18-Fluorine fluorodeoxyglucose positron emission tomography in the pretreatment evaluation of thymic epithelial neoplasms: a metabolic biopsy confirmed by Ki-67 expression†

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OBJECTIVES: To investigate the usefulness of 18-fluorine fluorodeoxyglucose (18F-FDG) positron emission tomography/computed tomography (PET–CT) in the pretreatment evaluation of thymic epithelial neoplasms (TENs). We previously demonstrated that the ratio between standardized uptake value of the tumour and aortic arch (SUV T/M) correlates with World Health Organization (WHO) classification. We now focused our evaluation on thymomas only, excluding carcinomas. We also searched for the expression of a pathological biomarker, Ki-67, that gained both diagnostic and prognostic relevance for various solid tumours. Its correlation with SUV T/M and WHO classification was evaluated.

METHODS: We performed a retrospective dynamic cohort study of data from January 2006 to December 2012, on 23 consecutive patients with pathologically proven TEN, excluding thymic carcinomas, evaluated with PET–CT. For each patient, SUV T/M was calculated. The patients were then categorized, according to WHO classification, into two groups (low-risk: 3 A, 9 AB, 5 B1; high-risk: 5 B2, 1 B3) and Ki-67 labelling index (LI) was defined. We employed the Spearman rank non-linear correlation coefficient (ρ) to estimate the correlations between variables.

RESULTS: SUV T/M proved to be significantly higher for high-positive Ki-67 samples, indicating a strong correlation between SUV T/M and Ki-67 LI (ρ = 0.8). Furthermore, high Ki-67 LI samples correlate with the higher-risk WHO subgroup (ρ = 0.9).

CONCLUSIONS: FDG PET–CT can provide a useful tool in the preoperative work-up of TEN, reflecting its proliferation capacity, as described also by the Ki-67 expression. In particular, SUV T/M could provide a ‘metabolic biopsy’ to divide TEN into high-risk and low-risk neoplasms.

Keywords: PET–CT • Thymoma • Ki-67
fluorodeoxyglucose positron emission tomography/CT (18F-FDG PET–CT) was then evaluated in various experiences [8, 9]. 18F-FDG uptake by thymomas reflects enhanced glycolysis of tumour cells and overexpression of glucose transporter 1, the transporter used also by 18F-FDG [10]. Endo et al. found a progressive amount of uptake when employing a three-cohort grouping of TEN (low-risk, high-risk and thymic carcinomas). The authors also describe a novel index to describe metabolic activity, the ratio of the maximum standard-ized uptake value (SUV_{max}) and the mean SUV of the mediasti-num at the level of the aortic arch, or T/M ratio [11]. In our previous experience, we employed 18F-FDG PET–CT in the preoperative work-up for thymomas and carcinomas, identifying the T/M ratio as a predictor of risk group according to WHO [12]. Now we decided to concentrate our analysis of preoperative role of PET–CT on a more specific group of neoplasms, excluding carcinomas from our evaluation. Furthermore, we searched for correlation of CT–PET results with Ki-67, a marker of neoplastic aggressiveness, to suggest a biological basis for the imaging observation.

Ki 67 is a histological marker of proliferation used as an index of biological aggressiveness in a variety of solid tumours. Some experiences suggested that Ki-67 labelling index (LI), defined as the fraction of Ki-67-positive cells within the examined tumour sections, is associated with the histology and biological behaviour of thymomas [13, 14]. Ki-67 LI was therefore determined on specimens of patients operated between 2006 and 2012.

PATIENTS AND METHODS

Patients

The study is a retrospective analysis of data collected over a period of 7 years, between January 2006 and December 2012. We focused only on tumours that undertook complete resection or debulking, because of the possibility of intratumoural variation of histology described for thymomas, and because in those cases an adequate amount of tissue was granted for immunohistochemical staining. The sample comprised 23 consecutive patients, who underwent PET–CT for an anterior mediastinal mass and subsequently surgical resection for thymoma. Thymic carcinomas were excluded because of their different oncological and clinical behaviour. From our previous experience [12], 6 new patients, operated between June 2010 and December 2012, were added. All patients underwent adequate physical examination, chest X-ray, contrast-enhanced CT of the chest and PET-CT scan. Biopsy was performed when needed to differentiate invasive thymoma from lymphoma or other malignancies, in order to define the need for preoperative chemotherapy.

We applied the Masaoka-Koga staging system [15] by accurate evaluation of the operative and pathology reports. WHO classification was defined on the operative specimen. Then the types were grouped into low-risk thymomas (A, AB, B1) and high-risk thymomas (B2, B3). Both Masaoka-Koga stage and WHO type were reviewed by two pathologists, experienced in thymic pathology.

18-Fluorine fluorodeoxyglucose positron emission tomography

The 18F-FDG PET–CT images (GE, Discovery LS PET–CT system, Waukesha, WI, USA) were acquired within 55–70 min from injection of 330–400 MBq of 18F-FDG (4 min/bed; total field view, 150–165 cm; single slice overlapping). CT images were coregistered before emission scanning. The presence of hypermetabolic spots was evaluated by two experienced physicians, blinded to the clinical data. The SUV_{max} was then calculated for the medias-tinal lesion. In order to define the metabolic activity of the mediastinal ‘background’, the aortic arch was taken as reference region [16]. Then the tumour SUV_{max}/mediastinum SUV (T/M ratio) was calculated. The T/M ratio was then defined for the two groups.

We decided to use the T/M ratio in order to avoid the bias derived from the use of the SUV_{max}, which is the most common way of representing metabolic activity in a tissue. It represents the highest voxel value within the region of interest. It is independent from the definition of region of interest, but is susceptible to distorting factors such as blood glucose level of the patient, uptake time and respiratory motion. Also technical factors (inter-scanner variability image acquisition and reconstruction parameters) could alter the SUV_{max}. The T/M ratio could reduce the variability introduced by all those ‘non-tumour’-dependent factors, therefore allowing a more precise match of data.

Operative procedures

All surgical procedures were carried out by median sternotomy. Operative procedures consisted of extended thymectomy in all cases.

Ki-67 labelling index

Tissue samples from the surgical specimens were fixed in 10% formaldehyde and embedded in paraffin according to the standard histological procedure. Five-micrometre-thick sections were stained with the routine ematoxylin–eosin method for the pathological diagnosis. Additional sections were prepared for immunohistochemistry. Staining was performed using an automated slide preparation system (Benchmark XT, Ventana, Tucson, AZ, USA) and prediluted monoclonal antibody Ki-67 (Ventana, Tucson, AZ, USA) available commercially. Detection involved Ventana’s ultraView Universal DAB Detection Kit that utilized a cocktail of enzyme-labelled secondary antibodies that locate the bound primary antibody. The complex was then seen with hydrogen per-oxide substrate and a 3, 3’-diaminobenzidine tetrahydrochloride chromogen. No biotin was involved. Antigen retrieval on the machine was the Ventana CC1, EDTA-Tris, pH 8.0, solution.

We determined the replicative fraction of neoplastic cells using the immunohistochemical detection of Ki-67 antigen [17]. All fields with positive cell predominance were viewed and at least 1000 cells were evaluated for Ki-67 nuclear staining with a ×40 objective (Fig. 1). Five different areas were selected in all samples and the percentage of positive cells was counted.

Statistical analysis

Two-tailed t-test (normally distributed data) or Wilcoxon–Mann–Whitney U-test (not normally distributed data) was used to test for the significance of differences in the mean values for the groups. Correlation between variables was analysed using the Spearman rank correlation test (ρ). P < 0.05 was deemed significant.
We employed the R software for statistical analysis (http://www.r-project.org).

RESULTS

Patients and tumour characteristics

Among the 23 study patients (14 males, 9 females; mean age: 52 ± 11 years), 17 were with low-risk thymoma (3 A, 9 AB, 5 B1) and 6 with high-risk thymoma (5 B2, 1 B3). Transverse diameter varied from 2.5 to 12 cm (mean: 5.80 ± 2.20 cm). Masaoka-Koga Stage was I in 9 cases, II in 9 cases. Four cases had Stage III due to infiltration of the lung (n = 1), pericardium (n = 2), pericardium and brachiocephalic vein (n = 1). One case was in Stage IVa due to pleural implants. Paraneoplastic syndromes were myasthenia gravis in 10 cases and pure red cell aplasia in 1 case.

PET–CT scan confirmed the CT findings in all cases. In 3 cases, CT imaging could not rule out infiltration of surrounding organs (pericardium in 2 and lung in 1 case), and the patients were referred to first-line surgery. We did not routinely use magnetic resonance imaging (MRI). Table 1 describes the mean SUVmax, mean SUV M and T/M ratio according to WHO classification, and Table 2 describes the same according to Masaoka-Koga stage. Low-risk thymomas showed a mean SUVmax of 4.01 ± 0.72 and a mean T/M ratio of 1.91 ± 0.21. High-risk thymomas showed a mean SUVmax of 7.60 ± 2.38 and a mean T/M ratio of 3.73 ± 0.76.

In 2 patients, surgical biopsy was performed before surgery for advanced clinical stage thymoma (Stage III with diffusion to great vessels and Stage IVa due to multiple pleural implants) because the tumour was not considered completely resectable. Preoperative chemotherapy with a platinum-based regimen was then provided. Operative procedures comprised extended thymectomy in 22 cases and subtotal resection with residual macroscopic residual tissue in 1 case with infiltration of the left subclavian artery. Associated procedures were limited wedge resection of the lung (n = 1), resection of a portion of pericardium (n = 2) and subsequent prosthetic reconstruction (n = 1), and tangential resection and direct suture of the innominate vein (n = 1).

Intraoperative and postoperative (30 days) mortality was 0%. Postoperative (30 days) morbidity included major bleeding requiring reoperation in 1 case, anaemia requiring blood transfusion in 3 cases, atrial fibrillation in 2 cases and pneumonitis in 1 case. Surgery achieved complete resection in all but 1 case (Stage IVa tumour). After a median follow-up of 40 months (range: 9–81 months), 3 patients relapsed. All patients were alive at the last follow-up.

T/M ratio, World Health Organization and staging

The difference between mean SUVmax and T/M ratio proved to be significant between low-risk and high-risk tumours (P = 0.001 and = 0.001, respectively). We tried to correlate PET–CT indicators with stage. Because prediction of the Stage is the cornerstone for planning therapy, we calculated T/M ratio and SUVmax for each stage, but we did not observe a correlation between T/M ratio and Masaoka-Koga stage (ρ = 0.3).

Standardized uptake value T/M and Ki-67 labelling index

The expression of Ki-67, defined as Ki-67 LI, distributed by WHO type is presented in Table 1. Ki-67 LI was found to be significantly higher in high-risk thymomas (P = 0.0002, Fig. 2) and shows a correlation with WHO classification (ρ = 0.8). Interestingly, Ki-67 LI variability was more pronounced in the low-risk group. Furthermore, T/M ratio proved to be significantly higher for strong positive Ki-67 LI tumours. This implies a correlation between T/M ratio and Ki-67 LI (ρ = 0.9, Fig. 3).
DISCUSSION

Many factors have been studied to evaluate the prognosis for TENs. A large series identified histology, completeness of resection and Masaoka-Koga stage as independent prognostic factors [3]. Attempts to predict the impact on survival of WHO types brought to definition of classes of risk, or subgroups. In particular Okamura grouped together A, AB and B1 as ‘low-risk thymomas’, with low invasiveness and better prognosis, and B2 and B3 as ‘high-risk thymomas’, more aggressive [5]. This grouping was employed in other large series and again a significant difference in prognosis between the two groups was demonstrated [6].

A predictor of histology or, more generically, of risk group should be useful in the pretreatment work-up to define the need for pre-operative therapy and even the surgical approach, in the era of minimally invasive surgery.

The core of the pretreatment evaluation relies essentially on CT scan of the thorax, and MRI. Those methods are essential for defining the presence and extent of invasion in the surrounding structures. However, there are many difficulties in predicting the class of risk.

PET–CT has progressively been studied in the last years for characterization of mediastinal masses and for thymic tumours in particular. Kumar et al., in the only prospective study on the predictive value of PET–CT, used SUVmax to differentiate thymic hyperplasia, thymomas and thymic carcinomas. Anyway, no difference in PET–CT features was observed between low-risk and high-risk thymomas [8]. Other authors stated that PET–CT features of carcinomas allowed their differentiation from thymomas, although a differentiation between classes of risk within thymomas was not identified [18]. In these studies, only the parameter SUVmax was used. Derived descriptors of metabolic features of TENs evolved to provide a more homogeneous indicator that could reduce technical and metabolic bias of the simple SUVmax.

In the present study, we grouped A, AB and B1 thymomas into the low-risk group and B2 and B3 in the high-risk group.

Table 1: Distribution of patients according to WHO classification system and tumour risk group, and features of PET–CT scan

<table>
<thead>
<tr>
<th>WHO classification</th>
<th>No. of patients (26)</th>
<th>SUVmax</th>
<th>SUV M</th>
<th>T/M ratio</th>
<th>Ki-67 LI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk group</td>
<td></td>
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<tr>
<td>Type A</td>
<td>3 (13%)</td>
<td>3.4 ± 0.93</td>
<td>1.86 ± 0.36</td>
<td>1.82 ± 0.71</td>
<td>30 ± 22.63</td>
</tr>
<tr>
<td>Type AB</td>
<td>9 (39%)</td>
<td>3.86 ± 0.73</td>
<td>1.99 ± 0.22</td>
<td>1.92 ± 0.25</td>
<td>29.22 ± 18.73</td>
</tr>
<tr>
<td>Type B1</td>
<td>5 (22%)</td>
<td>4.64 ± 2.06</td>
<td>2.32 ± 0.53</td>
<td>1.96 ± 0.47</td>
<td>50 ± 36.83</td>
</tr>
<tr>
<td>High-risk group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type B2</td>
<td>6 (26%)</td>
<td>7.60 ± 2.38</td>
<td>2.13 ± 0.82</td>
<td>3.73 ± 0.76</td>
<td>88.67 ± 8.86</td>
</tr>
<tr>
<td>Type B3</td>
<td>1 (4%)</td>
<td>6.52 ± 1.34</td>
<td>1.76 ± 0.44</td>
<td>3.82 ± 0.89</td>
<td>90.4 ± 10.46</td>
</tr>
</tbody>
</table>

Table 2: Distribution of patients according to Masaoka-Koga stage

<table>
<thead>
<tr>
<th>Masaoka-Koga stage</th>
<th>No. of patients (%)</th>
<th>SUVmax</th>
<th>SUV M</th>
<th>T/M ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>9 (39%)</td>
<td>3.78 ± 0.52</td>
<td>1.91 ± 0.23</td>
<td>2.06 ± 0.67</td>
</tr>
<tr>
<td>Stage II</td>
<td>9 (39%)</td>
<td>4.94 ± 1.37</td>
<td>2.01 ± 0.29</td>
<td>2.52 ± 0.73</td>
</tr>
<tr>
<td>Stage III</td>
<td>4 (18%)</td>
<td>5.58 ± 2.47</td>
<td>2.10 ± 0.74</td>
<td>2.61 ± 0.80</td>
</tr>
<tr>
<td>Stage IVa</td>
<td>1 (4%)</td>
<td>13</td>
<td>4</td>
<td>3.25</td>
</tr>
</tbody>
</table>

SUVmax: maximum standardized uptake value; SUV M: mean standardized uptake value of mediastinum; T/M ratio: the ratio of the SUVmax of the tumour and mean standardized uptake value of mediastinum; Ki-67 LI: Ki-67 labelling index.

Figure 2: Ki-67 expression whisker plot stratified by risk groups: low-risk thymomas (n = 17) and high-risk thymomas (n = 6) demonstrating a significant difference in Ki-67 LI (P = 0.0002).
metastases, not evidenced in the PET indicators, Ki-67 LI showed a strong correlation with SUVmax. The use of PET–CT (SUVmax and T/M ratio), followed by histological confirmation, should help defining the subsequent clinical management. Actually, when facing a mediastinal mass, T/M ratio may suggest the need for a preoperative biopsy, especially for little masses with no signs of gross invasion of the surrounding structures. In this case, biopsy is generally avoided, and first-line surgery is a generally accepted option. Thus, T/M ratio may help define the surgical approach: in particular, a high SUV T/M ratio should discourage a minimally invasive approach.

Our study is flawed by some limitations. In particular, it is limited to a small number of patients with TEN who underwent preoperative work-up and surgery in a single institution. A multicentric study on a larger series would allow better understanding of the usefulness of PET–CT in the clinical scenario of thymic malignancies. Because of the short follow-up time, we did not attempt a survival evaluation in order to define any prognostic role of the PET indicators.

CONCLUSIONS

The use of PET–CT in the pretreatment evaluation of anterior mediastinal masses can differentiate between high-risk and low-risk thymomas. The strong correlation of T/M ratio with Ki-67 expression underlines the possibilities of PET–CT to predict aggressiveness and clinical behaviour of TENs. In particular, high T/M ratio identifies a subset of high-risk patients within clinical Stages I and II, who deserve a more thorough preoperative work-up, which should also include biopsy. In case of upfront surgery, an open approach with extended thymectomy should be preferred.

Conflict of interest: none declared.

REFERENCES


APPENDIX. CONFERENCE DISCUSSION

Dr K. Athanassiadi (Athens, Greece): In this paper the authors wanted to investigate the usefulness of PET in predicting prognosis. They have already mentioned in their study that the numbers are really small so we cannot make firm conclusions safely. You started with the idea of calculating the ratio between tumour and mediastinum some years ago in lung cancer, so why did you choose the part near the aortic arch?

Dr Viti: It was decided because it was used in our previous experience and we find it easy to calculate the ratio because the region of interest of the thymus, of the thymoma and of the aortic arch, were quite near, so for a visual reason, mainly for the radiologist.

Dr Athanassiadi: If you took another point in the mediastinum, it could be different?

Dr Viti: Yes

Dr Athanassiadi: Okay. A thymoma is near, or sometimes invading, the aortic arch, so that’s why I was a little bit curious whether it’s correct to do this.

Dr Viti: Because at the aortic arch root it is easy to calculate the conventionally used area, 3 cm².

Dr Athanassiadi: And my second question is: You operated on all these patients, correct?

Dr Viti: Yes

Dr Athanassiadi: So you have Ki-67 proliferation marker from the histology in all these patients?

Dr Viti: Yes

Dr Athanassiadi: What’s the clinical implication of this study? Did you change anything in your clinical practice? Because you have a PET, okay, you have a PET ratio, you calculated everything, but you do operate on these patients and you yourself state in your paper that PET didn’t really help radiologically because it couldn’t describe the extension of the disease.

Dr Viti: Not so surprisingly we found a weak relation between the T/M ratio and Masaoka. We decided to use other indicators such as a volumetric indicator, that is the total glycolytic volume, that resulted in a more suitable indicator for the stage, to decide to adopt surgery as an upfront strategy instead of neoadjuvant chemotherapy. So for stage we prefer to use other indicators.

Dr Athanassiadi: If you have a proliferation marker that is high, that you can find out from PET, and the thymoma is resectable, are you going to propose neoadjuvant chemotherapy?

Dr Viti: No. At the moment, no.

Dr Athanassiadi: So what’s the implication?

Dr Viti: I agree with you, the clinical implication is very limited at the moment. We think that in case of an elevated T/M ratio, as I said, when you have to decide between the surgical approaches, you should decide for an open approach, in our opinion, avoiding minimally invasive surgery. This is one of the clinical options.