EGFR driving mutation in non-small-cell lung cancer influences the incidence and characteristics of related brain metastases

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Case Report

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Abstract

Background: Brain metastases (BMs) are the most common intracranial tumors in adults and the brain is one of the most frequent metastatic sites for non-small-cell lung cancer (NSCLC). Some studies have hypothesized that EGFR expression in the primary tumor may result in clinically radiological and prognostic changes in related brain metastases, comparable to EGFR's correlation with prognosis in primary brain tumors. There are no current studies that clinically and radiologically demonstrate a difference between BMs from EGFR-mutated and wild-type NSCLC.

Methods: We performed a retrospective study to identify prognostic factors for the survival of patients with NSCLC-BMs by exploring the role of driving mutations in NSCLC, focusing on EGFR mutated status, and comparing all morphological, radiological, and clinical features of NSCLC-BMs with their outcome parameters in a cohort of surgically treated patients.

Results: The final cohort consisted of 81 patients. The overall survival of the cohort was 15±17 months. The tumor-related edema was associated with neurological symptoms at the clinical onset of the disease (p=.048). We found that EGFR and ALK mutation status did not reach significant associations with age, sex, and the morphology of the lesions. EGFR mutation in the primary tumor is positively associated with higher edema and tumor volume (respectively 22.38±21.35cm3 versus 7.68±8.44cm3 and 72.44±60.71cm3 versus 31.92cm3 p=.046 and p=.028). Moreover, EGFR mutation is associated with a clinical debut of seizures (p=.004).

Conclusions: The role of EGFR and ALK mutations of NSCLC on prognostic characteristics of BMs is still to be fully clarified; however the results retrieved from the present study suggest that the presence of EGFR mutations significantly correlates with bigger edema volume and higher incidence of seizures. In recent studies, while EGFR mutation status did not affect the immune pathway scores of primary lung tumors, the overall immune pathway scores in related BMs indicate a peculiar immunogenic phenotype that could explain a large amount of edema volume. This study could be considered the first clinical and radiological demonstration of this immunological phenotype.

Introduction

Brain metastases (BMs) are the most common intracranial tumors in adults and are an important cause of poor outcomes; BMs develop in approximately 5–30% [1–5] of advanced non-small-cell lung cancer (NSCLC) patients at initial diagnosis and about 40–50% of NSCLC patients develop them during the course of the disease [6, 7] with a significant negative impact on their quality of life (QoL) and prognosis [8]. Although the brain is one of the most frequent metastatic sites for lung cancer, there are few treatment options [9–12].

Today, clinical research focuses on discovering novel molecular features of NSCLC to identify possible biological targets to facilitate early diagnosis, prognosis prediction, and drug treatment [13]. More specifically, the understanding of NSCLC has been transformed with the identification of molecular

subtypes of some important oncogenic drivers, such as epidermal growth factor receptor (EGFR), anaplastic lymphokinase (ALK), and PD-L1 [14]. Indeed, it has been observed that the incidence of BM is higher in patients with ALK fusions [15–17] and EGFR mutation [15–18]. Moreover, it was recently argued that EGFR positivity or ALK-1 rearrangements (which are mutually exclusive in their expression) are associated with different prognoses in terms of OS and OR [19–21]. Appropriate EGFR gene mutation testing is essential in selecting NSCLC patients for therapy with tyrosine kinase inhibitors of EGFR (TKIs EGFR). TKIs EGFR constitutes effective first- and subsequent-line management in advanced NSCLC patients with EGFR mutations [22].

Some studies [23–25] have hypothesized that EGFR expression in the primary tumor may result in clinically radiological and prognostic changes in related brain metastases, comparable to EGFR correlates with prognosis in primary brain tumors.

To our knowledge, However, There are currently no studies that clinically and radiologically demonstrate a difference between brain metastases from EGFR-mutated and wild-type NSCLC.

In the present study, we retrospectively reviewed a consecutive series of patients who underwent surgery to resection BMs from NSCLC between January 2015 and January 2019 in the department of Neurosurgery of Policlinico Umberto I of Rome and Hospital Molinette of Turin.

We performed a retrospective study to identify prognostic factors for the survival of patients with NSCLC brain metastases by exploring the role of driver mutations in NSCLC, focusing on EGFR mutated status, and comparing all morphological, radiological, and clinical features of NSCLC brain metastases with their survival parameters in a cohort of surgically treated patients. We measured the relationship between lesion volume and edema volume measured on FLAIR sequences, impacting prognosis and recurrence as a secondary objective.

Methods

We performed a retrospective study on surgically treated patients affected by BMs from diagnosed NSCLC. The patients were considered eligible for surgical treatment if they had a good preoperative performance status measured before surgery (Karnopsky performance status, KPS >50) and estimated overall survival of >3 months (according to the radiation therapy oncology group and the grade prognostic assessment rankings) [26,27]. The estimated target of the surgical procedure was the gross-total, near-total- or subtotal resection of the lesions. The molecular analysis of EGFR and ALK mutations was carried out on the brain lesion. Patients were included if, in the postoperative period, they could undergo an adjuvant chemo-radiotherapy and a follow-up program. We excluded all cases with incomplete or incorrect data on clinical, radiological, surgical, and follow-up records.

All patients underwent a general medical, neurological, and oncologic evaluation at admission. For all the included patients, we recorded patient-related variables such as sex, age, peri and post-operative KPS, clinical presentation, survival, antiepileptic prophylaxis and treatment, the incidence of postoperative

seizures, and tumor- and surgery-related variables: number, location, and side of the lesions, tumor and edema volume, morphology, onset about the primary tumor and molecular profile (EGFR, ALK, and PD-L1). In particular, the specimens used in this study were examined for EGFR and ALK mutations. Immunohistochemistry with CDX-2, CK7, CK20, TTF-1 and Napsin-A expression was routinely performed in the Department of Neuropathology of our University Hospitals. All patients were submitted to a standardized and shared preoperative and operative Protocol reported in all studies referred to intracranial tumors [28-32].

Overall Survival (OS) was recorded in months; it was measured from the date of diagnosis to the date of death or the date of the last contact if alive. Clinical information was obtained by the respective institutions' digital database, whereas telephone interviews obtained OS data. A particular focus was centered on the performance status expressed as KPS results in a dichotomy data (> and < 70): such parameter was considered, as previously observed, as associated with Survival [28] to the presentation of BM [33] and in general as a protective factor when >70 [34-36]. In particular, it was recorded in three different moments:

- Before surgery at the time of diagnosis

- At 30 days after surgery (early post-operative evaluation and

- At the end of the adjuvant treatment (the moment of the last outpatient evaluation).

Data sources and Quantitative variables

The extent of resection (EOR) was determined by comparing the MR images obtained before surgery and the first early MRI after surgery, following RANO criteria. EOR was coded in a 3-step ordinal variable as reported elsewhere [11]: Gross-Total Resection (GTR) <2 mm3 residual lesions; Near-Total Resection (NTR) (≧2 to <5 mm3), and Subtotal Resection (STR) (≧5 mm3).

In the case of GTR, “tumor progression” was defined as the first MRI scan demonstrating the presence of pathologically enhancing tissue characterized by an MRI pattern (mainly relying on Perfusion Weighted Imaging) inconsistent with a cerebral radiation injury (which is, in fact, a “pseudo-progression”). In incomplete resections (NTR/STR), a volumetric increase of the residual disease detected at the first postoperative MRI scan was considered disease progression. A close-range dedicated neuro-imaging follow-up program was routinely performed in our Institutions [37]. This program included:

A standard early (maximum 24 hours after surgery) postoperative volumetric brain MRI.

At approximately one month from surgery (25-35 days), a volumetric brain MRI scan was repeated for a first step follow-up control and information for the radiation treatment planning. A volumetric brain MRI scan was performed every three months at the end of irradiation. We performed a complete outpatient clinical and neurological outpatient re-evaluation at every radiological reevaluation.

Size, statistics, and a potential source of Bias

The study size is given by the selection of the inclusion criteria. As previously stated, we addressed no missing data because incomplete records were an exclusion criterion. The sample was analyzed with SPSS v18 (SPSS Inc., Released 2009, PASW Statistics for Windows, Version 18.0, Chicago, Illinois, USA) to outline potential correlations between the investigated variables. Comparisons between nominal variables have been made with the Chi2 test. EOR, OS, and PFS mean, edema, and lesions volume, and their correlations with ALK and EGFR mutations were compared with One Way and Multivariate ANOVA analysis and Contrast analysis and Post-Hoc Tests. Kaplan-Meier survival analysis assessed survival. Continuous variables correlations have been investigated with Pearson's Bivariate correlation. The threshold of statistical significance was considered $p<.05$. It is expected that when the underlying pathology is known and the BM is diagnosed based on radiological follow-up, the Pathological Anatomy laboratory does not carry out the molecular analysis of EGFR and ALK again to have a substantial loss of data. To avoid methodological biases, we, therefore, have to consider them as missing data rather than insert the molecular data found from other sources (e.g., the molecular data on the primary tumor)

Ethical issue

The Institutional Review Board approved the informed consent of our Institution. Before the surgical procedure, all the patients gave informed, written explicit consent after appropriate information. Data reported in the study have been completely anonymized. No treatment randomization has been performed. This study is perfectly consistent with the Helsinki Declaration of Human Rights in Medical Research.

Results

In a period ranging from January 2015 to January 2019, 81 patients suffering from NSCLC brain metastases have been operated on in our Neurosurgical Departments. 27 patients were female (33.3%), and 54 were male (66.7%), with a 1:2 ratio. The average age of the cohort was 62.1 years ± 10.9. In this cohort, brain metastasis favored frontal (32 patients, 39.5%) and cerebellar (18 patients, 22.2%) localization; in general, the lesions were more commonly found in the supratentorial compartment (77.8%). This data is statistically significant for frontal involvement ($p<.001$). Thirty-six patients had a right lesion, 43 left, while just 2 involved the midline. No statistically significant preference of side has been outlined.

The diagnosis and clinical presentation were respectively more commonly synchronous (60.5%) rather than metachronous and with sensory-motor dysfunction (41.9%) or with seizures (27.2%). In 59 cases (72.8%), a GTR was achieved. A total of 67 patients presented a preoperative KPS over 70 before surgery, whereas 73 had the same performance status at the 30th postoperative day reevaluation ($p=.001$). The overall survival of the cohort was 15±17 months (data reassumed in **Table 1**).

Table 1

Patient's demographics

	N=81 patients	
Sex	Male N=54 – 66.7%	
	Female N=27 – 33.3%	
Age	62,1 years± 10.9	
KPS at admission	> 70 = 67 - 82.7%	
	< 70 = 14 - 17.3%	
KPS after surgery (30d)	> 70 = 73 - 90,1%	
	< 70 = 9 - 11.1%	
KPS at last Evaluation	> 70 = 49 - 60.5%	
	< 70 = 32 - 39.5%	
Dead 68/81 pts at 09/20	48 dead	
	20 alive	
Overall Survival	15 ± 17 months	
Volume ($cm^{3)}$	14.62 ± 18.5	
Edema Volume ($cm^{3)}$	54.21 ± 45.76	
Periventricular	11 pts - 15.1 %	
Location	Supratentorial = 63 - 77.8%	
	Subtentorial = 18 - 22.2%	
Major Lobe involved	Frontal	32 (39.5%)
	Temporal	5 (6.1%)
	Occipital	10 (12.34%)
	Parietal	16 (19.75%)
	Cerebellar	18 (22.22%)
Side	Left	43 (53.1%)
	Right	36 (44.4 %)
	Midline	2 (2.47 %)
Symptoms at onset	Seizures	22 (27.16 %)
	Sensory-Motor Dysfunction	34 (41.9 %)
	Asymptomatic (follow-up)	25 (30.8 %)

Antiepileptic Profilaxis and Treatment	43 pts (53.1%)
Post-operative Seizure	25 pts (30.86%)
Surgical Resection	GTR = 59 (72.84%)
	STR = 22 (27.16%)
Morphology of Tumors	Solid = 50 (61.73%)
	Cystic = 16 (19.75%)
	Hemorragic = 8 (9.87%)
	Mixed = 7 (8.6%)
Onset	Synchronous = 49 pts (60.5%)
	Metachronous = 32 pts (39.5%)
Immunohystochemical/ molecular features.	
EGFR mutation	Expressed = 56,25%
	Not expressed = 43,75%
ALK mutation	Expressed = 17%
	Not expressed = 83%
PDL-1 mutation	Expressed = 54%
	Not Expressed = 46%

PFS: Progression Free Survival**; OS**: Overall Survival; **SVZ:** Subventricular Zone, **KPS:** Karnofsky performance status, **EOR:** Extent of Resection, **GTR:** Gross Total Resection, **NTR/STR:** Near Total/Subtotal Resection.

On radiological analysis of data, we confirm that tumor volume and edema demonstrated a significant reciprocal association (r=.369, p=.010), and the more significant edema was associated with the supratentorial locations (p=.034, Fig 1a). The average volume of the lesions and perilesional edema were respectively 14.62 ± 18.5 cm3 and 54.21 ± 45.76 cm3. The tumor morphology was solid primarily, compact (61.7%), whereas BMs presented as cystic lesions in 19.7% of cases.

The tumor-related edema demonstrated a significant association with the neurological symptoms at the beginning of the disease (p=.048) rather than with the lesion volume per se (p=0.89), indicating that the tumor-associated edema is more commonly responsible for the neurological symptoms rather than a greater tumor volume than itself. Moreover, a greater tumor volume was associated with a higher incidence of complications (p=0.03, Fig 1b), which, in turn, was also associated with significantly shorter survival (p=.018 Fig 1c). This finding is exciting when observing, on a multivariate ANOVA analysis, that complications, per se, negatively affect survival, independent of the tumor volume (p=.002, Fig 1d). Furthermore, on a Repeated measures ANOVA analysis, edema was demonstrated to play a statistically

significant role (p=.049, Fig 2a) in impacting the early postoperative period: patients with an edema volume greater than 30cm^3 proved to have a poorer 30th postoperative day KPS when compared to the preoperative and late follow up functional status.

We focused our study on the role of the main driving mutation expressed in our series. We found that EGFR and ALK mutation status did not reach significant associations with age, sex, the morphology of the lesions, and total volume of the lesions.

ALK mutation expressed in primary tumors demonstrated an independent association with survival parameters: the cumulative survival of patients presenting an ALK mutation was 30.0±18.36 months compared to 12.88±8.31 months of those showing a wild-type ALK phenotype (p=.015 Fig 2c). These data should be interpreted cautiously since ALK mutation is associated with better survival only in patients harboring smaller lesions, possibly with smaller edema volumes. In more significant lesions (>10 cm3) determining a more significant lesion-associated edema (>30cm3), the survival advantage disappears (Fig 2d).

Interestingly, EGFR mutation in the primary tumor is positively associated with higher edema and tumor volume in BM (respectively 22.38±21.35cm3 versus 7.68±8.44cm3 and 72.44±60.71cm3 versus 31.92cm3 p=.046 and p=.028 Fig 2b). Moreover, EGFR mutation expressed in brain-metastatic NSCLC is associated with a clinical debut of seizures (p=.004).

Discussion

In recent decades, the prognosis of patients with BM's from NSCLC has not changed [1,2, 38-42]. Factors that are independently associated with a poor prognosis for patients with BM include age, poor performance status, lymph node involvement, and increasing primary tumor size, and lymphovascular space invasion [1,43,44]. If treatment protocols in NSCLCs have been radically changed by discovering molecular targets such as ALK and EGFR and the subsequent development of TKI, the same is not be said for patients affected by BMs where is not fully understood their role in the dissemination and because this group of patients is usually excluded from clinical studies, especially when neurologically symptomatic [8,45,46].

In the present series, several factors (functional status, general health conditions, morphological and histological features of the lesions, prognostic indices) have been investigated to analyze their association with the risk of death at 12 weeks and one year and among these. We confirmed that only the KPS score (> 70%) and a time-reasonable RT application appear to be strongly significant protective factors since it is typical and shared by all intracranial malignant pathologies [29-32].

To date, the current treatment option for BMs is represented by RT (or radiosurgery), or microsurgery followed by RT, since this approach resulted in being more efficient than chemotherapy alone [20,47]. Treatment of both the primary site and the BMs are not contraindicated solely by a single BM, and complete resection of all diseases should be attempted whenever safe and feasible [48]. In fact, given the

encouraging results in terms of survival, primary tumor resection and treatment (neurosurgical intervention or irradiation) for synchronous lung and brain lesions appear to be justified [14,41,34-36,40,49]. Most single BMs are manageable to total resection, performed on 72,84% of the patients with low mortality and morbidity rates, in line with data reported in the literature [48]. Nevertheless, the surgical indication in debilitated patients with advanced systemic disease should be carefully considered because the morbidity and deaths in our study were primarily due to systemic and infectious complications. Since prophylactic cranial irradiation has been abandoned for important series of complications such as cognitive disorders and neurological deficits in affected patients [50], accurate approaches to the identification of lung cancer patients with an increased risk of developing BMs are required for more effective individualized and, eventually, preventive therapy. In this scenario, the role of EGFR and ALK could be relevant in the immediate future. Recent findings suggest that driver mutations in NSCLC, at least in part, would be associated with the development of BMs in NSCLC [51]. Similar to our results, different studies demonstrate the high initial incidence (nearly 25%) and subsequent prevalence of BM in patients with advanced EGFR-mutated NSCLCs treated with TKIs [52-54]. Further, patients with NSCLC who present with BM's have varied treatment responses, and these seem to be affected by the molecular alterations that characterize the primitive tumor. Patients with these characteristics have a significantly longer PFS [24,43,55]. Our results did not show that response to radiotherapy varies as per tumor molecular status, but further clinical studies on more extensive series are required.

The most exciting finding in our study on EGFR expression concerns its significant positive correlation between metastatic intracranial lesion characteristics and related edema. It appears that patients with BM from EGFR-mutated NSCLC have more edemigenous lesions at diagnosis. Clinical onset also seems to be more correlated with seizures.

Suppose in literature the presence of the EGFR-mutation in BM has been described as a possible prognostic factor [55]. In that case, the correlation between significant volumes of brain edema and cerebral lesions is still to be clarified. Based on our result, we suppose that it may depend on the different abilities of BM with mutated EGFR to induce changes in the peri-tumoral microenvironment.

In a recent study by Song SG et al. [56] on the immunophenotypic expression of BMs from NSCLC, it was found that, while EGFR mutation status did not affect the immune pathway scores of primary lung tumors, the overall immune pathway scores in related BMs were higher in the EGFR-mutated cases, indicating a peculiar immunogenic phenotype. Therefore, it was hypothesised that the effect of EGFR mutation on the tumor immune-microenvironment may vary among organs, and the EGFR mutation of a tumor might shape the tumor immune-microenvironment of metastases. In another study [23] that investigated the immunologic landscape of primary lung cancer and related BM to assess the effect of tumor-involved organs and EGFR mutation status on the tumor immune-microenvironment, it was found that contrary of what happened in the primary tumor, BM with an expression of EGFR there is an increase of macrophages infiltration around the lesion with a reduced infiltration by T cells [23]. BMs showed an immunosuppressive phenotype in terms of immune-related pathways, and the composition of immune cell infiltrates with a large part of macrophagic infiltration could explain a large amount of edema

volume. This study could be considered the first clinical and radiological demonstration of this immunological phenotype.

BMs from NSCLC had a unique immune cellular component and gene expression, with immunotherapeutic implications. The differences in the immune reaction of BMs from NSCLC according to EGFR mutation status could be considered when treating patients with EGFR-mutated lung cancer with BMs [23]. Still, new and different genetic alterations would be considered during their characterization. Previous studies, indeed, comparing primary lung cancer and BM and if they showed good agreement in terms of mutations of major oncogenic drivers and different copy number alterations of key genes, it was found [57]. Finally, as described in the Results section, this study outlines how larger volumes of edema are associated with a higher incidence of neurological symptoms such as seizure. It might be reasonable to explain why BMs with positivity for EGFR mutations are more likely to develop more extensive lesions and earlier neurological symptoms.

Study limitations and further study

The main limitation of the present paper consists of its retrospective nature. Moreover, the current retrospective investigation was conducted on a selected subset of BMs patients, who met the surgical indication criteria and who had relatively good functional status. Therefore the generalisability could probably be affected when extending the conclusions to all the patients suffering from BMs from NSCLC. Nevertheless, the reported findings are supported by statistically robust results, thus providing exciting clues concerning the potential role of EGFR in the further stratification of such particular patients.

Conclusion

BM are present in 10% of non-small cell lung cancer (NSCLC) patients at diagnosis, and this number is expected to increase with the standardization of brain imaging in asymptomatic patients, particularly in those with specific molecular alterations, such as EGFR mutations or ALK rearrangements. According to the result of the present study, the presence of EGFR-mutated NSCLC seems to correlate with more enormous edema volumes of the lesion when metastasizing to the brain and, consequentially, with a higher incidence of seizures. Brain metastases from EGFR-mutated NSCLC are significantly more edemigenous than wild-type forms, demonstrating clinically that EGFR mutated in NSCLC can modify the tumor microenvironment in metastasis. Given the dense scientific debate on the role of EGFR mutation in NSCLC, aimed studies on BMs derived from this specific family of lung cancer should be carried out to explore their impact on diagnosis and treatment prognosis.

Abbreviations

Brain Metastases (BMs), Non-small Cell Lung Cancer (NSCLC), Epidermal Growth Factor Receptor (EGFR), Anaplastic Lymphoma Kinase (ALK), Tyrosine Kinase Inhibitors (TKI), Overall Survival (OS), Quality of Life (QoL), Whole-Brain Radiotherapy (WBRT), Gross-Total Resection (GTR), Near-Total Resection (NTR),

Subtotal Resection (STR) Fluid Attenuated Inversion Recovery (FLAIR), Isotropic Volumetric T1-weighted Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE)

Declarations

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Ethical approval:

All procedures performed in studies involving human participants were by the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any authors.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

The patient has consented to submitting this review article to the journal.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that all have approved the order of authors listed in the manuscript of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work. There are no impediments to publication, including the timing of publication, concerning intellectual property. In so doing, we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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Research Data Policy and Data Availability Statements:

datasets are not deposited in publicly available repositories. The database from Sapienza will be uploaded on repository IRIS, Sherpa-Romeo.

Author Contributions

Alessandro Pesce, Daniele Armocida: conceived of the presented idea, developed the theory, and performed the computations.

Mauro Palmieri, Giuseppe Di Perna, Giuseppe Palmieri: developed the theoretical formalism, performed the analytic calculations, and performed the numerical simulations.

Fabio Cofano, and Sara Mantovani: contributed to the interpretation of the results.

Paola Cassoni, Manila Antonelli: verified the analytical methods

Maurizio Salvati, Giancarlo D'Andrea: contributed to sample preparation

Marco Anile, Antonio Santoro, Diego Garbossa, Alessandro Frati: supervised the project.

All authors discussed the results and commented on the manuscript.

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Figures

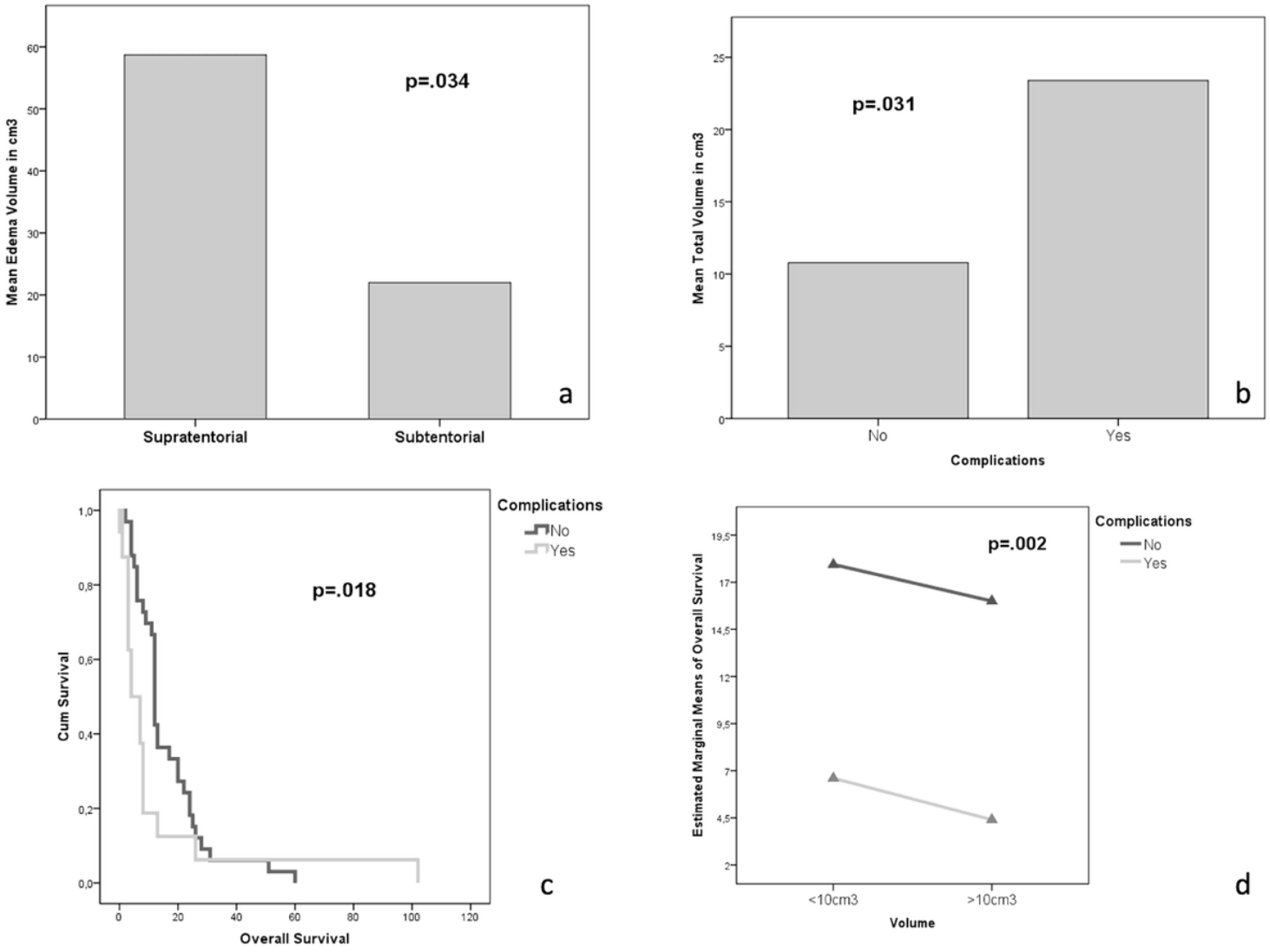

Figure 1

a. One Way ANOVA analysis demonstrating the association between edema volume and the intracranial compartment. **b**. One Way ANOVA analysis demonstrating the association between total volume and the incidence of complications. **c.** Kaplan-Meier survival curve demonstrating the impact of complications on

survival. **d.** Multivariate ANOVA analysis demonstrating the association between complications and survival independently from the volume of the lesion

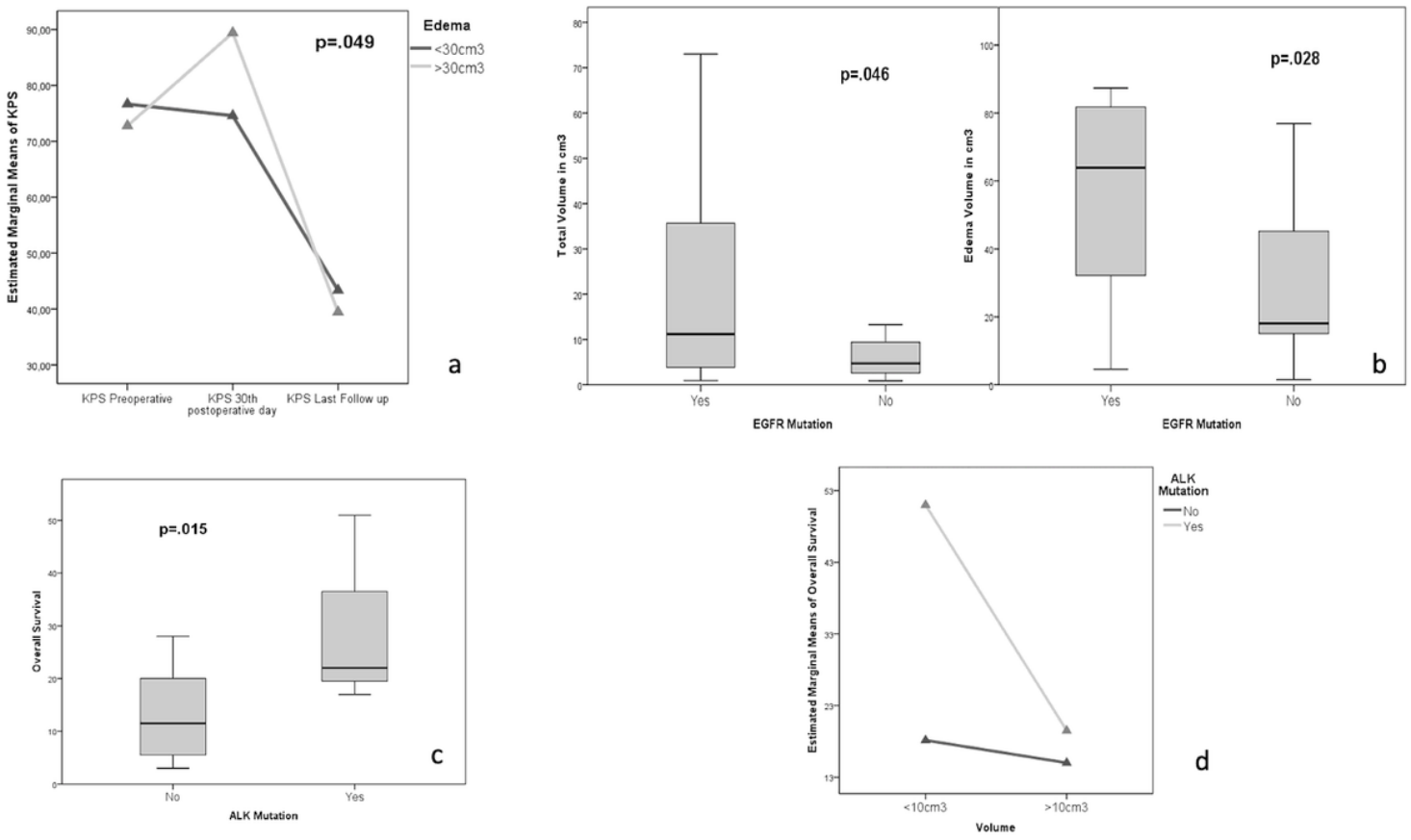

Figure 2

a. Repeated Measures ANOVA analysis demonstrating the impact of edema on the functional status **b.** One Way ANOVA analysis demonstrating the association between EGFR mutation status and the lesion and edema volumes. **c.** One Way ANOVA analysis demonstrating the association between ALK mutation status and Overall Survival. **d.** Multivariate ANOVA analysis demonstrating the association between ALK mutation status and survival in respect to the volume of the lesion: the survival advantage disappears for the greater lesions.