# Original Article

# Paraoxonase-1 (PON1) Status in Pancreatic Cancer: Relation to Clinical Parameters

(pancreatic cancer / paraoxonase 1 (PON1) polymorphisms / PON1 arylesterase/lactonase activities / malnutrition)

M. VECKA[1], M. JÁCHYMOVÁ[2], L. VÁVROVÁ[1], J. KODYDKOVÁ[1], J. MACÁŠEK[1], M. URBÁNEK[1], T. KRECHLER[1], A. SLABÝ[1], J. DUŠKOVÁ[3], A. MURAVSKÁ[2], A. ŽÁK[1]

[1]4th Department of Internal Medicine, [2]Institute for Clinical Biochemistry and Laboratory Diagnostics, [3]Institute of Pathology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic

**Abstract. Human paraoxonase 1 (PON1) has been shown to decrease the level of systemic oxidative stress, which is thought to contribute to cancer development. The aim of this study was to examine the interrelationships between PON1 status and some clinical characteristics in patients with pancreatic cancer (PC). A group of 73 consecutive patients with PC (stage II–IV) and 73 control subjects were examined. Laboratory studies included five polymorphisms of the *PON1* gene (L55M, Q192R, -108C/T, -126C/T, and -162A/G), PON1 arylesterase (PON1-A) and lactonase (PON1-L) activities, as well as some markers of protein metabolism, insulin resistance, and oxidative stress. In comparison with the control group, no difference in the distribution of the *PON1* polymorphisms was found in cancer patients, both arylesterase and lactonase activities being significantly lower (-33, -47 %, respectively, both P < 0.001). There was neither statistically significant association of *PON1* polymorphisms with tumour stages nor with diabetes mellitus connected with PC. The genotype distribution of L55M and -108C/T differed only in a subgroup of patients presenting clinically relevant malnutrition ($\chi^2$ = 6.50, 6.25, respectively, both P < 0.05). In the PC group, PON1-A and PON1-L activities correlated with Nutritional Risk Index (r = 0.351, 0.409, respectively, both P < 0.01), PON1-L with mid-arm muscle circumference (r = 0.328, P < 0.05), and PON1-A and PON1-L with serum albumin (r = 0.352, 0.391 respectively, both < 0.01). Our results suggest that PON1 plays an important role in PC, especially in cancer-associated malnutrition.**

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Corresponding author: Marek Vecka, 4th Department of Internal Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic. Phone: (+420) 224 964 300; e-mail: marek.vecka@lf1.cuni.cz

Abbreviations: BMI – body mass index, CCS – cancer-related cachexia syndrome, CD-LDL – conjugated dienes in precipitated LDL, DM – diabetes mellitus, HOMA-IR – homeostatic model assessment of insulin resistance, HWE – Hardy-Weinberg equilibrium, LDL – low-density lipoprotein, LDL-C – LDL cholesterol, NRI – Nutritional Risk Index, OS – oxidative stress, PC – pancreatic cancer, PCDM+ – PC with DM, PCDM- – PC without DM, PCMN+ – PC with clinically relevant malnutrition, PCMN- – PC without malnutrition, PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism, PON1 – paraoxonase 1, PON1-A – PON1 arylesterase, PON1-L – PON1 lactonase, RONS – reactive oxygen and nitrogen species, SAA – serum amyloid A, SNP – single-nucleotide polymorphism.

## Introduction

Pancreatic cancer (PC), one of the most fatal human malignancies, ranks as the fourth cancer-related cause of deaths in Europe as well as in the Czech Republic. In 2008, its incidence in the Czech Republic reached 19.4 cases per 100,000 inhabitants in men, 17.9 cases in women (Ferlay et al., 2010; IHIS, 2011). The factors contributing to the development of PC include age, genetic risk factors, pre-existing diseases [chronic pancreatitis, diabetes mellitus (DM), obesity, and other insulin resistance conditions], as well as several lifestyle and environmental factors (diet, cigarette smoking and heavy alcohol consumption) (Hassan et al., 2007).

Particular interest has been devoted to the role of increased oxidative stress (OS), proinflammatory cytokines, reactive oxygen and nitrogen species (RONS), as well as the end products of lipid peroxidation in cancerogenesis (Valko et al., 2006). The concomitant presence of diabetes (pancreatic cancer-associated hyperglycaemia and/or type 2 DM) has been found in a substantial proportion of PC patients at the time of diagnosis (Pannala et al., 2008), in our experience reaching up to 40 % (Krechler et al., 2011). The most common symptom in patients with PC is malnutrition which can

progress to cancer-related cachexia syndrome (CCS) characterized by extensive muscle wasting. OS has been considered a mechanism greatly involved in the pathogenesis of this syndrome, as well as significantly associated with DM and its complications (Maritim et al., 2003; Silvério et al., 2011).

An important role in the defence against toxic effects of increased systemic OS is played by paraoxonase 1 (PON1), an anti-oxidative enzyme associated with high-density lipoprotein. PON1 has many functions, among which are preventing oxidative modification of low-density lipoprotein (LDL) particles and eliminating cancerogenic lipid-soluble radicals (Précourt et al., 2011). The activity of PON1 varies widely among individuals, which is partly related to polymorphisms in the *PON1* gene. Reduced activities of PON1 have been implicated in the pathogenesis of a number of disorders, including cardiovascular, neurodegenerative and autoimmune diseases, DM, and various types of cancer (Goswami et al., 2009). Several functional polymorphisms have been described in the promoter region of the *PON1* gene (-108C/T, -162A/G, -824G/A, -907G/C) which can affect the expression of the enzyme and its serum concentrations (Leviev and James, 2000). In the coding region of the *PON1* gene, two single-nucleotide polymorphisms (SNPs) (L55M, Q192R) are known to influence the enzyme activities in different substrates (Mackness et al., 1997). Polymorphisms of the *PON1* gene have been studied in patients with a large number of malignancies. However, no studies on *PON1* polymorphisms in PC patients have yet been published.

The aim of this study was to examine the relationship between *PON1* polymorphisms, arylesterase/lactonase activities, and some clinical parameters (cancer stages, presence of DM and malnutrition) in PC patients.

## Material and Methods

### *Subjects*

The study included 73 patients (M/F, 39/34) with PC aged 64.8 ± 9.7 years and 73 control subjects (M/F, 32/41) aged 56.5 ± 10.2 years (mean ± SD). The PC group was recruited from the consecutive patients hospitalized at the 4th Department of Internal Medicine between 2008 and 2011, the control group included outpatients with functional gastrointestinal disorders and members of the medical staff. DM and other disorders of glucose metabolism were diagnosed according to WHO criteria (Alberti and Zimmet, 1998); nutritional status was assessed using the Nutritional Risk Index (NRI) (McMillan, 2009). The diagnosis of PC was confirmed in all patients (based on histological examination of endoscopic ultrasonography-guided aspiration cytology). Cancer staging was performed according to the TNM system developed by the International Union Against Cancer and the American Joint Committee on Cancer (UICC/AJCC 2003) (Fleming et al., 1997). The study protocol was approved by the Joint Ethical Committee of the First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague. Written informed consent was obtained from each study participant.

### *Laboratory analyses*

Blood samples were taken after 12 h of fasting. Routine biochemical and haematological analyses were performed immediately; samples for special analyses were stored at -80 °C until use. Routine biochemical parameters were determined by Modular analyzer (Roche Diagnostic, Mannheim, Germany). Concentrations of conjugated dienes in precipitated LDL (CD-LDL) were determined spectrophotometrically, and expressed in CD-LDL/LDL cholesterol (LDL-C) ratios (Ahotupa et al., 1996). The levels of serum amyloid A (SAA) and oxidized LDL (ox-LDL) were established using sandwich ELISA kits (Invitrogen, Carlsbad, CA; Mercodia, Uppsala, Sweden; respectively).

The PON1 arylesterase activity (PON1-A) was determined by the spectrophotometric kinetic method as described earlier (Kodydková et al., 2009). The lactonase activity of PON1 (PON1-L) was measured according to the modified method described earlier (Draganov et al., 2000) using dihydrocoumarin (final concentration 1 mM) as a substrate. Kinetic rates were converted to enzyme activities using molar absorbance coefficients of the reaction product 3-(2-hydroxy-phenyl) propionate.

*Table 1. Analytical conditions for detection of polymorphisms in the PON1 gene*

| **Polymorphisms in *PON1* gene** | **allelic variants** | **sense primers (5′→ 3′) antisense primers (5′→ 3′)** | **annealing temperature (°C)** | **restriction enzyme** | **fragment size (bp)** |
|---|---|---|---|---|---|
| Leu55Leu/Val/Met (T→G/A) | rs854560 | ccagtttcaagtgaggtgtga tttatttgaaagtgggcatgg | 59.3 | *Hin*1II *Tai*I | T - 251 A - 142+109 G - 143+108 |
| Gln192Arg (A→G) | rs662 | aaggctccatcccacatctt atacttgccatcgggtgaaa | 60.6 | *Alw*I | A - 320 G - 187+133 |
| -108C→T -126G→C -162A→G | rs705379 rs705380 rs705381 | agttggaaggagcaaaatgg aagaagactggtggttcctga | 59.0 | - | 303 |

Legend and abbreviations: Gene polymorphisms and allelic variants were denominated according to the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov), accessed on January 10, 2008.
A – adenine, Arg – arginine, bp – base pair, C – cytosine, G – guanine, Gln – glutamine, Leu – leucine, T – thymine, Val – valine

The isolation of DNA was performed according to standard desalting procedures (Miller et al., 1988). The DNA concentration and its purity were assessed (Nanodrop ND 1000, Thermo Scientific, Wilmington, DE) as prerequisite parameters for subsequent methods. Polymerase chain reaction (PCR) conditions, forward and reverse primers, annealing temperature, restriction endonucleases, and digested fragment sizes are given in Table 1. The restriction products were directly separated by electrophoresis in 3% agarose gel, and visualized in UV light after ethidium bromide staining. Fragment sizes were assessed using the NebCutter V2.0 program (http://tools.neb.com/NEBcutter2). For primer design the Primer3 Input web application was used (http://tools.neb.com/NEBcutter2). Forward and reverse primers were further used for cycle sequencing to verify the results of the PCR restriction fragment length polymorphism (PCR-RFLP) method. The SNPs in the *PON1* promoter were determined using direct sequencing. Sequencing was performed using an automated DNA capillary sequencer (Model SEQ 8000, supplied by Beckman Coulter, Prague, Czech Republic).

Malnutrition was categorized into mild, moderate, and severe form according to the NRI (McMillan, 2009). NRI was calculated according to the formula: NRI = (1.519*albumin + 0.417*current body weight/usual body weight*100) and the classification was: normal nutrition: NRI > 100; mild malnutrition: NRI 97.5–100; moderate malnutrition: NRI 83.0–97.4; severe malnutrition: NRI < 83.0.

### *Statistical analyses*

The data were processed with the STATISTICA® software for Windows. Categorical data were summarized by absolute and relative frequencies, continuous data using means and standard deviations. The distributions of genotypes were tested for Hardy-Weinberg equilibrium (HWE) (with df = N-2). Pearson $\chi^2$-test was employed for testing the differences in the distribution of genotype frequencies in both groups (Yates' correction for small numbers in 2 × 2 tables). Statistical significance was defined as $P < 0.05$.

## Results

Basic clinical and laboratory characteristics of the study groups are shown in Table 2. Because of the statistically significant age difference between the PC patients and control subjects (64.6 ± 10.6 vs. 56.5 ± 10.2, $P < 0.001$) we compared all other parameters after adjustment for this variable. Both groups had a similar sex ratio ($\chi^2 = 1.34$, $P = 0.247$), but differed in smoking prevalence ($\chi^2 = 6.24$, $P = 0.0125$). In the PC group, there were 29 patients with DM and 34 subjects with clinically relevant malnutrition. In the PC group, 12 of patients were diagnosed with stage II, 33 with stage III, and 28 with stage IV. Among the patients with PC, 23 were characterized as normal nutrition, six as mild malnutrition, 25 with moderate, and 10 with severe malnutrition. Both the serum PON1-A and PON1-L activities were decreased (-33.1 %, -46.8 %, respectively; both $P < 0.001$), and the drop in the activities persisted after adjustment to concentrations of serum albumin, glycated haemoglobin, and concentration of conjugated dienes in CD-LDL as covariates (Table 2). Both groups did not differ in fat mass, mid-arm muscle circumference, levels of total cholesterol, triglycerides, and apolipoprotein (apo) B.

Genotype frequencies of *PON1* polymorphisms in PC patients and control subjects are shown in Table 3. Almost all genotype frequencies were compatible with HWE. Only one polymorphism (Q192R; rs662) did not conform to HWE in the control group ($P < 0.002$) and was therefore excluded from the analyses. When comparing both study groups, we failed to prove any significant differences in the frequencies of *PON1* polymorphisms. In the PC group, no association was found between the distribution of the *PON1* polymorphisms and the presence of DM. On the contrary, a significant association of the polymorphisms L55M ($\chi^2 = 6.50$, $P < 0.05$) and -108 C/T ($\chi^2 = 6.25$; $P < 0.05$) was found in PC patients with clinically relevant malnutrition (NRI score < 97.4). No significant association could be established between the *PON1* polymorphisms and cancer stages ($\chi^2 = 0.20$; $P = 0.905$), while PON1-A activities correlated negatively with the increasing stages (ANOVA contrast analysis, $P < 0.05$).

The PC patients with DM (PCDM+) only had, when compared to PC without DM (PCDM-), increased body mass index (BMI) (27.1 ± 5.3 vs. 24.1 ± 4.9 kg/m$^2$; mean ± SD, PCDM+ vs. PCDM-; $P < 0.05$), plasma glucose [8.5 (6.4–10.9) vs. 5.9 (5.1–7.2) mmol/l, $P < 0.05$], and levels of glycated haemoglobin [6.0 (5.1–6.9) vs. 4.2 (3.2–4.5), mmol/mol, $P < 0.01$].

The patients with clinically important (moderate to severe) malnutrition (subgroup PCMN+) had, when compared to PC patients with normal or mild malnutrition (subgroup PCMN-), lower BMI (23.9 ± 5.6 vs. 26.6 ± 4.6 kg/m$^2$; mean ± SD, PCMN+ vs. PCMN-; $P < 0.05$), fat mass [20.9 (14.9–29.9) vs. 25.7 (21.9–28.9) kg; median ($25^{th}$–$75^{th}$ percentile), $P < 0.05$], mid-arm muscle circumference (21.0 ± 2.9 vs. 23.6 ± 3.4 cm; mean ± SD; $P < 0.01$), NRI (86.4 ± 7.9 vs. 105.4 ± 5.2 score; mean ± SD; $P < 0.001$) as well as albumin (35.2 ± 5.0 vs. 44.6 ± 3.1 g/l; mean ± SD; $P < 0.001$), prealbumin, (0.14 ± 0.09 vs. 0.21 ± 0.06 g/l; mean ± SD; $P < 0.001$), homeostatic model assessment of insulin resistance (HOMA-IR) [1.5 (0.9–4.3) vs. 2.5 (1.6–3.6) ratio; median ($25^{th}$–$75^{th}$ percentile), $P < 0.05$], HDL-C (0.74 ± 0.35 vs. 1.10 ± 0.34 mmol/l; mean ± SD; $P < 0.001$), and apo A1 (0.74 ± 0.30 vs. 1.07 ± 0.28 g/l; mean ± SD; $P < 0.001$). The PC patients with clinically relevant malnutrition had, when compared to PC patients without important malnutrition, higher concentrations of C-reactive protein (CRP) [24.1 (10.6–70.1) vs. 8.6 (5.2–27.4) mg/l; median ($25^{th}$–$75^{th}$ percentile), PCMN+ vs. PCMN-; $P < 0.05$], and SAA [114.6 (46.9–140.4) vs. 40.9 (28.0–109.3) µg/ml; median ($25^{th}$–$75^{th}$ percentile); $P < 0.05$]. Both

*Table 2. Clinical and laboratory characteristics of pancreatic cancer and control group*

| Parameter | Pancreatic cancer | Control group |
|---|---|---|
| Number of subjects (M/F) | 73 (39/34) | 73 (32/41) |
| Age at diagnosis (years) | 64.8 ± 9.7 [b]*** | 56.5 ± 10.2[a] |
| Duration of symptoms (months) | 3.0 (1.5 – 6.0) | - |
| Non-smokers and ex-smokers/smokers | 25/48[+] | 40/33 |
| Weight loss (kg/previous 3 months) | 11.4 ± 8.6 [b]*** | -0.1 ± 0.7 |
| Body mass index (kg/m$^2$) | 25.3 ± 5.3 | 26.1 ± 3.7 |
| Nutrition Risk Index (score) (N = 70) | 96.1 ± 11.6 [b]** | 113.8 ± 3.7 |
| Albumin (g/l)<br>Prealbumin | 40.0 ± 6.3 [b]***<br>0.18 ± 0.08 [b] *** | 46.8 ± 2.9<br>0.26 ± 0.04 |
| CA 19-9 (kU/l)<br>CA 72-4 (kU/l) | 209.9 (39.6–2481.5) [b]***<br>3.6 (1.8–17.0) [b]* | 8.7 (6.1–10.7)<br>1.44 (1.02–3.86) |
| Glucose (mmol/l)<br>Insulin (mIU/l)<br>HOMA-IR (ratio)<br>Glycated haemoglobin (mmol/mol) | 7.8 ± 3.5 [b]***<br>7.1 (4.5–11.1)<br>2.2 (1.3–4.1)<br>5.4 ± 2.0 [b]*** | 5.09 ± 0.49<br>7.1 (5.4–9.0)<br>1.64 (1.07–1.92)<br>3.9 ± 0.3 |
| Total cholesterol (mmol/l)<br>LDL-cholesterol (mmol/l)<br>HDL-cholesterol (mmol/l)<br>Triacylglycerols (mmol/l)<br>FFA (mmol/l)<br>Apo A1 (g/l)<br>Apo B (g/l) | 5.6 ± 2.4<br>3.7 ± 2.4 [b]*<br>0.91 ± 0.37 [b]***<br>1.7 (1.4–2.2)<br>0.72 (0.47–0.91) [b]**<br>0.92 ± 0.33 [b]***<br>1.28 ± 0.51 | 5.34 ± 0.97<br>3.30 ± 0.83<br>1.54 ± 0.33<br>1.06 (0.79–1.34)<br>0.40 (0.28–0.67)<br>1.53 ± 0.30<br>1.00 ± 0.25 |
| WBC (× 10$^9$/l) | 8.6 ± 2.9 [b]*** | 6.4 ± 1.7 |
| PLT (× 10$^9$/l) | 273 (203–330) [b]* | 239.1 ± 61.5 |
| Haemoglobin (g/l) | 133.7 ± 17.1 [b]*** | 149.5 ± 10.4 |
| CRP (mg/l) | 16.6 (6.7–55.5) [b]*** | 2.1 (1.0–4.9) |
| SAA (μg/ml) | 92.7 (34.4–130.4) [b] *** | 12.7 (4.6–26.6) |
| CD/LDL (ratio) | 0.017 (0.014–0.020) [b]* | 0.015 ± 0.004 |
| Ox-LDL/LDL-C (U/mmol) | 37.3 ± 12.8*** | 28.6 ± 8.4 |
| PON1 arylesterase activity (U/ml) | 106.1 ± 33.2 [b, c ,d, e]*** | 158.6 ± 40.4 |
| PON1 lactonase activity (U/ml) | 16.0 ± 6.0 [b, c, d, e]*** | 30.1 ± 8.3 |

Legend and abbreviations: [a/] the values are given as mean ± SD or median (25$^{th}$–75$^{th}$ percentile). Statistical analysis: ANCOVA (adjusted to age [b], albumin [c], CD/LDL [d], and glycated haemoglobin [e] as covariates): * P< 0.05; ** P< 0.01, *** P < 0.001; $\chi^2$ test: [+]P < 0.05. apo – apolipoprotein, CA – carbohydrate antigen, CRP – C-reactive protein, FFA – free fatty acids, HDL – high-density lipoprotein, ox-LDL – oxidized LDL, PLT – platelets, WBC – white blood cells. The group of PC patients categorized according to the nutritional status comprises only 70 patients because of no available data concerning weight loss in three PC patients.

*Table 3. Genotype distribution of the PON1 polymorphisms in control subjects and pancreatic cancer patients (further categorized according to the presence of diabetes mellitus and malnutrition)*

| ***PON1*** **SNP (genotype)** | **CON (73)** [a] | **PC (73)** | **PCDM+ (29)** | **PCDM- (44)** | **PCMN+ (34)** [c] | **PCMN- (36)** [c] |
|---|---|---|---|---|---|---|
| L55M (TT/TA/AA) | 28/37/8 [b] | 24/39/10 | 10/13/6 | 14/26/4 | 14/19/1 [1] | 9/19/8 |
| Q192A (AA/AG/GG) | 40/20/13 [d] | 40/28/5 | 15/13/1 | 25/15/4 | 18/12/4 | 20/15/1 |
| -108C/T (CC/CT/TT) | 22/35/16 | 19/38/16 | 10/11/8 | 9/27/8 | 10/21/3 [2] | 8/16/12 |
| -126G/C (GG/GC/CC) | 61/12/0 | 62/11/0 | 25/4/0 | 37/7/0 | 28/6/0 | 31/5/0 |
| -162 (GG/GA/AA) | 39/31/3 | 36/35/2 | 16/12/1 | 20/23/1 | 14/18/2 | 20/16/0 |

Legend and abbreviations: CON – control group, MN – malnutrition; PCMN+ – PC with clinically relevant MN [subjects with moderate malnutrition (NRI 83.0–97.4) and severe malnutrition (NRI < 83)]; PCMN- – PC without clinically relevant malnutrition [subjects with normal nutrition (NRI > 100) and mild malnutrition NRI (97.5–100.0)]

[a/] values in parentheses represent numbers of subjects in individual studied groups

[b/] values represent numbers of cases with specific genotypes in individual studied groups

[c/] subgroup of PC patients categorized according to the nutritional status comprising only 70 patients because of no available data concerning weight loss in three PC patients

[d/] the genotype distribution of *PON1* Q192R did not conform to HWE (P < 0.002)

Pearson $\chi^2$ tests: [1/] – genotype $\chi^2 = 6.5$ (P = 0.040); [2/] – genotype $\chi^2 = 6.25$ (P = 0.044).

groups did not differ in sex ratio, age of diagnosis, prevalence of smoking, and DM, as well as in frequencies of tumour stages. We found no significant differences in the levels of glucose, insulin, glycated haemoglobin, and concentration of CD/LDL and ox-LDL. The subgroup of PC patients with malnutrition had decreased activity of PON1-A (97.4 ± 35.7 vs. 115.3 ± 29.2 U/ml; mean ± SD, PCMN+ vs. PCMN-; $P < 0.05$) and PON1-L (13.9 ± 557 vs. 18.7 ± 6.0 U/ml; $P < 0.01$).

In the PC group the PON1-L activity correlated with mid-arm muscle circumference ($r = 0.328$, $P < 0.05$), NRI ($r = 0.409$, P ($P < 0.01$), albumin ($r = 0.391$, $P < 0.01$), and apo A1 ($r = 0.302$, $P < 0.05$). In the PC group, PON1-A activities correlated with NRI ($r = 0.351$, $P < 0.01$), albumin ($r = 0.352$, $P < 0.01$), prealbumin ($r = 0.374$, $P < 0.01$), and apo A ($r = 0.272$, $P < 0.05$). We did not find statistically significant correlations of PON1-A (PON1-L, respectively) with BMI, fat mass, tumour markers, as well as with markers of oxidative stress and inflammation in PC patients. There were no statistically significant correlations of the above-mentioned parameters with PON1-A/PON1-L activities in the control subjects.

## Discussion

Paraoxonase-1 (*PON1*) gene polymorphisms and their influence on paraoxonase 1 activities and/or mass have been studied in various types of cancer. Wide variations in PON1 activities (up to 13-fold) were described even among individuals with the same genotype (Deakin and James, 2004). At present, arylesterase activity is considered to be one of the PON-1 functions and the most reliable indicator of the antioxidant/anti-inflammatory efficacy. The lactonase activity of PON1, which is more sensitive to oxidation, was shown to protect against the progression of atherosclerosis (James, 2006) and to play a role in the immunologic defence against bacterial infections. The *PON1* gene L55M polymorphism is associated with lower serum activities and concentrations of the enzyme. L55 isoforms are more stable and resistant to proteolysis; this phenomenon partly explains their association with higher PON1 levels. However, one study found that LL homozygotes had significantly impaired glucose disposal, compared with the LM+MM genotypes (Deakin et al., 2002). The Q192R polymorphism of the *PON1* gene can also modify the affinities and catalytic activities of the enzyme PON1; the QQ192 genotype was associated with elevated markers of systemic OS (Bhattacharyya et al., 2008).

Several studies have shown that the *PON1* polymorphism L55M contributes to the development of various cancers, especially those developing in the breast, lungs, prostate and ovaries, while the polymorphism Q192R can decrease the risk of breast and ovarian cancers (Lee et al., 2005; Hussein et al., 2011; Fang et al., 2012).

A number of studies have shown decreased arylesterase and/or paraoxonase activities of PON1 in different malignancies, e.g. gastroesophageal, laryngeal, ovarian, endometrial, lung, breast, prostate, and cervix cancer (Goswami et al., 2009; Karaman et al., 2010; Samra et al., 2011). In published studies, PON1 activities correspond to the severity of oxidative stress/inflammation, and some of them showed a relation of PON1 activities to the clinical status as well as to biochemical and haematological findings (for review see Goswami et al., 2009; Arioz et al., 2009).

To our knowledge, no studies on *PON1* polymorphisms in patients with PC have yet been published. In one study, paraoxonase activity was determined in a group of 20 patients with pancreatic cancer; it was found to be significantly decreased in comparison with age- and sex-matched healthy controls (Akcay et al., 2003).

PON1 levels and activities can be modified by genetic polymorphisms and acquired factors such as diet, lifestyle and disease. It is supposed that 25–40 % of variance in PON1 levels (activities, respectively) is dependent on genetic polymorphisms and the rest of variation is attributable to acquired factors (Soran et al., 2009). Under conditions of systemic inflammation and/or OS, several mechanisms are implicated in the fall of PON1 activities. Among the most important are displacement of PON1 from its linkage to apo A1 in HDL by SAA, down-regulation of liver PON1 by lipopolysaccharides and cytokines (TNFα, IL-1) via IL-6 (Deakin and James, 2004), and inhibition of PON1 activity by oxidized phospholipids (Tavori et al., 2011). All these mechanisms can be related to cancer-related decrease in PON1 activities. The fall in activities of PON1-A and PON1-L in the PC group persisted after adjustment to concentrations of visceral proteins (albumin, prealbumin) and of CD/LDL. Therefore, the mechanistic link of decreased PON1 to decreased liver proteosynthesis and systemic OS seems not to be plausible.

We failed to prove any difference between the study groups in the distribution of five *PON1* polymorphisms, which is in disagreement with the published results of research on other types of cancer. If our finding is confirmed in other studies, it could be interpreted as evidence that *PON1* gene polymorphisms do not play any important role in the pathogenesis of pancreatic cancer, and the reduced PON1 activities can be considered a consequence of the disease itself.

The PC patients displayed significantly increased markers of lipid peroxidation – concentrations of CD/LDL, and ox-LDL, respectively. In the group of PC patients, the activities of PON1-A negatively correlated with the tumour stages; the more severe was the disease, the lower the arylesterase activities.

Activities of PON1 can be considerably reduced in patients with poorly controlled DM, due to glycation of the PON1 protein (Hendrick et al., 2000). This mechanism probably did not operate in our study, because decreased PON1 activities persisted after adjustment to the glycated haemoglobin level. In cancer-free type 1 and type 2 DM, significant associations were found between PON1 activities and *PON1* polymorphisms L55M and Q192R (Flekač et al., 2008). The CODAM Study

surprisingly revealed that the RR192 genotype prevailed in subjects with newly diagnosed DM (Van den Berg et al., 2008). When comparing the subgroups of PC patients with and without DM in our study, we failed to find any difference in the distribution of *PON1* gene polymorphisms. The activities of PON1-A and PON1-L in PC patients did not significantly differ in relation to the presence of DM.

Almost all patients with PC were losing weight, and half of them presented with clinically symptomatic malnutrition at the time of diagnosis. Subsequently, CCS may evolve, characterized by extensive muscle wasting. Chronic systemic inflammation, partly caused by cytokines, as well as an increased OS contributes to sarcopoenia and cachexia. RONS are supposed to be the pivotal players in muscle catabolism by stimulating the ubiquitin-proteasome-dependent system, which increases proteolysis and apoptosis of myofibres (Lenk et al., 2010). Lowered PON1 activities were found in marantic children as compared to control subjects (Ece et al., 2007).

In our study, a significantly higher prevalence of the *PON1* gene polymorphisms L55M and -108C/T was found in PC patients with clinically relevant malnutrition; these patients also had significantly lower activities of PON1-A and PON1-L than those with normal or slightly reduced values of NRI. The PON1 activities correlated positively with the nutritional status and concentrations of visceral proteins. However, the physiological mechanisms by which these polymorphisms influence the development of malnutrition remain to be determined.

## References


Ahotupa, M., Ruutu, M., Mäntylä, E. (1996) Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. *Clin. Biochem.* **29**, 139-144.

Akcay, M. N., Polat, M. F., Yilmaz, I., Akcay, G. (2003) Serum paraoxonase levels in pancreatic cancer. *Hepato-Gastroenterology* **50**, **Suppl II,** ccxxv-ccxxvii.

Alberti, K. G., Zimmet, P. Z. (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabet. Med.* **15**, 539-553.

Arioz, D. T., Camuzcuoglu, H., Toy, H., Kurt, S., Celik, H., Erel, O. (2009) Assessment of serum paraoxonase and arylesterase activity in patients with endometrial cancer. *Eur. J. Gynaecol. Oncol.* **30**, 679-682.

Bhattacharyya, T., Nicholls, S. J., Topol, E. J., Zhang, R., Yang, X., Schmidt, D., Fu, X., Shao, M., Brennan, D. M., Ellis, S. G., Brennan, M. L., Allayee, H., Lusis, A. J., Hazen, S. L. (2008) Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA* **299**, 1265-1276.

Deakin, S., Leviev, I., Nicaud, V., Gustini, P., Grison, E., Pizzolo, F., Friso, S., Trabetti, E., Pignatti, P.F., Corrocher, R., Olivieri, O., Girelli, D. (2002) Paraoxonase-1 L55M polymorphism is associated with abnormal oral glucose tolerance test and differentiates high risk coronary disease families. *J. Clin. Endocrin. Metab.* **87**, 1268-1273.

Deakin, S. P., James, R. W. (2004) Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. *Clin. Sci. (Lond.)* **107**, 435-447.

Draganov, D. I., Stetson, P. L., Watson, C. E., Billecke, S. S., La Du, B. N. (2000) Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. *J. Biol. Chem.* **275**, 33435-33442.

Ece, A., Gürkan, F., Celik, F., Boşnak, M., Yel, S., Balik, H., Erel, O. (2007) Paraoxonase, total antioxidant activity and peroxide levels in marasmic children: relationships with leptin. *Clin. Biochem.* **40**, 634-639.

Fang, D. H., Fan, C. H., Ji, Q., Qi, B. X., Li, J, Wang, L. (2012) Different effects of paraoxonase 1 (PON1) polymorphisms on cancer risk: evidence from 25 published studies. *Mol. Biol. Rep.* **39**, 6801-6809.

Ferlay, J., Parkin, D. M., Steliarova-Foucher, E. (2010) Estimates of cancer incidence and mortality in Europe in 2008. *Eur. J. Cancer.* **46**, 745-781.

Flekač, M., Škrha, J., Zídková, K., Lacinová, Z., Hilgertová, J. (2008) Paraoxonase 1 gene polymorphisms and enzyme activities in diabetes mellitus. *Physiol. Res.* **57**, 717-726.

Fleming, I. D., Cooper, J. S., Henson, D. E., Hutter, R. V. P., Kennedy, B. J., Murphy, G. P., O'Sullivan, B., Sobin, L. H., Yarbro, J. W. (eds.) (1997) *AJCC. Cancer Staging Manual.* 5th ed. Philadelphia-New York, Lippincott-Raven.

Goswami, B., Tayal, D., Gupta, N., Mallika, V. (2009) Paraoxonase: a multifaceted biomolecule. *Clin. Chim. Acta* **410**, 1-12.

Hassan, M. M., Bondy, M. L., Wolff, R. A., Abbruzzese, J. L., Vauthey, J. N., Postere, P. W., Evans, D. B., Khan, R., Chou, T. H., Lenzi, R., Jiao, L., Li, D. (2007) Risk factors for pancreatic cancer: case-control study. *Am. J. Gastroenterol.* **102**, 2696-2707.

Hendrick, C. C., Thorpe, S. R., Fu, M. X., Harper, C. M., Yoo, J., Kim, S. M., Wong, H., Peters, A. L. (2000) Glycation impairs high-density lipoprotein function. *Diabetologia* **43**, 312-320.

Hussein, Y. M., Gharib, A. F., Etewa, R. L., ElSawy, W. H. (2011) Association of L55M a Q192 polymorphisms in paraoxonase 1 (PON1) gene with breast cancer risk and their clinical significance. *Mol. Cell. Biochem.* **351**, 117-123.

Institute of Health Information and Statistics of the Czech Republic (IHIS) and Council of the National Oncological Registry of the Czech Republic (NOR) (2011) *Cancer Incidence 2008.* ÚZIS and NOR ČR. Prague.

James, R. W. (2006) A long and winding road: defining the biological role and clinical importance of paraoxonase. *Clin. Chem. Lab. Med.* **44**, 1052-1059.

Karaman, E., Uzun, H., Papila, I., Balci, H., Ozdilek, A., Genc, H., Yanardag, H., Papila, C. (2010) Serum paraoxonase activity and oxidative DNA damage in patients with laryngeal squamous cell carcinoma. *J. Craniofac. Surg.* **21**, 1745-1749.

Kodydková, J., Vávrová, L., Zeman, M., Jirák, R., Macášek, J., Staňková, B., Tvrzická, E., Žák, A. (2009) Antioxidative enzymes and increased oxidative stress in depressive women. *Clin. Biochem.* **42**, 1368-1374.

Krechler, T., Zeman, M., Vecka, M., Macášek, J., Jáchymová, M., Zima, T., Žák, A. (2011) Leptin and adiponectin in pancreatic cancer: connection with diabetes mellitus. *Neoplasma* **58**, 58-64.

Lee, C. H., Lee, K. Y., Choe, K. H., Hong, Y. C., Kim, Y. D., Kang, J. W., Kim, H. (2005) Effects of oxidative DNA damage induced by polycyclic aromatic hydrocarbons and genetic polymorphism of paraoxonase-1 (PON-1) gene on lung cancer. *J. Prev. Med. Publ. Health* **38**, 345-350.

Lenk, K., Schuler, G., Adams, V. (2010) Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. *J. Chachexia Sarcopenia Muscle* **1**, 9-21.

Leviev, I., James, R. W. (2000) Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arterioscler. Thromb. Vasc. Biol.* **20**, 516-521.

Mackness, B., Mackness, M. I., Arron, S., Turkie, W., Durrington, P. N. (1997) Effect of molecular polymorphisms of human paraoxonase (PON1) on the rate of hydrolysis of paraoxon. *Br. J. Pharmacol.* **122**, 265-268.

Maritim, A. C., Sanders, R. A., Watkins, J. B. (2003) Diabetes, oxidative stress, and antioxidants: a review. *J. Biochem. Mol. Toxicol.* **17**, 24-38.

McMillan, D. C. (2009) Systemic inflammation, nutritional status and survival in patients with cancer. *Curr. Opin. Clin. Nutr. Metab. Care* **12**, 223-226.

Miller, S. A., Dykes, D. D., Polesky, H. F. (1988) A simple salting out extraction DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.

Pannala, R., Leirness, J. B., Bamlet, W. R., Basu, A., Petersen, G. M., Chari, S. T. (2008) Prevalence and clinical profile of pancreatic cancer-associated diabetes mellitus. *Gastroenterology* **134**, 981-987.

Précourt, L. P., Amre, D., Denis, M. C., Lavore, J. C., Delvin, E., Seidman, E., Levy, E. (2011) The three-gene paraoxonase family: physiologic roles, action and regulation. *Atherosclerosis* **214**, 20-36.

Samra, Z. Q., Pervaiz, S., Shaheen, S., Dar, N., Athar, M. A. (2011) Determination of oxygen derived free radicals producer (xanthine oxidase) and scavenger (paraoxonase 1) enzymes and lipid parameters in different cancer patients. *Clin. Lab.* **57**, 741-747.

Silvério, R., Lavino, A., Rossi Fanelli, F., Seelaender, M. (2011) L-carnitine and cancer cachexia: clinical and experimental aspects. *J. Cachexia Sarcopenia Muscle* **2**, 37-44.

Soran, H., Younis, N. N., Charlton-Menys, V., Durrington, P. (2009) Variation in paraoxonase-1 activity and atherosclerosis. *Curr. Opin. Lipidol.* **20**, 265-274.

Tavori, H., Aviram, M., Khatib, S., Musa, R., Mannheim, D., Karmeli, R., Vaya, J. (2011) Human carotid lesion linoleic acid hydroperoxide inhibits paraoxonase 1 (PON1) activity via reaction with PON1 free sulfhydryl cysteine 284. *Free Radic. Biol. Med.* **50**, 148-156.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., Mazur, M. (2006) Free radicals, metals, and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* **160**, 1-40.

Van den Berg, S. W., Jansen, E. H., Kruijshoop, M., Beekhof, P. K., Blaak, E., van der Kallen, C. J., van Greevenbroek, M. M., Feskens, E. J. (2008) Paraoxonase 1 phenotype distribution and activity differ in subjects with newly diagnosed Type 2 diabetes (the CODAM Study). *Diabet. Med.* **25**, 186-193.