BACKGROUND
Recently, it has been demonstrated that subclinical inflammation and the con-centration of inflammatory markers, such as cytokines, correlate strongly to cardiovascular mortality and morbidity in both healthy subjects and in those with known coronary artery disease (CAD) [1,2]. Furthermore, vascular inflammation plays a critical role in the initiation, evolution and rupture of atherosclerotic plaque [3].

Systemic inflammation is a normal response to altered homeostasis and has an important role in several pathophysiological processes, such as infection or trauma. It is characterized by the endocrine release of different cytokines such as tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1), IL-4, IL-6, IL-10 and many others, normally confined to para-crine regulation of a local inflammatory response [4,5]. As part from their involvement in local and systemic inflammation, cytokines may induce activation of brain-derived neuroendocrine immunomodulatory responses. Neuroendocrine pathways, such as hypothalamo-pituitary-adrenal (HPA) axis and both the sympathetic and parasympathetic divisions of the autonomic nervous system (ANS) are powerful modulators of inflammation, typically through an anti-inflammatory balancing mechanism [6,7].

The pathophysiological link to the communication between the Central Nervous System (CNS) and the immune-regulated inflammation is the capability of the brain to monitor and affect at the same time the immune status. The first mechanism relies upon activation of vagus nerve afferent fibers that signal the brain that inflammation is occurring. Different kind of mediators such as cytokines can activate visceral vagus afferent fibers which terminate within the dorsal vagal complex (DVC) of the medulla oblongata. The DVC consists of the nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus (DMN) and the area postrema (AP) [8]. Ascending projections from the NTS reach hypothalamic paraventricular nucleus (PVN), which is associated with the synthesis and release of corticotropin releasing hormone (CRH). This factor induces the production of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which is the main inducer of the synthesis of immuno-suppressive gluccocorticoids from the adrenal cortex. Projections from NTS are connected to the DMN, which is the major site of origin of preganglionic vagus efferent fibers and to rostral ventrolateral medulla (RVLM). This region increases firing of the noradrenergic preganglionic neurons in the spinal cord [9].

The brain can affect the immunological status through the activation of the HPA axis and the increased outflow of sympathetic (SNS) and parasympathetic nervous system. The SNS activation during the early stages of stress induces local inflammatory response through α2-subtype adrenoreceptor stimulation by norepinephrine (NE), whereas stimulation of β2-α2 subtype adrenoreceptor-cAMP-protein kinase A pathway is associated with an inhibition of pro-inflammatory cytokines production [10-12]. It seems that SNS activation protects the organism from the detrimental effects of pro-inflammatory cytokines, while it can increase local inflammatory response [13]. Apart from the SNS, a link between the parasympathetic part of the ANS and immunoregulatory processes has been suggested. It has been de-monstrated that acetylcholine decreases TNF-α production by endotoxin-stimulated human macrophage cultures, through α7-subunit of the nicotinic acetylcholine receptor [14,15]. The vagus nerve cholinergic signaling interacts with the above receptor on immune cells in the spleen and inhibits TNF-α production and release into the circulation [16]. Acetylcholine is also effective in suppressing other pro-inflammatory cytokines such as IL-1β, IL-6, and high mobility group box 1 (HMGB1) [17]. This so-called ‘cholinergic anti-inflammatory pathway’ (Figure 1) is responsible for a ‘hard-wired’ connection between the nervous and immune systems and is considered, according to Tracey, as the primary component of the ‘immunoreflex’ or ‘inflammatory reflex’ (Figures 2&3). Its knowledge can yield insight into both pathophysio-logical pathways and therapeutic strategies in many pathological processes, including infections, sepsis and cardiovascular diseases [9,18].
Figure 1. The cholinergic anti-inflammatory pathway. Efferent vagus activity produces release of acetylcholine (ACh) in several organs including the liver, the spleen and the gastrointestinal track (GI track). Ach binds to nicotinic receptors that contain the specific α7 subunits on tissue macrophages and inhibit release of pro-inflammatory cytokines, such as TNF-α, IL-1 and high mobility group box-1 (HMGB-1) protein, which is considered as a ‘dangerous’ signal released from cellular nucleus. Adapted from reference 18.

Figure 2. The ‘immunoreflex’. Inflammatory signals derived from damaged tissues in the periphery activate directly or indirectly the nucleus tractus solitarius (NTS), which subsequently inhibits cytokine synthesis through the vagal efferent effect upon peripheral damaged organs or tissues. Furthermore, activation of the hypothalamus and the dorsal vagal complex (DVC) stimulate the release of ACTH, activating a humoral anti-inflammatory pathway. Release of catecho-lamines and glucocorticoids can also suppress the inflammatory response. Adapted from reference 18.
In conclusion, there is strong evidence that CNS controls body’s systemic response to inflammation. Recently, a limited number of clinical studies investigating a possible association between ANS outflow and various inflammatory indices in patients with heart diseases have appeared in the literature [19-27]. Their aim was to measure ANS activity through a set of different ‘physiomarkers’ and correlate them with various biomarkers that can indirectly assess inflammatory response in different clinical scenarios, such as CAD [19-25] and heart failure [26,27].

The best such ‘physiomarkers’ that can be studied through analysis of heart rate signals are the heart rate variability (HRV) that is the variability of R-R series in the electro-cardiogram (ECG), and its frequency components that estimate the sympathovagal balance [28,29]. Beat-to-beat fluctuations reflect the dynamic response of the cardiovascular control systems to a host of naturally occurring physiological perturbations. A variety of animal and human research has established two clear frequency bands in heart rate and blood pressure signals. These bands include oscillations associated with respiration between 0.2 to 0.4 Hz (high frequency-HF) and bands with a lower frequency range, below 0.15 Hz. The latter has often been subdivided into low-frequency (LF) range below 0.09 Hz and mid frequency range as well (0.09-0.15 Hz), [29]. Akselroad introduced in 1981 power spectrum analysis of heart rate fluctuations in order to quantify beat-to-beat cardiovascular control. Power spectrum density (PSD) analysis provides the basic information of how power (variance) distributes as a function of frequency [30,31]. In 1996, the Task Force of the European Society of Cardiology and the Northern American Society of Pacing and Electrophysiology published guidelines regarding standardization of nomenclature, specification of methods of measurement, definition of physiological and pathophysiological correlates, description of clinical applications and identification of different areas for future research [29].

The association of higher risk of post-infarction mortality with reduced HRV was first shown by Wolf in 1977 [32]. The clinical importance of HRV became appreciated in the late 1980s, when it was demonstrated that low HRV was a strong and independent predictor of mortality after an acute myocardial infarction [33,34].

**MEASUREMENT OF HRV**
The RR variations may be evaluated by a number of methods:

**Time domain methods**
Time domain methods determine heart rate or RR intervals in continuous ECG records. Each QRS complex is detected and the normal-to-normal (NN) intervals (all intervals between adjacent QRS complexes) are calculated. Other time domain variables include the mean NN interval, the mean heart rate or the difference between the longest and the shortest NN interval as well. There are also more complex statistical methods being used, particularly from heart rate signals being recorded for more than 24 hours. The simplest from these metrics is the standard deviation of the NN intervals (SDNN), which is the square root of the variance. However, it should be emphasized that the shorter the monitoring period the less accurate becomes the SDNN variable. The most commonly used time domain methods are the square root of the mean squared differences of successive NN intervals (RMSSD), the number of interval differences of successive NN intervals greater than 50 ms (NN50) and the proportion derived from dividing NN50 by the total NN intervals (pNN50) [29,30]. (Table 1)
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**SDANN** - Standard deviation of the average N-N intervals for each 5-min period over 24 hours

**NN50** - Number of N-N intervals differing by > 50 msec from the preceding interval

**pNN50** - Percentage of adjacent cycles that are > 50 msec apart

**RMSSD** - Root mean square of successive differences in milliseconds

**Table 1. HRV metrics in time domain**

**Frequency domain methods**
Spectral analysis of heart rate signals provides their power spectrum density (PSD). It displays in a plot the relative contribution (amplitude) of each frequency. Methods that are mostly used include Fast Fourier Transformation (FFT) and autoregressive modeling. This plot includes at least three peaks. Fast periodicities in the range 0.15-0.4 Hz [high frequency (HF)] are largely due to the influence of the respiratory phase on vagal tone. Low-frequency periodicities (LF), in the region of 0.04-0.15 Hz, are produced by baroreflex feedback loops, affected by both sympathetic and parasympathetic modulation of the heart and very low frequency periodicities (VLF), in the frequency range less than 0.04 Hz have been variously ascribed to modulation by chemoreception, thermoregulation and the influence of vasomotor activity. The area under the power spectral curve in a particular frequency band is considered to be a measure of HRV at that frequency whereas the LF/HF ratio reflects sympathovagal balance. According to the report of the Task Force, the analyzed ECG signals must satisfy several technical requirements in order to obtain reliable information. The optimal sampling frequency range should be between 250 to 500 Hz. Ectopic beats, arrhythmic events, missing data and noise effects should be properly filtered and omitted. Frequency domain methods must be preferred in cases of short term investigations. The recordings should last for at least 10 times the wavelength of the lower frequency bound, thus recordings of approximately 1 minute can assess the HF component of HRV while 2 minutes are needed for the LF component. In conclusion, 5-minute recordings are preferred, unless the aim of the study dictates a different design [28-31] (Table 2).

**Table 2. HRV metrics in frequency domain**

**CLINICAL IMPLICATIONS OF ALTERED HRV**
The first large prospective population study that proved the significant prognostic value of low HRV after an acute myocardial infarction was the Autonomic Tone and Reflexes After Myocardial Infarction Study (ATRAMI), and included 1284 patients with a recent (<28 days) myocardial infarction [35]. A 24 h Holter recording was done to quantify HRV (using SDNN values) and ventricular arrhythmias. Low values of HRV (SDNN<70 ms) carried a significant multivariate risk of cardiac mortality. Furthermore, the association of low SDNN with left ventricular ejection fraction (LVEF)<35% carried a relative risk of 6.7, compared with patients with LVEF above 35%. Investigators from the Framingham Heart Study studied HRV time and frequency domain measures in 736 patients and correlated them with all-cause mortality during 4 years of follow-up [36]. They concluded that HRV offers prognostic information independent of that provided by traditional risk factors. During the Zutphen study, 885 middle-aged (40-60 years old) and elderly Dutch men (aged 65-85) were followed from 1960 until 1990, whereas SDNN was determined from the resting 12-lead ECG. It was shown that low HRV is predictive of mortality from all causes, indicating that it can be used as an index of compromised health in the general population [37]. It seems that the predictive value of low HRV is independent of other factors such as depressed left ventricular ejection fraction and presence of late potentials [33,34].

**HRV AND INFLAMMATORY BIOMARKERS IN CARDIOVASCULAR DISEASES**
The relationship between HRV and inflammation has been studied mainly in healthy controls, patients with acute or stable CAD, chronic heart failure (CHF) and metabolic syndrome with impaired glucose tolerance [38]. The inflammatory biomarkers that were used included C-reactive protein (CRP), TNF-α, IL-6 and white blood cell count.

**Patients with coronary artery disease**
Hamaad et al tested the linkage between time and frequency domain indices of HRV and circulating IL-6, high sensitivity CRP (hs-CRP) and white cell counts, in a sample of 100 patients with proven acute coronary syndrome [19].

In addition, they compared these with healthy controls (n=49) and estimated these relationships on repeated measures at 4 months in recovery (n=51). They found modest negative correlations between all inflammatory bio-markers and mainly SDNN, VLF and LF power. The strongest associations were seen between white cell counts and SDNN index (r= -0.351),
whereas these associations did not persist on multivariate analyses after a 4-month period. According to the authors, the correlations were observed largely among HRV indices reflecting sympathetic activity, suggesting that the inflammatory response in acute coronary events may be associated with sympathetic activation instead of vagal withdrawal. Furthermore, leukocytosis observed in these patients seems to be a potential source of pro-inflammatory cytokines within the atheromatous plaque and might induce a potential rupture.

In another study, Lanza and colleagues assessed HRV and measured CRP serum levels within 24 hours of admission in 531 patients with unstable angina pectoris [20]. They found a significant negative correlation between CRP levels and all HRV metrics, with the highest r coefficient with SDNN (r=-0.23) and VLF (r=-0.22). After categorizing patients into 4 subgroups according to CRP quartile levels, significantly lower HRV values were found in the upper CRP quartile. The subsequent multivariate analysis revealed that SDNN and VLF were the most significant predictors of increasing CRP whereas CRP itself was proven to be a strong predictor of impaired ANS activity as well.

Psychari et al also reported a strong inverse association between CRP and several HRV indices (SDNN, SDNN index, HF and LF) in patients after acute myocardial infarction after adjustment for left ventricular function [23].

In a study including patients with suspected CAD, Madsen et al enrolled 269 subjects referred for elective coronary angiography [21]. They found that SDNN, SDNNindex and SDANN were significantly higher in the lower CRP quartile compared to the upper one, whereas these associations were proved to be stronger for patients with a previous myocardial infarction and with significant coronary stenoses.

In a similar study, Nolan reported a negative correlation between CRP and HRV but also found decreased HF power (reflecting vagal tone) in the high CRP quartile, compared to the lowest one [22]. Finally, in the study of Janszky that included only female patients who survived hospitalization for acute myocardial infarction and were evaluated 1 year after the event, levels of IL-6 showed an inverse relation with all HRV measures except for HF [25]. This relation along with the association between CRP levels and IL-1 receptor antagonist (IL-1ra) with HRV indices were also inverse but weaker and nonsignificant.

An excellent overview of the current state of knowledge on the association between HRV and different inflammatory biomarkers in patients with CAD has recently been published by Haensel [39].

**Patients with heart failure**

In 2001, Aronson evaluated for the first time the relation between HRV metrics derived from both the time and frequency domain and different biomarkers such as IL-6, TNF-α and serum levels of norepinephrine, in 64 patients admitted for decompensated chronic heart failure [26]. TNF-α levels did not correlate with any of the HRV indices. IL-6 inversely correlated with SDNN and SDANN (r=-0.36 and r=-0.39 respectively) and with total power and ULF (r=-0.37 and r=-0.43 respectively). No correlation was found between IL-6 and indices of vagal activity.

Malave et al examined HRV in relation to circulating levels of TNF-α, TNF receptors and norepinephrine in 10 controls, 15 patients with mild CHF and 14 subjects with moderate heart failure [27]. There was a significant inverse linear correlation between increased levels of all biomarkers and SDNN, SDANN, LF and HF power among CHF patients. In addition, LF power was more closely correlated with circulating levels of TNF-α than was the HF component, whereas multiple linear regression analysis showed that TNF-α was a stronger predictor of reduced HRV than was the circulating levels of norepinephrine. The authors concluded that overexpression of TNF-α and subsequent loss of β-adrenergic responsiveness contributes to the decrease in HRV observed in heart failure. According to recent experimental studies, TNF-α might inhibit β-adrenergic signal transduction through either activation of Gi proteins or impairment of activation of Gs proteins [40], something that could be viewed as an adaptive mechanism in the early stages of CHF through protection of cardiac myocytes from the deleterious actions of catecholamines. However, in the more advanced stages of the disease, this mechanism could become maladaptive, leading to a reduction in cardiac output [41].

Finally, in a small prospective study that included 34 patients with CHF followed for a 2-year period with monthly CRP measurements and 24-hour Holter recordings, it was shown that five unexpected deaths that occurred were preceded by progressive increases in both CRP serum levels and autonomic dysfunction (low HRV indices) [42].

**Healthy controls**

As a part of the Copenhagen Holter study, which aimed to assess the value of 24-hour Holter recording in the risk assessment of men and women aged 55, 60, 65, 70 and 75 years with no apparent heart disease, Sajadieh et al investigated the associations between time domain components of HRV, CRP and white blood cell count in 643 healthy men and women [43]. They found that both SDNN and SDANN were negatively correlated with smoking, inflammatory indices, blood sugar, triglyceride concentration, female gender and diabetes. Moreover, in multivariate regression analysis, increased heart rate and reduced HRV were significantly related to white blood cell count and CRP. Since SDNN and SDANN reflect both sympathetic and parasympathetic modulation of heart rate, their reduction was attributed to sympathetic predominance, whereas lack of any association between inflammation and pNN50, which is considered as a marker of vagal activity, indicates that reduced HRV is mainly due to increased sympathetic activity rather than vagal withdrawal.

From the Whitehall II cohort, a multicenter epidemiologic investigation of over 5000 subjects, two studies used subsamples to examine the relation between HRV indices derived from the frequency domain and inflammation in healthy subjects. Sloan found in a sample of 757 people an inverse correlation between CRP and IL-6 with both LH and HF components of HRV power spectrum [44], whereas Owen and Steptoe did not reveal any association between IL-6, TNF-α and time domain measures of HRV in a group of 211 healthy adults [45].

Albert reported a strong positive association between CRP levels and the long-term risk of sudden cardiac death in case-control analysis among healthy individuals followed for 17 years in The Physician’s Health Study [47]. Men in the upper CRP quartile had a 2.8 fold increased risk of sudden cardiac death compared to men in the lower quartile. According to the authors, a low-grade inflammation involved in atherosclerosis shifts ANS balance toward sympathetic activation, making
individuals more prone to ventricular arrhythmias and sudden cardiac death.

A significant confounding factor of HRV analysis that has to be considered in these studies includes the presence of depressive symptoms and anxiety. It has been estimated that approximately 12 to 20% of hospitalized cardiac patients suffer from major depression, whereas 15% of subjects following acute myocardial infarction exhibit a posttraumatic stress disorder [48,49]. In a 2-year follow up prospective observational study, Pizzi et all investigated the relation between time domain HRV indices, IL-6, TNF-α, CRP and depression in a cohort of 415 subjects free of CHD, with at least two CHD risk factors (age, male gender, current smoking, hypertension, dislipidaemia) [50]. All HRV and inflammatory indices were significantly associated with depression whereas logistic regression showed that depressive individuals were more likely to have a higher CRP and IL-6 and altered HRV (lower SDANN). In conclusion, in patients without heart disease, depression seems to be associated with HRV imbalance and inflammation.

**CONCLUSIONS**

In conclusion, recent experimental and clinical data from studies involving patients with coronary artery disease, heart failure and healthy subjects with increased risk factors for heart diseases suggest that there is a continuous ‘cross-talk’ between inflammation and ANS activity, estimated through HRV analysis. In such cases, an inverse correlation between ANS and immune system has been found, particularly in most severe cases. The discovery of the so-called ‘cholinergic anti-inflammatory pathway’ has expanded our understanding of how the nervous system modulates the inflammatory response through an immunoreflex. However, Goldberger has observed that HRV increases with augmented vagal tone, followed by a plateau and a subsequent decrease with increased parasympathetic activity [51]. Moreover, sympathetic outflow can either induce or inhibit inflammatory activity. Thus it has been suggested that newer non-linear methods

**Table 3.** Summary of several clinical studies investigating a possible association between HRV indices and inflammation in patients with CAD, CHF and healthy individuals.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Population</th>
<th>Duration and HRV measures</th>
<th>Inflammatory indices</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamaad et al [19]</td>
<td>100 patients with acute CAD vs 29 healthy controls</td>
<td>20 min time and frequency domain</td>
<td>CRP, IL-6</td>
<td>Negative correlation with SDNN, SDNN index, VLF and LF</td>
</tr>
<tr>
<td>Lanza et al [20]</td>
<td>531 patients with unstable CAD</td>
<td>24-h time and frequency domain</td>
<td>CRP</td>
<td>Inverse correlation between CRP with SDN and VLF</td>
</tr>
<tr>
<td>Madsen et al [21]</td>
<td>269 patients with suspected CAD</td>
<td>24-h time domain</td>
<td>CRP</td>
<td>Upper CRP quartile negatively correlated with SDNN, SDNN index and SDANN</td>
</tr>
<tr>
<td>Nolan et al [22]</td>
<td>29 patients with CAD</td>
<td>5-min time frequency domain</td>
<td>CRP</td>
<td>HF decreased in high CRP group</td>
</tr>
<tr>
<td>Psychari et al [23]</td>
<td>98 patients with acute CAD</td>
<td>24-h time frequency domain</td>
<td>CRP</td>
<td>Inverse relation between CRP and HRV</td>
</tr>
<tr>
<td>Yue et al [24]</td>
<td>52 patients with CAD</td>
<td>5-min time frequency domain</td>
<td>CRP</td>
<td>No association between CRP and HRV</td>
</tr>
<tr>
<td>Aronson et al [26]</td>
<td>64 patients with CHF</td>
<td>24-h time frequency domain</td>
<td>TNF-α, IL-6</td>
<td>IL-6 inversely correlated with SDNN, SDANN, ULF</td>
</tr>
<tr>
<td>Malave et al [27]</td>
<td>10 healthy controls, 15 patients with mild CHF, 14 patients with moderate CHF</td>
<td>24-h time frequency domain</td>
<td>TNF-α, TNF soluble type 1 and 2 receptors</td>
<td>Inverse correlation between inflammatory measures, SDNN, SDANN, LF and HF in CHF patients</td>
</tr>
<tr>
<td>Sajadieh et al [43]</td>
<td>643 subjects without CHF</td>
<td>24-h time domain</td>
<td>CRP, white blood cell count</td>
<td>Inverse correlation between SDNN, SDANN with CRP and white blood cell count SDNN predictor of CRP</td>
</tr>
<tr>
<td>Sloan et al [44]</td>
<td>757 young healthy adults</td>
<td>10-min time frequency domain</td>
<td>CRP, IL-6</td>
<td>CRP and IL-6 inversely correlated with HF and LF</td>
</tr>
<tr>
<td>Owen and Steptoe  [45]</td>
<td>211 healthy subjects</td>
<td>20-30 min time domain</td>
<td>TNF-α, IL-6</td>
<td>No relation between both TNF-α and IL-6 with HRV</td>
</tr>
<tr>
<td>Kon et al [46]</td>
<td>823 healthy individuals</td>
<td>2-min time domain</td>
<td>CRP</td>
<td>CRP independently predicted low SDNN index</td>
</tr>
</tbody>
</table>

Abbreviations: CRP: C-reactive protein, CAD: coronary artery disease, CHF: chronic heart failure
derived from chaos theory should be implemented in the assessment of ANS output, such as fractal analysis of heart rate signals [52]. Further-more, according to Haensel, the explanatory power of HRV analysis can be limited by time variation throughout the day, similarly with estimation of inflammatory activity through bio-markers measurements from a single blood sample [39]. Finally, the presence of significant confounders of HRV, such as depression and anxiety, has to be controlled in future studies.

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CV of Author
- Professional status: Lecturer in Intensive Care Medicine, Democritus University of Thrace, Alexandroupolis University Hospital, Dpt of Intensive Care Unit
- Clinical education: Certified from the Greek Association of Anesthesiology. Certified from the Greek Association of Intensive Care Medicine.
- Clinical interests: Hemodynamics and ECHO ultrasound of heart-lung-cerebral vasculature (TCD) in critically ill patients. Education in UK and Germany.
- Scientific interests: Biosignal analysis and application of chaos systems theory in critical illness.
- Major publications: Over 16 publications in international peered review journals. Over 29 abstracts presented in International Medical Conferences and published (15) in international peered review journals. Over 10 chapters in Medical books (Greek).