The prognostic value of serum and bronchoalveolar lavage levels of adiponectin in advanced non-small-cell lung cancer

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Practice points

- Adiponectin (APN) is a candidate biomarker of prognosis in various solid tumors.
- Previous data on the prognostic value of circulating APN in non-small-cell lung cancer (NSCLC) are extremely limited.
- APN levels in biological fluids other than peripheral blood samples have not been previously investigated in cancer.
- We aimed to explore the prognostic implications of serum and bronchoalveolar lavage (BAL) levels of APN in advanced NSCLC.
- Twenty-nine patients with stage IV NSCLC were prospectively enrolled.
- APN levels in the serum and BAL were measured prior to initiation of treatment.
- APN values were correlated with overall survival and various clinicopathological parameters, including BMI and weight loss.
- Statistical analysis results failed to reveal any associations between APN levels and all variables evaluated.
- Our results suggest lack of a prognostic significance of serum and BAL APN levels in advanced NSCLC.
- Larger prospective series are warranted to confirm these findings.

Aim: We aimed to explore the prognostic implications of adiponectin (APN) levels in the serum and bronchoalveolar lavage (BAL) of patients with advanced NSCLC. **Materials & methods:** 29 newly diagnosed patients with stage IV NSCLC were prospectively enrolled. Baseline serum and BAL levels of APN were assayed by ELISA and correlated with various clinicopathological parameters, including overall survival. **Results:** No statistically significant correlations were observed between the serum or BAL levels of APN and the clinicopathological parameters evaluated. The only prognostic factor identified, both by univariate and multivariate survival analyses, was performance status. **Conclusion:** The results of our cohort failed to reveal any prognostic significance of serum and BAL levels of APN in stage IV NSCLC.

First draft submitted: 15 November 2016; Accepted for publication: 10 July 2017; Published online: 17 November 2017

Keywords: adiponectin • bronchoalveolar lavage • NSCLC • serum

NSCLC is the commonest histological subtype of lung cancer, comprising the overwhelming majority (80%) of all lung cancer cases. A striking feature of NSCLC is its considerable inter- and inpatient heterogeneity, which carries a significant clinical impact on prognosis and treatment options [1,2]. Despite recent treatment advances, mainly with regard to the advent of novel targeted therapies, NSCLC remains a major cause of cancer-related mortality and morbidity worldwide [3]. More accurate stratification of patients, based on robust biomarkers of prognosis and prediction of treatment response and treatment-related toxicity, is, therefore, urgently needed for the development of more effective and better tolerated personalized therapies.

Adiponectin (APN) is the most abundant adipose tissue-derived adipokine in the plasma, and a potent hormone with a crucial involvement in variable physiologic functions, including, among others, regulation of lipid

metabolism, insulin sensitization, apoptosis and inflammation [4,5]. Accumulating preclinical data have further demonstrated that, in addition to its above effects, APN may also represent an important modulator of key molecular pathways underlying carcinogenesis, such as those involved in tumor cell proliferation, invasion and migration, angiogenesis, evasion of apoptosis and immune system surveillance [6–10]. Notably, both positive and negative effects of APN on tumor growth and progression have been demonstrated, suggesting the complex biological role of this molecule in cancer, while the exact pathophysiologic mechanisms underlying its cancer-suppressive or cancer-promoting effects remain poorly understood [2,6–14].

A considerable body of research has investigated the potential value of circulating APN as a prognostic and/or predictive biomarker in various tumors and its association with cancer cachexia, but published results are not consistent and often contradictory [15–33]. Relevant data on lung cancer in particular are, nevertheless, extremely limited, while, to the best of our knowledge, APN levels in biological fluids of NSCLC patients other than peripheral blood samples have not been previously evaluated.

The primary objective of this study was to prospectively investigate the prognostic value of APN levels – with regard to overall survival (OS) – in the serum and bronchoalveolar lavage (BAL) of patients with advanced NSCLC. Secondary objectives were to explore potential correlations of APN levels with various clinicopathological features, including gender, age, BMI, weight loss and performance status (PS) of patients at baseline, and histology, grade and size of the primary tumor.

Materials & methods

Patient population & study design

This study was a prospective analysis of patients with newly diagnosed stage IV NSCLC, referred to the Oncology Unit GPP of Sotiria Athens General Hospital for diagnostic evaluation, consultation and/or treatment, between January 2008 and February 2010. The study protocol was approved by the institutional ethics committee; written informed consent was obtained from all participants. Inclusion criteria were as follows: histologically confirmed diagnosis of NSCLC, disease stage IV, age more than 18 years, Eastern Cooperative Oncology Group PS ≤ 2 , availability and suitability of BAL fluid samples for analysis and signed informed consent. Patients who had received, prior to enrollment, any treatment for NSCLC, patients with significant life-limiting co-morbidities (such as end-stage cardiac, renal, pulmonary or liver disease) or a second synchronous malignancy, patients without biopsy confirmation of their diagnosis and those lost to follow-up were excluded from the study.

PS, BMI (calculated as weight/height²) and weight loss (unintentional progressive weight loss more than 10% of body weight over the preceding 6 months) were assessed at baseline, in other words, prior to initiation of any therapeutic intervention. Patients were staged according to the TNM classification system (6th edition) developed by the American Joint Committee on Cancer and the International Union against Cancer. Histological subtyping and grading of tumors was done according to the latest WHO histological classification. Peripheral blood samples and BAL specimens were also collected in all cases at baseline and processed for the assessment of APN levels; results were correlated with gender, age, PS, weight loss and BMI of patients, size, histology and grade of the primary tumor, and OS.

All patients were treated with standard-of-care chemotherapy regimens (platinum- or taxane-based) with or without palliative radiotherapy, as determined in each individual case by the attending physician. Follow-up evaluations of patients were carried out at 8-week intervals, and typically included physical examination, CT scan of the chest and abdomen and routine laboratory investigations. OS was defined as the time from diagnosis to the date of death by any cause.

Assessment of APN levels

Peripheral venous blood samples were drawn from patients after an overnight fast, directly collected into centrifuge tubes – without anticoagulant – and allowed to clot at room temperature for 30 min. Clotted blood samples were promptly centrifuged at 3000 rpm for 15 min. BAL fluid samples were collected during fiberoptic bronchoscopy using standard procedures. BAL aliquots were placed on ice after collection and promptly (within an hour) transported to the laboratory for further processing. BAL was centrifuged at 1200 rpm for 7 min. Serum and BAL fluid supernatants were stored at -70°C until tested.

APN levels were determined using a commercially available ELISA kit (Human Adiponectin ELISA, Biovendor), according to the manufacturer's instructions. Briefly, this assay is a sandwich ELISA, measuring native human APN only. It is based on concurrent capture of APN molecules from tested samples to a microtiter plate coated with

Table 1. Demographics, clinicopathological features, adiponectin levels and biochemical test results of our patient population.

	N (%)
Sex	
– Women	5 (17.2)
– Men	24 (82.8)
Age (years), mean (SD)	65.6 (10.1)
Performance status	
– 0	17 (58.6)
– 1–2	12 (41.4)
BMI (kg/m ²), mean (SD)	25.7 (5.2)
BMI	
– Normal	14 (48.3)
– Overweight	8 (27.6)
– Obese	7 (24.1)
Weight loss	
– No	19 (65.5)
– Yes	10 (34.5)
Histological type	
– Squamous cell carcinoma	11 (37.9)
– Adenocarcinoma	18 (62.1)
Tumor grade	
– II	5 (17.2)
– III	10 (34.5)
– IV	14 (48.3)
Tumor size (cm)	
– 1	2 (6.9)
– 2	9 (31.0)
– 3	3 (10.3)
– ≥4	15 (51.7)
Adiponectin levels in serum, median (IQR; ng/ml)	17,710.0 (13,540.0–20,315.0)
Adiponectin levels in BAL, median (IQR; ng/ml)	911.5 (527.9–1217.1)
HbA1c, mean (SD)	5.5% (1.3%)
Total cholesterol, mean (SD) mg/dl	210.4 (48.8)
HDL cholesterol, mean (SD) mg/dl	42.6 (11.1)
LDL cholesterol, mean (SD) mg/dl	139.9 (34.8)

BAL: Bronchoalveolar lavage; HbA1c: Glycated hemoglobin; HDL: High-density lipoprotein; IQR: Interquartile range; LDL: Low-density lipoprotein; SD: Standard deviation.

monoclonal antihuman APN antibodies, followed by binding of a second APN antibody to the captured molecules, removal of unbound materials by washing, binding of streptavidin-horseradish peroxidase enzyme conjugates to the immobilized biotinylated antibodies and quantification of the final immobilized antibody–enzyme conjugates after washing of excess of free conjugates. The sensitivity (the lowest level of APN detected by this method) was 0.2 ng/ml. All assays were run in duplicate.

Statistical analysis

Continuous variables are presented with mean and standard deviation (SD) or with median and interquartile range (IQR). Qualitative variables are presented with absolute and relative frequencies. The Mann–Whitney test was used for the comparison of median values between two groups. The association of each study variable with survival was assessed by univariate and multiple Cox regression analyses. Hazard ratios (HR) with 95% CI were computed from the results of Cox regression analyses. The assumption of proportional hazards was evaluated by testing for interaction with a continuous time variable. Kaplan–Meier survival estimates for survival were graphed over the

Table 2. Adiponectin levels in serum in association with demographic and clinicopathologic variables.

	Adiponectin levels in serum (ng/ml)		P Mann-Whitney test
	Mean (SD)	Median (IQR)	
Sex			
– Females	17,710.0 (0.0)	17,710.0 (17,710.0–17,710.0)	1.000
– Males	17,361.5 (8631.6)	17,710.0 (10,677.5–22,915)	
Age, r	-0.11		0.571
Performance status			
– 0	17,555.7 (6563.7)	17,710.0 (17,710.0–19,790.0)	0.838
– 1–2	17,231.6 (9648.7)	17,710 (8332.5–25,000.0)	
BMI, r	-0.06		0.756
BMI (kg/m ²)			
– Normal	17,541.8 (7221.4)	17,710.0 (15,625.0–19,790.0)	0.946
– Overweight/obese	17,309.5 (8601.5)	17,710.0 (11,460.0–22,915.0)	
Weight loss			
– No	17,626.8 (8727.5)	17,710.0 (9895.0–22,915.0)	0.906
– Yes	17,031.8 (6153.1)	17,710.0 (13,540.0–19,790.0)	
Histological type			
– Squamous cell carcinoma	17,283.0 (9275.8)	17,710.0 (6770.0–19,790.0)	0.926
– Adenocarcinoma	17,506.4 (7085.1)	17,710.0 (13540.0–22,915.0)	
Tumor grade			
II–III	17,778.5 (7313.3)	17,710.0 (13,540.0–20,315.0)	0.686
– IV	17,039.3 (8599.6)	17,710.0 (11,460.0–22,915.0)	
Tumor size (cm)			
– 1–2	19,034.5 (3488.0)	17,710.0 (17,710.0–22,915.0)	0.355
– ≥3	16,435.9 (9537.4)	17,710.0 (6770.0–20,315.0)	

IQR: Interquartile range; SD: Standard deviation.

follow-up period. All reported p-values are two-tailed. Statistical significance was set at $p < 0.05$ and analyses were conducted using SPSS statistical software (version 19.0).

Results

Demographics, clinicopathological features, APN levels & biochemical test results

A total of 29 patients with stage IV NSCLC were finally included for analysis. Baseline demographics, clinicopathological characteristics of patients and APN levels are summarized in Table 1. Mean age of patients was 65.6 years (SD = 10.1 years) and the majority were males (24/29, 82.8%). PS was 0 in 17/29 (58.6%) and 1–2 in 12/29 cases (41.4%). BMI was normal in 14/29 patients (48.3%), while none of the patients was underweight. Weight loss more than 10% was observed in 10/29 patients (34.5%). The histological type of the primary tumor was adenocarcinoma in the majority of cases (18/29, 62.1%) and squamous cell carcinoma in the remaining cases (11/29, 37.9%). The majority of cases had large (≥ 4 cm) and poorly differentiated tumors (grade III and IV). None of our patients had received recent immunosuppressive treatment or had chronic obstructive pulmonary disease (COPD) or active infection at the time of peripheral blood or BAL fluid collection. Furthermore, none of our patients was under active treatment for diabetes or hyperlipidemia during peripheral blood or BAL fluid collection.

Mean and median APN levels in the serum were 17,421.6 ng/ml (SD = 7824.2 ng/ml) and 17,710 ng/ml (IQR: 13,540–20,315), respectively. Mean and median APN levels in the BAL were 1036.3 ng/ml (SD = 910.1 ng/ml) and 911.5 ng/ml (IQR: 527.9–1217.1 ng/ml), respectively.

Serum levels of glycated hemoglobin, total cholesterol, high-density lipoprotein and low-density lipoprotein were also measured in all patients; peripheral blood samples for these measurements and those for APN were collected at the same day. Mean value of glycated hemoglobin was 5.5% (SD = 1.3%), of total cholesterol 210.4 mg/dl (SD = 48.8 mg/dl), of high-density lipoprotein 42.6 mg/dl (SD = 11.1 mg/dl) and low-density lipoprotein 139.9 mg/dl (SD = 34.8 mg/dl).

Table 3. Adiponectin levels in bronchoalveolar lavage in association with demographic and clinicopathologic variables.

	Adiponectin levels in BAL (ng/ml)		P Mann–Whitney test
	Mean (SD)	Median (IQR)	
Sex			
– Females	708.9 (404.5)	527.9 (527.9–527.9)	0.379
– Males	1104.5 (975.6)	925.3 (527.9–1221.2)	
Age, r	0.02		0.933
Performance status			
– 0	1145.8 (1132.7)	910.6 (527.9–1225.4)	0.702
– 1–2	881.1 (444.5)	912.0 (527.9–1107.0)	
BMI, r	0.06		0.765
BMI (kg/m²)			
– Normal	949.6 (574.5)	911.1 (527.9–1077.4)	0.756
– Overweight/obese	1117.1 (1155.7)	938.0 (527.9–1225.4)	
Weight loss			
– No	1103.1 (1012.7)	938.0 (527.9–1225.4)	0.576
– Yes	909.3 (706.0)	719.3 (527.9–1217.1)	
Histological type			
– Squamous cell carcinoma	1231.5 (1270.8)	911.5 (527.9–1217.1)	0.616
– Adenocarcinoma	917.0 (612.1)	720.2 (527.9–1225.4)	
Tumor grade			
– II–III	819.7 (474.0)	527.9 (527.9–1171.5)	0.278
– IV	1268.4 (1195.8)	1060.0 (527.9–1432.5)	
Tumor size (cm)			
– 1–2	764.8 (482.5)	527.9 (527.9–1225.4)	0.338
– ≥3	1202.2 (1072.6)	990.3 (527.9–1217.1)	
BAL: Bronchoalveolar lavage; IQR: Interquartile range; SD: Standard deviation.			

BAL: Bronchoalveolar lavage; IQR: Interquartile range; SD: Standard deviation.

Table 4. Spearman correlation coefficient of serum and bronchoalveolar lavage adiponectin levels in relation to age, BMI and biochemical test results of patients.

		Serum APN levels	BAL APN levels
Age	r	-0.11	0.02
	P	0.571	0.933
BMI	r	-0.06	0.06
	P	0.756	0.765
HbA1c	r	0.02	-0.21
	P	0.911	0.285
Total cholesterol	r	0.14	-0.12
	P	0.454	0.548
HDL	r	-0.06	0.08
	P	0.765	0.674
LDL	r	0.07	-0.05
	P	0.735	0.783

APN: Adiponectin; BAL: Bronchoalveolar lavage; HbA1c: Glycated hemoglobin; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Clinicopathological correlates of APN levels & survival analysis

The associations of serum and BAL levels with clinical, pathological features and biochemical test results of patients are displayed in Tables 2–4, respectively. No statistically significant associations were observed between APN levels (both in serum and BAL) and the examined variables.

All our patients died of progressive disease after a mean survival time of 7.1 months (standard error [SE] = 0.69) and a median survival time of 6 months. Kaplan–Meier survival estimates for OS according to serum and BAL

Figure 1. Kaplan–Meier estimates by adiponectin levels in serum (categorized into low and high according to median values).

levels of APN are displayed in [Figures 1 and 2](#), respectively (log-rank p-values = 0.947 and 0.384, respectively). Univariate Cox regression analysis ([Table 5](#)) showed lack of any statistically significant association between APN levels (both in the serum and BAL) and OS. The only variable that was found to be predictive for OS was PS. More specifically, patients with PS 1–2 had 2.75-times greater hazard for death as compared with those with PS equal to zero (HR [95% CI]: 2.75 [1.17–6.46]; $p = 0.02$). Similar results were obtained when multiple Cox regression analysis was conducted, confirming the independent prognostic value of PS in our cohort.

Discussion

In the present cohort of patients with metastatic NSCLC, we failed to reveal any association between serum APN levels and the demographic, clinical and pathologic variables evaluated. Survival analysis failed to show any correlation of serum APN with OS either, arguing against a potential prognostic significance of circulating APN in NSCLC, at least in the setting of advanced disease stage. In accordance with our present observations, previous studies, investigating the prognostic and/or predictive relevance of circulating APN in lung cancer, almost invariably concluded that baseline serum levels of APN do not seem to predict survival or to be associated with standard clinicopathological predictors of prognosis – such as disease stage – or treatment response [34–36].

In another prospective cohort of advanced NSCLC patients, by Karapanagiotou *et al.* [34], serum levels of the adipokines APN, leptin and resistin at diagnosis were not predictive of OS or disease progression. Kerenidi *et al.* [36] reported lack of statistically significant association between circulating APN concentrations and clinicopathological characteristics of patients or treatment response. Gioulbasanis *et al.* [35], in the larger series to date, including 115 patients with metastatic NSCLC, similarly failed to demonstrate any correlation between circulating APN and

Figure 2. Kaplan–Meier estimates by adiponectin levels in bronchoalveolar lavage (categorized into low and high according to median values).

prognosis although leptin was found to be independently associated with OS. Notably, studies on hematological malignancies and solid tumors other than NSCLC have shown that circulating APN may be useful as a marker of prognosis, and lower baseline levels of this biomarker have been associated both with favorable [37–41] and with adverse prognostic parameters [23,29,42].

Although the clinical relevance of APN as a diagnostic or prognostic biomarker in lung cancer has yet to be documented, previous preclinical, genetic and epidemiological data have supported the potential implication of APN in lung cancer pathogenesis. Sun and Lodish [43] reported that APN deficiency may promote growth of lung cancer cells in APN knockout mice, by reducing infiltration of tumors by macrophages. Furthermore, specific single-nucleotide polymorphisms within the structural gene of APN (*ADIPOQ*), such as the single-nucleotide polymorphism rs2241766 [44], have been associated with circulating APN levels and increased susceptibility to NSCLC, while chronic administration of antidiabetic drugs, increasing APN synthesis, has been linked to a decreased risk for the development of lung cancer [45,46]. Notably, recent *in vitro* data suggested that APN may exert a direct antiproliferative and anti-inflammatory effect on lung epithelial cells, thus further supporting a potential protective role of this molecule against lung cancer [47].

Although blood-based techniques are the method of choice for biomarker analysis, due to their convenience of use, minimal invasiveness, high safety profile, repeatability and low cost, use of other biological samples for the assessment of various biomarkers is desirable, as it may add clinically relevant and independent information, both in the clinical and the research setting. Similarly to the results obtained with serum APN levels, we failed to observe any statistically significant association between BAL levels of APN and the clinicopathological variables studied, including OS. To the best of our knowledge, aside from peripheral blood samples, the prognostic value of APN

Table 5. Results of univariate Cox regression survival analyses.

	HR (95% CI)*	p-value
Sex		
– Females	1.00**	
– Males	1.40 (0.53–3.73)	0.496
Age	1.01 (0.97–1.04)	0.682
BMI	1.02 (0.91–1.14)	0.760
BMI		
– Normal	1.00	
– Overweight/obese	0.93 (0.44–1.98)	0.852
Weight loss		
– No	1.00	
– Yes	0.93 (0.40–2.13)	0.857
Histological type		
– Squamous cell carcinoma	1.00	
– Adenocarcinoma	1.48 (0.68–3.22)	0.319
Tumor grade		
– II–III	1.00	
– IV	0.66 (0.31–1.43)	0.291
Tumor size (cm)		
– 1–2	1.00	
– ≥3	1.01 (0.47–2.18)	0.979
Performance status		
– 0	1.00	
– 1–2	2.75 (1.17–6.46)	0.020
Adiponectin levels in serum	0.98 (0.93–1.03)	0.473
Adiponectin levels in BAL	0.69 (0.43–1.11)	0.123

*Hazard ratio (95% CI).

**Indicates reference category.

BAL: Bronchoalveolar lavage.

levels in BAL or other body fluids of patients with NSCLC – or patients with other solid tumors, for that matter – has not been previously evaluated, hence, no safe conclusions can be drawn and this issue remains for further study.

A secondary objective of our study was to further investigate the potential association between APN and cachexia-related markers. Negative results were again obtained, showing no correlations between serum and BAL levels of APN and BMI or weight loss, thus confirming most earlier reports. In accordance with our findings, Jamieson *et al.* [48], Karapanagiotou *et al.* [34] and Gulen *et al.* [49] found no association between circulating APN levels and weight loss in NSCLC patients, while in two additional cohorts of patients with various solid tumors, including NSCLC, serum APN was not correlated with the presence of cancer cachexia or body weight changes [50,51].

APN measurement in at least one point after antineoplastic treatment could strengthen the conclusions of the present study in terms of correlation with cachexia that can be exacerbated by chemotherapy, however, follow-up measurements of APN were not technically feasible at the time of this writing. As described earlier in this report, all our patients died of progressive disease and since repeat APN measurements were not included in the initial study design, there are no stored peripheral blood or BAL samples available for this purpose. Furthermore, evaluation of APN levels in earlier-stage patients would be of interest, however, prospective evaluation of APN levels in stage I/II NSCLC patients would require a different study design and should be explored in future studies.

Interestingly, limited previous data have further suggested that the biologically active, high-molecular weight form of APN may represent a more accurate and robust biomarker of increased risk – as compared with total APN – for the development of solid tumors other than NSCLC (mainly breast cancer) [52]. In contrast, other researchers have failed to observe any significant difference between high-molecular weight and total APN, as regards their risk-prediction value [53]. This controversial issue remains to be further studied and elucidated in NSCLC.

The findings presented herein, should, nevertheless, be interpreted with caution and in the light of our study limitations. Our small sample size – albeit sufficient for the performance of statistical analysis – may have reduced the statistical power of the observed correlations, and the reported results may not be necessarily applicable to larger patient populations. Furthermore, lack of a control group precluded us from performing a comparative assessment of APN levels in subgroups of patients and healthy controls and, thus, from further investigating the potential value of APN as a diagnostic biomarker. The prospective design, homogeneity of the studied population, stringent inclusion criteria – such as enrollment of histologically confirmed cases only – and complete follow-up data, are, nevertheless, important strengths of our study and should also be acknowledged.

Conclusions & future perspective

In conclusion, the results of our prospective cohort of patients with stage IV NSCLC failed to reveal any significant correlations of APN levels (both in the serum and BAL) with OS, as well as with several clinicopathological parameters of prognosis or markers of cachexia, in accordance with most previous observations. Although current clinical data on the potential utility of circulating APN as a prognostic biomarker in NSCLC are largely discouraging and on the negative side, the small number of published studies and their methodological limitations – mainly pertaining to their small sample size, the heterogeneity of the populations studied and the lack of robust statistical analysis – must be noted and the need for additional large-scale prospective studies emphasized.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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