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The cancer anorexia/weight loss syndrome: exploring associations with single nucleotide polymorphisms (SNPs) of inflammatory cytokines in patients with non-small cell lung cancer

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Abstract

Objective—The cancer anorexia/weight loss syndrome commonly occurs in patients with non-small cell lung cancer (NSCLC) and is characterized by loss of weight and appetite as well as diminished survival. The current study explored whether any of 22 single nucleotide polymorphisms (SNPs) of certain previously implicated inflammatory cytokines (interleukin-1 beta, interleukin-1RN, interleukin-6, and tumor necrosis factor) are associated with this syndrome.

Patients and Methods—All NSCLC patients who had been enrolled in the Mayo Clinic Lung Cancer Cohort, had completed a health-related questionnaire approximately 6 months after enrollment, and had blood drawn were included in this study, thus yielding a sample size of 471 patients.

Results—Sixty-six (14%) patients manifested weight loss shortly after diagnosis, and 152 (32%) reported appetite loss. Only tumor necrosis factor alpha rs800629 was associated with anorexia (odds ratio: 0.46; 95% confidence interval: 0.29, 0.72; $p<0.001$); patients who were heterozygous and minor homozygous were less likely to suffer anorexia. Otherwise, there were no statistically significant associations between any of the other 21 SNPs and weight loss and/or anorexia. In univariate analyses, weight loss, anorexia, more advanced cancer stage, and interleukin-1 beta rs1143627 were associated with a worse survival, and interleukin-6 rs2069835 was associated with better survival. However, in multivariate analyses, cancer stage and patient age were the only statistically significant predictors of worse survival.

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Conclusion—No specific SNP was associated with all aspects of the cancer anorexia/weight loss syndrome, but rs800629 may merit further study in cancer-associated anorexia.

Keywords

Cancer-associated anorexia; Single nucleotide polymorphism; Non-small cell lung cancer

The cancer anorexia/weight loss syndrome is characterized by loss of weight, diminished appetite, inferior quality of life, and a shortened survival. The latter is a salient aspect of this syndrome, as indicated by the four studies described below.

First, evaluating 116 patients with relatively early stage lung cancer, Jeremic and others observed that patients with weight loss died early and that this sign was one of the most robust predictors of early demise [1]. Second, in a landmark study, Dewys and others evaluated 3,047 patients, with a variety of malignancies, including non-small cell lung cancer [2]. These investigators found once again that weight loss predicted early death and that this predictive capability occurred independently of performance status and tumor burden. Third, this prognostic effect is not confined to weight loss that occurs immediately prior to a diagnosis of cancer. Chuang and others evaluated 356 late-stage cancer patients, all of whom were candidates for hospice and many of whom had received prior cancer therapy [3]. Lung cancer was the most common cancer type within this group. Evaluating patient-reported weight loss in the preceding 3 months, these investigators observed that even under these challenging circumstances and well after a patient's original cancer diagnosis, weight loss continued to be a powerful predictor of early death. Finally, the symptom of anorexia also predicts early death. In a 1,115-patient North Central Cancer Treatment Group study, in which approximately half the cohort had lung cancer, patients were assessed for anorexia in a prospective fashion. These investigators observed that this one symptom carried a statistically significant negative prognostic effect [4]. Hence, these four studies, as well as others, underscore the fact that weight loss and/or anorexia in cancer patients signals a poor outcome.

What mediates this weight loss and anorexia and thereby leads to early death? Although ongoing preclinical research suggests that a complex series of factors are at work, previous studies point to a cascade of inflammatory cytokines that ultimately lead to loss of appetite, wasting of functional lean tissue, weight loss, and shortened survival [5–10]. These cytokines include interleukin-1 beta (IL-1 beta), interleukin-1RN (IL-1RN), interleukin-6 (IL-6), and tumor necrosis factor (TNF). Therefore, the current study was conducted to focus specifically on functional single nucleotide polymorphisms (SNPs) of these inflammatory cytokines in an effort to explore whether associations exist between these potential mediators and the symptom complex of anorexia, weight loss, and poor survival in patients with non-small cell lung cancer.

Methods

Overview

This study was undertaken within the Mayo Clinic Lung Cancer Cohort, a prospectively conducted study that has attempted to recruit consecutively all lung cancer patients seen at the Mayo Clinic in Rochester, Minnesota since January 1, 1997. The Mayo Clinic Lung Cancer Cohort is reviewed at least annually by the Mayo Clinic Institutional Review Board, and previously published studies provide details of the cohort. All patients had provided written informed consent prior to enrollment.

Ascertainment of clinical data

After enrollment, each patient's medical record was reviewed by trained personnel, and extensive demographic information on the patient's health, cancer status, and subsequent treatment were entered into the database. The Revised Tumor–Node–Metastasis staging system for non-small cell lung cancer was used to assign a cancer stage to each patient [11]. Thereafter, all patients were asked to complete a series of health-related surveys. The first of these were mailed to patients within 6 months of enrollment. Subsequently, surveys were mailed to patients on an annual basis in an effort to acquire follow-up information and, as particularly relevant to the current report, to ascertain vital status.

The survey, which had been sent within 6 months of enrollment, asked patients to record their current weight. This weight was then compared to the weight in the medical record recorded in the peri-diagnostic period; the percent change in weight over this time interval was then calculated. Greater than 10% weight loss during this period indicated weight loss of clinical concern. This threshold was chosen because of its negative prognostic significance and because it is indicative of nutritional compromise that often merits nutritional support [12].

To gain a broader spectrum of symptomatology relevant to weight loss, patients were asked at approximately the 6-month time point about their appetite. A previously validated questionnaire item [13], “How would you compare your appetite now to what it was before your present illness?” provided patients with five response options, “increased,” “same,” “slightly reduced (about 75% of normal),” “moderately reduced (about 50% of normal),” and markedly reduced (about 25% of normal or less).”

Vital status had been ascertained by means of follow-up surveys. Moreover, within the Mayo Clinic Lung Cancer Cohort, vital status is continuously verified every year by means of the following sources: the Mayo Clinic registration clinical database, other next-of-kin reports, death certificates, documentation of death in the clinical record, and/or documentation of vital status within the Mayo Clinic Tumor Registry.

Single nucleotide polymorphism analyses

Blood was drawn at baseline and utilized for genotyping the SNPs for the following inflammatory cytokines: IL-1 beta, IL-1RN, IL-6, and TNF. These cytokines were chosen because of prior research which has implicated them in cancer-associated weight loss and anorexia.

A deliberate decision was made not to assay and assess serum cytokine concentrations as such levels can be highly variable, often do not correlate with clinical endpoints, and are not reflective of cytokine concentrations at the tissue level where wasting occurs [14]. Thus, the current study focused exclusively on the SNPs of these inflammatory cytokines.

Genotyping was performed in the Mayo Clinic Genomic Shared Resources. Two genotype approaches were used, SNPstream and TaqMan. Each was run according to the manufactures' protocol, as described previously [15, 16]. For SNPstream panels and Taqman assays primer sets were designed using software specific to each platform. Control CEPH DNAs (n=4–12) and no template controls (n=8) were included in each 384 well plate to assure accuracy and reproducibility across the project. In addition, sample duplicates (2%) were included in the plate design. Genotyping was successful for all samples with an average SNP call rate of 99.8%.

Statistical Analyses

Demographic data and frequencies of each SNP are presented descriptively. Patient-reported anorexia was categorized as a dichotomous variable. Patients were considered to have anorexia if they responded that their appetite was “slightly,” “moderately,” or “markedly” reduced. Similarly, patients were categorized as not having anorexia if they had reported that their appetite was “increased” or the “same.” As mentioned earlier, weight loss was defined as a 10% drop in weight at 6 months after diagnosis.

The polymorphisms of interest were assessed and, in this case, confirmed for Hardy–Weinberg equilibrium. Then cytokine polymorphisms were categorized based on whether they were major homozygous, heterozygous, and minor homozygous SNP alleles. For the analyses, the minor homozygous SNP alleles with frequencies of <5% were grouped together with the heterozygous SNP alleles. SNPs with minor allele frequencies of <5% were excluded from the analyses. This approach was necessary to ensure the stability of the models described below.

Logistic regression models were used to assess associations between the various genetic polymorphisms and weight loss and anorexia. Odds ratios and 95% confidence intervals are reported as appropriate. The Bonferroni method for *p* value adjustment was used for multiple comparisons. In view of the fact that 22 SNPs were examined, a *p* value of <0.002 was deemed statistically significant in the logistic regression models that assessed associations between these clinical variables of weight loss and anorexia and these polymorphisms. Gene–gene interactions and other two-way interactions of factors were not investigated in view of the relatively limited patient sample size and the exploratory nature of these analyses.

This study utilized a landmark survival analysis that included the postdiagnosis, baseline survey completion data as the baseline time-point. Overall survival was defined as the time from this baseline time-point to death from any cause. The distribution of overall survival was estimated by the Kaplan–Meier method. Univariate Cox proportional hazard models were then used to explore associations between each polymorphism within the context of the clinical factors of weight loss, anorexia, cancer stage, age, gender, and overall survival. Factors with a *p* value of <0.1 in the univariate analysis were then examined in a multivariate model. Cancer treatment was not utilized in this model because of collinearity with stage. Hazard ratios and 95% confidence intervals are reported as appropriate. A *p* value of <0.05 is considered statistically significant. In these two, separate multivariate models for weight loss and anorexia, a *p* value of <0.025 was considered statistically significant.

A sample size of 471 patients provided greater than 90% power to detect an association assessed by an odds ratio of 0.48 for a two-level SNP polymorphism with a prevalence, for example, of 34% versus 66% (two-sided chi-square test, alpha = 0.05). A sample size of 471 patients also provided approximately 90% power to detect an effect assessed by a hazard ratio of 1.5 for this two-level factor with a prevalence of 34% versus 66% (two-sided logrank test, alpha level = 0.05) using the actual enrollment rates for the present data and assuming an exponential distribution for survival with a minimum of 2-years follow up for each patient.

Results

Demographics

A total of 471 patients with non-small cell lung cancer completed the 6-month health-related questionnaire on weight loss and anorexia and had blood available for genotyping. These patients are the focus of this report. Patient demographics are shown in Table 1.

Weight loss, anorexia, and associations with SNPs

Sixty-six patients (14%) had lost weight between the time of their diagnosis and their 6-month postdiagnosis assessment, and 152 (32%) described appetite loss (Table 2). Of incidental note, a total of 12 patients (2.5%) manifested weight gain of 10% or greater. SNP polymorphism frequencies are shown (Table 3).

With one exception, there were no statistically significant associations between any of these 22 SNPs and weight loss and/or anorexia. Only TNF polymorphism rs800629 was associated with anorexia (odds ratio: 0.46; 95% confidence interval: 0.29, 0.72; $p<0.001$). Patients who were heterozygous and minor homozygous were less likely to suffer anorexia compared to those who were major homozygous.

Survival

At the time of these analyses, 237 patients (50%) had died. The median follow-up time for the living patients is 5.1 years (range: 1.0, 8.2 years) with 95% of patients followed beyond 2 years.

In univariate analyses, weight loss, anorexia, and advanced cancer stage were associated with a worse survival (Table 4). The IL-1 beta rs1143627 SNP was also associated with worse survival. In contrast, IL-6 rs2069835 was associated with an improved survival. However, in multivariate analyses, cancer stage and age were the only statistically significant factors that predicted a worse survival.

Discussion

The current study explored whether SNPs from the inflammatory cytokines, IL-1 beta, IL-1RN, IL-6, and TNF are associated with weight loss, anorexia, and diminished survival. One statistically significant association emerged, that between TNF rs800629 and the presence of anorexia. This observation merits further investigation. However, the current study did not find that a single SNP or group of SNPs is associated with all aspects of this cancer anorexia/weight loss syndrome; and, therefore, this study did not generate compelling clinical evidence to suggest that these SNPs act alone as key mediators of the cancer anorexia/weight loss syndrome.

Do such negative findings demonstrate that SNPs of IL-1 beta, IL-1RN, IL-6, and TNF do not in any way mediate this syndrome? We believe the answer to this question is “no,” and cite the following two explanations. First, a growing consensus suggests that SNPs in general may exert their effects by means of interactions with multiple other environmental and genetic factors [17]. This opinion has been invoked to explain why other previous studies that have focused on other SNPs have also often yielded negative results. In the cancer anorexia/weight loss syndrome, the SNPs studied in this investigation may in fact give rise to this syndrome, but other factors, which remain undiscovered, must perhaps act in concert for them to do so. Thus, we cannot conclude with certainty that these SNPs are not mediators of the cancer anorexia/weight loss syndrome; at best, we can only conclude that,

when acting alone, these SNPs do not appear to be robust key mediators, and that further study of their role is warranted.

Second, from a logistic standpoint, this study examined weight loss that occurred from the time of cancer diagnosis to approximately 6 months afterwards. Many of these patients might have been suffering weight loss as a result of cancer treatment or other comorbid conditions, not necessarily from the cancer itself. Although previous studies have shown that the inflammatory cytokines are implicated in cancer-associated weight loss, it remains unknown if these inflammatory cytokines mediate weight loss that occurs in cancer patients who may have other non-cancer-related causes of weight loss. Again, further study of weight loss and its pathophysiology in cancer patients is warranted.

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Table 1

Baseline characteristics; *N*=471[a]

Age at diagnosis, median (range)	**67 (18, 89)**
Gender	
Female	241 (51)
Male	230 (49)
Tumor histology	
Adenocarcinoma	244 (52)
Squamous	119 (25)
Bronchioalveolar	23 (5)
Large cell	12 (2)
Adenosquamous	9(2)
Other, including non-small cell, unspecified	64 (13)
Cancer stage[b]	
I	224 (48)
II	45 (10)
III	110 (24)
IV	89 (19)
Cancer therapy reported on baseline Questionnaire[b]	
Surgery	337 (73)
Radiation	143 (31)
Chemotherapy	223 (48)

[a] Numbers in parentheses refer to the percentage of the cohort unless otherwise specified

[b] Not all percentages sum to 100% either because of rounding, as seen with cancer stage, or patients' falling into more than one category, as seen with cancer therapy

Table 2

Baseline weight loss and anorexia data; *N*=471[a]

Weight loss of >10% since diagnosis	
Yes	66 (14)
How would you compare your appetite now to what it was before your present illness?	
Increased	73 (15)
Same	246 (52)
Slightly reduced (about 75% of normal)	100 (21)
Moderately reduced (about 50% of normal)	26(6)
Markedly reduced (about 25% of normal or less)	26 (6)

[a]Numbers in parentheses denote the percentage of the entire cohort unless otherwise specified

Table 3

SNP frequencies

Gene	SNP	Homozygous common allele	Heterozygotes *N*(%)	Homozygous rare allele *N*(%)	Harding-Weinberg equilibrium (*p*value) *N*(%)
IL1B	rs1143627	216(45.9)	205(43.5)	50(10.6)	0.90
	rs1143630[a]	417(88.5)	51(10.8)	3(0.6)	0.30
	rs1143633	191(40.6)	219(46.5)	61(13)	0.89
	rs1143634	269(57.2)	168(35.7)	33(7)	0.34
	rs2853550[a]	398(84.5)	68(14.4)	5(1.1)	0.28
IL1RN	rs3087263[a]	381(81.1)	87(18.5)	2(0.4)	0.20
	rs3087266[a]	345(73.3)	118(25.1)	8(1.7)	0.56
	rs315952	236(50.2)	197(41.9)	37(7.9)	0.64
	rs380092	214(45.4)	208(44.2)	49(10.4)	0.88
	rs397211	221(46.9)	203(43.1)	47(10)	0.97
	rs4252041	434(92.3)	36(7.7)	-	1.00
IL6	rs1800795	144(30.6)	243(51.6)	84(17.8)	0.29
	rs2066992	425(90.2)	46(9.8)	-	0.62
	rs2069835[a]	421(89.4)	49(10.4)	1(0.2)	0.73
	rs2069840	205(43.6)	218(46.4)	47(10)	0.32
	rs2069843[a]	443(94.3)	25(5.3)	2(0.4)	0.07
	rs2069852	441(93.8)	29(6.2)	-	1.00
	rs2069857[b]	458(99.1)	4(0.9)	-	1.00
	rs2069860[b]	465(98.7)	6(1.3)	-	1.00
	rs2069861[a]	375(79.8)	91(19.4)	4(0.9)	0.55
TNF	rs1800629[a]	302(66.2)	133(29.2)	21(4.6)	0.20
	rs1800630[a]	334(70.9)	124(26.3)	13(2.8)	0.72
	rs3093661	433(92.1)	37(7.9)	-	1.00
	rs3093662	410(87.1)	61(13)	-	0.13
	rs3093665[b]	455(96.6)	16(3.4)	-	1.00
	rs3093671[b]	448(95.3)	22(4.7)	-	1.00

Gene	SNP	Homozygous common allele	Heterozygotes *N*(%)	Homozygous rare allele *N*(%)	Harding-Weinberg equilibrium (*p*value) *N*(%)
	rs4645843[b]	470(100)	-	-	1.00

[a] Due to low frequencies (<5%), the homozygous rare alleles of these SNPs were grouped together with heterozygotes for the stability of statistical models

[b] Due to low frequencies (<5%) of the heterozygotes and absent of homozygous rare alleles, these SNPs were not included the analyses

Table 4

Relationships between relevant variables and survival

		Univariate models		Multivariate models for weight loss		Multivariate models for anorexia	
Factor	**Categories**	**Hazard ratio (95% confidence interval)**	***p*value**	**Hazard ratio (95% confidence interval)**	***p*value**	**Hazard ratio (95% confidence interval)**	***p*value**
>10% weight loss	Yes versus no	1.48 (1.05, 2.08)	0.02	1.36 (0.97, 1.92)	0.08	-	-
Anorexia	Yes versus no	1.56 (1.20, 2.02)	<0.001	-	-	1.37 (1.05, 1.78)	0.02
Cancer stage	II versus I	1.78 (1.09, 2.90)	0.02	1.89 (1.16, 3.09)	0.01	1.82 (1.11, 2.98)	0.02
	III versus I	2.54 (1.81, 3.57)	<0.001	2.84 (2.00, 4.03)	0.001	2.80 (1.97, 3.97)	<0.001
	IV versus I	6.77 (4.87, 9.43)	<0.001	7.37 (5.23, 10.4)	<0.001	7.14 (5.07, 10.1)	<0.001
Age	10 year increments	1.14 (1.00, 1.30)	0.046	1.28 (1.13, 1.46)	<0.001	1.28 (1.13, 1.45)	<0.001
rs1143627 (IL1B)	Heterozygous vs major homozygous	1.50 (1.14, 1.96)	<0.001	1.14 (0.86, 1.51)	0.36	1.14 (0.86, 1.50)	0.36
	Minor homozygous vs major homozygous	1.25 (0.80, 1.96)	0.33	1.08 (0.68, 1.69)	0.75	1.07 (0.68, 1.69)	0.76
rs2069840 (IL6)	Heterozygous vs major homozygous	1.19 (0.92, 1.56)	0.19	1.15 (0.88, 1.50)	0.32	1.14 (0.87, 1.49)	0.35
	Minor homozygous vs major homozygous	0.56 (0.33, 0.97)	0.04	0.59 (0.34, 1.01)	0.05	0.56 (0.33, 0.97)	0.04