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METABOLIC TUMOR VOLUME OR TOTAL LESION GLYCOLYSIS QUANTIFIED ON [^{18}F]FDG PET/CT. WHICH HAS MORE PROGNOSTIC VALUE IN NON-SMALL CELL LUNG CANCER PATIENTS?

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THESIS OUTLINE

This Master thesis in Medicine consists of an Original Scientific Article, written between September 2018 and October 2019, with bibliographic research until March 2019.

This article was written with the goal of submission to the European Journal of Nuclear Medicine. Therefore, the instructions for the authors of this journal were followed and are presented in Appendix 1.

RESUMO

INTRODUÇÃO

O carcinoma do pulmão é a maior causa de mortalidade relacionada com cancro a nível mundial, sendo o carcinoma do pulmão de não pequenas células (CPNPC) o tipo histológico mais frequente. A tomografia por emissão de positrões com tomografia computarizada com 18-fluor-2-deoxi-D-glucose (PET/CT [^{18}F]FDG) é considerada uma ferramenta fundamental para o diagnóstico, estadiamento, planeamento terapêutico e avaliação de resposta terapêutica no CPNPC. A PET/CT [^{18}F]FDG pode ser interpretada segundo parâmetros quantitativos tais como volume metabólico tumoral (MATV) e glicólise total da lesão (TLG). O MATV é obtido somando os volumes de todas as lesões metabolicamente ativas na PET/CT [^{18}F]FDG. O TLG resulta do produto do MATV com o grau de captação do [^{18}F]FDG, refletindo a carga tumoral total. Esta definição sugere que o TLG poderá ser mais sensível na predição da sobrevivência global em doentes com CPNPC.

OBJETIVO

Comparar o valor prognóstico dos parâmetros MATV e TLG em doentes com CPNPC, para melhor estadiar estes doentes.

MÉTODOS E RESULTADOS

Fez-se o estadiamento de 334 doentes com CPNPC, sem metástases cerebrais ou história de outras neoplasias, entre janeiro de 2011 e agosto de 2018, de acordo com a oitava edição do estadiamento TNM para CPNPC. O estudo incluiu 92 (27.5%) mulheres e 242 (72.5%) homens, com idades entre os 33 e os 38 anos (média 66,16 ± 10,21 anos), que foram avaliados retrospetivamente. O MATV e o TLG foram quantificados, com recurso à PET/CT [^{18}F]FDG. O tempo de sobrevivência foi analisado pelo método de Kaplan-Meier, usando o teste do Log-Rank quando apropriado, tendo a regressão de Cox sido aplicada para identificar preditores daquele e avaliado o C Index dos marcadores de prognóstico. Os testes foram avaliados ao nível de significância de 5%. Foram calculados intervalos de confiança a 95% após *bootstrapping*.

Os doentes foram seguidos entre 0,50 e 98,3 meses (média 42,40 $\pm$ 2,27 meses e mediana 22,63 $\pm$ 2,61). A sobrevivência global ao ano e aos cinco anos foi, respetivamente, 97,0% e 85,4%, com uma taxa de mortalidade global de 84,6%.

Os resultados revelaram que quer o MATV quer o TLG, ajustados a idade do doente, conjuntamente com o estadiamento TNM, têm um valor prognóstico elevado, e que o valor prognóstico do TLG nunca é inferior ao do MATV.

CONCLUSÃO

O MATV e o TLG são ambos fortes preditores de sobrevivência global nos doentes com CPNPC. Sendo que o TLG, por definição, faz mais sentido em termos clínicos, prova-se que o seu valor prognóstico não é inferior ao do MATV.

Palavras-chave: [^{18}F]FDG PET/CT; quantificação; carga tumoral; valor prognóstico; cancro do pulmão

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ABREVIATIONS

NSCLC non-small cell lung cancer

TNM tumor, nodes, metastases

[^{18}F]FDG PET/CT Positron Emission Tomography/Computed Tomography (PET/CT) with ^{18}F-labeled 2-deoxy-D-glucose ([^{18}F]FDG)

SUV standardized uptake value

MATVwb whole-body metabolic active tumor volume

TLGwb whole-body total lesion glycolysis

HR Hazard Ratio

ABSTRACT

PURPOSE

To compare the prognostic value of the parameters whole-body metabolic active tumor volume (MATVwb) and whole-body total lesion glycolysis (TLGw) in non-small cell lung cancer (NSCLC) patients, to allow their further staging stratification.

METHODS

Initial TNM staging of 334 NSCLC patients was performed, in patients without brain metastases or history of other malignancies, recruited between January/2011 and August/2018, staged according to the eighth edition of TNM staging system for NSCLC, in similarity with the previous article by *Lapa et al*. [1]. The study included 92 (27.5%) women and 242 (72.5%) men, aged between 33 and 88 years (66.16 ± 10.21), who were retrospectively evaluated. MATVwb and TLGwb were quantified using [^{18}F]FDG PET/CT.

Survival time was analyzed through the Kaplan-Meier method, applying the log-rank test whenever appropriate, and the Cox regression was applied in order to identify predictors of overall survival. The C Index was obtained for identified predictors. Statistical tests were evaluated at a 5% significance level and 95% confidence intervals were obtained after bootstrapping.

RESULTS

Patients' follow-up time ranged between 0.50 and 98.3 months (mean 42.40 $\pm$ 2.27, median 22.63 $\pm$ 2.61 months). The one and five-years survival rate were 97.0% and 85.4%, with an overall mortality rate of 84.6%.

The results revealed that either MATVwb or TLGwb, adjusted for patients age, along with stage or sub-stage of the disease have a high prognostic value on patient´s overall survival time. Furthermore, TLGwb does not have an inferior prognostic value than MATVwb.

CONCLUSION

MATVwb and TLGwb are strong predictors of overall survival in NSCLC patients. Moreover, having the TLGwb more sensibility, by definition, it always provides at least the same prognosis value as MATVwb thus, its use is more accurate, in a clinical perspective.

KEY WORDS: [^{18}F]FDG PET/CT; quantification; tumor burden; prognostic value; lung cancer

INTRODUCTION

Lung cancer remains the leading cause of cancer mortality worldwide, representing close to 1 in each 5 cancer deaths (18.4%) [2]. The main histological type is non-small cell lung cancer (NSCLC), comprising 80-85% of all histological types among lung cancer patients. The prognosis of NSCLC is generally poor, with a five-year overall survival rate that goes from 73% (stage IA) to 13% (stage IV) [3].

The American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) have developed the TNM (tumor, nodes, metastases) classification system for NSCLC patients staging. The TNM system is used to choose the best treatment and to predict the prognosis. Additional prognostic factors influencing treatment decisions include age, gender, previous treatments and tumor histology, but the clinical TNM (cTNM) is still considered the most important prognostic factor [4, 5].

The recently revised eighth edition of the TNM staging system for NSCLC (January/2017) defines new T and M descriptors and updates stage groupings, in the effort of improving prognostic accuracy [6]. However, even with the new revised edition, the TNM system does not contain volumetric tumor burden information [4]. Therefore, despite the efforts in improving prognostic value, much remains to be done [7-9].

Positron Emission Tomography/Computed Tomography (PET/CT) with ^{18}F-labeled 2-deoxy-D-glucose ([^{18}F]FDG) combines metabolic and morphologic data, and is an extremely useful tool in diagnosis, initial staging, restaging, recurrence, and treatment response assessment in NSCLC [10, 11].

Currently, in clinical practice, [^{18}F]FDG PET/CT is interpreted mostly by a qualitative (visual) image analysis to serve the purposes of the cTNM staging. However, to get a better prognosis prediction, some quantification parameters may be useful [12, 13].

The standardized uptake value (SUV) is the most commonly used parameter. Maximum SUV (SUV_{max}) represents the voxel with the maximum [^{18}F]FDG uptake in the region of interest (ROI) [7, 14]. This value reflects metabolic grade and lesion aggressiveness [15, 16]. However, many studies have already suggested that whole-body metabolic active tumor volume (MATVwb) and whole-body total lesion glycolysis (TLGwb) have better prognostic value than SUV_{max} in NSCLC patients, independent of tumor stage, with low inter-observer variability [8, 14, 17-20].

The volume-based parameters, MATVwb and TLGwb, reflect tumor aggressiveness with more accuracy [21, 22]. MATVwb, measured in cm^3, obtained through the some of the volumes of all metabolically active lesions (primary tumor and metastatic lesions), reflects the metabolically active tumor mass. TLGwb (the product SUV_{mean} x MATVwb) relates the intensity of [^{18}F]FDG uptake to the metabolically active tumor volume, reflecting the tumor burden. This

definition suggests it might be more sensitive in the prediction of life expectancy in NCSLC patients. As already demonstrated in the previous study by *Lapa et. al*, MATVwb quantified on initial staging [^{18}F]FDG PET/CT is an independent, statistically significant predictor of overall survival [1].

We aim to compare the prognostic value of MATVwb and TLGwb, investigating which parameter has higher overall survival predictive power in NSCLC patients and, therefore, higher potential ability to further stratify these patients.

MATERIAL AND METHODS

STUDY SAMPLE

This work was approved by the Institutional Ethics Committee. This type of retrospective study did not require formal consent, once the data were anonymized.

Three hundred thirty-four patients diagnosed with NSCLC who performed [^{18}F]FDG PET/CT for initial staging, between January/2010 and July/2018, were retrospectively evaluated. Ninety-two (27.5%) were female and two hundred forty-two (72.5%) were male, aged between 33 to 88 years (mean ± SD: 66.2 ± 10.2). None of the patients included in the study had brain metastases (excluded by magnetic resonance) or a history of other malignancies. The PET/CT scans were performed within 17 days of diagnosis and before any therapeutic intervention. The histological types and cTNM stages found in the study sample are described in table 1.

Table I: Histological characterization and cTNM stage of the study sample

Histological type	n (%)	cTNM stage	n (%)	cTNM substage	n (%)
Adenocarcinoma	194 (58.1%)	**I**	45 (13.5%)	**IA**	32 (9.6%)
Epidermoid carcinoma	80 (24.0%)			**IB**	13 (3.9%)
Adenosquamous carcinoma	25 (7.5%)	**II**	41 (12.3%)	**IIA**	10 (3.0%)
Adenomucinous carcinoma	17 (5.1%)			**IIB**	31 (9.3%)
Pleomorphic carcinoma	9 (2.7%)	**III**	124 (37.1%)	**IIIA**	47 (14.1%)
Sarcomatoid carcinoma	6 (1.8%)			**IIIB**	51 (15.3%)
Large cell carcinoma	2 (0.6%)			**IIIC**	26 (7.8%)
Bronchoalveolar carcinoma	1 (0.3%)	**IV**	124 (37.1%)	**IVA**	53 (15.9%)
				IVB	71 (21.3%)

After histological characterization and cTNM staging of the lung tumor, patients were treated according to the therapeutic strategies most appropriate to their clinical situations, respecting the current good practice guidelines [3].

[^{18}F]FDG PET/CT ACQUISITION PROTOCOL

For this monocentric study the [^{18}F]FDG PET/CT scans were conducted according to the institution's current protocol: All patients fulfilled a 6-hour fast, blood glucose levels were evaluated and required to be below 151 mg/dL before administration of [^{18}F]FDG, intravenously. The administered activities ranged from 181.3 to 658.6 MBq (mean ± SD: 341.9 ± 78.7). Images were acquired 44 to 201 minutes after intravenous injection (mean ± SD: 79.3 ± 23.0). There where variations observed in the administered activities and times of biodistribution related to the usual conditions of clinical practice [23]. Whole-body images of each patient (in dorsal decubitus position, with arms above the head) were acquired, using a PET/CT scanner General Electric Discovery ST (GE Healthcare, Waukesha, WI, USA). A CT scan was then performed, for attenuation correction of PET data and for anatomical mapping, using the following acquisition parameters: 120 kV of voltage, smart mA (with current values between 10 and 200 mA and noise index 35), pitch 1.5:1, rotation 0.5 s and slice thickness 3.75 mm and 90 mA of tube current. Finally, the PET emission study was obtained at 3-minute acquisition time per table position, in a 3-dimensional mode with a Field of View diameter of 70 cm, following the manufacturer's recommendations. The attenuation-corrected PET images using the CT data were reconstructed using the VUE Point 3-D iterative reconstruction algorithm (35 subsets, two iterations), and 256 × 256 matrix. A 4-mm full-width at half maximum post-reconstruction filter was applied.

AQUISITION METHODOLOGY

The cTNM stage assigned to each patient was recorded. Given the limited number of patients included in the study, patients belonging to the nine cTNM substages were also grouped in stages: stage I (IA and IB) (n = 45); stage II (IIA and IIB) (n = 41); stage III (IIIA, IIIB and IIIC) (n = 124); stage IV (IVA and IVB) (n = 124). Both stage and sub-stage were considered as factors in the analysis.

The [^{18}F]FDG PET/CT scans were retrospectively re-evaluated on an Advanced post-processing Workstation (Advanced Windows 4.4 GE Medical Systems, Milwaukee, USA). Each patient's primitive lesions were delineated and evaluated using the Volume Computer Assisted Reading (PET_VCAR) software (version vxtl_8_3_65). A pre-defined threshold SUV value of 2.5 was defined and this software generated whole-body 3-D regions of interest. Two

nuclear medicine specialists assessed the [^{18}F]FDG PET/CT images and manually excluded the regions corresponding to physiological uptake and/or uptake in benign lesions. Subsequently, 3-D regions of interest were obtained, corresponding to the primary lung tumor and all metastatic lesions. To calculate MATVwb and TLGwb, quantitative analysis was performed. A representative example from a patient with metastatic NSCLC is shown on figure 1.

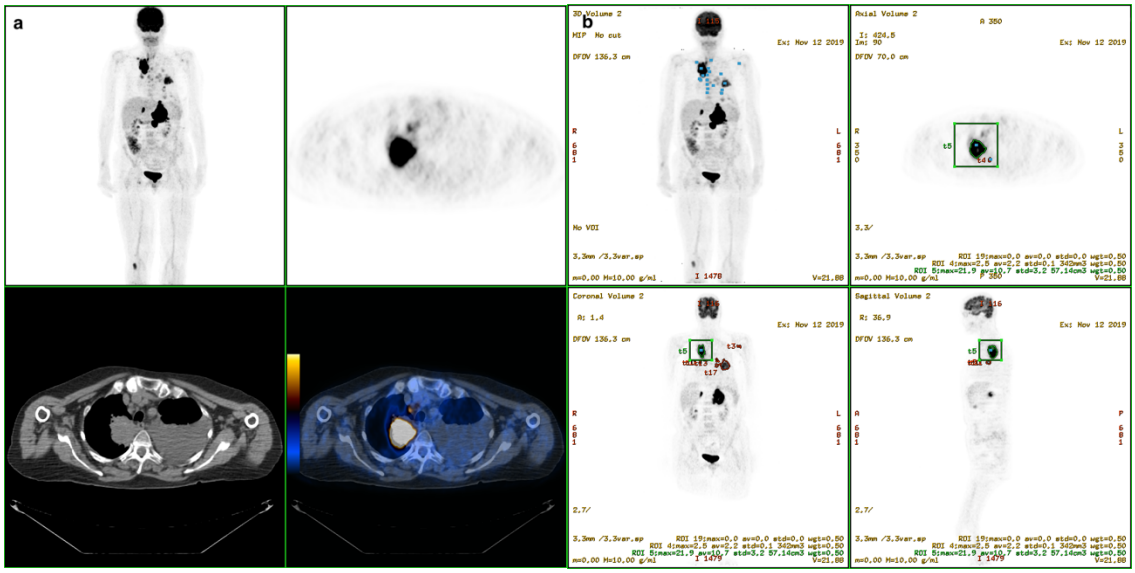

Figure 1 – **a.** [^{18}F]FDG PET/CT in a 77-year-old patient with stage IV lung adenocarcinoma with lung, pleural, supra-renal and bone metastases. **b.** program obtaining of quantitative parameters on the same patient.

STATISTICAL ANALYSIS

Quantitative data were presented with minimum-maximum, mean ± standard deviation or median (inter quartile range), while qualitative data were presented by its absolute and relative frequencies.

The follow-up time was calculated, in months, for each patient, since the date of his/her initial staging [^{18}F]FDG PET/CT scan to the date of his/her death, if it has occurred; otherwise, the patients' follow-up time was counted until the 29th of august 2018.

The eligible method for this type of analysis is Cox regression and Survival analysis, particularly using the Kaplan-Meier estimator, and the Harrel's C Index to assert each variable or set of variables prognostic value, as well as its Hazzard ratio (HR). In order to increase confidence in the results, one thousand random sub-samples with approximately 80% of the original sample size were obtained and analysis was repeated for each one, which allowed us to obtain results with a 95% confidence interval instead of a single estimated value.

In each one of the 1000 samples, a Cox regression with each one of the variables considered (MATVwb, TLGwb, SUV_{max}, SUV_{mean}, Stage, Substage, Age, Administered Activity, Gender, Histology and Biodistribution interval) was performed.

The variables were then ordered according to its importance for survival (HR) and its prognostic value (CI), after computing its Z score based on the 1000 simulations. The C Index Z score was computed comparing it to a random concordance (50%). The variables that were simultaneously statistically significant (based on absolute Z scores > 1.96) for the HR and the prognostic value for survival were considered into a multiple Cox regression model, considering absence of multicollinearity between independent variables.

Multicollinearity was assumed to exist between stage and sub-stage, for obvious motives. For all the other quantitative variables, the presence of multicollinearity was assumed whenever they presented an absolute Spearman's correlation coefficient above 0.8.

Therefore, four models were obtained, and were compared in terms of the 95% confidence interval for the model C-Index. All the models included the variable age and SUV_{max}, combined with TNM stage or sub-stage as well as MATVwb or TLGwb.

Analysis were conducted using R (R Foundation for Statistical Computing, Vienna, Austria) software throughout R Studio and were interpreted at a 5% significance level.

RESULTS

MATVWB AND TLGWB AS PREDICTORS OF OVERALL SURVIVAL

The MATVwb, TLGwb, SUV_{max} and SUV_{mean} values calculated are presented in Tables II to V, respectively, either for the total sample as for TMN stages and TMN sub-stages of the disease. When comparing those values between TNM stages or between TNM sub-stages in each TNM stage, we found statistically significant differences in all the parameters (all the comparisons presented a *p* value below 0.001).

Table II: MATVwb values, in cm^3, measured in the total sample and in the groups defined by cTNM stages I, II, III and IV, and substages IA, IB, IIA, IIB, IIIA, IIIB, IIIC, IVA and IVB.

	N	Mean±SD	Min	Max	Median	Q1-Q3
Total	334	107.1±170.0	0.1	1181.0	46.8	11.1-119.9
Stage I	**46**	**10.1±12.7**	**0.2**	**54.6**	**5.0**	**2.1-14.3**
IA	33	4.4±5.5	0.2	31.5	3.6	1.1-5.3
IB	13	24.4±14.5	3.0	54.6	23.6	17.3-27.1

Stage II	**41**	**37.0±43.3**	**0.1**	**176.3**	**24.1**	**5.4-56.5**
IIA	10	37.0±17.5	5.5	62.8	39.4	26.6-46.5
IIB	31	37.0±49.1	0.1	176.3	11.2	2.5-60.9
Stage III	**123**	**84.2±99.9**	**0.2**	**485.1**	**48.7**	**16.7-99.8**
IIIA	47	60.0±84.1	0.2	485.1	36.2	17.4-66.3
IIIB	50	90.6±103.1	0.7	462.6	63.4	13.3-131.8
IIIC	26	115.7±112.4	7.2	398.1	86.0	32.2-194.9
Stage IV	**124**	**189.0±235.0**	**0.2**	**1181.0**	**100.4**	**42.8-241.7**
IVA	53	125.4±207.4	0.2	1181.0	55.8	21.9-124.0
IVB	71	37.0±43.3	12.1	1093.2	168.1	65.4-307.3

N-*number of patients*; SD-*standard deviation*; Min-*minimum*; Max-*maximum*; Q1 – 1st quartile; Q3 – 3rd quartile

Table III: TLGwb values, measured in the total sample and in the groups defined by cTNM stages I, II, III and IV, and substages IA, IB, IIA, IIB, IIIA, IIIB, IIIC, IVA and IVB.

	N	Mean±SD	Min	Max	Median	Q1-Q3
Total	334	579.8±884.1	0.3	5419.2	262.5	48.8-727.3
Stage I	**46**	**55.6±80.1**	**0.7**	**309.2**	**21.6**	**6.4-74.0**
IA	33	19.1±25.1	0.7	138.6	12.7	3.9-25.5
IB	13	148.3±96.8	9.8	309.2	124.3	89.2-208.7
Stage II	**41**	**249.8±320.5**	**0.3**	**1377.1**	**141.7**	**17.4-387.4**
IIA	10	256.4±199.7	17.1	729,2	220.7	141.7-277.9
IIB	31	247.6±353.5	0.3	1377.1	42.9	7.2-409.0
Stage III	**123**	**506.6±593.4**	**0.5**	**3065.5**	**270.7**	**75.7-674.0**
IIIA	47	380.1±573.5	0.5	3065.5	219,87	82.4-432.4
IIIB	50	554.5±595.4	1.9	1950.6	335,45	55,00-938.5
IIIC	26	643.2±603.8	24.5	2005.1	509,15	144,00-1271.2
Stage IV	**124**	**956.1±1201.6**	**0.6**	**5419.2**	**497.0**	**195.7-1139.5**
IVA	53	718.8±1151.4	0.6	5419.2	293.5	84.5-803.9
IVB	71	1133,28±1215.6	43.2	5247.3	738.1	372.0-1397.6

N-*number of patients*; SD-*standard deviation*; Min-*minimum*; Max-*maximum*; Q1 – 1st quartile; Q3 – 3rd quartile

Table IV: SUV_{max} values, measured in the total sample and in the groups defined by cTNM stages I, II, III and IV, and substages IA, IB, IIA, IIB, IIIA, IIIB, IIIC, IVA and IVB.

	N	Mean±SD	Min	Max	Median	Q1-Q3
Total	334	13.4±7.3	2.9	59.6	12.7	8.4-16.6
Stage I	**46**	**8.9±4.9**	**2.9**	**29.7**	**7.6**	**5.1-12.6**
IA	33	7.2±3.0	2.9	13.4	6.8	4.9-9.1
IB	13	13.0±6.4	4.4	29.7	13.7	9.1-15.1
Stage II	**41**	**13.3±10.1**	**3.0**	**59.6**	**11.6**	**5.4-17.9**
IIA	10	19.3±15.5	4.5	59.6	16.3	10.9-17.9
IIB	31	11.4±6.9	3.0	23.7	9.3	4.5-17.9
Stage III	**123**	**14.1±6.7**	**3.3**	**41.9**	**12.9**	**9.5-17.5**
IIIA	47	14.6±8.3	3.5	41.9	12.7	8.0-18,10
IIIB	50	13.6±6.0	3.3	32.2	12.8	10.0-16.5
IIIC	26	14.5±4.7	5.2	26.2	15.8	10.5-17.2
Stage IV	**124**	**14.3±7.0**	**3.0**	**45.6**	**13.4**	**10.0-17.3**
IVA	53	14.5±9.4	3.0	45.6	13.0	8.0-17.5
IVB	71	14.1±5.0	5.8	33.1	13.8	11.1-16.0

N-*number of patients*; SD-*standard deviation*; Min-*minimum*; Max-*maximum*; Q1 – 1st quartile; Q3 – 3rd quartile

Table V: SUV_{mean} values, measured in the total sample and in the groups defined divided by cTNM stages I, II, III and IV, and substages IA, IB, IIA, IIB, IIIA, IIIB, IIIC, IVA and IVB.

	N	Mean±SD	Min	Max	Median	Q1-Q3
Total	334	5.1±1.9	2.5	16.3	4.8	3.9-6.1
Stage I	**46**	**4.5±1.6**	**2.7**	**9.4**	**3.9**	**3.4-5.0**
IA	33	3.9±0.9	2.7	6.1	3.8	3.3-4.4
IB	13	5.8±2.1	3.3	9.4	5.7	4.0-6.9
Stage II	**41**	**5.3±2.2**	**2.7**	**11.6**	**5.0**	**3.3-6.3**
IIA	10	6.2±2.3	3.1	11.6	5.6	5.0-7.2

IIB	31	5.1±2.1	2.7	9.8	4.6	3.0-6.3
Stage III	**123**	**5.6±1.9**	**2.5**	**12.5**	**5.4**	**4.2-6.4**
IIIA	47	5.8±2.3	2.5	12.5	5.4	4.1-6.7
IIIB	50	5.6±1.9	2.7	11.1	5.5	4.1-6.9
IIIC	26	5.4±1.3	3.0	8.9	5.4	4.5-6.3
Stage IV	**124**	**4.9±1.7**	**2.5**	**16.3**	**4.6**	**4.0-5.3**
IVA	53	5.2±2.3	2.5	16.3	4.8	3.8-6.1
IVB	71	4.7±1.0	3.0	7.6	4.6	4.0-5.2

N-*number of patients*; SD-*standard deviation*; Min-*minimum*; Max-*maximum*; Q1 – 1st quartile; Q3 – 3rd quartile

Age, Gender, Histology, Stage, Substage, MATVwb, TLGwb, SUV_{max}, SUV_{mean}, Administrated Activity, and Biodistribution interval were considered one at a time in a Cox regression model and the mean hazard ratio and concordance index of the 1000 simulations were computed and transformed into Z scores. The importance of each one of the previous independent variables for overall survival, measured in terms of Z scores, were plotted in a bar graph.

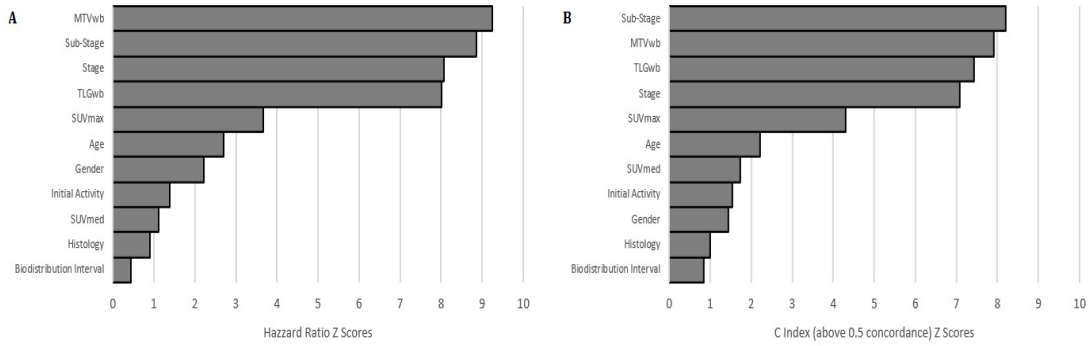

Figure 2 – Relative importance of each independent variable for overall survival based on the Z scores for the hazard ratio (plot on the left) or for the C Index above 0.5 (plot on the right)

As observed in figure 2, the common independent variables with prognosis value to patients' overall survival (Z score > 1.96, corresponding to statistically significant values) based on both criteria are: age, SUVmax, MATVwb and TLGwb, and stage or sub-stage of the disease. Thus, multiple Cox regression should be performed, taking into account the interactions between variables.

Table VI: Hazard ratio (HR) and Concordance Index statistic (C Index), both presented with its standard error (SE) and *p*-value (HR compared to value 1; C Index compared to value 0.5)

Variable	HR ± SE (p)	C Index ± SE (p)
Age	1.023 ± 0.007 (0,002)	0.567 ± 0.024 (0.027)
Gender (M)	1.511 ± 0.168 (0.014)	0.544 ± 0.018 (0.149)
Histology	1.053 ± 0.049 (0.297)	0,531 ± 0.023 (0.316)
Stage	2.360 ± 0.095 (< 0.001)	0.699 ± 0.019 (< 0.001)
Substage	1.450 ± 0.037 (< 0.001)	0.724 ± 0.019 (< 0.001)
MTVwb	1.003 ± $3.00x10^{-4}$ (< 0.001)	0.718 ± 0.020 (< 0.001)
TLGwb	1.001 ± $5.85x10^{-5}$ (< 0.001)	0.707 ± 0.020 (< 0.001)
SUV_{max}	1.033 ± 0.008 (< 0.001)	0.627 ± 0.024 (< 0.001)
SUV_{mean}	1.042 ± 0.032 (0.204)	0.553 ± 0.024 (0.083)
Initial Activity	0.950 ± 0.034 (0.127)	0.547 ± 0.024 (0.125)
Biodistribution interval	1.001 ± 0.003 (0.628)	0.526 ± 0.023 (0.398)

As observed in figure 2 and in table VI, males present a higher risk for mortality than females (HR = 1.511; *p* = 0.014), keeping in account all the other characteristics. However, estimation of the survival time is not accurate when it is only based on gender. Independent variables that are simultaneously predictors of survival and that may be used to accurately predict the risk of mortality, when used one at a time, are: stage; substage; MATVwb; TLGwb; SUV_{max} and, as previously mentioned, age.

Therefore, a model considering those independent variables together, with more information, should perform better than models that use only one of the variables alone. Thus, multiple Cox regression is justified, considering the five variables that present predictive prognostic value. However, it is not possible to perform a multivariate analysis when two variables have a strong correlation between them (r > 0.8). Thus, due to strong correlation between the TLGwb and the MATVwb (r = 0.957; *p* < 0.001) (Table VII), different models were tested to avoid multicollinearity.

Table VII: Pearson Correlation between variables, measured in the total sample (334 patients).

		MTVwb	TLGwb	SUV_{max}	IDADE
MTVwb	r	1	0.957	0.279	-0.101
	p		< 0.001	< 0.001	0.065
TLGwb	r	0.957	1	0.425	-0.084
	p	< 0.001		< 0.001	0.125
SUV_{max}	r	0.279	0.425	1	-0.057

	p	< 0.001	< 0.001		0.303
IDADE	r	-0.101	-0.084	-0.057	1
	p	0.065	0.125	0.303	

r – *Pearson correlation coefficient*

DEVELOPMENT OF MODELS FOR SURVIVAL PREDICTION

Four multiple Cox regression models were tested, using Stage/Substage, MATVwb/TLGwb and SUVmax as predictors, adjusted for age, either tested as main effects or tested as interaction or even full factorial. Only main effects models produced statistically significant results thus, interaction or full factorial models were not presented in these results.

The models in analysis considered the following independent variables:

Model 1 - Age, Stage, MATVwb, SUV_{max}

Model 2 - Age, Stage, TLGwb, SUV_{max}

Model 3 - Age, Sub-Stage, MATVwb, SUV_{max}

Model 4 - Age, Sub-Stage, TLGwb, SUV_{max}

Models were developed and evaluated in 1000 random samples of size 80% of the total study sample, and mean concordance index (C Index) as well as mean Akaike Information Criteria (AIC) were obtained for the simulations. The mean standard error for C Index was also obtained and used to determine the 95% confidence interval for the C Index statistic. Results may be observed in figure 3 and we may assume that all the models perform quite well, as each one of them is expected to present values of C Index above 70%, which denotes good prediction.

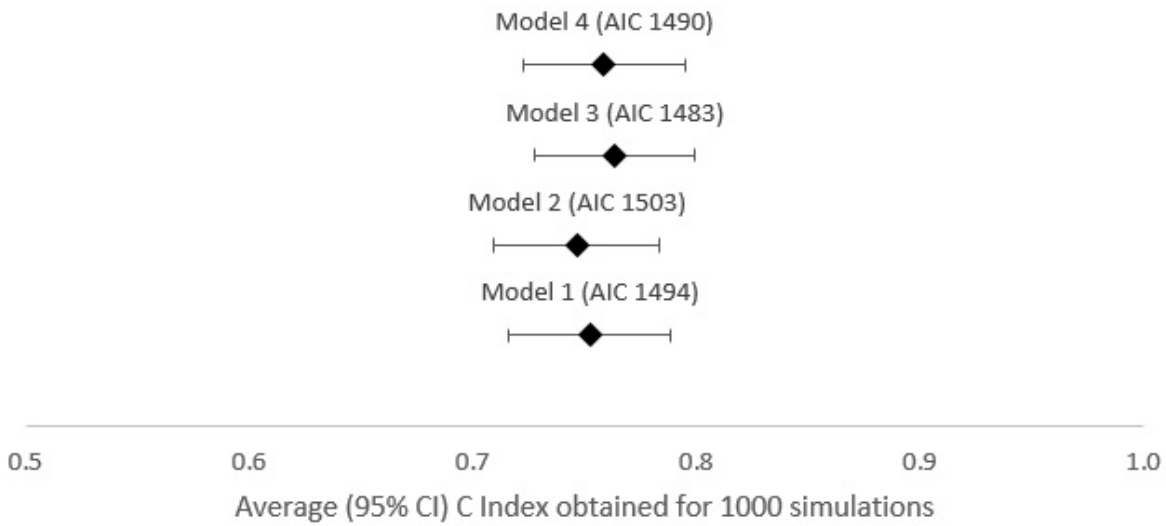

Figure 3 - Average C Index for each one of the models and its respective 95% confidence interval (the mean Aikaike Information Criteria (AIC) for each one of the models is also referred)

Furthermore, predictions using the cTNM substage perform slightly better than models that use the cTNM stage, but there were no statistically significant differences in the C Index statistic between models 1 and 3 (non-adjusted $p = 0.546$), or between models 2 and 4 (non-adjusted $p = 0.508$). The same conclusion applies to the comparison of models 1 and 2 (non-adjusted $p = 0.754$), or models 3 and 4 (non-adjusted $p = 0.799$), where the dissimilarity term is the use of the MATVwb or the TLGwb for prediction (figure 3 and table VIII). It should also be noted that the mean standard error for each model is very similar, differing only after the third decimal place, which denotes accuracy between the 1000 simulations.

Table VIII: Mean Concordance Index statistic (C Index) obtained for the 1000 simulations, its mean standard error (SE), confidence interval and *p*-value (C Index compared to value 0.5)

Model	C Index ± SE (p)	95% Confidence interval
1	0.752 ± 0.019 (< 0.001)	0.716 – 0.789
2	0.747 ± 0.019 (< 0.001)	0.710 – 0.783
3	0.764 ± 0.018 (< 0.001)	0.728 – 0.799
4	0.759 ± 0.018 (< 0.001)	0.723 – 0.795

Concerning the coefficients for each model, aging is always considered as a bad prognostic marker, and increasing stage or sub-stage, and increasing MATVwb or TLGwb values are considered as risk factors for mortality. (table IX)

Table IX: Mean Hazard Ratio (HR) obtained for the 1000 simulations and mean *p*-value between brackets

Model	Age	Stage	Sub-stage	MTVwb	TLGwb	SUVmax
1	4.821 (< 0.001)	6.825 (< 0.001)	-	5.700 (< 0.001)	-	0.570 (0.569)
2	3.387 (0.002)	7.104 (< 0.001)	-		4.364 (< 0.001)	0.514 (0.676)
3	3.883 (< 0.001)	-	7.489 (< 0.001)	4.953 (< 0.001)	-	1.889 (0.095)
4	3.599 (< 0.001)	-	7.905 (< 0.001)	-	3.764 (< 0.001)	1.096 (0.349)

We also have determined the 95% confidence limits for the *p*-values. They were not presented for maintaining table IX simplicity; however, all statistically significant values presented the upper 95% confidence limit within statistically significance (the highest 95% upper confidence

for *p*-value was achieved for age in model 2: 0.008). Non-statistically significant *p*-values were obtained only for SUV_{max}, where all the 95% confidence intervals ranged from 0 to 1, indicating lack of statistically significant predictive value.

Therefore, models should be adjusted excluding SUV_{max} and recalculating HR for the remaining variables, comparing the performance of this new models using the same methodology presented on figure 3 and tables VIII and IX. These results are presented in tables X and XI and figure 4.

Final models:

1 - Age, Stage, MATVwb

2 - Age, Stage, TLGwb

3 - Age, Sub-Stage, MATVwb

4 - Age, Sub-Stage, TLGwb

Table X: Mean C Index statistic obtained for the 1000 simulations for the final models, its mean standard error (SE), confidence interval and *p*-value (C Index compared to value 0.5)

Final model	C Index ± SE (p)	95% Confidence interval
1	0.750 ± 0.019 (< 0.001)	0.713 – 0.786
2	0.745 ± 0.019 (< 0.001)	0.708 – 0.782
3	0.759 ± 0.019 (< 0.001)	0.722 – 0.795
4	0.756 ± 0.019 (< 0.001)	0.719 – 0.792

As observed in table X, the four models present similar C Index values, all statistically significant, demonstrating moderate to good prognostic value as the lower limit of each 95% confidence interval for the C Index are all above 0.700. The interception of those confidence intervals and the high statistically significance for the hazard ratios (table XI) of each variable in each model indicate that either one of the models may be used to predict these patients' survival.

Table XI: Mean Hazard Ratio (HR) obtained for the 1000 simulations of the final models and mean *p*-value

Final Model	Age	Stage	Sub-stage	MTVwb	TLGwb
1	4.821 (< 0.001)	6.825 (< 0.001)	-	5.700 (< 0.001)	-
2	3.362 (0.002)	7.115 (< 0.001)	-		5.467 (< 0.001)
3	3.738	-	7.499	5.837	-

	(< 0.001)		(< 0.001)	(< 0.001)	
4	3.538 (< 0.001)	-	7.894 (< 0.001)	-	5.100 (< 0.001)

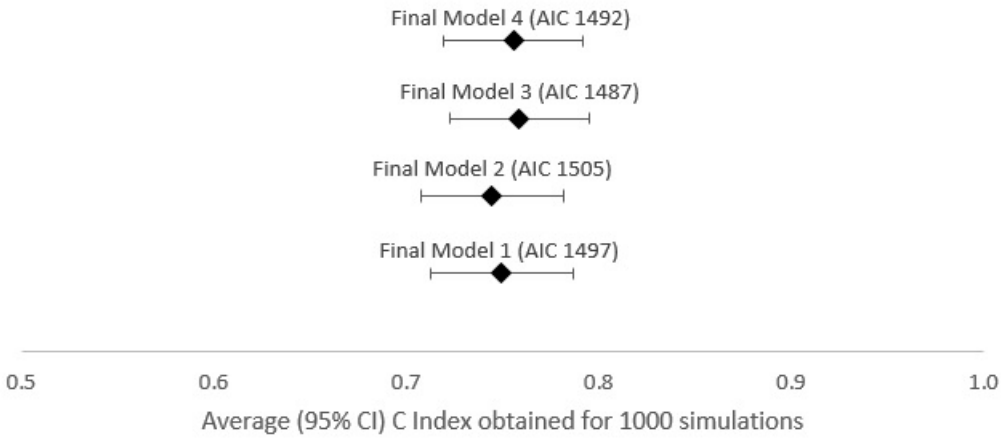

Figure 4 - Average C Index for each one of the final models and its respective 95% confidence interval (the mean Aikaike Information Criteria (AIC) for each one of the models is also referred)

These results reveal that stage or sub-stage of the disease, together with MATVwb or TLG-wb, adjusted for patients age, have a high prognostic value on both patient´s survival rate and survival time (Figure 4). Moreover, either one of these models can be used to accurately predict patient's overall survival thus, using the parameter TLGwb of the [^{18}F]FDG PET/CT is not significantly inferior to using the MATVwb.

DISCUSSION

This study investigates the role of MATVwb and TLGwb measured by [^{18}F]FDG PET/CT as indicators of overall survival. To our knowledge, this is the first study that compares TLGwb and MATVwb in terms of their prognostic value in NSCLC.

The TNM staging system is widely used in NSCLC patients, as it is considered the most important factor for tumor staging and prognosis information [4, 5, 17]. Nevertheless, it does not account for the metabolic active tumor burden of each patient. As a result, there are still substantial differences in the overall survival in patients in the same TNM stage, with similar pathological and clinical characteristics. Therefore, it is important to further stratify these patients, to get them the best possible treatment and outcome.

The National Comprehensive Cancer Network (NCCN) guidelines recommend whole-body [^{18}F]FDG PET/CT for the management of all NSCLC patients (stages I-IV) [24], as it is an established radiological modality and it provides parameters for the substaging of patients in the same TNM stage who have different prognoses [25].

The SUV_{max} has been widely used, partly due to its convenience, as an indicator of tumor metabolic activity [21]. However, SUV_{max} is not a reliable parameter because its value depends on many factors such as the body weight, serum glucose level of the patient, and various technologic factors (the model of scanning equipment used, image resolution, the attenuation correction method and image reconstruction algorithm, radiopharmaceutical activity, fasting time and uptake time). Furthermore, SUV_{max} measures a single highly metabolic focus that may not reflect the whole tumor metabolic activity and it does not take the volume of the tumor into account. This may be a significant limitation because lung tumor volume is a well-known prognostic determinant [5, 19, 26].

In contrast, metabolic volumetric parameters measured by [^{18}F]FDG PET/CT, such as MATVwb and TLGwb, incorporating both tumor volume and metabolic activity, should be more sensitive than SUV in predicting patients' prognosis in NSCLC [17, 21, 26, 27].

Recently, MATVwb and TLGwb have been widely studied for prognostic analysis of the NSCLC and studies have confirmed that these are better indices of patient survival than SUV_{max} or SUV_{mean} [17, 27-29].

As previously explained, the MATVwb is the some of the volumes of all metabolically active lesions of the [^{18}F]FDG PET/CT (primary tumor and metastatic lesions), thus reflecting the metabolically active tumor mass. The TLGwb, being a product SUV_{mean} x MATVwb, simultaneously represents the degree of [^{18}F]FDG uptake and the size of the metabolically active tumor. Thus, theoretically, it is the ideal parameter of the tumor burden [26]. Thereby, considering a clinical point of view, the TLGwb is a more relevant parameter to use in the evaluation of NSCLC patients' overall survival.

Nevertheless, there have been apparently stronger results with the use of MATVwb [21, 22], leading to several studies addressing it. *Pu et al.* developed and validated a MATVwb risk stratification system, with independent prognostic value, to supplement the TNM staging in NSCLC [8]. *Zhang et al.* proposed a PET/CT volumetric prognostic index (PVP index) using TNM staging and MATVwb [30], *Finkle et al*. later updated this PVP index for NSCLC, using the eight edition TNM staging system, MATVwb and age, and found it to be a prognostic indicator superior to TNM stage or MATVwb alone [4].

In our study, we concluded that using the substage is slightly better than using the stage and that there is no significant statistical difference in using TLGwb or MATVwb. This result was consistent with the study by *Hyun et al*, demonstrating that TLGwb and MATVwb are

independent prognostic factors for overall survival and promising tools for better prediction of outcome in NSCLC patients [5].

Moreover, our results are concordant with existing studies, demonstrating that, even though SUV_{max} alone is an independent predictor of overall survival [15, 16, 28], in the multivariate analysis, the SUV_{max} was no longer a good predictor [19], indicating that the inclusion of this parameter on the model does not improve the predictive capacity of survival, meaning, what SUV_{max} predicts can be predicted better by the other variables.

The principal finding of this study was that either one of the four models can be used to accurately predict prognosis in NSCLC. Interestingly, the prognostic value of the parameter TLGwb of the [^{18}F]FDG PET/CT is not significantly inferior to prognostic value of MATVwb. A new prognostic stratification based on the TNM staging and the TLG_{wb} may help optimize patient care by providing more accurate prognostic.

The limitations of the study comprise: (1) the fact that it is a retrospective study and its relatively small size. (2) using a tomograph with a spatial resolution of about 6 mm and detection sensitivity of 0.2%, when the state-of-the-art equipment can achieve a spatial resolution of about 4 mm and a detection sensitivity of 0.9%. A device with more favourable spatial resolution and detection sensitivity values would allow more precise quantitative analysis [31].

In conclusion, the present study showed that MATVwb and TLGwb are strong predictors of overall survival in NSCLC patients, both independently and included in a predictive model. Moreover, having the TLGwb more sensibility, by definition, we found it always provides at least the same prognosis value as MATVwb thus, its use is more accurate, in a clinical perspective. Additional studies with large patient numbers are needed to validate the results of our study.

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APPENDICES

APPENDIX 1

4 Manuscript Preparation

To guarantee a smooth publication process and a seamless transformation of your manuscript into the final layout and various electronic formats (e.g., HTML for online publication, ePub for e-book readers), the manuscript needs to be structured as follows:

- **Front Matter:** Title page, Dedication (optional), Foreword (optional), Preface (optional), Table of Contents, List of abbreviations (optional).
- **Text Body:** It comprises the chapters containing the content of the book, i.e., text, figures, tables, and references. Chapters can be grouped together in parts.
- **Back Matter:** After the last chapter, the back matter can contain an appendix, a glossary, and/or an index, all of which are optional.

4.1 Front Matter

The title page and the **table of contents** precede the actual content of a book.

The preface (optional) should be about the book: why it was written, who it is for, its organization, or the selection of contributors. An **introduction** to the subject of the book, however, should appear as the first chapter of the book.

Other optional items in the front matter at the beginning of a book are e.g., **dedication**, a **foreword** or a **list of abbreviations**.

4.1.1 Title Page

- Please include all author names (for contributed books, the editor names) and their affiliations, the book title and subtitle. Ensure that the sequence of the author names is correct and the title of your book is final when you submit your manuscript.
- Please also supply all the email addresses and telephone numbers and in case of multiple authors or editors, clearly indicate the corresponding author or editor.

- Once the manuscript has been delivered to Springer Production, changes to title or authorship are no longer possible.

4.1.2 Foreword (optional)

- If you intend to include a foreword, please submit it with the manuscript.

Tip

– A foreword is usually written by an authority on the subject, and serves as a recommendation of the book.
– The name of the foreword's contributor is always given at the end of the foreword; affiliations and titles are generally not included, but the date and place of writing may be.

4.1.3 Preface (optional)

- A preface should not contain a reference list.
- An introduction to the **subject** of the book should not be confused with a preface. The introduction does not belong in the front matter, but should appear as the first chapter of the book.

Tip

– The preface should be about the book: why it was written, who it is for, its organization, or the selection of contributors.
– Acknowledgments of support or assistance in preparing the book can be included as the last paragraph(s) of the preface. If the acknowledgment is more than one page long, it should start on a separate page under the heading **Acknowledgments**.

4.1.4 Table of Contents

- List all parts, chapters, and back matter material (e.g., an index) in the final sequence.
- If your chapters are numbered, use **Arabic** numerals and number the chapters consecutively throughout the book (Chapter 1, Chapter 2, etc.), i.e., do not start anew with each part.
- If there are parts, use **Roman** numerals for parts (Part I, Part II, etc.).

Key Style Points: Table of Contents

4.1.5 List of Abbreviations (optional)

Tip

– A **list of abbreviations** and/or symbols is optional but it may be very helpful if numerous abbreviations and special symbols are scattered throughout the text.

4.2 Chapters

Chapters contain the actual content of the book, i.e., text, figures, tables, and references. Chapters can be grouped together in **parts**; subparts are not possible. Only one chapter (i.e., an introduction) may precede the first part and would be the first chapter.

- Decide the numbering style for the chapters and apply this style consistently to all chapters: consecutively numbered (monographs or textbooks) or unnumbered (contributed volumes).
- If an introduction to the subject of the book (historical background, definitions, or methodology) is included, it should appear as the first chapter and thus be included in the chapter numbering. It can contain references, figures, and tables, just as any other chapter.

4.2.1 Language

- Either **British** or **American** English can be used, but be consistent within your chapter or book. In contributed books chapter-wise consistency is accepted.
- Check for consistent **spelling** of names, terms, and abbreviations, including in tables and figure captions.

Tip

– For American spelling please consult *Merriam–Webster's Collegiate Dictionary*; for British spelling you should refer to *Collins English Dictionary*.
– If English is not your native language, please ask a native speaker to help you or arrange for your text to be checked by a professional editing service. Please insert their final corrections into your data before submitting the manuscript.

▶More about language editing

4.2.2 Chapter Title and Authors

- For contributed volumes, please include each chapter's authors' names (spelled out as they would be cited), affiliations and e-mail addresses and telephone numbers after the chapter title. (The telephone number will not be published but may be needed as contact information during the publishing process.)

Key Style Points: Chapter Title Page

4.2.3 Abstract

- **Begin each chapter with an abstract** that summarizes the content of the chapter in 150 to 250 words. The abstract will appear **online** at SpringerLink and be available with unrestricted access to facilitate online searching, using, e.g., Google, and allow unregistered users to read the abstract as a teaser for the complete chapter
- If no abstract is submitted, we will use the first paragraph of the chapter instead.
- Abstracts appear only in the **printed edition** of contributed volumes unless stipulated otherwise.

Tip

– Don't include reference citations or undefined abbreviations in the abstract, since abstracts are often read independently of the actual chapter and without access to the reference list.
– For further tips on writing an effective abstract, see the website on Search Engine Optimization.

4.2.4 Keywords (if applicable)

- Some books also publish keywords. Please check with the editor of your book or with the publishing editor to see if keywords are required.

Tip

– Each keyword should not contain more than two compound words, and each keyword phrase should start with an uppercase letter.
– When selecting the keywords, think of them as terms that will help someone locate your chapter at the top of the search engine list using, for example, Google. Very broad terms (e.g., 'Case study' by itself) should be avoided as these will result in thousands of search results but will not result in finding your chapter.

4.2.5 Headings and Heading Numbering

- Heading levels should be clearly identified and each level should be uniquely and consistently formatted and/or numbered.
- Use the **decimal system** of numbering if your headings are numbered.
- Never skip a heading level. The only exceptions are run-in headings which can be used at any hierarchical level.

Key Style Points: Headings

Tip

– In **cross-references**, for hyperlink purposes, refer to the chapter or section number (e.g., see Chap. 3 or see Sect. 3.5.1).
– In addition to numbered headings, two more (lower) heading levels are possible. Their hierarchical level should be identified with the help of Springer's templates or the standard Word or LaTeX heading styles.
– Another option for lower level headings is a run-in heading, i.e., headings that are set immediately at the beginning of the paragraph. Such headings should be formatted in bold or italics.

4.2.6 Terminology, Units and Abbreviations

- Technical terms and **abbreviations** should be defined the first time they appear in the text.
- Please always use internationally accepted signs and symbols for units, so-called **SI units**.
- **Numerals** should follow the British/American method of decimal points to indicate decimals and commas to separate thousands.

Key Style Points: Abbreviations, Numbers, Units and Equations

Tip

– If the manuscript contains a large number of terms and abbreviations, a list of abbreviations or a **glossary** is advised.

4.2.7 Formal Style and Text Formatting

- Manuscripts will be checked by a copy editor for formal style. Springer follows certain standards with regard to the presentation of the content, and the copy editors make sure that the manuscript conforms to these styles.

Key Style Points: Formal Style, Text formatting

Tip

– Remember not to make changes that involve only matters of style when you check your proofs. We have generally introduced forms that follow Springer's house style.

Emphasis and special type

Key Style Points: Formal Style, Text formatting

Tip

– **Italics** should be used for emphasized words or phrases in running text, but do not format entire paragraphs in italics.
– In addition, use italics for species and genus names, mathematical/physical variables, and prefixes in chemical compounds.
– **Bold** formatting should only be used for run-in headings and **small capitals** for indicating optical activity (D- and L-dopa).

– **Sans serif** (e.g., Arial) and **nonproportional font** (e.g., Courier) can be used to distinguish the literal text of computer programs from running text.

Boxes

- Do not set entire pages as boxes, because this affects online readability.

Tip

– Additional text elements for **professional and text books** such as examples, questions or exercises, summaries or key messages can be highlighted with Springer's manuscript preparation tool. If you do not use the tool, use a consistent style for each of these elements and submit a list of the styles used together with your manuscript.

4.2.8 Footnotes

- Always use footnotes instead of endnotes and never use footnotes instead of a reference list.
- Footnotes should not consist of a reference citation. Footnotes should not contain figures, tables and/or the bibliographic details of a reference.

Key Style Points: Formal Style, Text formatting

4.2.9 Equations and Program Code

- In **Word**, use the Math function of Word 2007 or 2010, MathType, or Microsoft Equation Editor with Word 2003 to create your equations, and insert the graphic into your text file as an object.
- In **LaTeX**, use the Math environment to create your equations.

Key Style Points: Abbreviations, Numbers, Units and Equations

Tip

– Prepare the whole equation in this way and not just part of it.

4.3 Tables

- Give each table a caption. Add a reference citation to the table source at the end of the caption, if necessary.
- Number tables consecutively using the chapter number (e.g. Table 1.1 for the first table in Chap. 1) and ensure that all tables are cited in the text in sequential order. Do not write "the following table".
- Use the table function to create and format tables. Do not use the space bar or multiple tabs to separate columns and do not use Excel to create tables as this can cause problems when converting your tables into the typesetting program and other formats.

Key Style Points: Tables and Lists

Tip

– Simple, one-column lists should not be treated as tables. Use the displayed list function instead.
– Save the tables in the same file as text, references, and figure captions.
– Do not manually insert table rules in the manuscript, because they cannot be retained.

4.4 Figures and Illustrations

4.4.1 Numbering

- Number the figures chapter-wise using the chapter number (e.g., Fig. 1.1 for the first figure in Chap. 1) and ensure that all figures are cited in the text in sequential order. Do not write "the following figure".

4.4.2 Figure Captions

- Give each figure a concise caption, describing accurately what the figure depicts. Include the captions at the end of the text file, not in the figure file.
- Identify all elements found in the figure in the figure caption and use boxes, circles, etc. as coordinate points in graphs instead of color lines.
- If a figure is reproduced from a previous publication, include the source as the last item in the caption.

Key Style Points: Figures and Illustrations

4.4.3 Figure and Illustration Files

A figure is an object that is drawn or photographed. It does not consist solely of characters and thus cannot be keyed.

- Do not submit tabular material as figures.
- **Graphics and diagrams** should be saved as EPS files with the fonts embedded. Microsoft Office files (Excel or PowerPoint) can be submitted in the original format (xls, xlsx, ppt, pptx). Scanned graphics in TIFF format should have a minimum resolution of 1200 dpi.
- **Photos** or drawings with fine shading should be saved as TIFF with a minimum resolution of 300 dpi.
- A **combination** of halftone and line art (e.g., photos containing line drawings or extensive lettering, color diagrams, etc.) should be saved as TIFF with a minimum resolution of 600 dpi.

Tip

- **Color figures** will appear in color in the eBook but may be printed in black and white. In that case, do not refer to color in the captions and make sure that the main information will still be visible if converted to black and white. A simple way to check this is to make a black and white printout to see if the necessary distinctions between the different colors are still apparent. Color illustrations should be submitted as RGB (8 bits per channel).
- Ensure consistency by using similar **sizing** and lettering for similar figures. Ideally, you should size figures to fit in the page or column width. For books in Springer's standard format, the figures should be 78 mm or 117 mm (3 or 4 1/2 inches) wide and not higher than 198 mm (7 3/4 inches).
- To add **lettering**, it is best to use Helvetica or Arial (sans serif fonts) and avoid effects such as shading, outline letters, etc. Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt). Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.

4.5 References

4.5.1 Reference Citations

- Cite references in the text with author name/s and year of publication in parentheses ("Harvard system"):

– One author: (Miller 1991) or Miller (1991)
– Two authors: (Miller and Smith 1994) or Miller and Smith (1994)
– Three authors or more: (Miller et al. 1995) or Miller et al. (1995)

- If it is customary in your field, you can also cite with reference numbers in square brackets either sequential by citation or according to the sequence in an alphabetized list:

– [3, 7, 12].

4.5.2 Reference List

- Include a **reference list at the end of each chapter** so that readers of single chapters of the eBook can make full use of the citations. References at the end of the book cannot be linked to citations in the chapters. Please do not include reference lists at the end of a chapter section, at the end of a book part, in a preface or an appendix.
- Include all works that are cited in the chapter and that have been published (including on the Internet) or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes as a substitute for a reference list.
- Entries in the list must be listed alphabetically except in the numbered system of sequential citation. The rules for alphabetization are:

– First, all works by the author alone, ordered chronologically by year of publication.
– Next, all works by the author with a coauthor, ordered alphabetically by coauthor.
– Finally, all works by the author with several coauthors, ordered chronologically by year of publication.

Tip

– For authors using EndNote software to create the reference list, Springer provides output styles that support the formatting of in-text citations and reference list.
▶ EndNote software: Springer reference styles
– For authors using BiBTeX, the style files are included in Springer's LaTex package.

4.5.3 Reference Styles

Springer follows certain standards with regard to the presentation of the reference list. They are based on reference styles that were established for various disciplines in the past and have been adjusted to facilitate automated processing and citation linking. This allows us, for example, to easily cross link the cited references with the original publication.

Tip

– Always select one of the reference list styles that are supported by **Springer** and suits your publication best or follow the instructions received from your book editor. There are, however, recommended styles depending on the **discipline**.
– The copy editor will check the references against the reference style applicable for the book and correct the format if necessary.