^{99m}Tc MIBI THYMIC SCINTIGRAPHY IN DIAGNOSIS AND THERAPEUTIC DECISION MAKING: WHAT IS BEHIND THE IMAGE?

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Abstract

Context. Despite CT being generally used in thymic pathology, in the case of regions with the same tissue density, only functional radioisotopic imaging can hint towards malignity.

Objectives. To assess the usefulness of ^{99m}Tc MIBI scintigraphy for diagnosis and treatment planning in thymoma, in relation with the radiotracer uptake mechanism.

Patients and methods. ^{99m}Tc MIBI thymic scans for 19 patients diagnosed with thymic disorders were assessed using tumor uptake ratio (UR). Specimens of thymectomies were examined and cytological assessments were correlated with the UR.

Results. The UR of all surgical patients was higher than 1.2, with a 1.5 cutoff between lymphoid hyperplasia and thymoma. The UR values were correlated with the histopathologic diagnosis (Pearson correlation 0.91, significant at $p < 0.01$). The highest UR was 3.24, found in the case of an AB thymoma where the rate lymphocytes/epithelial cells (L/E) was 1.6. In B1 thymoma UR was 1.14 and L/E was 2.46.

Conclusion. Phenotype differences between thymoma types correlate with ^{99m}Tc MIBI cellular uptake: lower rate L/E corresponds to higher UR, higher malignity potential and invasiveness. A thymic ^{99m}Tc MIBI UR higher than 1.5, corresponding to a CT tumoral image, is suggestive for a thymoma, requiring surgical treatment first.

Key words: Thymoma, Thymic Hyperplasia, ^{99m}Tc MIBI scan, Radiotracer Uptake Mechanism.

INTRODUCTION

Thymic pathology, either lymphoid hyperplasia or thymoma with malignant potential, associated or not with myasthenia gravis or other autoimmune disease such as hyperthyroidism or hemolytic anemia, could represent a real challenge for diagnosis and therapeutic choice. Thymoma is the primary mediastinal neoplasm

originated from the thymic epithelial cells (1), either benign or malignant. Cytologically benign thymomas have been classified as being lymphocyte rich, epithelial cell rich, or spindle cell type or, according with their architecture, as cortical, medullar, or mixed. Thymomas with malignant potential have, as a hallmark, a significant cytological atypia and mix of cells, as well as local invasive character (2).

Thoracic CT is considered the imaging modality of choice for evaluating thymic pathology (3), but, when tissue density is the same, it cannot sufficiently delineate between thymic lymphoid hyperplasia and thymoma. So, in clinical practice, on occasions, we faced unclear situations of thymopathy, when thorax CT oscillating between thymic lymphoid hyperplasia vs. thymoma or ectopic thymoma vs. other mediastinal mass or, postthymectomy, between tumor recurrence vs. mediastinal scar fibrous tissue. Before deciding the indication and the extension of surgery as the first step in the management of such a thymic pathology, it is important to make difference on CT images of a mediastinal mass especially between a thymic lymphoid hyperplasia and thymoma. In equivocal cases, thymic scintigraphy can make the difference.

Thymic radiotracers images, as ^{99m}Tc isonitrils (MIBI, tetrofosmin), ¹¹¹In DTPA octreotide scintigraphy, or, recently, ¹⁸F FDG PET-CT, all functional investigations, were demonstrated to add supplementary information to structural CT images (4-7). Between these, ^{99m}Tc MIBI scan is useful in diagnosis and therapeutic strategy of thymic lesions either when conventional imaging investigations fail to confirm a clinical diagnosis or in order to make evidence of the malignity of the thymic lesion. Based on hypothesis that understanding the radiotracer uptake can correlate with the cellular characteristics of the tumor, scintigraphy can be useful in elucidating the lesion type, with subsequent improvement on therapeutic decision and

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patient prognosis. The stage of the tumor at the time of diagnosis and the adequacy of the surgical excision are among the factors that influence the outcome of thymoma. The presence of clinical symptoms, large tumor size, local invasion or metastases at the time of the diagnosis and predominant epithelial features are poor prognostic factors. Surgery is the mainstay of the treatment in such cases. However, complete resection is sometimes not feasible because of local invasion of important structures and metastasis (8) and minimally invasive resection could be more appropriate (9). A multimodality approach that includes surgery, chemotherapy, and radiation therapy is better, but the therapeutic decision must be judged in relation with an appropriate initial characterization of the thymic lesion (10, 11).

AIMS OF THE STUDY

Our aims were to assess the usefulness of ^{99m}Tc MIBI scintigraphy functional images for the diagnosis and therapeutic decision of thymic pathology and to explain the tumoral radiotracer uptake degree in relation with cellular phenotype characteristics.

MATERIAL AND METHOD

The study included the patients presented at the Nuclear Medicine Laboratory, St. Spiridon Hospital, Iasi, for thymic scintigraphy, in the period 2007-2014, meaning 19 patients diagnosed with thymic disorders. All patients were informed about the study procedures and gave their informed, written consent. The institutional ethics committee agreed the study protocol. Patients were sent for scintigraphy when thorax CT results were equivocal, either preoperatively or when myasthenic symptoms recurred, postoperatively.

The patients were grouped in three categories: group 1 - surgery patients (male: female = 1:5, aged between 19 and 57, mean age 40.5), group 2 - non surgery patients (all females, aged between 19 and 57, mean age 38.33) and group 3 - patients with post-surgery myasthenic recurrence (male: female = 1:6, age between 31 and 64, mean age 46.42).

A thorax CT evaluation was realized for all patients before ^{99m}Tc MIBI scan, using a CT scanner Philips Brilliance 6, native and contrast media images, mediastinal window, 5 mm axial slices reconstruction. For the ^{99m}Tc MIBI scan, the patients received a standard radiotracer dose (7.4mCi/kgbw). Anterior/posterior planar 10 minutes images (256x256 matrix, 1.2

ZOOM), early and delayed (at 15 and 60 minutes) were performed, as well as delayed SPECT (60 projections over 360°, 20 seconds per projection, 64x64 matrix) acquisitions for a better localization of the tumor, when necessary. A Dual Head Siemens Gamma camera, with low energy – high resolution parallel collimators was used.

Qualitative and quantitative analysis were realized on the image with the highest uptake, between the early and delayed images. Qualitative analysis was based on the intensity of uptake and homogeneity of distribution of the radiotracer in the anterior mediastinum. Radiotracer uptake was classified in four categories: basal uptake (-), low uptake (+), moderate uptake (++) and high uptake (+++, when uptake was greater than heart uptake). Two independent observers evaluated the thymic lesion image by using the same four-point uptake scoring system. When uptake scores differed between the observers, a third observer was consulted.

Quantitative analysis was based on the uptake ratio (UR) calculation on the planar anterior images. Regions of interest (ROIs) were manually drawn around the entire area of uptake in the thymic lesion. The count densities (counts/pixel) of the ROIs were measured. The uptake ratio was then calculated by dividing the count density of the thymic lesion by the count density of a similar background region, considered at the lung level (D_t - thymic lesion counts density, D_l - lung background area counts density):

$$UR = D_t/D_l$$

The relation between UR and qualitative analysis is presented in Table 1.

Table 1. Relation between quantitative (UR) and qualitative assessment of the thymic lesion

UR	Qualitative analysis
<1	-
1 – 1.5	+
1.5 – 2.0	++
>2.0	+++

UR was analyzed among the surgery patients group, non-surgery group and post-surgery group. The scintigraphic images were defined in correlation with the histological findings in operated patients (n = 13 scintigraphic images). We described and assessed particularly the pathological characteristics of two thymomas types that had extreme uptake rates.

Morphology assessment of surgical specimen

Table 2. Group 1 patients – surgery patients (with preoperative scintigraphy) characteristics (?TLH = Thymic Lymphoid Hyperplasia observation, ?Ty = Thymoma observation, ABTy = AB thymoma, B1Ty = B1 thymoma)

Case	Sex	Age	MG (OSS)	CT	Uptake Intensity	UR	Pathology
1. UD	F	54	III	?TLH	+	1.4	TLH
2. UG	F	31	IIB	?TLH	+	1.32	TLH
3. AM	F	46	IIB	?TLH	+++ectopic	3.24	ABTy
4. PT	F	21	III	?Ty	+	1.31	TLH
5. IV	M	54	III	?Ty	++	1.54	B1Ty
6. CF	F	37	IIB	?Ty	+	1.4	TLH

was made with an optical microscope (Olympus CX41). The measurements were realized with a color image analysis system, QuickPHOTO MICRO 3.0. Morphologically, we have analyzed the tumor structure and cytology by usual and specific stains (hematoxylin eosin, elastic von Gieson, and Gordon Sweet for reticulin).

Quantitative morphometry was performed by appreciation of lymphocytes to epithelial cells ratio, microvessels density and measuring nuclear epithelial cell area. The measurements were expressed in mean values and frequencies.

Diagnosis of myasthenia gravis (MG) was confirmed on the clinical findings and pharmacologic, electrophysiologic, immunologic criteria. Clinical severity of MG was assessed by Osserman classification (OSS) (12).

RESULTS

Group 1- Surgery patients (with preoperative scintigraphy)

In this group, delayed index ratio was over 1.2, with a 1.5 cutoff between TLH and Ty (mean 1.358, standard deviation 0.049). Considering this index along with clinical severity of the disease (three cases OSS IIB and three cases OSS III) and/or suspicious thymoma on CT report (three cases), the therapeutic decision was surgery without delay.

In the biopsies from mediastinal masses, histopathological examination revealed features of thymoma and thymic lymphoid hyperplasia (TLH), as mentioned in Table 2. The histological characteristics and cytological analysis varied essentially between AB thymoma (case 3) and B1 thymoma (case 5).

Case 3 was a particular one, in which thymic scintigraphy was extremely rewarding as CT overlooked an antero-inferior mediastinal mass, giving, initially, as result, normal thymic region (Fig. 1a). Radiotracer uptake ratio was 3.24, drawing attention to an ectopic thymoma (Fig. 1c), identified further by a recall CT investigation (Fig. 1b). Surgical

specimen (Fig. 1e) pathological examination showed a nodular encapsulated tumor having capsular infiltration (Fig. 1d). The lymphocyte to epithelial cell ratio was of 1.6. Epithelial cells were medium sized to large and round to oval in shape, with an average nuclear area of 46.4 μm^2 . These tumoral epithelial cells are considered by literature to represent the tumoral neoplastic component. Angiogenesis with an average of 2 microvessels on HPF was found. The morphological features justified the diagnosis of AB thymoma (mix), including both type A areas (atrophied) and type B areas (bioactive).

In case 5 the uptake ratio was 1.54. Pathology examination showed the same dual cell population, but with more small lymphocytes, the lymphocyte to epithelial cell ratio being of 2.46. The most epithelial cells were small with polygonal shape and an average nuclear area of 18 μm^2 . Angiogenesis was greater than in AB thymoma, represented by 3 vessels on HPF (HE, x400). The histopathologic diagnosis was B1 thymoma.

Figure 1. Thymoma AB - case 3. **a-** initial normal CT image; **b-** recall CT, showing ectopic tumoral mass (arrow); **c-** ^{99m}Tc MIBI scan, showing high thymic uptake (arrow); **d-** histopathologic image showing invasiveness character and lymphocyte rich area; **e-** surgical resection specimen.

Figure 2. Thymoma B1- case 5. **a-** ^{99m}Tc MIBI scan, showing low thymic uptake (arrow); **b-** CT image, showing the tumoral mass; **c-** surgical resection specimen (the white arrow shows the part for radiotracer uptake); **d1-** lymphocyte rich-area; **d2-** densely packed, small lymphocytes (HE, x40).

Group 2- Non-surgery patients (selected for medical therapy)

In this group, the patients presented short history of MG mild forms (two cases OSS I and four cases OSS IIA). Usually thymectomy is indicated only when medical treatment of patients is not successful or when thymoma is suspected, the best results after thymectomy being encountered in young myasthenic patients with thymic lymphoid hyperplasia. This is why morphological and functional evaluation of the thymic lesion is important in planning the therapeutic strategy: medical or surgical treatment, first.

Thorax CT suggested, as thymic lesion, thymic lymphoid hyperplasia; at the ^{99m}Tc MIBI scan,

radiotracer uptake index ratios were all below 1.2, as presented in Table 3.

Based on these criteria the patients were treated with anticholinesterase drugs with good clinical response, being kept under observation.

Group 3- Post-surgery patients with recurrent MG

In this group, operated patients who presented moderate myasthenic recurrence had a scintigraphic examination to rule out a tumor recurrence or thymic ectopia left behind.

Low uptake index ($\text{UR}=0.79\pm0.09$, see Table 4), excluded these possibilities, myasthenic symptoms being attributable to extrathymic autoimmunity process. These patients were referred to Neurology Department for immunosuppressive treatment.

Considering a cutoff level of 1.5 for UR between TLH and thymoma, our study sustains the role of ^{99m}Tc MIBI scintigraphy for the differential diagnosis between these two entities. The Pearson correlation between UR values and histopathological diagnosis was a strong positive one of 0.91, the result being significant at $p<0.01$.

DISCUSSION

Even radiotracers functional images have been reported to be useful for thymoma diagnosis from a number of studies (13-17), the techniques and, consequently, the results are relatively heterogeneous and fewer authors explained clearly what the tumor

Table 3. Group 2 patients – non-surgery patients, selected for medical therapy characteristics

Case	Sex	Age	MG (OSS)	CT	Uptake Intensity	UR
1. RI	F	57	I	?TLH	+	1.18
2. TC	F	36	IIA	?TLH	+	1.15
3. FA	F	37	IIA	?TLH	+	1.1
4. AI	F	42	IIA	?TLH	+	1.2
5. PL	F	19	I	?TLH	+	1.06
6. MM	F	39	IIA	?TLH	+	1.12

Table 4. Group 3 patients (with recurrent, post-surgery, MG) characteristics

Case	Sex	Age	MG (OSS) (pre-surgery)	CT	Uptake Intensity	UR (thymic region)	Pathology (initial tumor)
1. TD	F	38	III	-	-	0.91	ABTy
2. CT	F	64	IV	-	-	0.74	B1Ty
3. MC	F	38	IIB	-	-	0.92	TLH
4. CC	F	42	IIB	-	-	0.67	TLH
5. GI	F	58	IIB	-	-	0.73	B1Ty
6. UG	F	31	III	-	-	0.76	TLH
7. IV	M	54	III	-	-	0.78	B1Ty

cells responsible for radiotracer uptake are, and what the relation of the radiotracer uptake degree with the tumor invasiveness is.

To explain the obtained results it is necessary to: a) start from the known data about the structure of ^{99m}Tc MIBI and its cellular uptake mechanism, and b) correlate these data with histopathological structure of the thymus, in order to understand why and when thymoma cells show high radiotracer uptake.

a) Essential data about ^{99m}Tc MIBI molecule biophysical characteristics

^{99m}Tc MIBI molecule has six alkyl radicals around technetium in the structure (18,19), realizing a lipophilic sphere with a singular positive electrical charge, that give the possibility of freely passage through the hydrophobic lipid biomembrane bilayer (Fig. 3).

As a lipophilic cation, ^{99m}Tc MIBI will cross passively the cellular membrane, depending on its electrochemical gradient, in relation with Nernst equations and with membrane fluidity (19). A number of in vitro studies on myocytes and some tumoral cell types (20, 21) have shown that, into the cell, the molecule neither is bound by a certain protein, nor is resting in a free cytosolic form for a long time, but it is driven further, through the mitochondrial membrane, depending also on the Nernst equations. If ^{99m}Tc MIBI intracytoplasmatic concentration was found, in some cells, five times greater than its extracellular one, the intramitochondrial accumulation can reach 300 times its cytosolic concentration, in the case of rat myocyte (19). This final intramitochondrial concentration depend both on the plasmatic and mitochondrial membrane potential. It can be demonstrated (20, 22) that the mitochondrial concentration, and, in fact, the whole cellular uptake of ^{99m}Tc MIBI is determined by cellular factors: directly, by plasma and mitochondrial

membrane potential and, indirectly, by mitochondrial surface and plasma membrane permeability differences for the radiotracer molecule. Together with these cellular factors, molecular factors (degree of lipophily, position of global electric charge, arrangement of atoms in the molecule and type of chemical connections which determine the shape of the molecule) will be all the factors to make differences between different types of isonitrils radiotracers uptake (19).

These data explains the high uptake of the ^{99m}Tc MIBI in cells with a high mitochondrial activity. In vitro studies have demonstrated that, between the normal cells, myocytes have the highest ^{99m}Tc MIBI uptake (20, 21), being considered reference value. This fact can be explained by their great number of mitochondria, as well as high membrane potential differences. Different tumoral cells were found to have high ^{99m}Tc lipophilic cationic complexes uptake that can be even related with cellular malignant characteristics (20, 22). This is not surprising how at least a 60mV potential difference was demonstrated between the mitochondrial membrane potentials of neoplastic and normal epithelial cells, for example (18). It was demonstrated that in some types of thymoma, medullary epithelial cells are larger and contain more mitochondria (23), so these could be the cells that uptake highly the isonitril radiotracer. However, no experimental data about the membrane or mitochondrial potential of the thymoma epithelial cells were related. Fibroblasts uptake this radiotracer in only a small proportion, considered the lowest among normal cells, as demonstrated in cell culture studies. Necrotic cells can not uptake radiotracer (18, 19). Cellular ^{99m}Tc MIBI efflux is, at least partially, mediated through the integral membrane protein, MDR1 glycoprotein (multi drug resistance protein) (18, 19).

b) Thymoma cellularity role in explaining tumor tissue radiotracer uptake

Thymoma is constituted by a mix of neoplastic and non-neoplastic cells (Fig. 4). Between these, sustained by the above presented data, not all will have the same ^{99m}Tc MIBI molecule uptake level (18, 19): viable neoplastic cells, proliferating and non-proliferating, will highly uptake ^{99m}Tc MIBI through specific mechanism but necrotic neoplastic cells do not uptake ^{99m}Tc MIBI and non-neoplastic cells can uptake ^{99m}Tc MIBI only at a very low level (particularly the fibroblasts).

At the neoplastic cellular level, the uptake of ^{99m}Tc MIBI will depend primarily on the characteristics

Figure 3. General structure of the ^{99m}Tc hexakis (metoxi isobutyl isonitrile) complex: molecular hexahedral configuration (19).

of malignant cells and could be considered to reflect various factors, including cellular metabolic activity, regional blood flow and the number of viable cells with high mitochondria content in the lesion (19).

Figure 4. ^{99m}Tc MIBI uptake in relation with the cellular types (19).

Quantitatively, the lymphocyte to epithelial cell ratio varies widely in thymomas, from predominantly lymphocytic to predominantly epithelial (12). Phenotypic characteristics vary also, related to malignancy. Mean nuclear area increased significantly with invasiveness degree (24). Morphological and morphometric studies demonstrated a significant difference in degree of malignancy between non-invasive and invasive thymomas (1). There appears to be a significant correlation between tumor angiogenesis and invasiveness (12). The epithelial cells represent the neoplastic component of thymomas. Microscopically, they can be larger and lighter, typically round or oval, but sometimes have a spindle-shaped nucleus (24). The non-neoplastic part of the tumor can be represented by different cells: the lymphocytes, small with dark nuclei, are considered non-neoplastic and, as in the normal thymus, are constituted mainly of T cells in various stages of maturation; the fibrous capsule, that surrounds the tumor and sends thick, fibrous septa, dividing the tumor into well-demarcated lobules. The malignant behavior of a thymoma is indicated also by microscopic or macroscopic invasion of the tumor capsule or of surrounding organs or by the presence of metastasis (25). Few thymic epithelial tumor cell lines have been established until now (26, 27), determining a lack in phenotypical thymoma direct cellular characterization and insufficient progress in understanding radiotracer imaging or developing further specific radiotracer therapy.

In our study, the different results found, morphometrically, between AB and B1 thymomas types, according with epithelial cell sizes, shapes

and their proportions reported to lymphocytes, could correlate both with differences in tumor invasiveness and radiotracer uptake ratio, as seen. Cell types were different from dimensions, shapes and, consequently, also, mitochondrial status. It can be stated that the cells that have higher ^{99m}Tc MIBI uptake were especially the larger neoplastic epithelial cells (like seen in case 3 thymoma), that have higher mitochondria content, giving the possibility of radiotracer concentration inside mitochondria, the final site of the radiotracer influx mechanism. The higher uptake ratio could be, finally, an expression of a higher content of the tumor in larger neoplastic epithelial cells.

Our data are consistent with other literature findings. The UR values calculated in our studies were in the same range with the values of Hashimoto *et al.* studies (13) and moderate higher then the values calculated by Fiorelli *et al.* (28) on a number of mediastinal tumors including thymoma. These different results between studies can be explained by particularities in image processing and quantification: firstly, if the UR is calculated on whole tumor mass, on planar images, or on SPECT slices, between maximum or mean uptake values; secondly, if the normal region is chosen on a lung region, having the lowest background or on a mediastinal region; thirdly, if the quantification is made on images acquired early or at 60 minutes, the last interval of time being demonstrated, by in vitro studies, to correspond to the highest ^{99m}Tc MIBI cellular uptake influx, for culture cells, like myocytes and different cancer cells (18, 19), so possibly also for thymus tumoral cells.

Our data sustain the statement that ^{99m}Tc MIBI thymic scan in thymus pathology is useful to be indicated after CT scan and before surgery, to complete, functionally, the initial diagnosis of a thymic lesion identified, structurally, on CT and to help therapeutic choice between surgery and medical treatment. In postsurgical status reevaluation, ^{99m}Tc MIBI thymic scan can be useful in the diagnosis of possible relapse, knowing that the recurrence rate after total resection of the thymoma ranges from 8% to 18% (14). Undoubtedly, nuclear medicine imaging, using different radiotracers (^{99m}Tc isonitrils, ^{201}Tl , ^{111}In Octreotide, ^{18}F FDG) can provide useful information in differential diagnosis between thymoma and TLH, staging and restaging (4, 5, 8) and therapeutic choice. However, the heterogeneity of radiotracer studies in terms of acquisition protocols, quantification and the low patients' number series, could be a reason of lack in clear statistical results in the literature, like also in

our study, based on the fact that small sample size could produce an imprecise estimate of accuracy with wide confidence interval (29). Due to the rarity, but also the gravity, of this pathology (30), small series studies, like ours, keep their importance.

In conclusion, ^{99m}Tc MIBI scintigraphy is a non-invasive imaging useful in diagnosis and therapeutic decision of thymic lesions especially either when conventional imaging investigations fail to confirm a clinical diagnosis or in order to make evidence of the malignancy of the thymic lesion. From the mix cellularity of thymoma, it seems that the larger epithelial cells are the ones that uptake ^{99m}Tc MIBI in higher quantities, determining intense positive scintigraphic images, in the case when the ratio lymphocytes:epithelial cells is lower. What is certain is that a hyperfixation of ^{99m}Tc MIBI, with a high uptake ratio, corresponding to a structural (CT) tumoral image, indicate a tumoral thymic lesion with malignity character that need appropriate surgical treatment and allow the differential diagnosis with a thymic lymphoid hyperplasia that can be treated non-surgical.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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