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Original Article

Brain metabolomic profiles of lung cancer patients prior to treatment characterized by proton magnetic resonance spectroscopy

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Abstract: Cancer patients without evidence of brain metastases often exhibit constitutional symptoms, cognitive dysfunction and mood changes at the time of clinical diagnosis, i.e. prior to surgical and/or chemotherapy treatment. At present however, there is limited information on brain metabolic and functional status in patients with systemic cancers such as lung cancer prior to initiation of treatment. Therefore, a prospective, observational study was conducted on patients with a clinical diagnosis of lung cancer to assess the cerebral metabolic status before treatment using proton magnetic resonance spectroscopy (^{1}HMRS). Together with neurocognitive testing, ^{1}HMRS was performed in the parietal and occipital cortices of patients diagnosed with a lung mass (N=17) and an age-matched control group (N=15). Glutamate concentrations in the occipital cortex were found to be lower in the patients compared to controls and the concentrations of creatine and phosphocreatine were significantly lower in the parietal cortex of the patients. The lung cancer patients were also characterized by greater fatigue scores (but not depression) prior to treatment when compared to controls. In addition, the serum concentration of interleukin-6 (proinflammatory cytokine) was higher in patients compared to controls; and the concentration of tumor-necrosis factor alpha ([TNF-α]) was positively correlated to the metabolic activity of the lung tumor as defined by the 2-deoxy-2-(^{18}F)fluoro-D-glucose (^{18}FDG) positron emission tomography (PET) derived maximal standardized uptake values (SUV_{max}). Finally, multivariate statistical modeling revealed that the concentration of N-acetyl-aspartate [NAA] in the occipital cortex was negatively associated with [TNF-α]. In conclusion, our data demonstrate that the cerebral metabolic status of patients with lung cancer is changed even prior to treatment. In addition, the association between inflammatory cytokines, SUV_{max} and [NAA] points towards interactions between the cancer's inherent metabolic activity, systemic subclinical inflammation and brain function.

Keywords: Lung cancer, proton magnetic resonance spectroscopy, brain, glutamate, proinflammatory cytokines, fatigue

Introduction

It is undisputed that cancer patients exhibit constitutional symptoms (e.g. weight loss, loss of appetite, fatigue and malaise) at the time of clinical diagnosis [1-3]. However, whether or not molecular signatures exist for these symptoms is far less clear. Even less information is available as to how these symptoms and their molecular equivalents (if existent) relate to brain metabolic function and ultimately, cancer outcomes. The importance of a cancer's direct effects on brain function is evidenced by recent prospective studies showing that as many as 20% of cancer patients can experience cognitive dysfunction and mood changes prior to treatment [4-6].

Brain imaging techniques such as positron emission tomography (PET) have documented significant changes in the resting cerebral metabolic rate of glucose (reflecting brain functional

activity) in patients with primary brain cancer or brain metastases and in breast cancer patients although after chemotherapy treatment [7-9]. However, there is very limited information in the literature on brain metabolic and functional status in patients with systemic cancers such as breast, prostate or lung cancer prior to treatment. This gap in knowledge is likely related to the complexity of conducting such studies in newly diagnosed cancer patients (without evidence of brain metastases), already overwhelmed by what is perceived as a time sensitive medical emergency with life threatening implications, requiring diagnostic testing procedures; and the often urgent need for surgical intervention.

To our knowledge, no imaging studies have documented brain metabolic status or neurochemical profiles prior to treatment in patients with lung cancer, although these patients typically present with significant constitutional symptoms and often advanced disease. We therefore conducted a study to investigate the feasibility of characterizing brain metabolic status by non-invasive proton magnetic resonance spectroscopy (^{1}HMRS) in parallel with brief assessment of neurocognitive and mood status as well as systemic inflammatory status in patients with a clinical diagnosis of lung cancer before treatment. Non-invasive ^{1}HMRS allows for an evaluation of the metabolic status of the brain in real time by tracking levels of metabolites involved in 'energetics' [10]. We hypothesized that patients with malignant lung cancers would display cerebral metabolic profile changes in comparison to non-cancer controls.

Materials and methods

Subjects

Eligible patients for this prospective, observational study included those who were referred to our surgical oncology clinic with a lung mass. Age-matched controls were recruited from the local community. We excluded subjects with severe psychiatric and neurological illness, vision or hearing impairment, liver or kidney failure, addiction to drugs of abuse and those who had been on any type of chemotherapy 6 months prior to study participation. All participants were subjected to the Mini-Cog and/or Mini Mental Status Exam for dementia screening. A score of 24 or higher on the Mini Mental Status Exam was required for eligibility. The verbal IQ of the subjects was also assessed by the Wechsler Test of Adult Reading. The subjects gave written informed consent and the study was approved by the local institutional review board.

Experimental design and data collection

All patients underwent 1) anatomical magnetic resonance imaging (MRI) followed by ^{1}HMRS, 2) neurocognitive testing and 3) blood sampling for analysis of two cytokines, TNF-α and interleukin-6 (IL-6) prior to treatment; in parallel with control subjects. Additional data collection for all subjects included laboratory screening tests, medical history and physical exam. For the patients the whole-body [^{18}F]fluoro-2-deoxyglucose positron emission tomography (^{18}FDG PET) scans and corresponding maximum standardized uptake values (SUV_{max}) of the lung mass obtained during the clinical work up were also acquired for analysis; as well as diagnosis of the lung mass by pathology attained in conjunction with surgical resection and/or biopsy.

^{1}HMRS scanning

MRI procedures for all patients and controls were conducted on a 3.0T Philips whole body scanner (Achieva system) equipped with a 12 channel phase array head coil; and included an initial T1 weighted 3D anatomical scan acquired with the following parameters: field of view =240mm, repetition time =8.5 ms, echo time=4 ms, Flip Angle=8°, 1.0 mm slice thickness with an acquisition matrix of 240×240, yielding a reconstructed isotropic voxel dimension of 1.00mm^{3}. ^{1}HMRS was performed by using a point-resolved spectroscopy sequence acquired in the parietal (15 x 15 x 15 mm^{3}) and in the occipital lobe (15 x 15 x 27 mm^{3}) with short echo time (32ms), repetition time of 2 seconds, receiver bandwidth=2000Hz, number of points=2048, and number of excitations = 256. Both shimming and water suppression routines were performed with automatic adjustments. A water unsuppressed scan was used to perform eddy current correction and to serve as a concentration reference for absolute quantification of metabolite concentrations.

Spectral data analysis

Data analysis of ^{1}HMRS spectra was performed

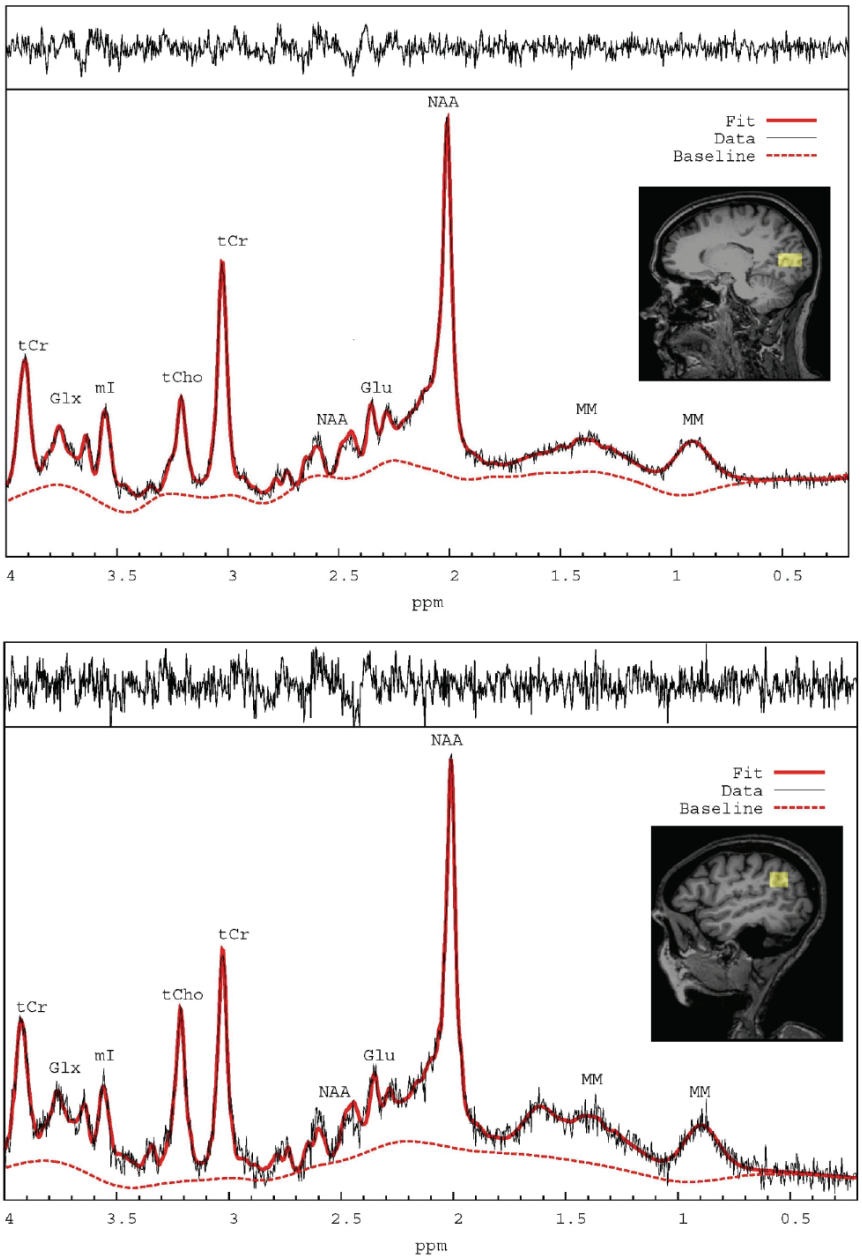

Figure 1. Localized ^{1}HMRS spectra processed by LCModel software from the occipital cortex (top) and parietal cortex (bottom) with the appropriate locations in parietal and occipital cortex shown on corresponding T1-weighted MR images. Labeling of the spectral signatures for glutamate (Glu), N-Acetyl-Asparate (NAA), Glutamate + Glutamine = GLX, total choline (tCho), total creatine (tCr), myo-inositol (mI) and macromolecules (MM). The raw (black) and fitted (solid red line) spectrum as well as the remaining baseline (stippled red line) are shown in each of the spectra.

using linear combination modeling (LCModel [11]) with prior knowledge of simulated spectral signatures for the following brain metabolites: Alanine, Aspartate, Creatine (Cr), Phosphocreatine (PCr), γ-aminobutyric acid, Glucose, Glutamine (Gln), Glutamate (Glu), Glycerophosphocholine (GPC), Phosphocholine (PCh), myo-Inositol, Lactate, N-Acetyl-Aspartate (NAA), N-acetyl-aspartyl-glutamate, Scyllo-inositol, Taurine and Guanidoacetate; in addition to lipid and macromolecules. No baseline correction, zero-filling or apodization functions were applied to the data prior to the analysis. **Figure 1** shows a typical processed ^{1}HMRS spectra from the parietal and occipital cortices and the respective voxel positions from a control subject. A quality analysis of all spectra was performed and included evaluation of the signal-to-noise ratio, spectral width (full-width half maximum), baseline and residual tracings derived from the LCModel analysis. All spectra with signal-to-noise ratio <8 and a full-width half maximum

>0.080 ppm were considered of poor quality and excluded from data analysis.

Neurocognitive testing

A brief test battery was administered by an experienced psychologist to all subjects. The domains assessed were the following: Fatigue (Profile of Mood States (POMS)) [12]; Depression (Profile of Mood States (POMS)) [12]; Long-term memory (Hopkins Verbal Learning Test-Revised); Short term memory (Digit Span) and Psychomotor Learning (Digit Symbol Coding) (WAIS-III) [13]; Verbal Fluency (semantic generation and response inhibition).

Analysis of blood for cytokines

Serum samples were prepared from freshly drawn blood that had been permitted to clot for 25-30 min. The samples were flash frozen in liquid N_2 and stored at -80 °C until analysis. (Quantikine and Quantikine HS immunoassay kits, R&D Systems, Inc, MN) were used to measure the serum levels of each cytokine in the samples according to the manufacturer's instructions. The color intensities were read at 450 nm (IL-6) or 490 nm (TNF-α), corrected at 540 nm, or 640 nm, respectively, corrected to the appropriate background absorbencies and compared to the corresponding cytokine standard curve. The cytokine concentration in each sample, expressed in pg/ml, was calculated from the standard curve equation derived from the linear fit to the standards. The assays had confirmed sensitivities of 1 and 0.2 pg/ml for IL-6 and TNF-α, respectively.

Statistical analysis

A two-sided independent t-test (Mann-Whitney-U test without normality) or Fisher's exact test, where appropriate were used to examine group differences in demographic and co-morbidity parameters at baseline. The neurocognitive and mood test scores between the two groups were compared using the non-parametric Mann-Whitney two-tailed test. Differences in cytokine concentrations between the two groups; and metabolite concentrations for each brain region calculated by LCModel for the two groups were analyzed by an independent t-test, and statistical significance was determined using a Type I error threshold of 0.05. Differences in metabolite concentrations between occipital and parietal cortices within groups were assessed using a paired, two-sided t-test. A multiple regression analysis was performed on the metabolic activity of lung tumors (SUV_{max}) and the corresponding levels of proinflammatory cytokines. Finally, the relation between inflammatory markers and LCModel quantified metabolites concentrations was explored using multivariate approach with a stepwise selection to select the significant metabolites in relation to the concentration of pro-inflammatory markers. Analysis was conducted using SAS software and XLSTAT (Version 2011.4.03).

Results

Subjects

A total of 37 subjects were assessed for eligibility; 17 controls (recruited from the local community) and 20 patients referred to the surgical oncology clinic with a lung mass. Two subjects in the control group and three in the patient group were unable to undergo MRI. One of the patients was found to have two metastases in the cerebellum. The demographics of the two groups are listed in **Table 1**; and show no differences, in age, gender, educational level, frequency of employment and verbal IQ. Differences in smoking history were significantly different between the groups. The average body mass index of the patients was slightly higher compared to controls at a significance level of 0.048 (**Table 1**). **Table 2** shows comorbidity data for the two groups and demonstrates that the frequency of chronic obstructive lung disease (COPD) and diabetes mellitus was significantly higher in the patient group when compared to controls. All of the control subjects and patients had hematocrits ≥ 35%, however the average hematocrits of the patients was slightly higher compared to controls (patient hematocrits: 41.8% ± 2.9% versus control hematocrits: 38.9% ± 3.8%, p=0.02) probably secondary to the higher frequency of tobacco use in the former group.

Pathology of lung tumors

Pathology revealed malignant lesions in 13 patients and benign lesions in 4 (**Table 3**). Seventy-seven percent of the patients with malignant lung lesions received a histological diagnosis of adenocarcinoma and 46% were classified as stage I, 31% stage II and 15% stage III according to the International Association for the Study of Lung Cancer staging system [14, 15]. Only

Table 1. Basic demographics and social history

Parameter		Controls (N=15)	Patients (N=17)	P-value
Age (Mean ± SD)		61.9 ± 9.0	59.4 ± 11.3	0.49
Gender	Male N, (%)	8, (53%)	8, (47%)	1.0
	Female N, (%)	7, (47%)	9, (53%)	
BMI		26.8 ± 3.1	30.1 ± 5.4	0.048
Smoking history	Active Smoker N, (%)	3, (20%)	10, (59%)	0.001**
	Former Smoker N, (%)	2, (13%)	6, (35%)	
	Never Smoked N, (%)	10, (67%)	1, (6%)	
Employed N, (%)		8, (53.3%)	8, (47%)	1.0
Education years (Mean ± SD)		15.6 ± 3.8	13.7 ± 2.7	0.18
Verbal IQ (estimated by WTAR)		108.9 ± 10.3	101.8 ± 11.9	0.09

Table 2. Co-Morbidity

Disease	Controls (N=15)	Pathints (N=16)	P-value
COPD N, (%)	0, (0%)	6, (35.3%)	0.019*
Hypertension N, (%)	6, (40%)	11 (64.7%)	0.287
Cardiac Disease N, (%)	2, (13.3%)	6, (37.5%)	0.229
Thyroid Disease N, (%)	4, (26.7%)	4, (23.5%)	1.0
Diabetes Mellitus N, (%)	0, (0%)	7, (41.1%)	0.008**
Prior Cancer N, (%)	1, (6.7%)	3, (18.7%)	0.603

one of the patients with a malignant lung cancer had evidence of metastases in the brain. **Table 3** also shows the corresponding ^{18}FDG PET SUV$_{max}$ characterizing the metabolic activity of the lung lesion, used to assess potential malignancy of the lung lesion prior to treatment.

Mood status and cognitive performance

Analysis of POMS fatigue scores were higher in patients compared to controls (Patients: 7.3 ± 4.2 versus Controls: 3.5 ± 3.3, p=0.009). However, there were no group differences in POMS depression scores (p=0.18). **Table 4** shows neurocognitive performance in patients and controls and demonstrates no differences in regards to memory, learning and psychomotor functioning between the two groups.

Brain metabolites

The average signal-to-noise ratio calculated based on the NAA peak was ~13 and ~20 for parietal and occipital spectra, respectively; and the average full-width half maximum ~0.055 ppm. The average Cramer-Rao-Lower-Bounds (CRLB), reflective of reliability of measurements, were consistently within acceptable range (<20%) across subjects for analyzing the concentrations of the following metabolites: Glutamate [Glu] (CRLB ~ 14%), N-acetyl-aspartate [NAA] (CRLB ~ 5%), Glutamate + Glutamine [Glu+Gln] (CRLB ~ 15%), Creatine + Phosphocreatine [Cr + PCr] (CRLB ~3%) and the total choline containing compounds Glycerophosphocholine and Phosphocholine [GPC + PCh] (CRLB ~5).

In control subjects the concentrations of [Glu], [NAA], [Cr+PCr] and [Glu+Gln] of the occipital cortex was similar to that measured in the parietal cortex; however [GPC+PCh] was higher in parietal compared to occipital cortex (**Table 5**). In the patient group [GPC+PCh] was also higher in the parietal cortex when compared to occipital cortex; and in addition [NAA] was lower in the parietal compared to occipital cortex (**Table 5**). The quantitative analysis further demonstrated that the occipital cortex [Glu] was significantly lower in the patients when compared to controls at baseline (Patients: 5.99 mM ± 0.78 versus Controls: 7.00 ± 1.04 mM, p=0.011, **Figure 2** and **Table 5**). This difference in [Glu] was slightly more significant if patients with benign lesions were excluded from the patient group (p=0.003); and also remained significant if the patient with brain metastases was excluded (p=0.005). There were no differences in the

Table 3. Surgical pathology of lung tumors and corresponding SUV_{max} values

Patient ID	Surgical Pathology	Staging	^{18}FDG SUV_{max}
P-001	Adenocarcinoma	IIIa	9.0
P-011	Aspergilloma	N/A	4.9
P-012	Carcinoid tumor	N/A	2.7
P-013	Squamous cell carcinoma	IIa	6.4
P-017	Squamous cell carcinoma	IIb	7.7
P-018	Adenocarcinoma	Ia	2.5
P-019	Adenocarcinoma	Ib	1.0
P-020	Nodule of chronic inflammation	N/A	3.6
P-021	Adenocarcinoma	Ia	7.6
P-023*	Adenocarcinoma	IV	15.7
P-024	Adenocarcinoma	IIb	6.0
P-026	Hamartoma	N/A	0.0
P-027	Adenocarcinoma	Ib	18.8
P-029	Adenocarcinoma	IIIa	8.4
P-030	Adenocarcinoma	Ia	1.6
P-036	Adenocarcinoma	IIb	6.0
P-037	Squamous cell carcinoma	Ib	13.6

*P-023 was found to have metastases in the cerebellum

Table 4. Mood status and cognitive performance

Measure	Domain	Control (N=15)	Patient (N=17)	P-value
Hopkins Verbal Learning Test-Revised (% Retained)	Long-Term Memory	92.2 (20.3)	90.0 (19.5)	0.716
Digit Symbol Coding (Age adjusted)	Learning & Psychomotor function	11.7 (3.2)	10.7 (3.2)	0.477
Digit Span (Age adjusted)	Working Memory	11.1 (2.8)	10.3 (2.7)	0.434
Verbal Fluency	Semantic Generation, Response Inhibition	36.2 (9.7)	39.2 (12.8)	0.582

Data are presented as mean ± (SD)

Table 5. Neurochemical profile of controls and patients

Metabolite	Control	Patient	Control	Patient
	Occipital Cortex (N=12)	Occipital Cortex (N=13)	Parietal Cortex (N=11)	Parietal Cortex (N=13)
[Glu]	7.00 (1.04)	5.99 (0.78)**	7.07 (1.13)	6.72 (1.52)
[NAA]	8.80 (0.72)	8.52 (0.37)	8.51 (0.86)	7.87 (0.97)#
[GPC + CPh]	1.04 (0.15)	1.09 (0.23)	1.47 (0.24)$^{₮}$	1.36 (0.25)#
[Cr + PCr]	6.89 (0.55)	6.76 (0.54)	6.79 (0.66)	6.28 (0.46)*
[Glu + Gln]	7.40 (2.22)	6.38 (0.92)	7.50 (1.28)	7.17 (1.59)

Data are presented as mean and (SD). **P=0.011 (comparison of occipital [Glu] between controls and patients); *p=0.035 (comparison of parietal [Cr + PCr] between controls and patients); $^{₮}$p < 0.001 (comparison of occipital versus parietal metabolites of control subjects); #p<0.02 (for comparison of occipital and parietal metabolites of patients)

concentrations of other occipital metabolites ([Cr+PCr], [NAA] and/or choline containing compounds) between the two groups. Since it has been documented that [Glu] in the brain is age-dependent [16] we also performed an one-way ANCOVA for [Glu] with age as a covariate including all subjects. This analysis demonstrated no age effect (p=0.92) but a significant group difference in [Glu] (p=0.02). As shown in **Table 5**, in the parietal cortex, the concentrations of [Cr+PCr] were lower in the patients compared to controls (p=0.035); and [NAA] also trended to be ~10% lower in the patients (p=0.09).

Proinflammatory markers, SUV_{max} of the lung mass and relation to brain metabolites

The proinflammatory cytokines TNF-α and IL-6

from the patients with malignancy were compared with the controls without cancer. The average [TNF-α] of all patients was within normal range reported in the literature (< 15 pg/ml, [17]) and not different from controls. The serum concentration of IL-6 ([IL-6], was very variable among the patients but the average [IL-6] was significantly higher compared to control subjects (7.24 ± 6.83 pg/ml versus 2.23 ± 2.68 pg/ml, p=0.038).

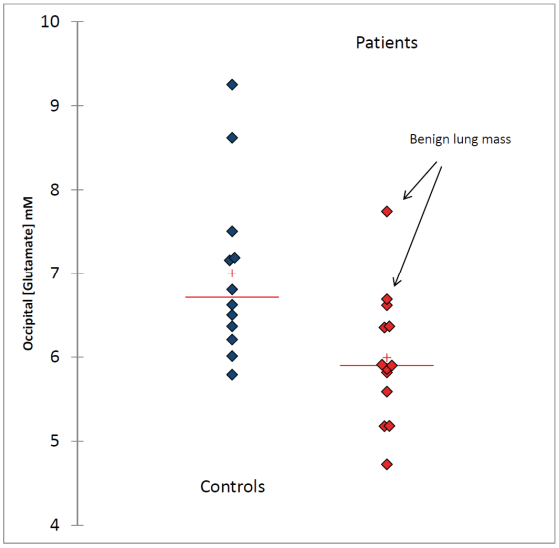

Figure 2. The scattergram shows occipital [Glu] from individual subjects in the control and patient groups and demonstrates that the mean concentration of [Glu] in the patients is lower compared to controls. The arrows point to two patients with the highest levels of [Glu] who were both found to have benign lung lesions.

A linear regression analysis revealed a trend (though not statistically significant) towards a positive relationship between [TNF-α] and SUV_{max} (R^2=0.299, p=0.066) and [IL-6] and SUV_{max} (R^2=0.24, p=0.12) suggesting that the greater the metabolic activity of the lung mass the greater the inflammatory response.

To examine the potential interaction between inflammatory markers and brain metabolites we performed a multiple regression analysis on [IL-6] and [TNF-α] of all subjects (both patients and controls) using all metabolites. A stepwise selection was used to select the significant metabolites in relation to the magnitude of the inflammatory response. Interestingly, in the occipital cortex, after stepwise selection a significant relation between [NAA] and [TNF-α] was found. The model derived was as follows: [TNF-α] = 15 - 1.5*[NAA] (p=0.036); indicating that an elevated [TNF-α] will decrease occipital [NAA] (**Figure 3**). No statistically significant relationship between inflammatory markers and parietal cortex metabolites were observed.

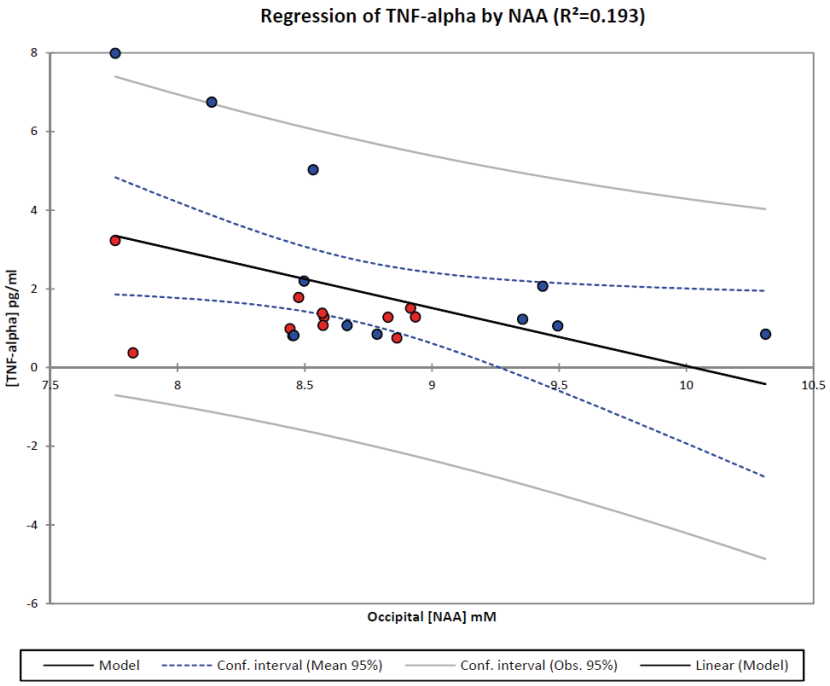

Figure 3. A multiple regression analysis was performed on [TNF-α] of all subjects (both patients and controls) using all metabolites in occipital cortex. A stepwise selection was used to select the significant metabolites in relation to the magnitude of the inflammatory response. After stepwise selection a significant relation between [NAA] and [TNF-α] was found. The model derived was as follows: [TNF-α] = 15 - 1.5*[NAA] (R^2=0.193; p=0.036); indicating that an elevated [TNF-α] will decrease occipital [NAA]. The patients and controls are marked by a red and blue circle, respectively.

Discussion

Our data provide evidence that the cerebral metabolic status of lung cancer patients is altered prior to treatment when compared to an age-matched control group. Specifically we showed that [Glu] in the occipital cortex of the lung cancer patients was approximately 10% lower than controls; even in patients without evidence of brain metastases. The lung cancer patients were also characterized by more fatigue and higher levels of IL-6 when compared to controls.

To our knowledge, the finding of cerebral metabolic status changes in lung cancer patients prior to treatment is novel and not previously documented. However, several prospective studies have documented the effects of chemotherapy or hormone treatment on cognition and revealed significant deterioration in neuropsychological test scores when compared to baseline performance in women with breast cancer [18] and in men with prostate cancer [19, 20]. Functional MRI studies have also supported evidence of cognitive dysfunction by demonstrating that treatment results in differences of task-related neural activation patterns in patients with prostate cancer in comparison to controls [20]. Further, women treated with tamoxifen to reduce the risk of breast cancer display changes in brain metabolites including choline containing compounds [21]. Measurements of the cerebral metabolic rate of glucose using ^{18}FDG PET have also shown that patients with breast cancer (but no metastases) can display abnormalities following treatment [22]. Another recent study demonstrated that 23% of patients with breast cancer exhibit cognitive impairment prior to treatment [23] suggesting that having a diagnosis of cancer affects brain function. In our patients the documented changes in occipital [Glu] were not associated with changes in cognitive performance in comparison to controls as evaluated by the brief battery of neurocognitive testing, possibly due to the fact that our groups were well matched at baseline in terms of estimated premorbid IQ (Wechsler Test of Adult Reading test done at screening). However, the lung cancer patients were characterized by higher fatigue scores which have also previously been demonstrated in cancer patients including lung cancer [24-27].

Spectroscopy studies using combined proton and ^{13}C-labeled precursors have shown that [Glu] is directly related to neuronal mitochondrial metabolism [neuronal tricarboxylic acid cycle (TCA) cycle rate (V_{TCAn})] in normal brain [28, 29]. Further, a decrease in V_{TCAn} in the elderly has been shown to correlate with decreases in [Glu] and [NAA], suggesting that mitochondria lose oxidative capacity with normal aging [10, 30, 31]. If one accepts, that [Glu] represents brain 'energy metabolism' and thereby indirectly brain function, the decrease in [Glu] observed in the lung cancer patients suggests that the presence of lung cancer itself reduces brain function prior to treatment. However, it is important to point out that the patients included in this study were heterogeneous with respect to their final lung mass diagnosis and included early as well as more advanced stages of lung cancer. Due to the small sample size it was not possible to correlate the cerebral metabolomic status specifically with tumor staging; and since our sample was dominated by early stage lung cancer patients it is likely that the overall impact of 'lung cancer' on [Glu] and potentially other brain metabolites may have been underestimated and might prove more significant in patients with more advanced disease. In support of this statement, **Figure 2** shows that the highest levels of [Glu] of the patient group belonged to two of the subjects with non-cancer. Future studies focused on characterizing a larger group of patients with lung cancer at various stages of progression will help address this issue.

It is important to also consider how the patient's other comorbidities might have interacted with metabolism and influenced [Glu]. For example, in contrast to controls a larger proportion of the lung cancer patients were diagnosed with COPD and it is possible therefore that this chronic condition is responsible for the change in [Glu] at baseline. However, none of the COPD patients were oxygen dependent and all had normal hematocrit and oxygen saturation at baseline. Further, a recent ^{1}HMRS study on oxygen-dependent and oxygen-independent COPD patients did not reveal metabolic differences in the brain when compared to controls [32], supporting our main hypothesis that the metabolic changes we observed in the cancer patients are caused by the cancer and not by COPD. Nonetheless, to further evaluate our preliminary findings and ascertain their independence of comorbidities such as COPD, it will be essential to enlarge our sample size and also include other

cancer types.

Previous quantitative ^{1}HMRS studies of the normal human brain have documented a heterogeneous distribution of [NAA] and [GPC+PCh] in the brain with higher [NAA] in the occipital cortex when compared to the parietal and frontal cortices; and higher [GPC+PCh] in the parietal compared to occipital cortex [33]. In contrast, [Glu] in grey matter is not region-dependent at least in normal, young human brain [33]. In our study, the quantitative profile of metabolites of control subjects also revealed higher concentrations of choline-containing compounds in the parietal compared to the occipital cortex (**Table 5**); but we did not observe higher [NAA] in the occipital compared to the parietal cortex as previously reported, which might be related to age-differences of the two control populations [33]. We also did not observe a [Glu] decrease in the parietal cortex of the patient group in comparison to controls, however, [NAA] trended to be lower in the patients ($p=0.09$). The relatively small sample size prohibits further interpretation and conclusions as to whether the effect of 'lung mass' or lung cancer exert a region-specific or global effect on cerebral metabolic status.

The measurements of proinflammatory cytokines revealed higher [IL-6] in the patients with a malignant lung mass compared to controls which is in agreement with previous reports [17, 34]. The normal range of [IL-6] in human serum is reported in the literature to be <15-20pg/ml [34]; and has been shown to increase in patients with lung cancer, although the increase varies greatly and is also dependent on tumor type and stage [17, 34, 35]. In our study the patient's average [IL-6] was still within the reported normal range probably because the majority of the patients were sampled at an early diagnostic stage (i.e. stage I-II). In the patient group, [TNF-α] was also within normal range and no different from controls; which also indicates the early stage of the cancer. When exploring the potential relation between the metabolic activity of the lung mass as evaluated by the SUV_{max} and inflammation, we found a positive association ($p=0.066$) between [TNF-α] and SUV_{max}. This finding indirectly supports previous data reporting that 1) TNF-α and other proinflammatory cytokines are produced locally in lung cancers [35, 36] and 2) the metabolic activity of the lung cancer is related to proliferative tumor activity [37] and increased tumor cell glycolysis secondary to local hypoxia [38].

It was intriguing that [TNF-α] was found to be negatively associated with [NAA] in the occipital cortex suggesting that 'inflammation'(regardless of cancer state) influences cerebral metabolic status, in agreement with previous reports. Thus, proinflammatory cytokines can cause cognitive dysfunction [39-41]; and elevations of cytokines have been found to co-occur with a reduction of the metabolic rate of glucose utilization in the brain [42-44].

Limitations of the study

The major limitation of the current study is the small sample size and as such the data presented are preliminary. The enrollment of patients for the study was difficult due to the often urgent need for extensive clinical work-up and the patient's emotional stress associated with the diagnosis and imminent need for surgery. The ability to perform ^{1}HMRS in conjunction with ^{18}FDG-PET would be ideal for this patient population since the study protocols could be carried out with less of a time-burden for the patient. This approach may be possible in the future with implementation of a combined MRI-PET imaging modality the clinical arena. It would also be important to compare ^{1}HMRS results with corresponding regional cerebral metabolic rate of glucose data in order to obtain more accurate spatial information. The latter will enable a better understanding of the neuronal networks involved in cerebral effects associated with cancer.

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