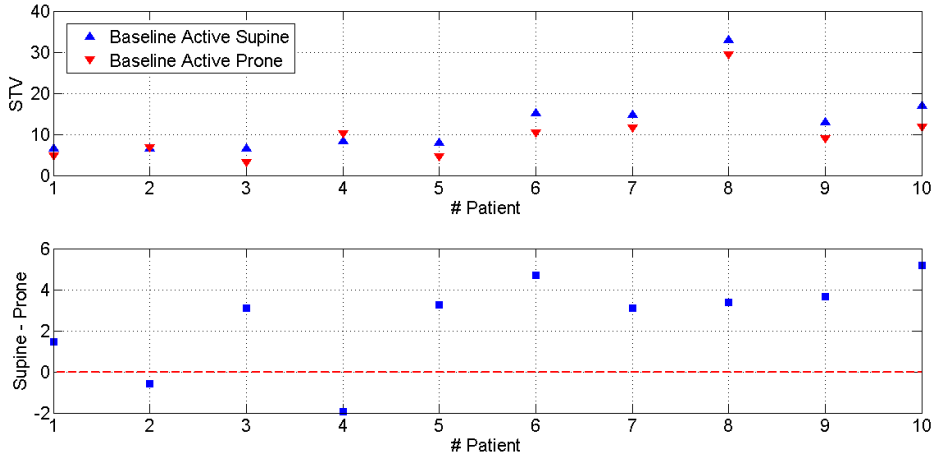


Figure 3.5.4: STV value for each patient for the Supine (blue) and Prone (red) Baseline is reported in the top figure. The difference between Supine and Prone is showed in the bottom figure, where the zero value is highlighted with the red dotted line.

3.5.2 Statistical analysis

To obtain the statistical analysis the Wilcoxon signed rank test is applied to see which parameter is significant for the research, interpreting as significant a parameter able to distinguish Supine and Prone position. As summarized in Table 3.5.4, only the MEAN of RR, RMSSD, STV and DI (as stressed by the star in the table) are significant, having a P-value under the imposed threshold of 0,05.

N° OF PATIENTS: 10	
PARAMETERS	P VALUE
MEAN	0,0488*
SDNN	0,6250
RMSSD	0,0371*
LTV	0,7695
STV	0,0137*
II	0,6250
DI	0,0371*
RMSM	0,6250
IIa	1
LTI	0,4921

Table 3.5.4: P-value for the time domain parameters, the star stresses the values under the threshold (0,05).

3.6 Frequency domain

3.6.1 Results

For every patient the power spectra and the power in the LF and HF bands are computed.

In Figure 3.6.1 the spectra of BSA vs. BPA can be seen, for patient ID 21, as a representative case. Differences between the two conditions are not observed.

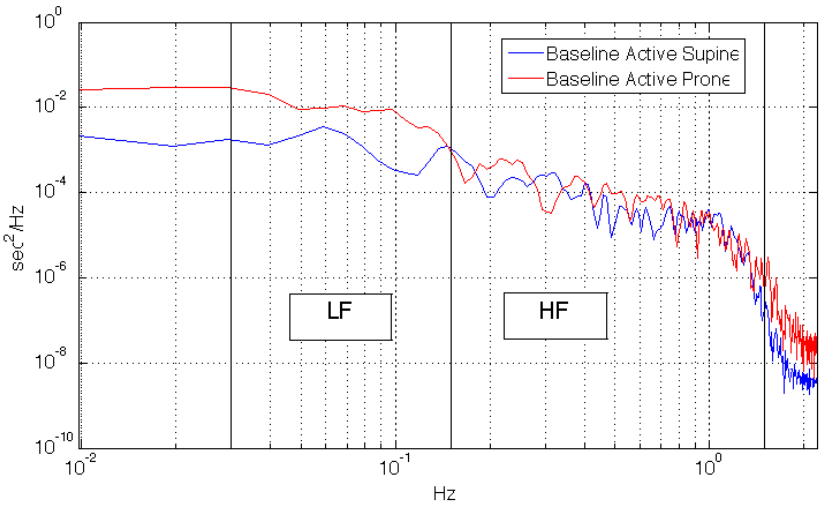


Figure 3.6.1: Power spectra for BSA (blue) and BPA (red) for the patient ID 21, the frequency bands LF and HF are highlighted.

It can be seen that in different patients the power within the selected bands is not consistently higher (or lower) for the Supine or Prone position.

In Tables 3.6.1 and 3.6.2 the value of the frequency domain parameters are reported, for the patient ID 21 and for the whole set of patients.

ID 21	SUPINE	PRONE
N° 3 MINUTES WINDOWS	1	2
LF	2,60E-04	6,89E-04± 0,0003
HF	2,06E-04	8,42E-05± 1,05E-05

Table 3.6.1: Frequency domain parameters for patient ID 21, 1 segment for the Supine Baseline, the mean of the 2 segments for the Prone Baseline, Active sleep.

N° OF PATIENTS: 10		
PARAMETERS	SUPINE	PRONE
LF	1,1691E-03 ± 8,27E-4	9,94E-04 ± 7,9E-04
HF	1,61E-4 ± 1,92E-04	1,24E-04 ± 1,66E-04

Table 3.6.2: Mean of the frequency domain parameters with the standard deviation of the 10 patients, for the Supine and Prone Baseline, Active sleep.

3.6.2 Statistical Analysis

The Wilcoxon Signed Rank Test is applied to assess any significant difference between the power in the two bands analyzed, the coupled populations P_{LF} BSA and P_{LF} BPA and also P_{HF} BSA and P_{HF} BPA. The P-values are reported in Table 3.6.3.

N° OF PATIENTS: 10	
COUPLED POPULATIONS	P-VALUE
P_{LF}	0,5566
P_{HF}	0,0839

Table 3.6.3: P-value for the frequency parameters, Supine vs Prone Active Baselines.

It is then shown in Figure 3.6.2 the values for each patient of the HF power and the difference Supine-Prone.

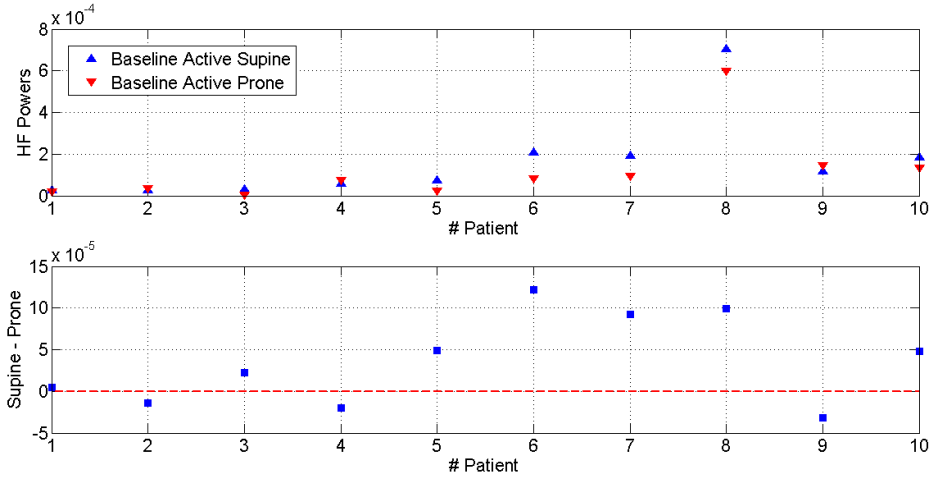


Figure 3.6.2: HF power value for each patient for the Supine (blue) and Prone (red) Baseline is reported in the top figure. The difference between Supine and Prone is showed in the bottom figure, where the zero value is highlighted with the red dotted line.

As discussed before there is no statistically significant difference between the Supine and Prone populations, both for P_{LF} and P_{HF}.

It is useful for a better understanding of this measures, to acknowledge that there is a high variability in the newborns and that respiration patterns are more irregular than adults, thus it is possible that these methodologies, originally developed to be used in an adult subject that is fully developed and stable, need more precautions when applied to newborns.

These results can also be partially, but significantly, explained considering the respiration spectra and the phenomenon of cardiac aliasing.

3.6.3 Cardiac aliasing

Cardiac aliasing can strongly affect spectral analysis, being the HR too low to "sample" the respiration, thus, HF power can be greatly underestimated. The spectra for respiration and the cross-spectra HR-Respiration are computed to assess whether or not cardiac aliasing could affect the measures. These findings show that cardiac aliasing happened often in newborns, thus possibly conducting to inconclusive results, as seen above.

Below are presented two significant examples, that prove the occurrence of cardiac aliasing.

In the first one (Figure 3.6.3) it can be seen that the peak for patient ID 16 in HF is found both in the HR and in the respiration spectrum; this means that the HR is “fast” enough to “sample” the breathing. In fact by analyzing the segment it is verified that the HR is more than double than the HF peak frequency, being then able to sample it:

$$HeartRate \geq 2 \cdot RespirationRate \tag{3.6.1}$$

$$2{,}45Hz \geq 2 \cdot 0.{,}65Hz = 1{,}3Hz \tag{3.6.2}$$

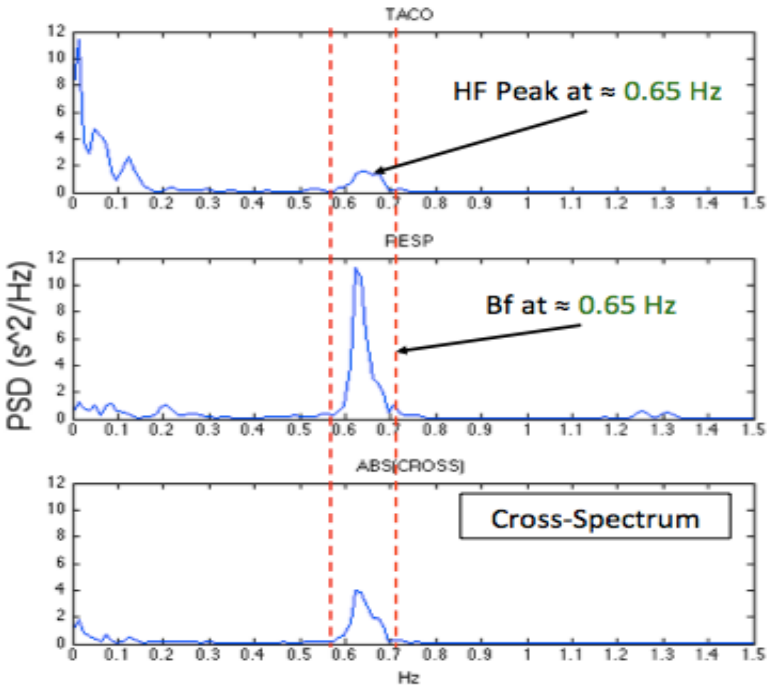


Figure 3.6.3: No cardiac aliasing: the peak in HF (Bf) found in the spectrum of respiration (in the middle) is also found in the HR spectrum for patient ID 16. The Nyquist frequency is 5 Hz, but the maximum frequency displayed is 1,5 Hz, since there were no significant components above.

Instead, in this other case (Figure 3.6.4) for patient ID 15, one can verify that even if a peak can be seen in the respiration's spectrum, this cannot be found in the relative segment of the HR. And coherently it is found that the HR in that segment is too low to sample the respiration rate:

$$HeartRate \geq 2 \cdot RespirationRate \tag{3.6.3}$$

$$1{,}97 \ngeq 2 \cdot 1{,}1Hz = 2{,}2Hz \tag{3.6.4}$$

this confirm the occurrence of cardiac aliasing for patient ID 15.

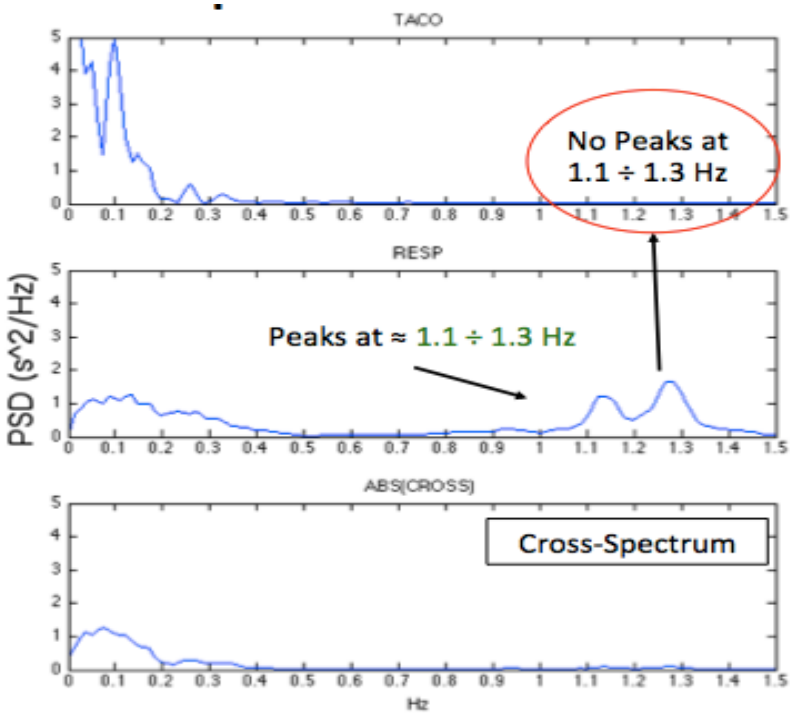


Figure 3.6.4: Cardiac Aliasing: HF peak visible in respiration spectrum but not in HR spectrum for patient ID 15. The Nyquist frequency is equal to 5 Hz, but the maximum frequency displayed is 1,5 Hz, because of the lack of components above that frequency.

In this case, in which the Shannon law for cardiac aliasing is not respected, the peak cannot be seen in the HR spectrum, as predicted. It can be then stated that, especially in newborns, the cardiac aliasing should be taken into account in the frequency power estimation in the spectral analysis of the HR, since, at least in some subjects, it can alter considerably the conclusions by distorting the PSD.

3.7 Entropy

3.7.1 Approximate and Sample entropy

Results

The value of Approximate Entropy and Sample Entropy is computed for each segment of 3 minutes length for m=1, m=2 and m=3, distinguishing Supine and Prone position (in both cases Active Sleep Baselines). Tolerance r is set equal to 20% of the standard deviation of the segment of the RR signal. Here the values of both the entropies for the subject ID 21 are shown in Table 3.7.1.

ID 21	ApEn		SampEn	
	Supine	Prone	Supine	Prone
n° 3 minutes windows	1	2	1	2
m = 1	1,7357	1,2919 ± 0,2414	1,7333	1,2352 ± 0,2536
m = 2	1,2506	1,0479 ± 0,1559	1,7064	1,1775 ± 0,2657
m = 3	0,5362	0,6993 ± 0,0386	1,6879	1,1459 ± 0,2780

Table 3.7.1: ApEn and SampEn values, with tolerance r set to r = 0.2*std (signal), for patient ID 21, for each embedded dimension m = 1,2,3 for both the Supine (1 segment) and Prone (mean of 2 segments) position, Active Baselines.

In the following Table (3.7.2) instead, the mean of the values of the 10 patients is reported.

n° of patients: 10				
	ApEn		SampEn	
	Supine	Prone	Supine	Prone
m = 1	1,1854 ± 0,3029	1,1079 ± 0,3023	1,1063 ± 0,3671	1,044 ± 0,3165
m = 2	0,9479 ± 0,1582	0,8564 ± 0,1623	0,9863 ± 0,3835	0,8983 ± 0,2929
m = 3	0,6076 ± 0,0813	0,5893 ± 0,1207	0,8614 ± 0,3676	0,7935 ± 0,2690

Table 3.7.2: Mean of the ApEn and SampEn parameters with their standard deviation of the 10 patients, for the Supine and Prone Baseline, Active sleep.

Statistical analysis

No significant difference in the population Prone vs. Supine is observed, in fact the P-value of the Wilcoxon signed rank test is above the significant level of 0,05 (Table 3.7.3).

N° OF PATIENTS: 10		
	P-VALUE APEN	P-VALUE SAMPEN
$m = 1$	0,5566	0,4316
$m = 2$	0,1308	0,3222
$m = 3$	0,6250	0,6250

Table 3.7.3: P-value for the ApEn and SampEn for each embedded dimension. None of the parameters is under the threshold of 0,05.

It can be seen how ApEn is more affected by the choice of m than SampEn; no significant difference is found between Prone and Supine position.

Here in the upper image of Figure 3.7.1 the values of ApEn are shown, for each patient, m=2 while at the bottom the difference between the Supine and Prone position for this parameter is portrayed.

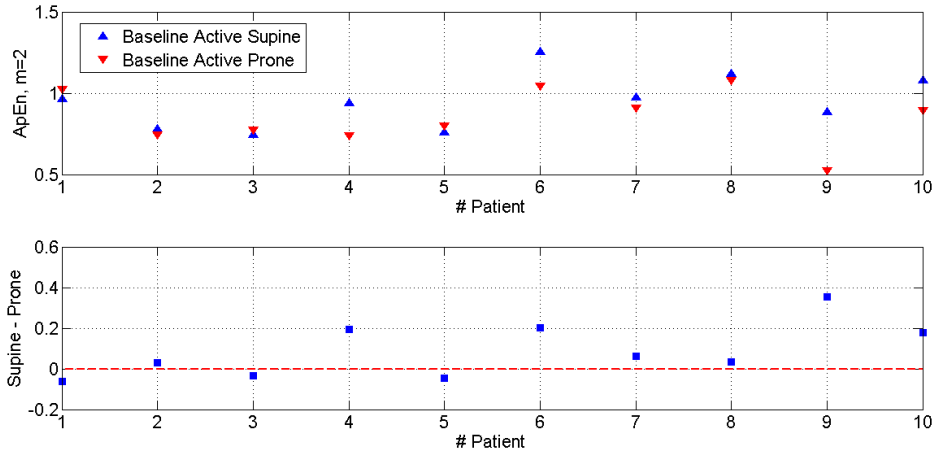


Figure 3.7.1: ApEn value for the embedded dimension $m = 2$, for each patient, for the Supine (blue) and Prone (red) Baseline is reported in the top figure. The difference between Supine and Prone is showed in the bottom figure, where the zero value is highlighted with the red dotted line.

These findings can be explained firstly through the comparison with the first study, since no significant difference is assessed even in that case, and also because being this study shorter, and, in general, having the babies lower HR, the 3 minutes segments had significantly less numerous time-series and that could affect even more the robustness of the measurement. Moreover the number of samples is different from a segment to another.

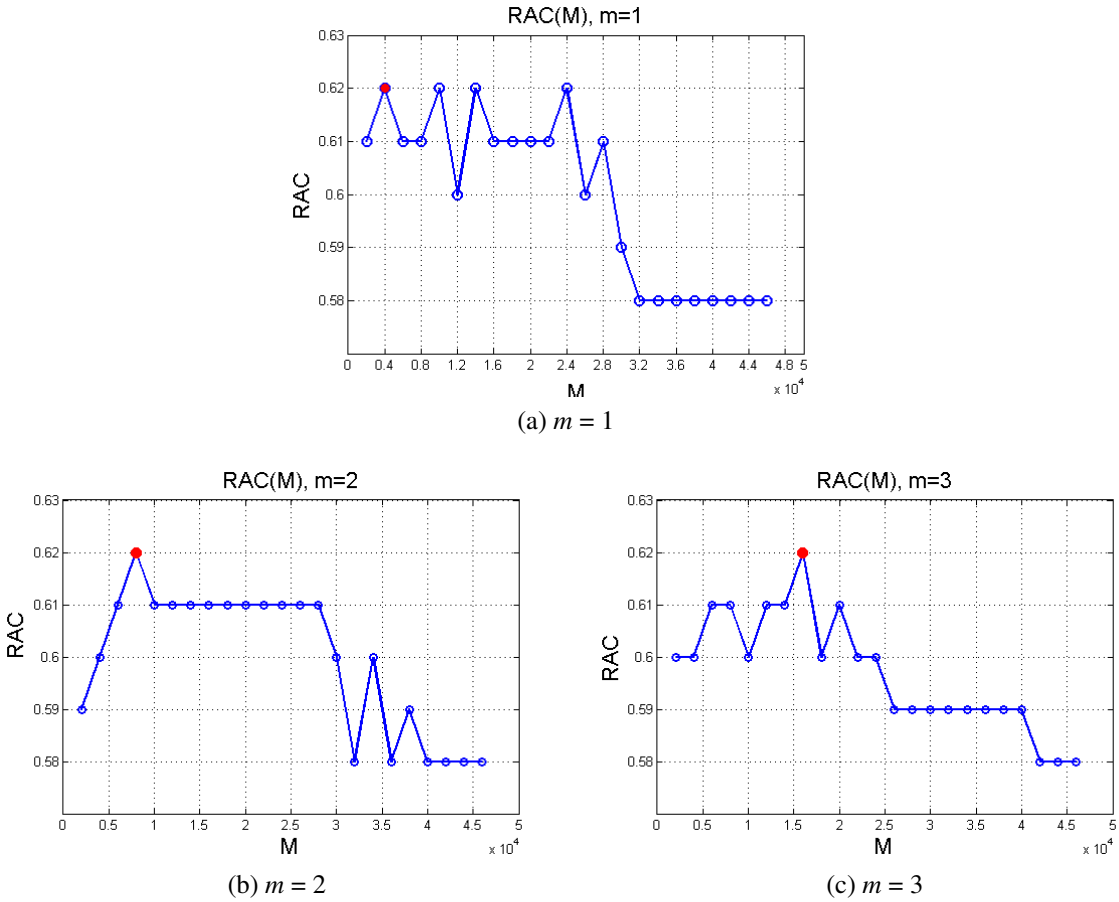
In the light of these considerations it has been chosen to implement a method that could overcome the limit of having segments with a low number of samples, that is computing the Quadratic Sample Entropy with the method to find the optimal minimum number of matches.

3.7.2 QSE

Follow up

Results

As described in Materials and Methods, the algorithm implemented evaluates, for each number of matches M selected, the area under the ROC curve (RAC) to find the $M_{optimum}$ and to compute the QSE. In the following figure (3.7.2) the value of RAC as a function of M is represented, for each embedded dimension m. From these images the so called $M_{optimum}$, stressed with a red dot, is selected, it represents the optimum number of matches that is for m=1 4'000, for m=2 8'000 and for m=3 16'000.



(a) m = 1

(b) m = 2

(c) m = 3

Figure 3.7.2: Area Under Roc (RAC) of the follow up study for the embedded dimension m = 1,2,3 as a function of the number of matches (M). The red dot is the first maximum found in each curve and it is representative of the $M_{optimum}$.

Once the $M_{optimum}$ is found, the value of the tolerance r, that allows to reach the $M_{optimum}$, is used to calculate the SampEn and finally the QSE value, for both the Supine and Prone position, for each 3 minutes window. The value of the QSE of the windows is averaged to obtain a single value. In the following Table (3.7.4) the value of the tolerance and the QSE

measure, obtained for each embedded dimension, for the Supine and Prone position, for the patient ID 21, are reported.

In the successive Table 3.7.5 there is the mean of the whole dataset.

ID 21	SUPINE		PRONE	
N° 3 MINUTES WINDOWS	1		2	
m	QSE	r	QSE	r
$m = 1$ (M = 4'000)	-2,9530	0,0062	-3,2354 ± 0,0879	0,0053 ± 0,0012
$m = 2$ (M = 8'000)	-2,8554	0,0141	-3,0931 ± 0,0926	0,0122 ± 0,0022
$m = 3$ (M = 16'000)	-2,7468	0,0220	-2,8839 ± 0,0811	0,0203 ± 0,0022

Table 3.7.4: QSE value and tolerance (r) associated, for patient ID 21, for each embedded dimension (m) and the optimum number of matches ($M_{optimum}$) for both the Supine and Prone position, Active sleep.

N° OF PATIENTS: 10				
	SUPINE		PRONE	
m	QSE	r	QSE	r
$m = 1$	-3,3073 ± 0,6183	0,0068± 0,0041	-3,5784 ± 0,6717	0,0054± 0,0029
$m = 2$	-3,1341 ± 0,5773	0,0143± 0,0087	-3,4214 ± 0,6510	0,0112± 0,0069
$m = 3$	-2,9090 ± 0,5506	0,0230± 0,0139	-3,1733 ± 0,6581	0,0188± 0,0118

Table 3.7.5: Mean of the QSE parameters with their standard deviation, of the 10 patients, for each embedded dimension *m,* for the Supine and Prone Baseline, Active sleep.

Statistical analysis

With the statistical test the following P-values, for each embedded dimension, are obtained.

N° OF PATIENTS: 10	
m	P-VALUE
$m = 1$	0,0488*
$m = 2$	0,0097*
$m = 3$	0,0195*

Table 3.7.6: P-value for the QSE, evaluated with $M_{optimum}$, for each embedded dimension; the star stresses the significance, under the threshold 0,05.

For all the *m,* the QSE parameter has a P-value under 0,05. In the following figure (Figure 3.7.3) it is represented, for the embedded dimension 2, for each patient, the value of the QSE for the Supine position (blue) and the Prone position (red). At the bottom is evident that the difference between Supine and Prone is generally above zero (higher for the Supine position), stressing the significance of this parameter in its capability to distinguish the two populations.

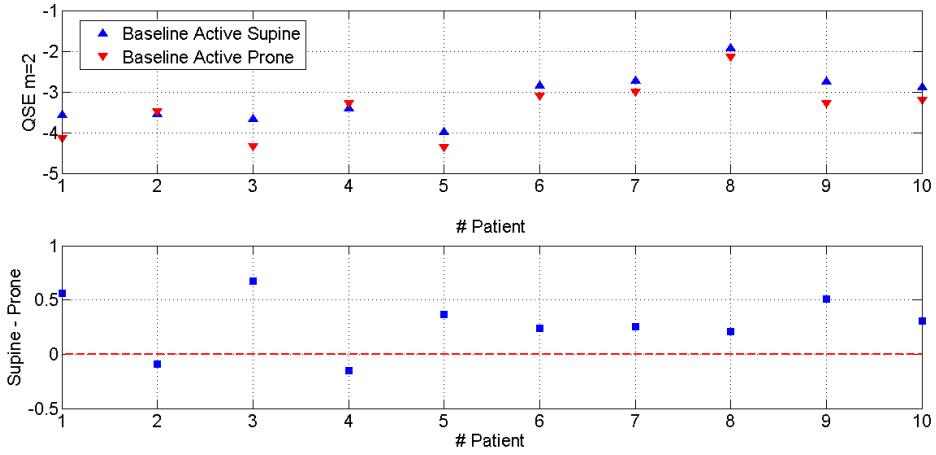


Figure 3.7.3: QSE value for the embedded dimension $m = 2$, for each patient for the Supine (blue) and Prone (red) Baseline is reported in the top figure. The difference between Supine and Prone is showed in the bottom figure, where the zero value is highlighted with the red dotted line.

Comparison between SampEn and QSE

In the Figure 3.7.4 the SampEn and the QSE are compared. At the top the trend of the SampEn value as a function of the number of matches can be seen, for patient ID 37, for the embedded dimension 2. At the bottom, instead, there is the trend of the QSE values. It can be seen that adding $ln(2r)$ allows the two curves of Supine and Prone to be more separated with the QSE parameter, resulting in higher significance, when the SampEn parameter is not significant. It can be seen that the SampEn decreases with the increase of M, approaching the zero line, while the QSE increases, reaching a plateau.

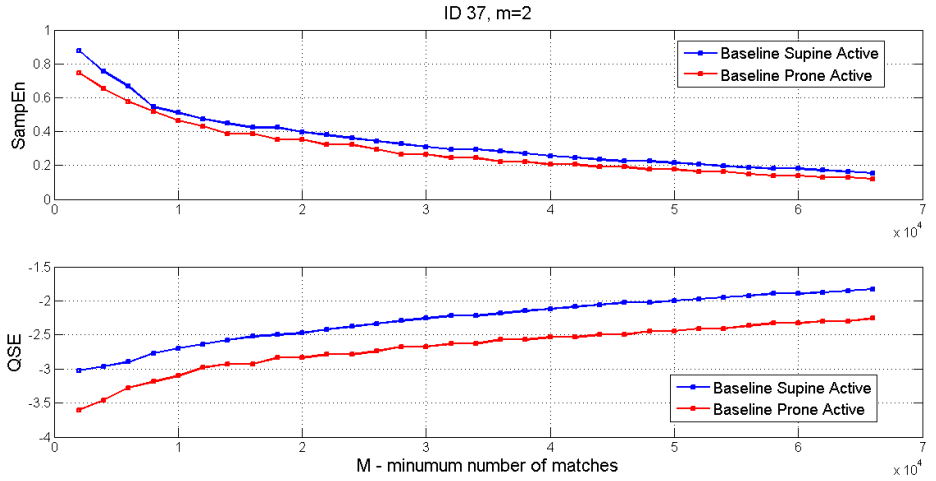


Figure 3.7.4: Comparison between SampEn and QSE as a function of the number of matches (M), the Supine (blue) and the Prone (red) Baseline are shown.

In Figure 3.7.5 instead the trend of the QSE and SampEn as a function of the length of the signal (N) is reported. It is evident that the QSE with a minimum counter of matches

ensures a good separation of the curves for short signals, while the values of SampEn are more overlapped for low N, distinguishing the curves only at higher values. Moreover it can be seen in the figure that the Supine curve for the QSE is higher than the Prone for all the N, showing that the QSE parameter is more consistent than the SampEn.

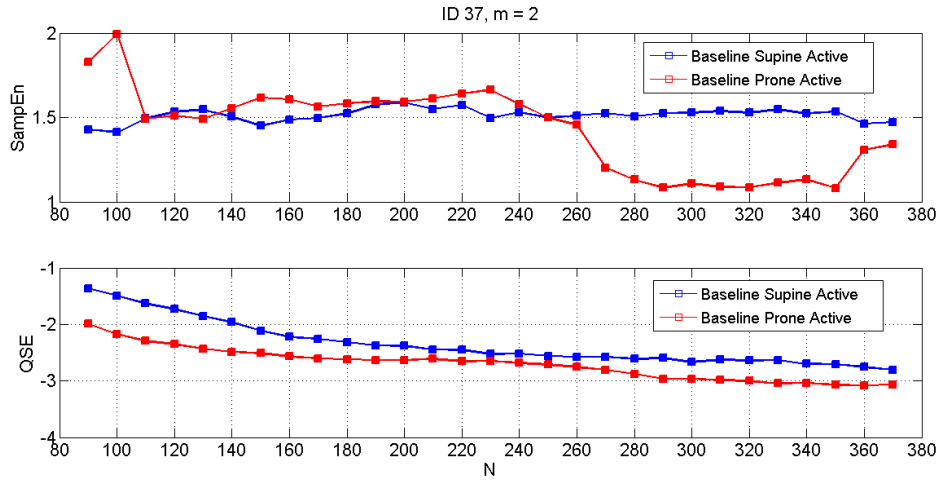


Figure 3.7.5: SampEn (above) and QSE of patient ID 37, $m = 2$, with the minimum counter of matches imposed to 8'000 (below), as a function of the number of samples (N). For the SampEn r is set to 15% of the standard deviation of the signal, as usually in literature, while for QSE is automatically set to achieve the minimum number of matches.

Gaussian noise

As described in Materials and Methods 3.7.6, the conversion of the QSE value to the standard deviation of Gaussian white noise with equivalent Entropy is a possible solution to better interpret this parameter. This way its peculiar unit of measure is converted to the physiologic measurement, thus it is simpler to interpret this parameter as a variability measure. The value of the QSE rate is $\frac{1}{2}ln(4\pi) + ln(\sigma)$ or $1.266 + ln(\sigma)$, where σ is the standard deviation of the Gaussian white noise.

The upper image in Figure 3.7.6 is the trend of QSE as a function of N, both for the Supine and Prone position (blu and red line). The lower image is the QSE converted in milliseconds, equal to the usual physiologic measurement of the RR series. These units could help the clinicians giving a meaning to the value of Entropy measured, since Entropy does not have a measure unit.

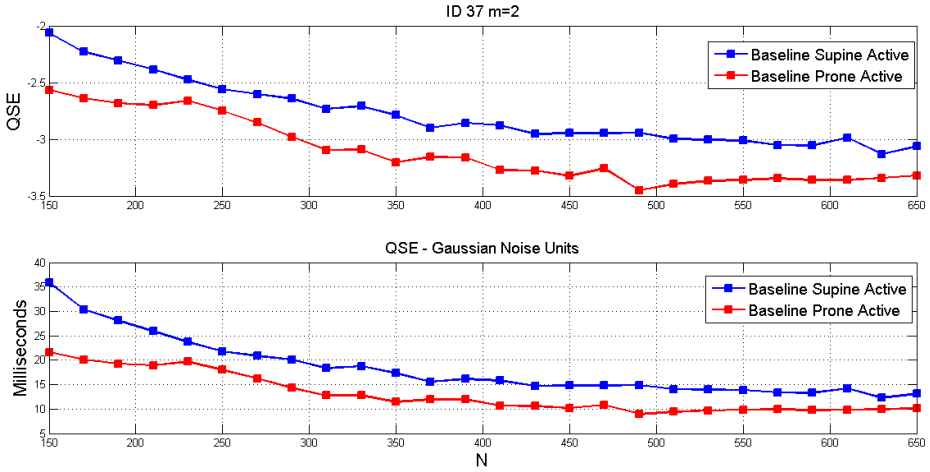
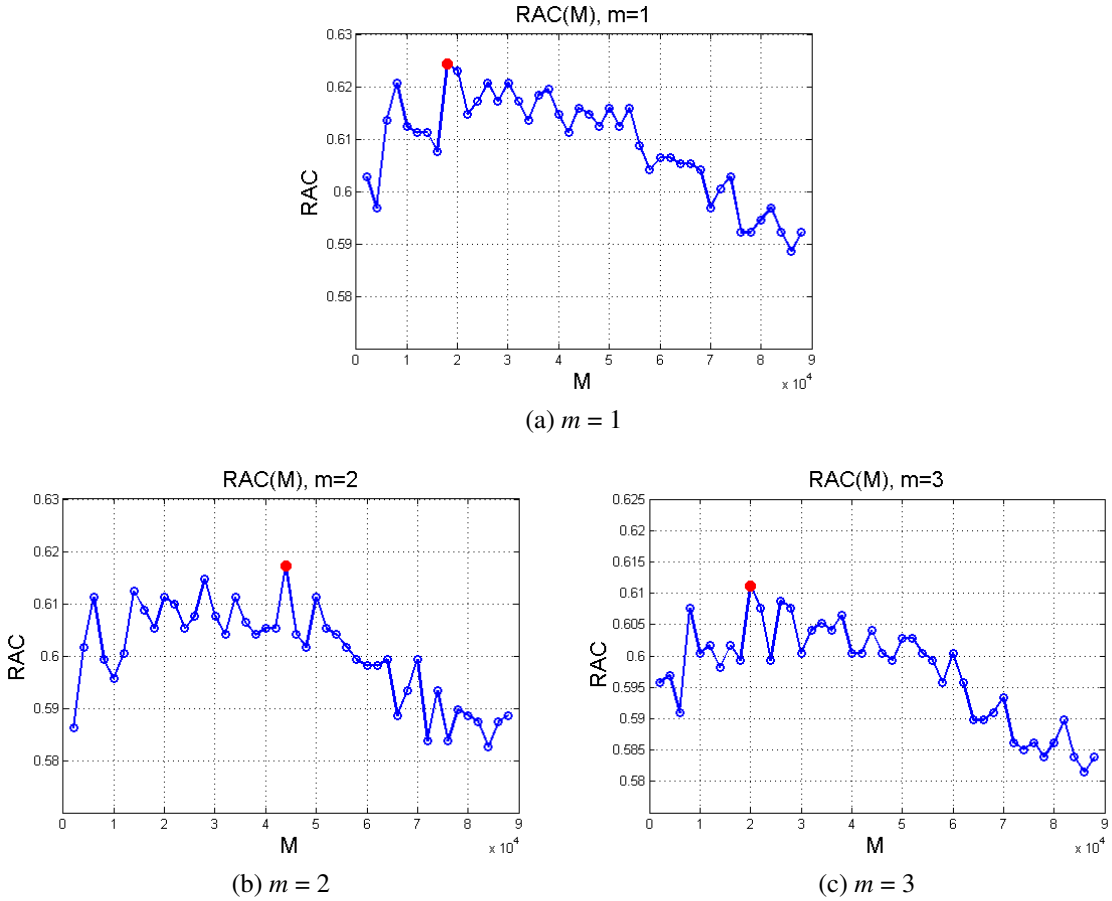


Figure 3.7.6: QSE converted to the standard deviation of Gaussian white noise with equivalent entropy, for the patient ID 37, $m = 2$. The Supine Baseline is in blue and the Prone Baseline in red. The unit of measure in the y axis is milliseconds.

First study

Results

The same approach is then implemented also in the first study dataset, to verify the results. Following the algorithm previously described, the RAC for each value of M (number of matches, from 1 to the maximum number of matches with step 2000) is evaluated. In the following figure (3.7.7) the trend of the value of the RAC can be seen and also, stressed with the red dot, the $M_{optimum}$ found is shown, in correspondence to the highest value of RAC, for the three embedded dimensions. For the embedded dimension $m = 1$ is 18'000, for $m = 2$ is 44'000 and for $m = 3$ is 20'000. These numbers are significantly higher than the ones found in the follow up study, probably because of the greater number of samples of the 3 minutes windows of the first study. In fact, as suggested in literature [41], the minimum numerator count increases and the tolerance r decreases as N (samples) becomes larger.



(a) $m = 1$

(b) $m = 2$

(c) $m = 3$

Figure 3.7.7: RAC of the first study for the embedded dimension $m = 1,2,3$ as a function of the number of matches (M). The red dot is the first maximum found in each curve and it is representative of the $M_{optimum}$.

In the table below (Table 3.7.7) there are the values of QSE and the tolerance associated for the patient ID 21, in both the Supine and Prone position, for each embedded dimension, evaluated using the $M_{optimum}$ found. The number of segments for this example is 1 in both the positions.

In the successive Table (3.7.8) the values of the mean of the QSE values for all the patients are reported.

ID 21	SUPINE		PRONE	
N° 3 MINUTES WINDOWS	1		1	
m	QSE	r	QSE	r
$m = 1$ ($M = 18000$)	-4,4816	0,0031	-4,6432	0,0031
$m = 2$ ($M = 44000$)	-3,9386	0,0080	-4,0051	0,0080
$m = 3$ ($M = 22000$)	-4,1521	0,0060	-4,3619	0,0051

Table 3.7.7: QSE value and tolerance (r) associated, of the first study, for patient ID 21, for each embedded dimension (m) and the optimum number of matches ($M_{optimum}$) for both the Supine and Prone Active Baseline.

N° OF PATIENTS: 29				
PARAMETERS	SUPINE		PRONE	
m	QSE	r	QSE	r
$m = 1$	-4,0425± 0,3036	0,0053± 0,0017	-4,2006± 0,4147	0,0047± 0,0020
$m = 2$	-3,6157± 0,3214	0,0119± 0,0041	-3,7666± 0,4414	0,0106± 0,0046
$m = 3$	-3,8725± 0,3103	0,0085± 0,0027	-4,0051± 0,4178	0,0077± 0,0032

Table 3.7.8: Mean of the QSE parameters with their standard deviation, of the 29 first study patients, for each embedded dimension, for the Supine and Prone Baseline, Active sleep.

Statistical analysis

With the statistical test, also for the first study, it is evident that the QSE parameter is significant for all the embedded dimensions, being predominantly higher for the Supine Position. In the Table 3.7.9 the P-values obtained from the statistical analysis are reported, they all are smaller than 0,05.

N° OF PATIENTS: 29	
m	P-VALUE
$m = 1$	0,0062*
$m = 2$	0,0110*
$m = 3$	0,0200*

Table 3.7.9: P-value for the QSE of the first study, evaluated with $M_{optimum}$, for each embedded dimension; the star stresses the significance, under the threshold of 0,05.

In Figure 3.7.8 the value of the Supine and Prone Baseline of each patient and the difference between these two values are reported.

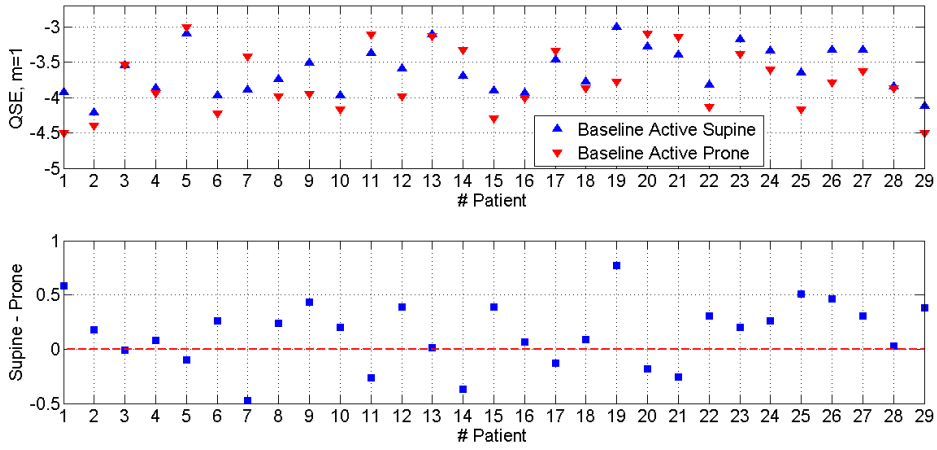


Figure 3.7.8: QSE value of the first study, for the embedded dimension $m = 1$, for each patient, for the Supine (blue) and Prone (red) Baseline is reported in the top figure. The difference between Supine and Prone is showed in the bottom figure, where the zero value is highlighted with the red dotted line.

Comparison between SampEn and QSE

In the following images (Figure 3.7.9), as done in the follow up study, the comparison between the SampEn and the QSE is reported for patient ID 24, m =2.

It is clear that the QSE parameter allows a considerable increase of the separation between Supine and Prone positions.

Particularly, for the $M_{optimum}$ 18'000, it can be seen that the curves of the two populations for the SampEn are overlapped, while the QSE is able to distinguish them.

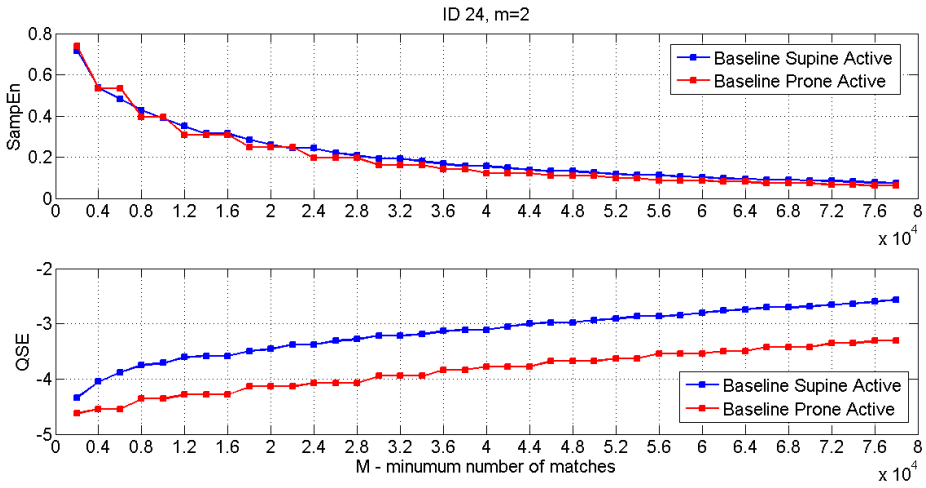


Figure 3.7.9: Comparison between SampEn and QSE as a function of the number of matches (M), for patient ID 24, m = 2. The Supine Active Baseline is in blue and the Prone Active Baseline in red.

The trend of SampEn and QSE is then shown as a function of the signal length (N). As in the follow-up for short lengths the two curves are slightly overlapped for the SampEn, but are well separated and consistently higher for the Supine in the QSE.

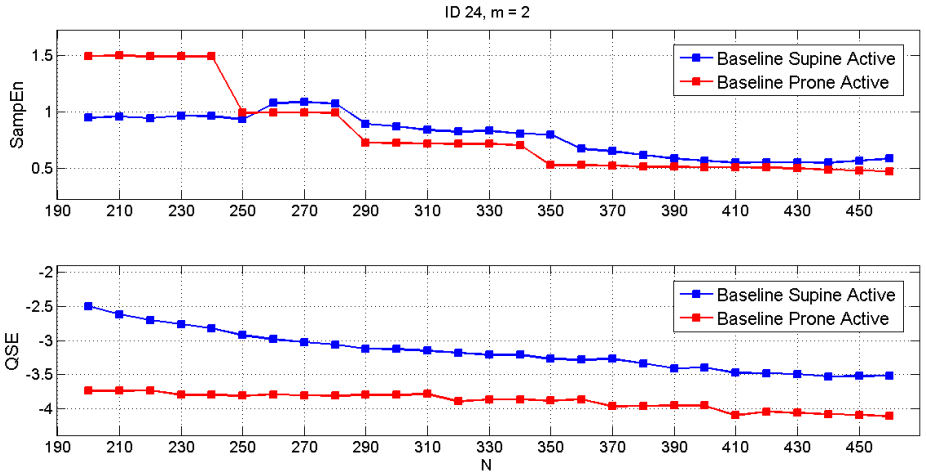


Figure 3.7.10: QSE and SampEn for increasing length of the time-series (N), for patient ID 24, m = 2. r is set as 15% of the std for SampEn while the minimum number of matches, equal to 20'000, is used for QSE.

Gaussian noise

Hereinafter, in Figure 3.7.11, the conversion of QSE to the standard deviation of the Gaussian noise with the same entropy is reported, following the formula described in 2.7.3. This conversion is useful to better understand the meaning of this parameter, using recognizable units of measure.

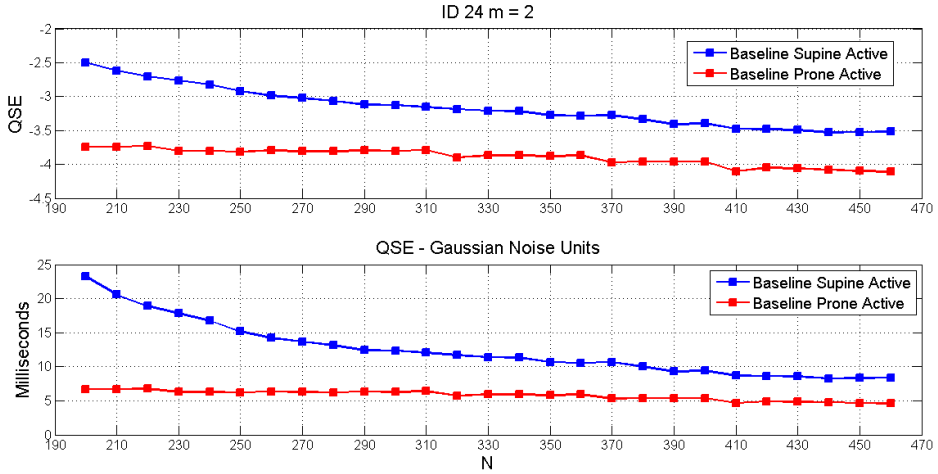


Figure 3.7.11: QSE converted to the standard deviation of Gaussian white noise with equivalent entropy for the patient ID 24, $m = 2$. The Supine is in blue and the Prone in red. The unit of measure in the y axis is milliseconds.

3.8 Correlation between parameters

In this section the linear correlation, computed between measures of different domains, are shown; this is done for those parameters which revealed to significantly distinguish the Prone and Supine conditions.

It can be seen in Table 3.8.1 that there is a strong linear correlation between some parameters of the time domain and the values of QSE for the same condition (Supine or Prone Baseline Active).

SUPINE	QSE m1	QSE m2	QSE m3
MEAN	0,9767*	0,9735*	0,9475*
RMSSD	0,6643	0,7036	0,7619
STV	0,9014*	0,9165*	0,8993*

(a) Supine

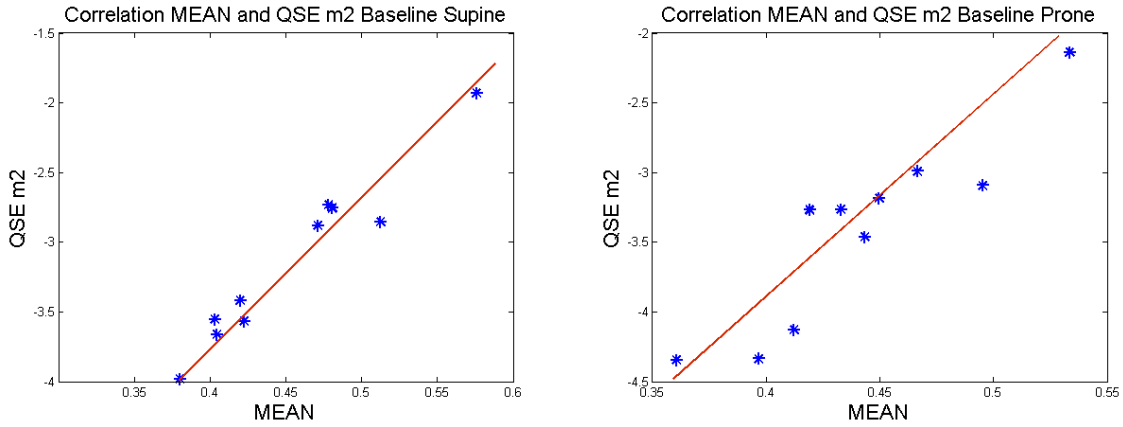
PRONE	QSE m1	QSE m2	QSE m3
MEAN	0,9055*	0,9158*	0,8892*
RMSSD	0,7359	0,7557	0,8136
STV	0,8970*	0,8944*	0,8712*

(b) Prone

Table 3.8.1: Correlation between Time Domain and QSE.

This is true for every embedded dimension m of the QSE and the correlation is especially evident for the MEAN of RR and the STV, since their higher values, as in the table.

It is then shown in Figure 3.8.1 the scatter-plot of the MEAN of RR parameter against the QSE values for the 10 patients for the Supine Active Baselines, with the regression line in red.



(a) Supine (b) Prone

Figure 3.8.1: Correlation between MEAN of RR and QSE $m = 2$

3.9 Comparison follow up and first study

As described in Materials and Methods, the dataset of this thesis is a follow up of a previous study, whose data was collected approximately 2 month before this one.

One of the aim of this research is to compare the value of parameters obtained on the same subjects at different ages, so that in could be possible to see the evolution and maturation of the babies.

In the following sections, for each field of analysis will be reported the results achieved from the comparison between the Baseline Supine Active of the first study and the follow up study and the Baseline Prone Active of the first study and follow up study.

The patients used are 9 among the 10 usually analyzed, because of the lack of the same Baseline for the patient ID 32 in the first study.

3.9.1 Time domain

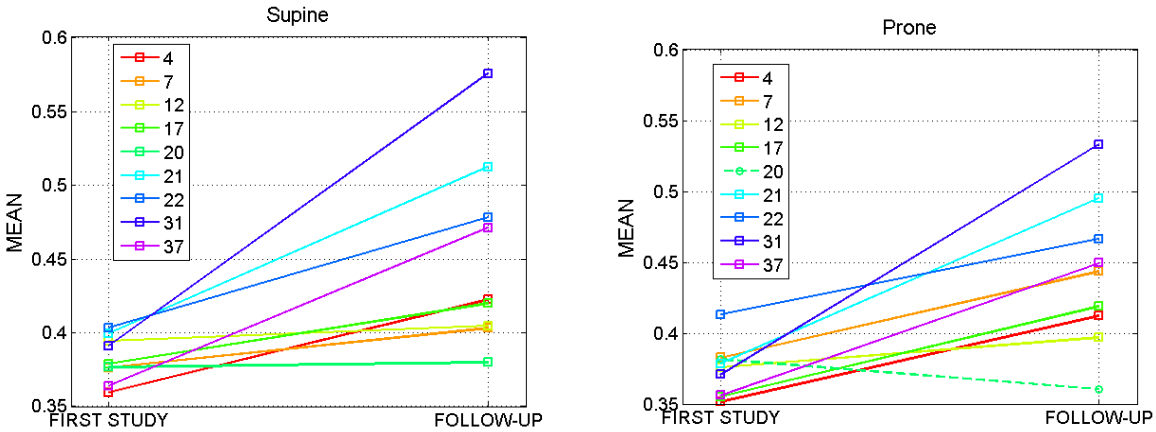
Results and statistical analysis

In Figure 3.9.1 an example of the time domain parameters is shown: the Mean of the tachogram for the Supine and Prone position.

Two considerations can be done: the first is that for each patient generally the value increases from the first study to the follow up study. This is what it was expected, because

the HR decreases with the PMA, as validated in section 3.1 and consequently the RR interval increases.

The second is that, in the first study, the value has a smaller variance than in the follow up, in which the parameter has a larger range of values.



(a) Supine

(b) Prone

Figure 3.9.1: MEAN of RR value of the first study and the follow up, for the Supine and Prone comparison. Each of the 9 patients compared has a different color as reported in the legend.

These considerations can be done more or less for all the parameters, with the exception of the IIa, which does not show a trend.

The statistical analysis is made to support the results visually discussed above. As expected, all the parameters, except the IIa, are significant, as presented in Table 3.9.1.

The analysis is done using the Wilcoxon Signed-Rank test and using as population the difference between the Supine position of the first study and follow up study and the Prone position of the first and follow up study.

It means that the great part of the time domain parameters shows a distinction between the two studies, in particular the increase of the values with the age of the babies.

BASELINE SUPINE ACTIVE (BSA)	
FOLLOW-UP VS. FIRST STUDY	
N° OF PATIENTS: 9	
PARAMETERS	P-VALUE
MEAN	0,0039*
SDNN	0,0039*
RMSSD	0,0039*
LTV	0,0039*
STV	0,0039*
II	0,0078*
DI	0,0039*
RMSM	0,0039*
IIa	0,8203
LTI	0,0117*

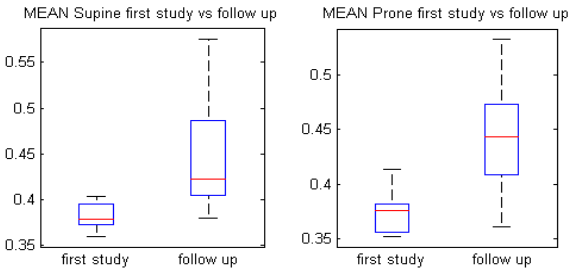
(a) Supine

BASELINE PRONE ACTIVE (BPA)	
FOLLOW-UP VS. FIRST STUDY	
N° OF PATIENTS: 9	
PARAMETERS	P-VALUE
MEAN	0,0117*
SDNN	0,0117*
RMSSD	0,0039*
LTV	0,0078*
STV	0,0039*
II	0,0117*
DI	0,0039*
RMSM	0,0117*
IIa	0,2500
LTI	0,0117*

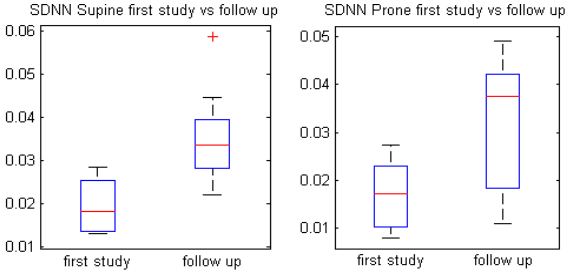
(b) Prone

Table 3.9.1: P-value for each time domain parameter, to the left the comparison between the BSA of the follow up and the BSA of the first study is portrayed, to the right the comparison between the BPA of the two studies.

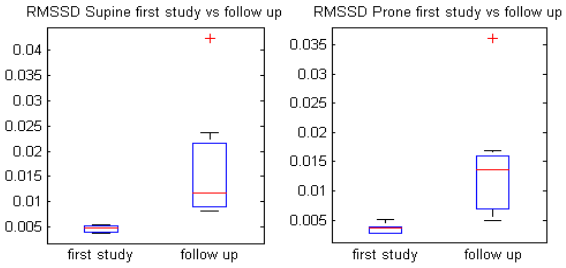
In the following box plots (3.9.2) all the time domain parameters are reported.



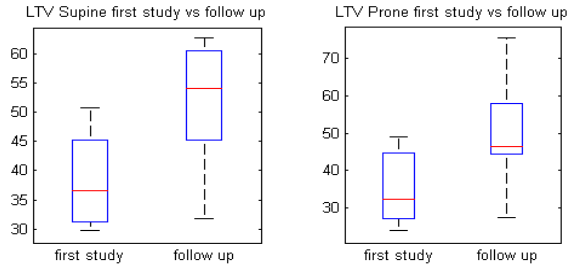
(a) MEAN Supine and Prone



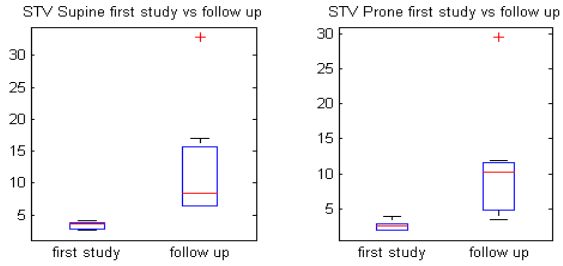
(b) SDNN Supine and Prone



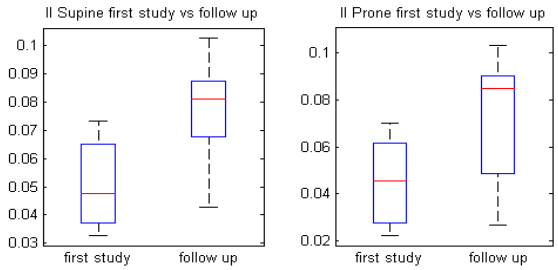
(c) RMSSD Supine and Prone



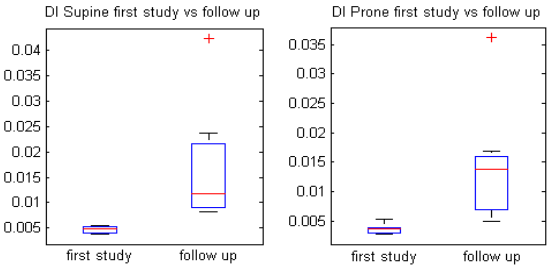
(d) LTV Supine and Prone



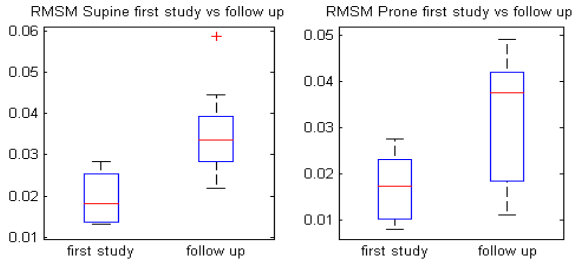
(e) STV Supine and Prone



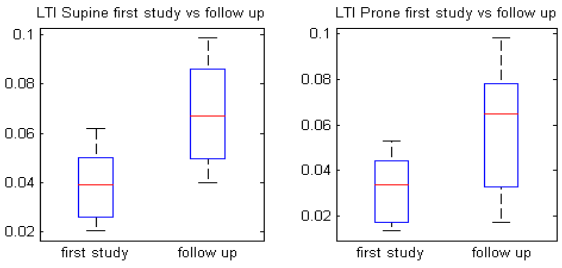
(f) II Supine and Prone



(g) DI Supine and Prone



(h) RMSM Supine and Prone



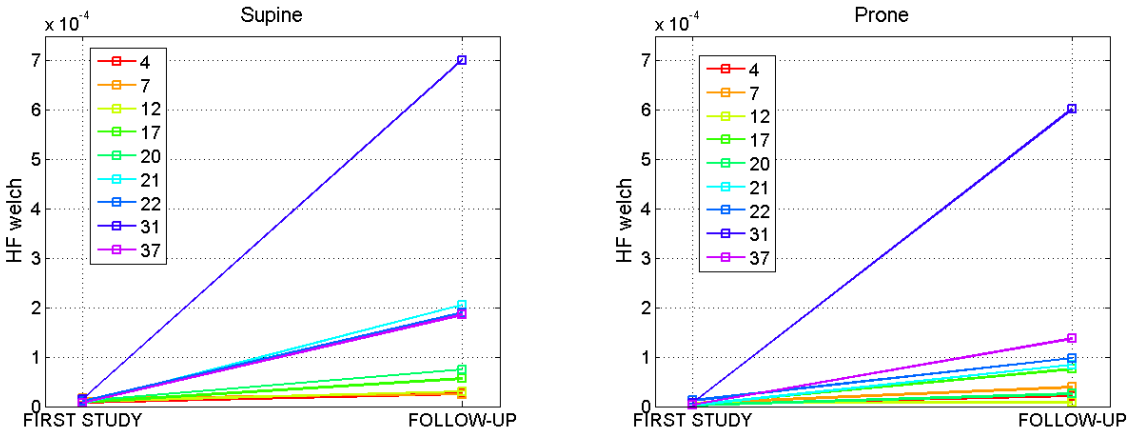
(i) LTI Supine and Prone

Figure 3.9.2: Box-plot time domain.

3.9.2 Frequency domain

Results and statistical analysis

The frequency domain parameters P_{LF} and P_{HF} of the two studies are compared. It can be seen in Figure 3.9.3 that the P_{HF} parameter, taken as example, increases from the first study to the follow up study, in the Supine and Prone position, for all the 9 patients compared, whit a relevant increase for the patient ID 31. The same happens for the P_{LF} parameter.



(a) Supine (b) Prone

Figure 3.9.3: P_{HF} value of the first study and the follow up, for the Supine and Prone comparison. Each of the 9 patients compared has a different color as reported in the legend.

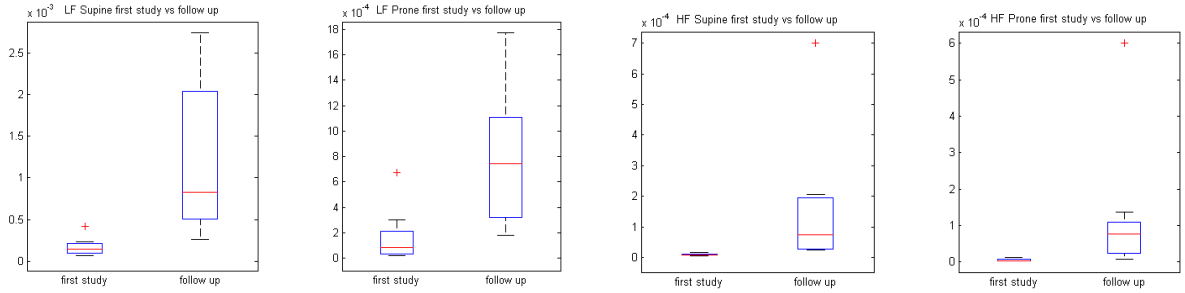
From the statistical analysis then, the significance of this increase is tested and in Table 3.9.2 the P-value is reported, that both for the comparison between the two Supine Baselines and the two Prone Baselines is under the threshold of 0,05 for the P_{LF} and P_{HF}.

BASELINE SUPINE ACTIVE (BSA)	
FOLLOW-UP VS. FIRST STUDY	
N° OF PATIENTS: 9	
PARAMETERS	P-VALUE
P_{LF}	0,0039*
P_{HF}	0,0039*

BASELINE PRONE ACTIVE (BPA)	
FOLLOW-UP VS. FIRST STUDY	
N° OF PATIENTS: 9	
PARAMETERS	P-VALUE
P_{LF}	0,0117*
P_{HF}	0,0039*

Table 3.9.2: P-value for each frequency domain parameter, to the left the comparison between the BSA of the follow up and the BSA of the first study is portrayed, to the right the comparison between the BPA of the two studies.

The summary box plots of the comparison in the frequency domain are here reported (Figure 3.9.4).



(a) LF Supine and Prone (b) HF Supine and Prone

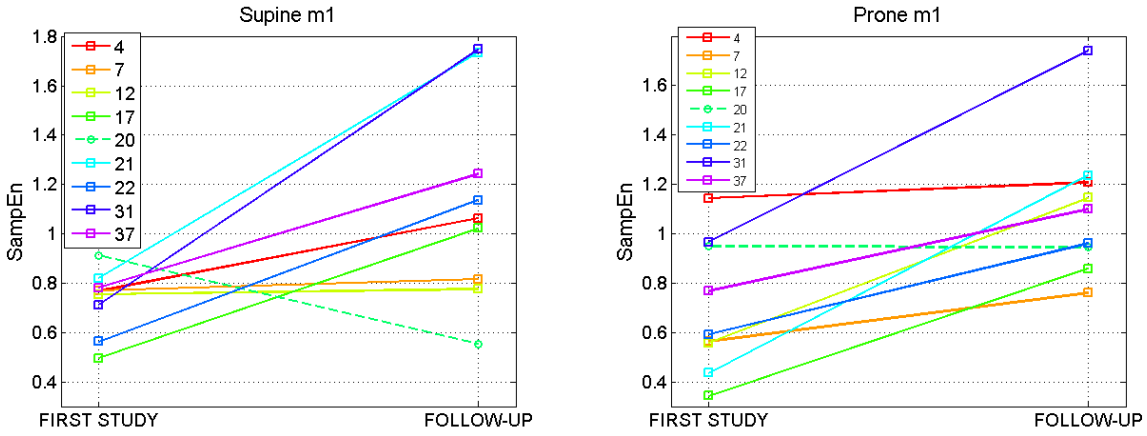
Figure 3.9.4: Box-plot frequency domain

3.9.3 Entropy

ApEn and SampEn

Results and statistical analysis

The values of ApEn and SampEn are compared for both BSA and BPA between the first and the follow up study. The value of SampEn for the embedded dimension $m = 1$ is shown as example in Figure 3.9.5, for both the conditions. An increase in its values between the two studies is evident.



(a) Supine (b) Prone

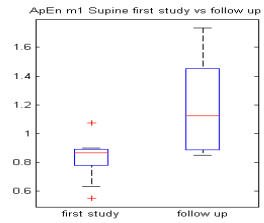
Figure 3.9.5: SampEn value of the first study and the follow up, for the Supine and Prone comparison. Each of the 9 patients compared has a different color as reported in the legend.

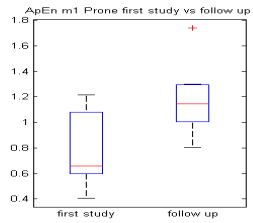
The increase of these parameters is significant for both BSA and BPA, for each embedded dimension, except for the Prone SampEn, as seen in Table 3.9.3.

BASELINE SUPINE ACTIVE (BSA)		
FOLLOW-UP VS. FIRST STUDY		
N° OF PATIENTS: 9		
m	P-VALUE APEN	P-VALUE SAMPEN
$m = 1$	0,0273*	0,0273*
$m = 2$	0,0195*	0,0390*
$m = 3$	0,4257	0,0742
BASELINE PRONE ACTIVE (BPA)		
FOLLOW-UP VS. FIRST STUDY		
N° OF PATIENTS: 9		
m	P-VALUE APEN	P-VALUE SAMPEN
$m = 1$	0,0078*	0,0078*
$m = 2$	0,0117*	0,0195*
$m = 3$	0,4257	0,0273*

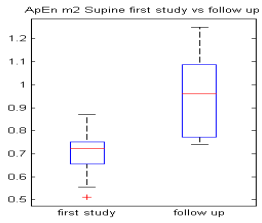
Table 3.9.3: P-value for ApEn and SampEn, to the left the comparison between the BSA of the follow up and the BSA of the first study is portrayed, to the right the comparison between the BPA of the two studies.

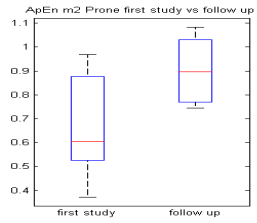
The box-plots for the different measures, that have been found significant, are here presented as summary (Figure 3.9.6).



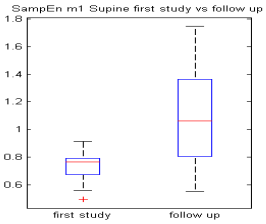


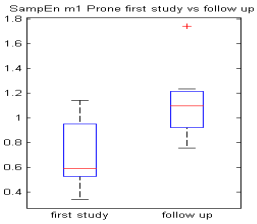
(a) ApEn m=1 Supine and Prone



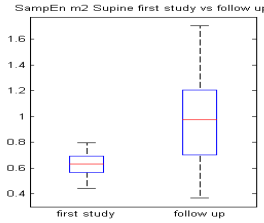


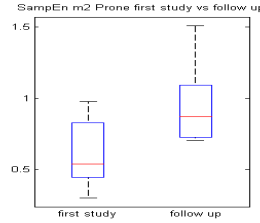
(b) ApEn m=2 Supine and Prone





(c) SampEn m=1 Supine and Prone





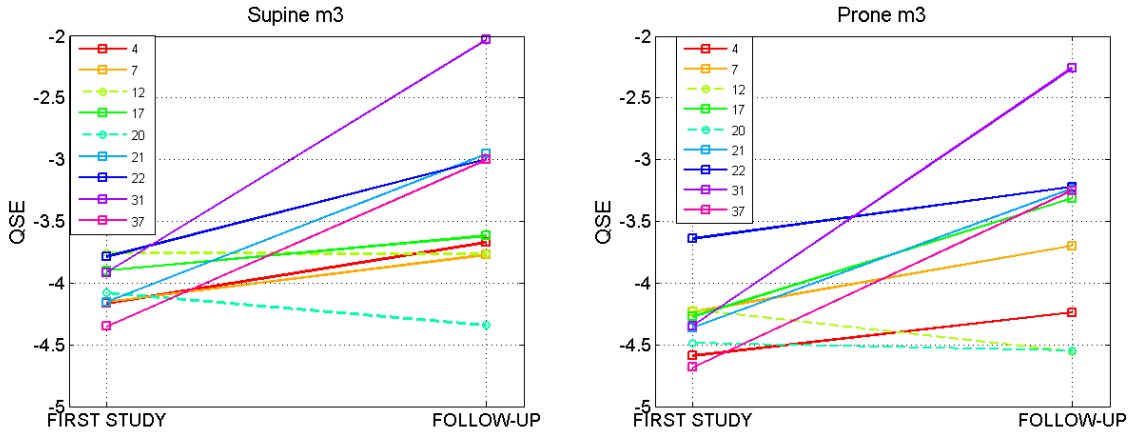
(d) SampEn m=2 Supine and Prone

Figure 3.9.6: Box-plot ApEn and SampEn

QSE

Results and statistical analysis

The values of QSE for $m = 3$ Supine Active Baseline are shown in Figure 3.9.7 for example. Again an increase among the patients between the two studies can be assessed; this is true for each embedded dimension, for both BSA and BPA (Table 3.9.4).



(a) Supine

(b) Prone

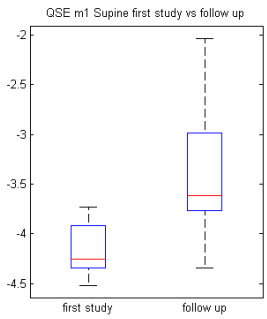
Figure 3.9.7: QSE value of the first study and the follow up, for the Supine and Prone comparison. Each of the 9 patients compared has a different color as reported in the legend.

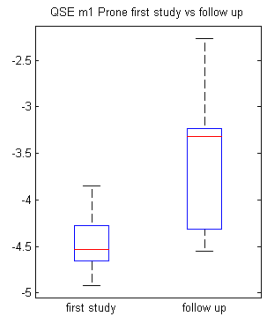
BASELINE SUPINE ACTIVE (BSA)	
FOLLOW-UP VS. FIRST STUDY	
N° OF PATIENTS: 9	
m	P-VALUE
$m = 1$	0,0078*
$m = 2$	0,0195*
$m = 3$	0,0039*

BASELINE PRONE ACTIVE (BPA)	
FOLLOW-UP VS. FIRST STUDY	
N° OF PATIENTS: 9	
m	P-VALUE
$m = 1$	0,0117*
$m = 2$	0,0391*
$m = 3$	0,0039*

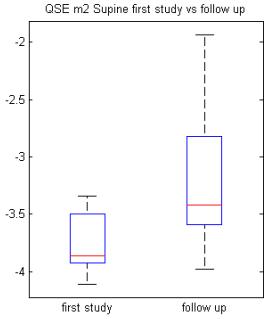
Table 3.9.4: P-value for QSE, to the left the comparison between the BSA of the follow up and the BSA of the first study is portrayed, to the right the comparison between the BPA of the two studies.

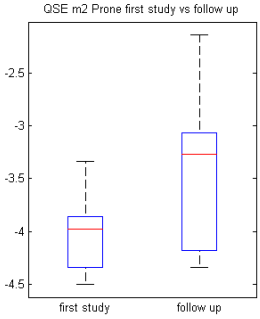
For summary the box-plots of the QSE parameters are here shown (Figure 3.9.8).



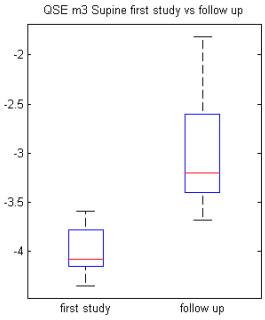


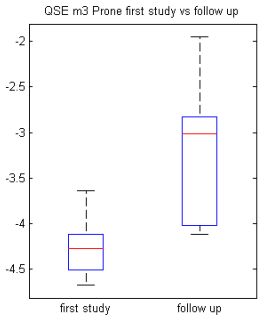
(a) QSE $m1$ Supine and Prone





(b) QSE $m2$ Supine and Prone





(c) QSE $m3$ Supine and Prone

Figure 3.9.8: Box plot QSE.

3.10 Summary of results

In the time domain the short term variability parameters have been found significant, it may be explained with the maturation of the parasympathetic system that has a higher velocity of action and a beat-to-beat response.

In the frequency analysis none of the parameters tested (LF and HF power bands) have been found significant.

The ApEn and SampEn too, have not resulted significant, while the new entropy parameter implemented, the QSE, has high significance, for each embedded dimension.

Results presented stress how the shortness of the signals, recorded for this study, influences some parameter, thus causing the lack of significance.

Furthermore, the results obtained suggest a relevant maturation of the babies with their PMA. In fact, from the first study to the follow up study (taken 2 months later) there is an evident increase of the parameters, more or less in every field of analysis.

For the time domain all the parameters increase, except for the IIa, for the frequency domain both LF and HF power bands increase; for the entropies the increase is both for the ApEn and SampEn with few exceptions for some embedded dimension, while the QSE increases for all the m.

Chapter 4

Discussion

This chapter offers the final considerations about the obtained results.

In particular, it summarizes what has been made all along the research path and tries to identify which are the most significant evidence obtained.

In the last section the conclusions will be drawn to understand the meaning of the results and the possible future developments will be presented.

The aim of this thesis has been to investigate changes in the HR of ex-premature babies, when an external stressor, in our case the Prone position, is imposed. The HRV analysis has been used to assess the ANS activity in a non-invasive way.

The recordings have been collected at the Columbia University Medical Center and the focus of this thesis is a follow up study. The follow up analysis comprehends 24 of the 37 babies of the first study and the recordings are 1 hour long, with respect to the 6 hours long of the first study. As described, the experimental protocol divided each recording in four phases: Supine Baseline (BS) , Supine Tilt (TS), Prone Baseline (BP) and Prone Tilt (TP). To have a meaningful analysis, the measures performed on the babies had to be made on segments in the same sleep state (Active sleep), both in the Supine and Prone Baseline. In light of this, 10 among the 24 patients have been selected for the analysis, as they satisfied the protocol requirements.

The minimum length for each segment was set to 3 minutes, that ensured a sufficient number of beats.

Methodologies applied in this study came from different approaches: time domain, frequency domain and the non-linear parameters were considered.

Parameters have been calculated for each Baseline Supine Active (BSA) and Baseline Prone Active (BPA). Then the Wilcoxon Signed-Rank test for paired differences was employed, in this way we were able to compare the significance of the parameters between our study and the previous one.

Short term variability parameters in time domain and the QSE have been found significant to divide the Supine and Prone sleep positions, while the frequency domain parameters and the ApEn and SampEn have not. Finally a comparison between our study and the previous one is done. The goal was to investigate the maturation of the babies' ANS in the 2 months passed between the two recordings.

4.1 Time domain measurements

Time domain parameters both from fetal and adult HRV analysis have been tested. The choice was taken because the condition of prematurity of the babies in this study can be considered as dynamic between fetal life and a mature newborn. We applied parameters generally proposed for HRV analysis in adults that we have adapted to newborns. They include the MEAN of the RR intervals, SDNN and RMSSD. From fetal analysis, we used LTV, STV, II, IIa, DI, RMSM and LTI. These parameters are related to HR variability at different time-scales. The entire parameter set has been computed for the 10 subjects. All values calculated in the 3 minutes intervals were averaged in BSA and BPA.

With statistical analysis, the capability of each parameter in distinguishing the Supine and Prone position is tested. MEAN of RR, RMSSD, STV and DI showed a P-value below 0,05. Their values are higher in Supine than in Prone position. Moreover we verified the presence of any correlation among all of them.

Our results confirm that there is an important difference with respect to the first study. In that study the most significant parameters were the long-term ones, as in our case the short-term ones show the largest change. This may be related to the prevalence of the sympathetic branch, while in the follow up the significance of the short-term variability parameters could be an effect of the maturation of the parasympathetic branch. It is known from literature that the sympathetic system is the first to develop during pregnancy, while the parasympathetic develops later and continue to develop even along the first months of life [22]. As presented in Chapter 1, the parasympathetic branch of the ANS could be responsible of the HR decrease; its faster response is manifested in a more rapid, beat-to-beat control of the HR and this emerged in the short-term variability parameters.

As also discussed in literature [15], HR in the Prone position is generally higher than in the Supine position, both for preterm and healthy babies. Analysis of our data confirms these findings as the MEAN of the RR (the inverse of the HR) is found higher in the Supine than in the Prone position.

As regards the RMSSD, we have observed an increase from Prone to Supine; this means that Supine position shows a higher degree of dispersion around the mean, thus a higher variability. This lower variability in the Prone position may be related to an increased sympathetic drive on the heart, bringing an unbalance in the ANS control.

To test differences between the two studies a statistical test was applied. Comparison was performed maintaining the same sleep position in Active sleep, with a population of 9 subjects having both BSA and BPA in the first and follow up studies. Excluding the IIa, all parameters of the time domain resulted significant with P-values smaller than 0,05. They show an increase from the first study to the follow up study. This increase may be associated to the maturation of the ANS that goes with the increase of the PMA. In fact the follow up population is 2 months older than the first study one.

4.2 Frequency domain analysis

Frequency domain analysis computed in our thesis uses the traditional Welch method to estimate the power spectral density (PSD) of the signals. Parameters drawn from PSD are studied because of their relationship with the estimation of sympatho-vagal balance [17]. Parameters selected for the analysis are the HF and LF powers of their relative frequency

bands, set adequately for the babies, in accordance to the first study. These powers are usually related respectively to the activity of the parasympathetic and sympathetic branches of the ANS.

Although its common use in the ANS investigation this analysis was extremely challenging in our database to draw conclusion in the comparison of the Active Sleep Baselines Supine and Prone. As a matter of facts, no difference was found between Supine and Prone position in terms of LF and HF powers. Nonetheless, after a deeper investigation, some insight has been reached regarding the poor performance of these measures.

It is known that respiration in newborns is weak and erratic, that means signals have lack of periodicity which could lead to a difficult detection of a peak in the HF band. Also, the HF components are spread on a wider range of values than adults, making it challenging to set appropriate boundaries for computing the power. Moreover, thanks to the cross-spectral analysis of the respiration signal, the occurrence of cardiac aliasing has been proven, at least in some subject. Cardiac aliasing is the distortion of the PSD that happens when HR is too slow to sample the respiration frequency content. Its presence is particularly crucial because it affects subjects that showed regularity in breathing, as a peak in the HF in the respiration spectrum, by hiding this regularity in the HR spectral analysis. In this cases, it is suggested to integrate the PSD analysis with the spectral analysis of respiration, since it can help preventing misinterpretations.

These reasons, together with the results obtained in first study, lead to the consideration that spectral analysis could be a difficult method to evaluate the ANS behaviors in newborns and could lead to possible inconclusive results.

The HF and LF powers, when compared between the two studies, show an increase, both for the Supine and Prone comparison. This may be related to the more stable regulation by the ANS, for instance a more regular respiration. Since the total power is equivalent to the variance of the signal, it is found that follow up has generally higher variability. These results may point to the already seen maturation of the ANS in these babies.

4.3 Entropy for complexity estimation

As described in literature, Entropy is a measure of complexity that might help to highlight differences between healthy and diseased populations. In fact Entropy estimates are able to quantify complexity properties that have been shown to change in normal vs. pathological subjects with heart diseases. For this reason the ApEn and SampEn were implemented. After an intensive analysis two main problems emerged: the shortness of the available recordings and the different number of samples in every 3 minutes windows. Even if ApEn and SampEn

were proposed for short data samples, it is difficult to reach stable results when too short data length are available. These factors clearly influenced results and statistical analysis and probably due to such a small number of samples (approximately 350), these parameters could not reach a stable Entropy estimate.

To face this problem QSE parameter was introduced. This new parameter is less influenced by the length of the signal so it is very useful in this work based on a limited number of samples. In this work the novel approach of the minimum count of matches was implemented to find the $M_{optimum}$ that can be used to compute the best value of QSE.

In our study the $M_{optimum}$ found are 4'000, 8'000 and 16'000 corresponding to the embedded dimension 1, 2, 3. Then the r value associated to any M found, is used to compute the SampEn and the QSE. The novelty of this algorithm consists in the up-to-date analytical and automatic method to evaluate the $M_{optimum}$. Moreover, it provides values that separates better Supine and Prone than the SampEn, so that the two populations can be distinguishable with the statistical test. The dependence from r is removed by computing it for each M and then set the final value to the one linked to the $M_{optimum}$. The results show that the significance is tested for each embedded dimension, validating this parameter as a good estimator for this kind of dataset. Particularly, the QSE in Supine position is generally higher than in Prone. As suggested in literature, low entropy values usually quantify more predictable and less random HR dynamics, suggesting significantly different HR regulation in the infant. This result might be linked to the higher risk for SIDS in the Prone position, that has been evidenced in several clinical studies.

To make a deeper comparison between the two studies, the QSE algorithm was implemented also for the first study, for which the number of samples in recordings was higher. For the first study the obtained $M_{optimum}$ was 18'000, 44'000 and 20'000; these numbers are significantly higher than previous ones, because of the greater number of samples. Also for the first study the QSE parameter shows significance and Supine values are higher than Prone values.

Comparison between the two studies has been done for each parameter of Entropy. It can be noticed that all parameter values increase from the first study to the follow up for each patient, both in Supine and Prone comparison. Larger entropy values correspond generally to greater randomness and unpredictability, smaller values instead, to more instances of recognizable patterns or features in data. The increase of the entropy values, associated to the increase of the PMA from the first to the follow up study, is the evidence of the maturation of the ANS in those babies. Their control system becomes more responsive, showing an higher HRV.

4.4 Conclusive remarks and future developments

The thesis work shows that some parameters have been found to well separate HRV signal of premature babies in Supine and Prone sleep position, during Active sleep. These measures belong to traditional analysis in Time Domain measures on HR (MEAN of RR, STV, RMSSD and DI) both in adults and fetuses and to recent methodologies proposed in the entropy field.

Time domain parameters showed that Supine position displays a higher HR combined with a higher short term variability than Prone position. Our thesis analysis have evinced that the follow up dataset was mainly described by short term variability parameters. The opposite happened for the first study, in which the more significant time domain parameters were in the long-time scale. This result may be explained by the maturation of the parasympathetic branch of the ANS that is strongly undeveloped at birth and operates on a shorter scale than the sympathetic. These hypotheses could be well confirmed by the observed decrease of the HR with the PMA.

Regarding the frequency analysis in newborns during Active sleep, results were inconclusive and do not allow to comment them. This analysis may require longer signals to be correctly made.

The newly implemented method of QSE instead, demonstrated itself useful to distinguish the two populations, being significantly higher in Supine position and assigning to Prone position a lower entropy value, i.e. a lower complexity. This method's results are also consistent across the two studies, providing significantly higher values in Supine position in both studies. Then QSE could be a useful tool especially in short length signals, where traditional entropy measures, such as ApEn and SampEn, perform worse and lack of consistency.

Results obtained in this thesis appear promising toward the building of a new monitoring system based on quantitative parameters with strong relation to physiological control mechanism. For this reason it would of clinical interest to extend the analysis on a wider population, to obtain more robust values. Because of the need of at least 3 minutes windows, in this study it has been possible to analyze only the Active Sleep Baseline, thus discarding the Quiet sleep. As the data length was insufficient, longer acquisitions, through new experimental protocols, can improve the chance to compare Active and Quiet sleep states and Supine and Prone Quiet Baselines, implementing the novel developed method of the QSE. Being the Prone position considered as one of the major risk factors of developing SIDS, this measure in full-term and preterm babies could allow to assess a possible risk condition related to the developing of SIDS.

The respiratory signal has been used in this work to draw some conclusion, but a deeper and more extended analysis would be suggested to confirm the results obtained. Other signals such as pulse-oximetry and blood pressure, could be integrated to the HR analysis, to build a multi-parametric classifier assessing changes in the physiological condition of the babies. The final aim should be to obtain a useful clinical tool to help the medical staff in diagnosing risky conditions for the baby, in order to help preventing the occurrence of SIDS.

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