



Vagal nerve activity predicts overall survival in metastatic pancreatic cancer, mediated by inflammation



Marijke De Couck^{a,*}, Raphaël Maréchal^b, Sofie Moorthamers^c, Jean-Luc Van Laethem^b, Yori Gidron^c

^a Mental Health and Wellbeing Research Group, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel (VUB), 103 Laarbeeklaan, 1090 Brussels, Belgium

^b Department of Gastroenterology, GI Cancer Unit Erasme University Hospital Université Libre de Bruxelles, 808, route de Lennik, 1070 Brussels, Belgium

^c Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel (VUB), 103 Laarbeeklaan, 1090 Brussels, Belgium

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ABSTRACT

Recent research findings suggest neuro-modulation of tumors. Finding new modifiable prognostic factors paves the way for additional treatments, which is crucial in advanced cancer, particularly pancreatic cancer. This study examined the relationship between vagal nerve activity, indexed by heart rate variability (HRV), and overall survival (OS) in patients ($N = 272$) with advanced pancreatic cancer. A “historical prospective” design was employed, where vagal activity and other confounders were retroactively obtained from medical charts at diagnosis, and subsequent OS was examined. HRV was obtained from 10 sec ECGs near diagnosis. Levels of C-reactive protein (CRP) were measured as an inflammatory marker. OS and survival date were obtained from medical charts and the Belgian national registry. Patients with high HRV (>20 msec) survived on average more than double the days (133.5) than those with low HRV (64.0). In a multivariate cox regression, higher initial HRV was significantly correlated with lower risk of death, independent of confounders including age and cancer treatments. This relationship was statistically mediated (accounted for) by CRP levels. Importantly, in patients who lived up to one month from diagnosis only, HRV was unrelated to CRP, while in patients surviving longer, HRV was significantly inversely related to CRP ($r = -0.20$, $p < 0.05$). These results are in line with possible vagal nerve protection in a fatal cancer, and propose that the mechanism may involve neuroimmuno-modulation. Future studies must test whether vagal nerve activation may help patients with advanced cancers.

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1. Introduction

Tumors progress under the influence of oxidative stress, inflammation and excessive sympathetic activity. For example, Voronov et al. [1] showed that in IL-1 beta knockout mice, lung metastases of B16 melanoma cells were not observed compared with wild-type mice. Furthermore, inherent oxidative stress also affects several functions in cancer cells or tumour tissues, such as cell proliferation, promotion of mutations and genetic instability, alterations in cellular sensitivity to anticancer agents, invasion, angiogenesis and metastasis [2]. A third mechanism linked to cancer progression is excessive sympathetic activity, which was found to promote tumors [3,4].

On the other hand, the vagus nerve, which is a major component of the parasympathetic nervous system, may slow tumor progression [5]. The vagus nerve can inhibit oxidative stress, inflammation and excessive sympathetic activity [6–8]. The vagus nerve represents an important channel for the bidirectional communication between the brain and the immune system [7]. Via the production of inflammatory cytokines, the immune system can activate sensory fibers of the vagus nerve expressing receptors for interleukin-1, that ascend to synapses in the nucleus tractus solitaries (NTS) in the brain stem. In return, the activated effector neurons of the vagus nerve can inhibit the production of peripheral pro-inflammatory cytokines via binding of acetylcholine on tissue macrophages. This negative feedback-loop system is the core of the nicotinic anti-inflammatory pathway [7], any may render the vagus tumor-protective roles. Indeed, tumor-bearing animals undergoing chemical or surgical vagotomy showed enhanced metastasis [9,10], while an anti-inflammatory drug (CNI-1493),

* Corresponding author.

E-mail address: marijke.decouck@vub.ac.be (M.D. Couck).

whose action depends on and stimulates the vagus nerve [11], reduced tumor size and metastases in tumor-bearing mice [12].

In cancer patients, high vagal nerve activity, indexed by heart rate variability (HRV) [13], significantly predicted lower tumor marker levels and longer overall survival (e.g. [14–16]). As mentioned above, inflammation promotes tumorigenesis [1,17], but it is strongly inhibited by vagal nerve activity [7]. However, the role of vagal neuroimmuno-modulation in cancer prognosis was not tested. This study tested whether HRV at diagnosis was independently correlated with overall survival in patients with advanced pancreatic cancer. Furthermore, we preliminarily investigated the role of inflammation in this relationship. We hypothesize that high baseline HRV would be associated with longer survival and that this relationship would be statistically mediated (accounted) by lower levels of inflammation. Finally, we examined whether the HRV-CRP relationship (reflecting neuro-immuno-modulation), is associated with survival time as well.

2. Patients and methods

2.1. Design

The present study used an “historical-prospective design”. We obtained existing archival data on patients’ medical background and HRV, and then examined the prospective relationship between these parameters measured at diagnosis and patients’ overall survival (OS). The design term “historical-prospective” is commonly used in reanalysis of existing longitudinal datasets.

2.2. Patient cohort

After approval of the Medical Ethics Committee, medical records of 620 patients with histologically proven advanced (locally advanced and metastatic) pancreatic cancer (PC) treated at the University Hospital Erasme, Brussels, between 1998 and 2011, were reviewed. Tumor staging was made based on chest and abdominal CT-scan, magnetic-resonance imagery, and endoscopic ultrasound findings. Exclusion criteria included chronic inflammatory disease, anemia and thyroid disease or lack of an ECG taken near the time of diagnosis. Following these exclusion criteria, $N=353$ patients were included, of whom survival data were certified for $N=272$ patients. There were no statistically significant differences in various background variables, treatments or HRV levels between patients with and without survival data ($p > 0.05$ for all). However, there were significantly fewer patients undergoing surgery (44.6% versus 62.5%, $p < 0.05$) in the sample with survival data than in those without survival data. Our study sample included 52.8% patients with locally advanced cancer and 47.2% with metastatic cancer and their mean age was 60.0 (± 11.5) years. The sample size of this study was anticipated to be sufficiently large, as it exceeded De Couck et al. [16] showing a significant correlation between HRV and survival time in $N=73$ lung cancer patients.

2.3. Variables

2.3.1. Background confounders

These included age, gender, treatments (radiotherapy, chemotherapy, surgery), locally advanced versus metastatic PC, and presence of cardiac disease. These data were obtained from patients’ electronic medical records.

2.3.2. Vagal nerve activity

Heart rate variability (HRV), the index of vagal nerve activity, was derived from patients’ 10 sec ECG near diagnosis. This represents efferent vagal nerve input to the heart. HRV has been

shown to be strongly correlated with and to be experimentally altered by vagal nerve activity [13,18]. The time domain parameters ‘standard deviation of normal R–R intervals’ (SDNN), in msec, and the root mean square of successive differences (RMSSD) between preceding R–R intervals, were derived. Such parameters from short ECGs have been found to correlate with ECGs of longer durations [19]. Furthermore, 10 sec SDNN measures have been shown to predict prognosis in cardiac disease [20], and in colon cancer [15]. A cut-off of 20 msec was used, as in past studies in cancer [15,20], to distinguish between patients with high versus low HRV. Furthermore, 20 msec has been shown to be the mean HRV in a big sample of cancer patients ($N=657$) [21].

2.3.3. Inflammatory marker

We obtained from patients’ medical records levels of C-Reactive Protein (CRP) near diagnosis, a systemic inflammatory marker of prognostic value in many cancers, including PC [22].

2.4. Outcomes

The primary outcome in the present study was overall survival (OS), obtained from medical charts and the Belgian national registry. We used as censorship date November 1st, 2012, the last date of inspecting patients’ medical files.

2.5. Statistical analysis

We first performed a multivariate Cox-regression analysis using all confounders, except for HRV, for predicting OS. This enabled all confounders to ‘compete’ in independently predicting OS. In this analysis, death (OS) was the dependent variable, and time till death or censorship date (for alive patients) was the time variable. The relationship between HRV and OS was tested with a univariate Cox-regression, followed by a multivariate Cox-regression with all other significant confounders. A two-tailed statistical significance of $p < 0.05$ was used. To determine statistically the mediating (explanatory) role of CRP, we performed two tests. First, we examined the relationship between CRP and HRV and OS. Then, we reexamined the relationship between HRV and OS, while statistically controlling for all confounders as well as for CRP in the Cox-regression. Second, we conducted a Sobel test on survival time (in days) and examined the Pearson’s correlation between HRV and CRP and between each with survival time. We then examined whether the HRV–survival time correlation remained significant, after statistically controlling for CRP. Then, we examined whether the CRP–Survival time relationship remained after statistically controlling for HRV.

Finally, to gain insight into the neuroimmuno-modulatory role of the vagus nerve in cancer, we examined the expected negative relationship between HRV and CRP in patients surviving up to one month versus in patients surviving over one month. Though the median survival time was 41 days, approximately one third of the sample survived up to one month, which was chosen as a clinically relevant and more tangible cut-off. A similar pattern was found when taking the median survival time.

3. Results

3.1. Sample characteristics

Table 1 depicts the characteristics of the present sample, on which survival data were available ($N=272$ patients). Patients’ mean age was 60 years, their mean HRV at diagnosis was very low (SDNN = 21.7 msec), yet similar to those in other cancers [16]. The mean CRP levels near diagnosis were elevated compared to some proposed cut-offs (e.g., 3 mg/L) [23]. Approximately half of the

Table 1
Study characteristics of the present sample.

a Continuous variables		
Variable	Mean	SD
Age	60.0	11.5
SDNN	21.7	15.3
RMSD	27.2	20.6
Baseline CA19-9	5420.2	19946.1
Bilirubin	4.3	9.7
CRP	4.1	16.4
b Categorical variables		
Variable%		
Gender		
Men	51.8%	
Women	48.2%	
Treatments		
Surgery	44.6%	
Radiotherapy	17.3%	
Chemotherapy	74.5%	
Location of metastasis		
Locally advanced	52.8%	
Metastatic	47.2%	
Cardiac problem	29.7%	

Note: SDNN = Standard deviation of R–R intervals; RMSD = root mean square of successive differences between proximal R–R intervals; CRP = C reactive protein.

patients were male, half had locally advanced PC, and three quarters received chemotherapy. Among the deceased patients, the mean survival time was 56.15 days, the median was 39 days, ranging from 0 to 896 days.

3.2. Relationship between study variables and OS

Of the 272 patients with survival data, 241 died and 31 survived during our follow-up. In the multivariate Cox-regression with all potential confounders considered together, surgery, chemotherapy, locally advanced versus metastatic PC and age were the only confounders significantly and independently correlated with OS. Radiotherapy, gender and cardiac disease were not significantly related to OS. A higher chance of OS was correlated to having surgery, having locally advanced tumors, receiving chemotherapy and a younger age. Nevertheless, we forced cardiac disease into the subsequent equations, due to its effects on HRV.

Using the cut-off for SDNN of 20 msec, SDNN was univariately significantly correlated with OS in a Cox-regression ($B = -0.494$, $p < 0.001$). Finally, as shown in Table 2, SDNN was also significantly correlated with OS, independent of all confounders ($B = -0.301$, $p = 0.047$). Patients with low SDNN survived on average only 64.05 days versus those with high SDNN who survived 133.52 days ($t(128.43) = 3.31$, $p = 0.001$). Fig. 1 depicts the relationship between HRV and OS.

3.3. The mediating role of CRP in the HRV-OS relationship

To test the mediating role of CRP in the relationship between HRV and OS, we conducted two separate analyses. First, in a

Table 2
Multivariate Cox-regression between all significant bivariate predictors and OS.

Predictor	B	SE	Sig.	H.R.	95% CI
Surgery	-0.94	0.17	0.000	0.39	0.28–0.55
Chemotherapy	-0.49	0.18	0.006	0.61	0.43–0.87
Location of invasion	-0.32	0.16	0.015	0.68	0.49–0.93
Cardiac problem	0.07	0.16	0.660	1.07	0.78–1.47
Age	0.02	0.01	0.005	1.02	1.01–1.03
Cat SDNN	-0.30	0.15	0.047	0.74	0.55–0.99

Note: SDNN = Standard deviation of R–R intervals; SE = Standard error of the mean; Sig. = Significance; H.R. = hazard ratio; 95% CI = 95% confidence interval.

univariate Cox-regression, log CRP was significantly correlated with OS ($B = 0.556$; $p < 0.001$). CRP levels were significantly and inversely related to HRV ($r = -0.15$, $p = 0.007$). Finally, when adding CRP into the multivariate Cox-regression, HRV was no longer correlated with OS ($B = 0.26$, $p = 0.116$), while CRP was still correlated with OS ($B = 0.44$, $p < 0.001$). This demonstrated that CRP statistically mediated (accounted for) the HRV–OS relationship. In a second analysis, we used the Sobel test to examine the mediating role of CRP between HRV and survival time. SDNN was inversely related to CRP ($r = -0.15$, $p = 0.007$) and positively related to survival time ($r = 0.18$, $p = 0.003$). In contrast, CRP was inversely related to survival time ($r = -0.38$, $p < 0.001$). After statistically controlling for CRP, SDNN was no longer significantly correlated with survival time ($r = 0.11$, $p > 0.05$). In contrast, when controlling for SDNN, CRP remained significantly correlated with survival time ($r = -0.36$, $p < 0.001$).

We found differences in HRV between patients with locally advanced and metastatic tumors (locally advanced had higher SDNN), and the locally-advanced variable predicted prognosis. However, since the sub-samples and number of alive patients declined very much, the statistical power was insufficient to conduct meaningful statistics in each sub-sample separately. Hence we conducted the main analyses in the full sample and statistically controlled for the effects of the variable of locally advanced versus metastatic tumors, as reported above.

3.4. A preliminary look at neuroimmuno-modulation and survival

In a second analysis, we tested whether neuroimmuno-modulation (the inverse HRV–CRP relationship) is stronger when OS is longer. In patients surviving up to one month (one third of the sample), SDNN was unrelated to CRP ($r = -0.07$, $p = 0.55$), while in patients surviving longer than one month, SDNN was significantly inversely related to CRP ($r = -0.17$, $p = 0.04$), statistically reflecting neuroimmuno-modulation.

4. Discussion

This study examined in a sample of patients with advanced pancreatic cancer the prognostic value of vagal nerve activity, indexed by HRV, and preliminarily one of its underlying mechanisms. Our results show that higher HRV near diagnosis was significantly correlated with longer OS, independent of important confounders including treatment and age. As hypothesized, we found that this relationship was statistically mediated (accounted for) by reduced levels of inflammation, indexed by CRP, using two statistical approaches. These results support those of previous studies in other cancers (e.g. [14–16]) and extend them to one of the most fatal cancers, and begin to point at a possible mechanism of vagal modulation in cancer.

Though the present study was performed on a sample of patients with a highly fatal cancer, the consistency of our results with past studies suggest that vagal nerve activity, indexed by HRV obtained from brief ECGs, may be a new independent prognostic factor in several cancers. Furthermore, the fact that patients above SDNN of 20 msec survived on average more than double the days of those with a lower SDNN, suggests that such a cut-off has statistical and clinical significance. Twenty milliseconds has also been shown to be the mean HRV in a big sample of cancer patients [21].

Two of our findings provide preliminary evidence for our hypothesized mechanism [5,6]. First, CRP was inversely related to HRV and to OS, and when adding CRP into the multivariate regression, HRV was no longer correlated with OS. Second, using the Sobel mediation test, CRP statistically mediated or accounted for the relationship between HRV and survival time. Though our

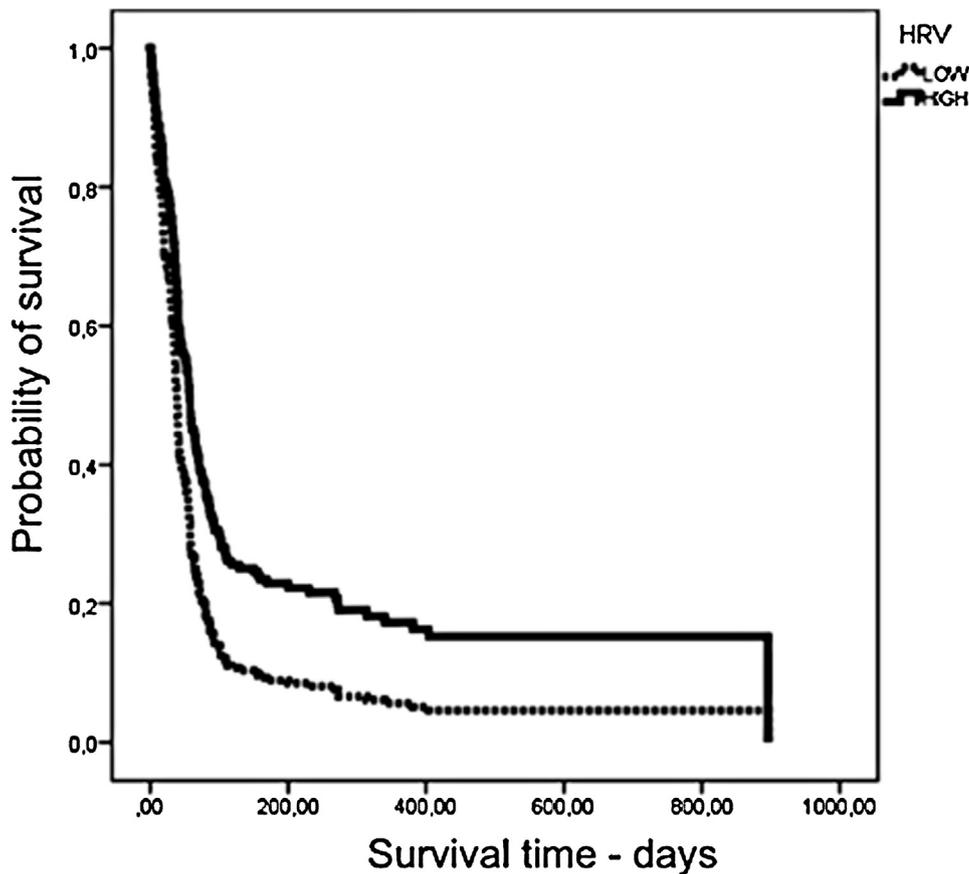


Fig. 1. The relationship between HRV and overall survival.

study design does not enable us to conclude any causal relationship between vagal nerve activity, reduced inflammation and better OS in cancer, these results statistically support a mediating role of inflammation between HRV and cancer prognosis.

Is vagal nerve inhibition of inflammation related to cancer prognosis? We observed in the present study that in patients surviving longer than one month, HRV was, as expected, inversely related to CRP, while no such relationship was observed in patients surviving up to one month from diagnosis. This pattern of findings suggests that in patients with neuroimmuno-modulation (inverse HRV–CRP relationship), survival time is increased.

Recent research suggests that the autonomic nervous system, and particularly the vagus nerve, may affect tumorigenesis. While excessive inflammation contributes to tumorigenesis [1,17], the vagus nerve may in contrast slow it down. The vagus nerve represents an important channel for the bidirectional communication between the brain and the immune system [7]. Via the production of inflammatory cytokines (interleukin-1), the immune system can activate sensory fibers of the vagus nerve that ascend to synapses in the nucleus tractus solitarius (NTS) in the brain stem. The brain can then process the information and, in return, activate effector neurons originating in the dorsal motor nucleus of the vagus nerve. These descending signals can then inhibit the production of peripheral pro-inflammatory cytokines via binding of acetylcholine, the principal neurotransmitter of the vagus nerve, to a nicotinic acetylcholine receptor on tissue macrophages. This negative feedback-loop system is the core of the nicotinic anti-inflammatory pathway [7]. However, the final anti-inflammatory pathway is still under investigation and includes activation of the hypothalamic-pituitary-adrenal axis which increases cortisol, and a descending vagal-sympathetic pathway activating T-cells

residing in the spleen, which then produce Ach that binds to monocytes to inhibit inflammation [35]. Several (though not all) experimental studies in mice show that vagotomised tumor bearing animals had more metastases [9,10]. Furthermore, one study demonstrated that an anti-inflammatory drug, which depends on the vagus nerve, reduced metastases in tumor-bearing mice [12]. These experimental studies clearly indicate the possible causal relationship between adequate vagal nerve activity and reduced tumor progression. The correlations observed in the present study are in line with such experimental studies and suggest that vagal inhibition of inflammation may partly account for such effects.

An important finding is that especially in advanced or metastatic cancer, vagal nerve activity may manifest its protective effects since during such a stage, inflammation promotes tumorigenesis [1,17]. Furthermore, it is possible that in earlier tumor stages, commonly provided treatments such as surgery and chemotherapy are successful in reducing the tumor burden, possibly leaving less of a margin for vagal nerve activity to slow the process.

Another possible mechanism requiring further testing is T-regulatory cells (T-Regs) and myeloid-derived suppressor cells (MDSC), which are both elevated in PC [24,25] and which suppress anti-tumor cytotoxic T-cells. Vagal nerve activation can reduce in some instances T-cell activity [26]. It is possible that in patients with higher HRV, adequate immune regulation by the vagus nerve may suppress such suppressors of cytotoxic T-cells, thus enable stronger anti-tumor cytotoxic T-cell activity, possibly resulting in higher OS [27]. Future studies must examine this pathway as well.

The present study had a few limitations. First, HRV was derived from brief 10-sec ECGs, rather than the advised 5 min [13]. Yet, our

results and past studies mentioned above inform us that even 10 sec ECGs, which prevail worldwide, have important independent prognostic value, when reflecting HRV. Second, this was an “historical prospective” study, hence, we lacked sampling and measurement control. Third, there were by far more deceased than living patients, due to the severe nature of this cancer, possibly influencing the confounder analysis. Nevertheless, most confounders were included in the multivariate analysis. Fourth, we only examined the mediating role of CRP, and thus more specific inflammatory cytokines with known roles in cancer prognosis, need to be tested. This may include Interleukin-6 which negatively influences prognosis [28] and Interferon γ which is used in some cancers as a treatment [29]. Finally, our study is correlational, and thus, to verify whether our observed possible statistically mediating role of CRP in the relationship between HRV and prognosis is causal, requires experimental testing in animals or via intervention studies in humans.

Given the accumulation of findings on the prognostic role of vagal nerve activity in cancer, future studies should test whether activating the vagus nerve can improve the prognosis of cancer patients. Such activation could be done via systematically practicing deep paced breathing [30], electrical vagus nerve stimulation or the anti-inflammatory vagal-dependent drug Semapimod [11] which was found to reduce tumor size and metastases in mice [12]. Furthermore, vagal nerve activation is inversely related to pain and to hopelessness (e.g. [31]), and it may reduce depression [32], all which independently predict poor prognosis in cancer as well [33,34]. Thus, future studies must test whether activating the vagus nerve in patients with metastatic cancer can increase their quality and length of life. This could offer a new manner of understanding and possibly treating cancer, via adjuvant neuroimmuno-modulation treatments.

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