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Analysis of inflammatory markers and hormones in old cancer patients: A descriptive study

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ABSTRACT

Advanced cancers are associated with a chronic inflammation, especially high interleukin-6 (IL-6) and with various levels of adipokines (leptin and adiponectin), while ghrelin counteracts the anorexigenic effect of leptin in cancer-induced anorexia-cachexia syndrome.

We aimed to understand how IL-6, adipokines and ghrelin plasma levels could be influenced by cancer on the one hand, and by age, frailty, and nutritional status in old cancer patients on the other hand.

Ninety-nine patients aged 79[76–83] years old were included. Sixty-six percent had advanced stages of cancer, and 34% had cachexia. Fifty percent were at risk of malnutrition, and 10% had overt malnutrition. None of the variables studied was significantly correlated with the advanced stage, or cachexia. In multiple regression, the only parameter significantly and positively associated with age was adiponectin ($p = 0.008$). Despite a high prevalence of frailty in our study, we did not find any independent association of frailty (assessed by G8) with IL-6, leptin, adiponectin, or ghrelin in multivariate analysis. We observed that a low albumin level was independently associated with a higher level of IL-6 ($p < 0.0001$), but not with the MNA score. However, leptin showed a positive correlation with BMI ($p < 0.0001$), confirming the persistence of a relationship between leptin and adiposity, even in older cancer patients. Finally, high IL-6 level was associated with a higher mortality rate ($p = 0.027$).

In conclusion, IL-6, leptin, adiponectin, and ghrelin are not associated with advanced stages of cancer or cancer-induced cachexia in older subjects with cancer, but they are significantly correlated with anthropometric factors and body composition.

1. Introduction

As population ages, the prevalence of cancer is growing, with patients older than 65 accounting now for two thirds of all cancers (Quaglia et al., 2009; Arnold et al., 2015).

In a number of previous studies, chronic inflammation was not only associated with typical inflammatory diseases but also with cancer (Volpato et al., 2001). Regardless of the type of tumor, a paraneoplastic cytokine pattern is associated with advanced stages, reflecting an immune response against tumor cells (Lippitz, 2013; Mantovani et al., 2008).

Because of its position in the hierarchy of the cytokine cascade, Interleukin 6 (IL-6) received special attention in the interaction between cancer and inflammation. Elevated serum concentrations of IL-6 are associated with tumor size, tumor stage, or presence of metastases, implicating that high IL-6 serum levels would reflect a late cancer stage, regardless of the tumor type (Lippitz, 2013).

Adipokines, circulating hormones produced by adipocytes and directly related to fat mass, are expected to contribute to carcinogenesis and tumor progression by enhancing inflammatory signaling, angiogenesis, and cellular proliferation. Among them, leptin, has been shown to stimulate cancer stem cells. In turn, cancer cells stimulate lipolysis, leading to the

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differentiation of adipocytes into fibroblast-like cells, providing additional inflammation, and to the release of leptin in the circulation (Lee et al., 2014). Leptin plays also a role in the development of cachexia in cancer, acting as an anorexigenic hormone, by suppressing appetite. Leptin levels are also significantly lower in patients with advanced cancer cachexia, signaling a starvation status (Mantovani et al., 2000).

Adiponectin (APN) modulates glucose regulation and fatty acid oxidation (Diez and Iglesias, 2003). The role of APN in cancer is controversial; while many studies find low APN serum levels in digestive, hematological, and hormone-dependent cancers, other studies suggest no difference in lung cancer or even an increase with cancer progression in pancreatic cancer (Hebbard and Ranscht, 2014). High APN levels may represent a general marker of chronic inflammation, as APN has a pro-inflammatory effect by enhancing the production of TNF-alpha and IL-6 and by increasing the synthesis of leptin (Hebbard and Ranscht, 2014; Dalamaga et al., 2012; Izadi et al., 2012).

Ghrelin, a peptide secreted by the stomach, increases appetite and reduces energy expenditure, exerting an antagonistic action to leptin (Cummings et al., 2001). Anorexia induced by cancer might largely be explained by the stimulating effect of the pro-inflammatory cytokines on leptin not counteracted by ghrelin secretion. Supra-physiological doses of ghrelin or selective agonists of the ghrelin receptor have therefore been introduced as a potential treatment for patients suffering from cancer anorexia-cachexia syndrome, with promising results (Khatib et al., 2018; Katakami et al., 2017).

IL-6, leptin, APN and ghrelin are modulated by age, frailty profile, body composition (including muscle and fat mass), and nutritional status, making the interpretation of their circulating levels difficult in older adults with cancer. IL-6 levels rise with age, in a context called “inflammaging”, defined as an increase in the body's proinflammatory status with advancing age, and have been associated with disability and mortality (Cohen et al., 1997; Franceschi and Campisi, 2014). The inflated production of cytokines by monocytes (IL-6 and IL-1ra) is also observed in many, but not all, frail older persons (Roubenoff et al., 1998). Centenarians, representing healthy ageing and longevity, maintain favorable adipokine profiles, particularly high levels of circulating APN (Nagasawa et al., 2018). Leptin and APN have been recognized recently as potential biomarkers of ageing and of frailty (Ng et al., 2018; Cardoso et al., 2018); although they do not seem to change with age, leptin and APN serum levels are significantly lower in old frail patients (Cardoso et al., 2018; Ma et al., 2018) and are positively correlated with muscle mass and function, through the IGF-1 signaling and energy metabolism pathway (Ma et al., 2018). Studies on ghrelin are more controversial: in humans, low ghrelin levels are associated with weight loss, and high levels with cognitive disorders, insulin-resistance, and cardiac diseases, but no correlation was found with sarcopenia, despite a positive action of ghrelin in preventing decline in muscle strength and endurance (Cardoso et al., 2018; Serra-Prat et al., 2010).

The present study aimed to understand how IL-6, adipokines and ghrelin could be influenced by cancer on the one hand, and by age, frailty, and nutritional status on the other hand.

2. Material and methods

2.1. Study design and participants

This study was performed in two university hospitals in Brussels, Belgium (Centre Universitaire de Bruxelles, Hôpital Erasme, and Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel), between June 2009 and December 2012.

Ninety-nine patients aged ≥70 years, newly diagnosed with solid cancer were included. Patients unable to provide informed consent, to perform anthropometric measurements, or having any inflammatory comorbidity were excluded.

The local Ethics Committees approved the study. Written informed consent was obtained from all subjects (Protocol P2009/130).

2.2. Data collection

Age, cancer characteristics (type, TNM staging (O'Sullivan et al., 2017)), and one-year mortality were registered.

All participants underwent a comprehensive geriatric assessment, including screening of frailty (G8) (Bellera et al., 2012), comorbidity (Cumulative Ilness Rating Scale-Geriatric, CIRS-G) (Wedding et al., 2007), activities of daily living (Katz) (Katz et al., 1963), cognition (Mini Mental State Examination, MMSE) (Folstein et al., 1975), and depression (Geriatric Depression Scale-4 items, GDS-4) (Yesavage and Sheikh, 1986). Nutritional status was assessed by the Mini Nutritional Assessment (MNA). Risk of malnutrition was defined by an MNA score between 17 and 23.5 points, overt malnutrition by an MNA score under 17 points (Vellas et al., 1999). Skeletal mass index (SMI) was assessed by BioImpedance Analysis, BIA (Bodystat ltd, UK). According to Janssen et al., muscle mass index is considered as low (sarcopenia) when < 8.5 kg/m^2 for males and < 5.75 kg/m^2 for females (Janssen et al., 2000). We estimated whole-body skeletal muscle mass (SMM) using the following formula: SMM = [(height^2 / BIA resistance × 0,401) + (gender × 3.825) + (age × −0,071)] + 5102 with height in cm, BIA resistance in Ohms, gender = 1 for male and 0 for female (age in years). Skeletal muscle mass index (SMI) was obtained by dividing SMM by height squared (m^2). Muscle strength was assessed by Martin' vigorimeter (Elmed, USA) (Bautmans and Mets, 2005). Thigh and calf circumferences corrected for height, and thigh and calf skinfold were also collected. Cachexia was defined according to Fearon et al. (associating a weight loss > 5%, or a weight loss > 2% in individuals and a body-mass index [BMI] < 20 kg/m^2 or a low SMI as described above) (Fearon et al., 2011).

2.3. Biological parameters

Fasting blood was sampled from EDTA-anticoagulated blood, and immediately stored at −80 °C until further treatment. Plasma levels of 25 cyto-/chemokines were measured simultaneously by a multiplex technique (Multiplex Bead Immunoassay, Biosource International, Nijvel, Belgium), according to the manufacturer's instructions and included: IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-17, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL11/Eotaxin, CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10, TNFα, IFN-α, IFN-γ and GM-CSF (for full names and sensitivities see Table 1). Plasma concentration of total ghrelin was assessed by Enzyme Linked Immunosorbent Assay (Sandwich ELISA) of Millipore (No cat. EZGRT-89K - USA). Plasma concentration of leptin, APN, insulin-like growth factor (IGF-1) and insulin were estimated using ELISA, following instructions provided by manufacturer (Invitrogen, USA).

Hemoglobin (Hb), albumin, and C-Reactive protein (CRP) were determined by standard laboratory methods.

2.4. Statistical analysis

Analyses were conducted using Stata software version 12 (Stata Corporation, USA).

Data were presented as means ± SD, or medians with interquartile ranges, or number and percentage. Values for cytokines, leptin, adiponectin, and ghrelin were log-transformed except when the number of available cytokines results was inferior to 75%, we used then the number of patients above the sensitivity cutoff (Talbert et al., 2018). Pearson's correlation coefficients were calculated to assess the relationship between levels of IL-6, adipokines, and ghrelin with cancer, geriatric, nutritional and biochemical characteristics. Forward stepwise multiple regression was then performed with clinically and statistically significant factors ($p < 0.05$) in order to determine which factors were independently predictive of IL-6, leptin, adiponectin and ghrelin levels. Two-sided p-values < 0.05 were considered as statistically significant.

Table 1
Fasting plasma levels of cytokines.

Cytokines (pg/mL)	Sensitivity	Mean ± SD - n(%)
IL-1 beta	< 15	LO
Log IL-1RA	< 1.3	5.4 ± 1.2
IL-2	< 15	LO
Log IL-2R	< 1.6	5.8 ± 0.6
IL-4	< 5	LO
IL-5 > 5	< 5	31(33)
Log IL-6	< 0.7	2.2 ± 0.9
IL-7 > 25	< 25	45(48)
IL-10	< 3	LO
Log IL-12	< 0.8	5.4 ± 0.6
IL-13 > 6	< 6	29(31)
Log IL-15	< 1.4	4.6 ± 0.8
IL-17	< 25	LO
TNF-α	< 20	LO
Log IFN-α	< 1.4	4.3 ± 0.7
IFN-γ	< 2	LO
GM-CSF	< 5	LO
Log MCP-1/CCL-2	< 0.9	5.0 ± 0.6
MIP1a/CCL-3 > 15	< 15	45(48)
Log MIP-1b/CCL-4	< 1	3.9 ± 0.9
RANTES/CCL-5	< 20	HI
Log Eotaxin/CCL-11	< 0.7	3.9 ± 0.6
IL-8/CXCL-8	< 3	LO
Log MIG/CXCL-9	< 1.3	5.3 ± 0.8
Log IP-10/CXCL-10	< 0.7	3.7 ± 0.5

IL = interleukin. IL-1RA = IL-1 receptor antagonist. IL-2R = IL-2 receptor. GM-CSF = granulocyte macrophage colony-stimulating factor. TNF-alpha = tumor necrosis factor alpha. IFN = interferon. For chemokines old and new names are indicated, separated by a forward slash. MCP = monocyte chemoattractant protein. MIP = macrophage inflammatory protein. RANTES = Regulated on Activation, Normal T Cell Expressed and Secreted. MIG = monokine induced by interferon gamma. IP-10 = interferon γ-inducible protein 10. LO = 66% below lower detection limit. HI = > 66% above upper detection limit. Results are expressed in means ± standard deviation and numbers (%) above the sensitivity cutoff when the number of samples was inferior to 75%.

3. Results

The general characteristics of the 99 participants are provided in Table 2.

Two third had advanced stages of cancer, and one third had cachexia. Muscle mass, handgrip strength, thigh and calf circumferences were significantly higher in men, although fat mass characteristics (skinfolds) and BMI were not different in men and women. Fifty percent were at risk of malnutrition, but only 10% had overt malnutrition.

Results for the determination of biological parameters are given in Tables 1 and 3. Insulin, leptin, and ghrelin were in the normal ranges, while APN was elevated.

IL-1ß, IL-2, IL-4, IL-10, IL-17, TNF-α, IFN-γ, GM-CSF levels were below the detection limit (LO) in > 66% participants, while serum levels of RANTES were above the upper detection limit (HI) in > 66% participants. Analyses thus concerned 15 cytokines/chemokines. Levels were similar in men and women (data not shown).

The relationships (univariate analysis) of insulin, leptin, adiponectin, and ghrelin with other parameters are given in Tables 4 and 5.

Table 6 summarizes predictive values from multiple regression for IL-6 (albumin, MCP-1, and one year-mortality: $F(5,79) = 10.30$, $p < 0.0001$, $R^2 = 0.39$); leptin (BMI, and IL-6: - $F(2,89) = 52$, $p < 0.0001$, $R^2 = 0.54$); adiponectin (age, and SMI; $F(2,84) = 7.75$, $p = 0.0008$, $R^2 = 0.16$); and ghrelin (thigh circumference, SMI, and IP-10: $F(3,78) = 7.21$, $p = 0.0002$, $R^2 = 0.22$). All variables adjusted added significantly to the predictions, and all p values were < 0.05. Of note is that we excluded CRP from multiple regression of IL-6, and BMI from multiple regression of adiponectin, as they showed multicollinearity.

Table 2
Cancer-related, nutritional, and geriatric characteristics.

	Total	Women	Males
Age (years)	79[76–83]	80[77–84]	78[76–82]
Gender (% women)	58%	*N* = 57	*N* = 42
1-year mortality	21(22)	11(20)	10(24)
Cancer types			
Lung	27(28)	11(20)	16(38)
Colon	19(19.5)	11(20)	8(19)
Breast	19(19.5)	19(34)	0
Upper digestive tract	8(8)	4(7)	4(10)
Kidney	5(5)	2(4)	3(7)
Pancreas	5(5)	5(9)	0
Urinary tract	5(5)	1(2)	4(10)
Prostate	3(3)	0	3(7)
Ovary	3(3)	3(5)	0
Skin	1(1)	0	1(2)
Others	3(3)	0	3(7)
Tumor staging (TNM)			
1	23(24)	13(24)	10(25)
2	9(10)	4(7)	5(12)
3a	22(23)	16(29)	6(15)
3b	4(4)	1(2)	3(8)
4	37(39)	21(38)	16(40)
Nutritional assessment			
MNA score	22.5[19.5–25]	21.5[19–24]*	23[21–26]
Risk of malnutrition (17 < MNA < 23.5)	47(50)	30(57)	17(41)
Overt malnutrition (MNA < 17)	10(10)	7(13)	3(7)
BMI (kg/m^2)	26 ± 5	26 ± 5	26 ± 4
Muscle assessment			
Handgrip strength (kgF)	46 ± 16	40 ± 14°	55 ± 15
Thigh circumference (cm)	43[38–49]	42[36–49]*	45[41–49]
Calf circumference (cm)	34[31–36]	32[29–36]*	35[31–36]
Skeletal muscle index (kg/m^2)	6.5[4.6–8.9]	3.3[1.3–8.7]°	9[8.5–9.7]
Fat assessment			
Thigh skinfold (mm)	9[2–15]	5[2–16]	11[3–15]
Calf skinfold (mm)	5[1–9]	3[1–9]	7[1 – 10]
Cachexia assessment			
Cachexia Fearon	33(34)	23(41)	10(24)
Geriatric assessment			
G8 frailty score	11 ± 3	11 ± 3**	12 ± 3
G8 score ≤ 14	86(89)	55(98)	31(76)
Katz score > 6	46(46)	31(54)*	15(36)
MMSE score	27[24–29]	26[21–28]°	28[26–29]
GDS-4 items score > 1	16(17)	13(24)*	3(7)
CIRS-G total score	16 ± 5	16 ± 5	16 ± 5
QOL29 overall health	5 ± 1	5 ± 2	5 ± 1
QOL30 quality of life	5 ± 1	5 ± 1	5 ± 1

MNA: Mini Nutritional Assessment – BMI: Body Mass Index – MMSE: Mini Mental State examination – GDS-4: Geriatric Depression Scale 4 items – CIRS-G: Cumulative Ilness Rating Scale – Geriatric – QOL: Quality of life questions 29 and 30 from the EORTC questionnaire. Results are expressed in means ± SD, medians [IQR] and numbers (%). Difference between females and males.
* $p < 0.05$.
** $p < 0.005$.
° $p < 0.001$.

4. Discussion

We collected data on an older (≥70 years) population with various, newly diagnosed cancer from various types. At the time of diagnosis, many participants had already either an advanced stage of cancer and/or cachexia, both related to a worse prognosis. IL-6 levels were elevated, as were many other cytokines and chemokines, indicating that patients had an inflammatory profile. Leptin and ghrelin were within normal ranges (respectively 4.1–25.0 ng/mL for women and 1.2–9.5 ng/mL for men for leptin (adjusted for a BMI between 18 and 25 kg/m^2); and 520–700 pg/mL for ghrelin); while APN levels were high (normal ranges: 5–10 ng/mL).

None of the variables studied was significantly correlated with an advanced stage, or cachexia, indicating that these markers are not primarily associated with cancer characteristics. APN level tended to be

Table 3
Biochemical characteristics.

	Total	Women	Males
Biological assessment			
Hemoglobin (g/dL)	12.5 ± 2	12 ± 1.9°	13.3 ± 1.5
Albumin (g/L)	40[35–44]	39[34–43]	41[37–44]
CRP (mg/L)	9[3–26]	7[3–26]	10[2−23]
Hormones and adipokines (fasting)			
IGF-1 (ng/mL)	106[76–147]	90[61–130]**	120[94–152]
Insulin (μIU/mL	8.6[5.1–16.4]	7.5[3.6–12]*	9.9[6–24]
Leptin (pg/mL)	8.7[4.0–18.6]	12.8[6.3–25.1]**	5.5[3.0–10.4]
Adiponectin(μg/mL)	29[19–43]	35[23–45]*	22[18–34]
Ghrelin (pg/mL)	439[283–797]	541[345–902]*	321[219–542]

CRP: C-Reactive Protein – IGF-1: Insulin-like Growth Factor. Results are expressed in means ± SD, medians [IQR] and numbers (%). Difference between females and males.
* $p < 0.05$.
** $p < 0.005$.
° $p < 0.001$.

Table 4
Univariate analysis of IL-6, leptin, adiponectin and ghrelin (general characteristics).

	IL-6	Leptin	Adiponectin	Ghrelin
Demographic and oncological data				
Age	−0.11	0.05	0.32**	0.18
TNM advanced stages (3,4)	0.07	0.13	−0.18	0.05
Mortality 1 year	−0.33**	0.26*	−0.12	0.08
Nutritional assessment				
MNA	−0.27*	0.16	−0.04	−0.13
BMI	−0.15	0.70°	−0.14	−0.26*
Muscle assessment				
Handgrip strength	−0.09	−0.09	−0.16	−0.17
Thigh circumference	−0.19	0.38°	−0.14	−0.33**
Calf circumference	−0.23*	0.58°	−0.19	−0.30**
Skeletal muscle index	0.27*	−0.14	−0.28**	−0.31**
Fat assessment				
Thigh skinfold	0.04	−0.02	0.03	0.16
Calf skinfold	0.09	−0.09	0.13	0.11
Cachexia assessment				
Cachexia Fearon	0.08	0.14	0.15	0.14
Geriatric assessment				
G8 frailty score	−0.13	0.10	−0.22*	−0.17
Katz score	0.08	0.17	−0.001	−0.19
MMSE score	−0.02	−0.21	0.10	0.14
GDS-4	−0.18	−0.04	0.04	0.02
CIRS-G total score	−0.04	0.06	−0.15	−0.17
QOL29 overall health	−0.29*	0.14	0.06	−0.03
QOL30 quality of life	0.17	0.03	0.07	0.02

MNA: Mini Nutritional Assessment – BMI: Body Mass Index – MMSE: Mini Mental State examination – GDS-4: Geriatric Depression Scale 4 items – CIRS-G: Cumulative Illness Rating Scale – Geriatric – QOL: Quality of life questions 29 and 30 from the EORTC questionnaire.
Data are expressed in Pearson correlation coefficient (r).
* $p < 0.05$.
** $p < 0.005$.
° $p < 0.001$.

lower in patients with advanced stages although the trend was not significant ($p = 0.090$).

4.1. Age and frailty

In multiple regression analysis, the only parameter significantly and positively associated with age was APN. This association was independent from BMI, and was already described by Obata et al. in old, healthy subjects after stepwise multiple regression for confounding factors, like renal function, diabetes, or medications (Obata et al., 2013). However, our study was not designed to detect the effect of age on biomarkers, as it concerned only old patients, which could have reduced the power of a potential influence of age.

Despite a high prevalence of frailty in our study, we did not find any association of frailty (assessed by G8) with IL-6, leptin, or ghrelin. Only one other study explored the relationship between IL-6 and cancer-

Table 5
Univariate analysis of IL-6, leptin, adiponectin and ghrelin (biochemical characteristics).

	IL-6	Leptin	Adiponectin	Ghrelin
Biological assessment				
Hb	−0.13	−0.09	−0.07	−0.10
Albumin	−0.29**	0.05	0.04	−0.07
CRP	0.42°	−0.06	−0.03	0.04
Hormones, adipokines and cytokines (fasting)				
Log IGF-1	−0.30**	0.14	−0.12	−0.19
Log insulin	−0.18	0.29**	−0.13	−0.41°
Log adiponectin	−0.04	−0.02	–	0.22*
Log leptin	−0.31**	–	−0.02	−0.27**
Log ghrelin	0.18	−0.27**	0.22*	–
Log IL1-RA	0.21*	−0.09	0.03	0.10
Log IL2-R	0.23*	−0.07	−0.01	0.06
IL5 > 5	0.21*	−0.03	0.01	0.03
Log IL-6	–	−0. 31**	−0.04	0.18
IL-7 > 25	0.16	−0.03	0.07	0.07
Log IL-12	0.16	−0.09	0.12	0.06
IL-13 > 6	0.27**	−0.11	0.08	−0.01
Log IL-15	0.30**	−0.26*	0.06	0.08
Log IFNα	0.20	−0.11	0.02	0.10
Log MIP-1a	0.22*	−0.20	−0.03	−0.01
Log MIP-1b	0.12	−0.11	0.05	0.04
Log eotaxin	−0.001	−0.03	−0.01	9.10
Log MCP-1	0.30**	−0.09	0.06	0.06
Log MIG	0.23*	−0.24*	0.02	0.13
Log IP-10	0.27**	−0.32**	−0.04	0.21*

Hb: hemoglobin – CRP: C-Reactive Protein – IGF-1: Insulin-like Growth Factor – IL: interleukin. IL-1RA: IL-1 receptor antagonist. IL2-R: IL-2 receptor. GM-CSF: granulocyte macrophage colony-stimulating factor. TNF-alpha: tumor necrosis factor alpha. IFN: interferon. MCP: monocyte chemoattractant protein. MIP: macrophage inflammatory protein. MIG: monokine induced by interferon gamma. IP-10: interferon γ-inducible protein 10.
Data are expressed in Pearson correlation coefficient (r).
* $p < 0.05$.
** $p < 0.005$.
° $p < 0.001$.

Table 6
Multiple regression of IL-6, leptin, adiponectin and ghrelin.

	Coefficient [CI 95%]	r^2	p
Interleukin-6 ($n = 85$)			
Albumin	−0.52[−0.79–−0.25]	0.39	< 0.0001
Mortality	0.53[0.06–0.99]		0.027
MCP-1	−0.42[−0.10 to −0.73]		0.010
Adiponectin ($n = 87$)			
Age	0.02[0.01–0.04]	0.16	0.008
SMI	−0.04[−0.08–−0.005]		0.029
Leptin ($n = 92$)			
BMI	0.15[0.12–0.19]	0.54	< 0.0001
IL-6	−0.24 [−0.40–−0.07]		0.005
Ghrelin ($n = 82$)			
TC	−0.04[−0.06–−0.008]	0.22	0.014
SMI	−0.08[−0.14–0.02]		0.007
IP-10	0.28[0.02–0.54]		0.033

MCP-1: monocyte chemoattractant protein – IP-10: interferon γ-inducible protein 10 – TC: thigh circumference – SMI: Skeletal Muscle Index. Data are expressed in coefficient of regression [confidence interval 95%], and coefficient of determination r^2.

related frailty, finding it also be unrelated (Brouwers et al., 2015). APN was significantly and positively associated with frailty in univariate analysis. This could be explained by the fact that G8 includes the body mass index in the criteria, indicating perhaps a collinearity problem. However, Nagasawa et al. recently described a significant increase in adiponectin levels in an old frail Japanese general population, using the Cardiovascular Health Study criteria of frailty of Fried et al., and suggested that APN could be used as a biomarker of frailty (Nagasawa et al., 2018). Frailty is a broad concept and the G8 tool has not a perfect sensitivity (estimated to be 87%), missing 13% of frail cases (Smets et al., 2014).

4.2. Nutritional status and body composition

We observed that a low albumin level was independently associated with a higher level of IL-6. Inflammation reduces albumin concentration by decreasing its rate of synthesis. One study focused on the relationship between the nutritional status and inflammatory markers in cancer, showing a significantly higher level of IL-6 in malnourished patients undergoing colorectal cancer surgery (Daniele et al., 2017). Albumin and IL-6 levels are both recognized as prognostic factors in cancer patients and are used to predict weight loss, post-operative complications and death in patients with gastrointestinal cancer (Costa MD de et al., 2016).

In a multiple regression analysis, IL-6, leptin, APN, and ghrelin were not associated with the MNA score, indicating that these biomarkers are not associated with the nutritional status, despite the high sensitivity (97%) of MNA in patients with cancer (Read et al., 2005). However, leptin showed a positive correlation with BMI, reflecting that leptin might be a good marker of adiposity even in older patients with cancer, but also reflecting the link between leptin and the carcinogenesis of solid cancers (Liao et al., 2013).

APN and ghrelin levels showed a negative correlation with muscle parameters, respectively with skeletal mass index and with thigh circumference. For APN, this inverse relationship is not in accordance with the existing literature, as cancer is classically associated with low levels of APN (Dalamaga et al., 2012; Ma et al., 2018). High APN levels have also been described in other chronic diseases, like heart failure and cardiac cachexia where APN level was inversely correlated with muscle mass and muscle strength in heart failure patients, but not in control group (Loncar et al., 2013). It was also associated with an increased risk of falls in old people (Huang et al., 2016). This association with chronic conditions might reflect the frailty status and the comorbidity of our sample, although the potential mechanism by which adiponectin affects skeletal muscle is not well understood.

Fasting ghrelin concentration was inversely associated with skeletal mass, and this finding concurs with other studies (Bertoli et al., 2006; Tai et al., 2009). Ghrelin has receptors on muscle cells membranes and may exert a negative feedback effect on ghrelin secretion, by maintaining energy homeostasis and promoting muscle function (Sever et al., 2016).

4.3. Other associations

High IL-6 levels were associated with a higher mortality rate, as described in a recent review from Lippitz et al. in late-stage cancers, regardless of the tumor type (Lippitz and Harris, 2016).

We also observed that MCP-1 (monocyte chemoattractant protein type 1) and IP-10 (interferon γ-inducible protein 10) were the only inflammatory markers significantly associated with IL-6, leptin, and ghrelin in a multiple regression analysis: MCP-1 was independently and negatively associated with IL-6, which is somewhat surprising, as we know that MCP-1 is a chemokine contributing to tumor progression via recruitment of macrophages and subsequent increase in inflammatory mediators (Talbert et al., 2018). MCP-1 genes are ubiquitously expressed, by skeletal and adipose tissue, and by the tumor itself. Conflicting results regarding its role in cancer progression have been reported: some authors found increased circulating MCP-1 in cancer cachexia (Cranford et al., 2017), while others did not (Lerner et al., 2016). It has been hypothesized that this lack of association was due to the possible blunting effect of chemotherapy and/or radiation treatment in these studies, which is not applicable to our treatment-naive patients. IP-10 was negatively associated with leptin and positively with ghrelin. IP-10 is expressed in a diverse range of human diseases, including advanced cancer through exacerbation of Th1 inflammatory response, causing significant tissue damage. IP-10 is involved in tumor progression and metastasis, and is associated with a poor prognosis in colorectal cancer patients. The combination of high IP-10 and high IL-6 levels associated with low leptin levels, also called “leptin-cytokin crosstalk”, could reflect the cancer progression and the processes of starvation and of cachexia. Of note that leptin was only associated with IL-6, but not with IP-10 in multivariate analysis. In parallel, the positive association between IP-10 and ghrelin would represent the phenomenon of ghrelin resistance induced by the stimulating effect of the pro-inflammatory cytokines on leptin, not sufficiently counteracted by ghrelin hypersecretion (Liu et al., 2011; Toiyama et al., 2012).

Our study has some limitations. We examined people with different types of cancers, and the total number of patients included is low, which could have underpowered our results. A strong aspect is that all participants were treatment-naive, and without inflammatory comorbidity, excluding confounding effects on inflammation, nutritional status, or body composition, often encountered in other studies. We considered also the possibility that our results could have been influenced by a lack in sensitivity in the multiplex platform. We used a high-sensitivity multiplex system, whose quantitation limits are approximately 10-fold lower than the standard multiplex. Also, the large number of parameters tested might have turned out some fortuitously significant *p*-values; however, all significant relationships are corroborated by the literature. Finally, our current dataset does not allow us to associate changes in adipose tissue mass with IL-6, adipokines and ghrelin, as we did not assess fat mass, which would have been interesting in the context of cachexia and the study of adipokines.

5. Conclusion

In conclusion, IL-6, leptin, APN, and ghrelin are not associated with advanced stages of cancer or cancer-induced cachexia in older subjects with cancer. However, they are significantly correlated with anthropometric factors and body composition, by a probable direct effect on muscle and adipocytes. Our results indicate that serum levels of adipokines and ghrelin could be used as potential biomarkers of frailty and body composition in old cancer patients, regardless of the cancer stage. In addition, cytokines like IL-6 and IP-10 could explain indirectly how adipokines and cancer interact with each other.

Statement of authorship and acknowledgement

Sandra De Breucker coordinated the study, recruited the patients of Erasme Hospital, made the statistics and wrote the manuscript. Sylvie Luce coordinated the National Cancer Plan in Erasme Hospital, Lore Decoster in Universitair Ziekenhuis Brussel. Rose Njemini performed the cytokine analyses. Sandra De Breucker, Ivan Bautmans, Tony Mets and Thierry Pepersack wrote the protocol. Sandra De Breucker, Tony Mets and Thierry Pepersack reviewed the manuscript.

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Declaration of competing interest

The authors have no disclosure of conflict of interest regarding this study.

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