Development of new radiotracers for diseases image diagnosis and evolution monitoring under treatment:

biophysical uptake mechanisms, in vivo biodistribution, image parameters

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# Summary

## Chapter

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CHAPTER I.

$^{99m}$Tc ISONITRILS FOR THE FUNCTIONAL EVALUATION OF PULMONARY TUBERCULOSIS (PTB)

I.1. In vitro studies

I.1.1. Experimental study of $^{99m}$Tc MIBI uptake on Mycobacterium Tuberculosis cultures

Hypothesis and Aims:

Hypothesis: In order to explain $^{99m}$Tc MIBI positive scintigraphic images in pulmonary tuberculosis (PTB) one hypothesis could be the uptake of this radiotracer in Mycobacterium Tuberculosis (MT). This can be verified in vitro.

Aims: The aim of this experimental work was to assess the uptake of a radiotracer, $^{99m}$Tc MIBI (metoxy-isobutil-isonitril) on Mycobacterium Tuberculosis cultures, and to compare this uptake level with the uptake of two normal cell types in culture (fibroblastes and myocites), chosen to represent the extremes uptake values of $^{99m}$Tc MIBI (data from the literature 15, 19, 20): fibroblastes have very low uptake and myocites have the highest known uptake in the normal cells range. The results could give informations to understand the Mycobacterium Tuberculosis $^{99m}$Tc MIBI uptake mechanisms.

Material and method:

Cells: Three types of cells were used: Mycobacterium Tuberculosis, fibroblastes, myocites.

Work protocol: Mycobacterium Tuberculosis cultures were realised on Löwenstein Jensen medium by standard methodology (22, 24, 30, 33), 21 days cultures being used in our experiment (Figure I.1).

- New born mouses hearts
  - separated
  - tripsinated
  - Cells in suspension (fibroblastes and myocites)
    - Differential cellular attachment technique
    - I. Nonmyocites (attached fibroblastes)
    - II. Myocites
  - Incubation with radiotracer
  - Mycobacterium Tuberculosis
    - Löwenstein-Jensen medium
    - 21 days incubation
    - 37˚C, one hour, in the day of the experiment
    - Incubation with radiotracer

Figure I.1. Experimental work protocol steps
The normal cells were neonatal rat heart myocytes and fibroblasts. Final plating was done to have an almost complete cell monolayer the day of the experiment, in culture dishes. The cells were incubated with a 1.85 kBq/µl concentration of $^{99m}$Tc MIBI, at 37°C, using three dishes for each cell type. A kinetic three point (15, 60 and 90 minutes) was realised for *Mycobacterium Tuberculosis*. The incubation was stopped by rapidly washing the dish cells, three times, with a 4°C physiological saline solution. Cells were then superficially scrapped and withdrawn in saline physiological solution for counting the uptaked radioactivity. The algorithm is presented in the next diagram (Figure I.2).

The incubation was stopped by rapidly washing the dish cells, three times, with a 4°C physiological saline solution. Cells were than superficially scrapped and withdrawn in saline physiological solution for counting the uptaked radioactivity. Protein concentration was determined using Lowry method.

Freely culture medium was also incubated with the radiotracer to exclude the possible influence on the cell data.

\[
\begin{align*}
\text{Confluent cells (monolayer)} \quad & \downarrow 37°C, 1h \\
& \quad \text{incubation, 2 ml } \text{NaCl 9‰} \\
& 99mTc \text{ MIBI: } 15, 60, 90 \text{ min} \\
& \quad (1.85 \text{ kBq/µl}) \\
& \downarrow \text{Stop the kinetics + wash} \\
& \quad (2x1 \text{ ml NaCl 9‰, at 4°C}) \\
& \downarrow \text{Cells scraping; prelevation in 2 ml NaCl 9‰} \\
& \downarrow \text{Tubes with radioactive cellular suspension: gamma counter}
\end{align*}
\]

*Figure I.2. The incubation with $^{99m}$Tc MIBI protocol*

**Results and discussions:**

Most important results were communicated and published (12, 22, 24, 25, 27, 28, 30).

Results were expressed in percent of the total added radioactivity amount, per µg proteins and like percentage in comparison with myocytes 60 minutes uptake, considered 100% (Figure I.3). Statistical comparisons were done using *t* student test for paired data (comparison vs fibroblasts uptake).
Figure 1.3. Relative uptake of $^{99m}$Tc MIBI on Mycobacterium Tuberculosis, fibroblastes and myocites (cpm/µg proteines, % of total added radioactivity, after different incubation time)

The results were also corrected for the precise amount of activity added and for the radioactivity decay of $^{99m}$Tc.

Table 1.1. Cellular uptake of the radotracer in different cell lines

<table>
<thead>
<tr>
<th>Cells</th>
<th>Incubation time interval</th>
<th>Relative uptake (cpm/µg proteines, % of total added radioactivity) Mean ± standard deviation</th>
<th>Relative uptake (%), in comparison with myocytes, considered 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium Tuberculosis</td>
<td>15 minutes</td>
<td>0.0395 ± 0.0057, p&lt;0.001</td>
<td>106.47</td>
</tr>
<tr>
<td></td>
<td>60 minutes</td>
<td>0.0181 ± 0.0026, p&lt;0.002</td>
<td>48.79</td>
</tr>
<tr>
<td></td>
<td>90 minutes</td>
<td>0.0206 ± 0.0042, p&lt;0.005</td>
<td>55.52</td>
</tr>
<tr>
<td>Myocites</td>
<td>15 minutes</td>
<td>0.0342 ± 0.0023, p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 minutes</td>
<td>0.0371 ± 0.0051, p&lt;0.001</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>90 minutes</td>
<td>0.0252 ± 0.0039, p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Fibroblastes</td>
<td>15 minutes</td>
<td>0.0149 ± 0.0025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 minutes</td>
<td>0.0151 ± 0.0019</td>
<td>40.70</td>
</tr>
<tr>
<td></td>
<td>90 minutes</td>
<td>0.0150 ± 0.0022</td>
<td></td>
</tr>
</tbody>
</table>
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 mechanisms on other cell type;

b) correlate these data with *Mycobacterium Tuberculosis* structure.

a) $^{99m}$Tc MIBI is a radiotracer whose molecule has six alkyl radicals around technetium, realizing a lipophilic sphere with a singular positive charge:

\[
\text{Chemical general formula:} \quad ^{99m}\text{Tc} - [\text{C} \equiv \text{N} \cdot \text{R}]^+ \\
\text{The alkyl radicals in the case of methoxy-isobutilisonitrile (}$^{99m}\text{Tc MIBI}$): \\
\text{R} = \text{CH}_2\text{C(\text{CH}_3)_2OCH}_3
\]

\[\text{Figure I.4. General structure of the} \quad ^{99m}\text{Tc hexakis (alkylisonitrile) complexes: molecular octaedric configuration}\]

All the $^{99m}$Tc compounds having the same general molecular characteristics - as lipophilic cations - cross the cellular membrane depending on their electrochemical gradient, in relation with Nernst equations (21). Hexakis (alkyl isonitrile) family is the most studied, with applications mostly in the cardiac as well as in oncologic scintigraphy.

**The transport across the plasmatic membrane**

$^{99m}$Tc MIBI molecule has a moderate lipophilicity between others $^{99m}$Tc compounds. The identical C≡N groups surround in a lipophilic "sphere" the metal, technetium - which is in a central position, like seen in *Figure I.4*. This character allows its transmembranar passive cross through the hydrophobic intramembranar medium. The molecule has also a global, but delocalised electric charge, which explain the dependence of the passage on the membranar potential, through the Nernst equations, as follows:

\[(\text{where:})
\]

- $\Delta \phi_{\text{plasma}} = \text{plasmatic membrane potential}$
- $\Delta \phi_{\text{mit}} = \text{mitochondrial membrane potential}$
- $\text{Tc-MIBI}_{\text{extracel}} = ^{99m}\text{Tc MIBI extracellular concentration}$
- $\text{Tc-MIBI}_{\text{cytoplas}m} = ^{99m}\text{Tc MIBI cytosolic concentration}$
- $\text{Tc-MIBI}_{\text{mitochondrial}} = ^{99m}\text{Tc MIBI mitochondrial concentration}$
- $RT/zF = \text{constant, depending on:}$
  - $z = \text{electron's number per mol}$
  - $F = \text{Faraday Number, 96500 Coulombs}$
  - $R = \text{perfect's gaz constant, 1,987 cal/mol/degree}$
  - $T = \text{absolute temperature}$)
\[ Tc\text{-MIBI}_{\text{cytoplasm}} = Tc\text{-MIBI}_{\text{extracel}} e^{-\Delta \phi_{\text{plasma}}/F/RT} \]  

(1)

so:

\[ \Delta \phi_{\text{plasma}} = RT/zF \times \ln \left( \frac{Tc\text{-MIBI}_{\text{extracel}}}{Tc\text{-MIBI}_{\text{cytoplasm}}} \right) \]  

(2)

or

\[ \Delta \phi_{\text{plasma}} = 2,3RT/zF \times \log_{10} \frac{Tc\text{-MIBI}_{\text{extracel}}}{Tc\text{-MIBI}_{\text{cytoplasm}}} \]  

(3)

but:

\[ 2,3 \text{ RT}/zF = 60 \text{ mV} \] (for a monovalent ion, at environmental temperature)

Equation (1) become:

\[ Tc\text{-MIBI}_{\text{cytoplasm}} = Tc\text{-MIBI}_{\text{extracel}} \times 10^{-(\Delta \phi_{\text{plasma}})/60\text{mV}} \]  

(4)

**Cytosolic concentration**

\(^{99}\text{mTc}\) MIBI intracytoplasmatic concentration was found five times greater than its extracellular one (6). A number of in vitro studies, on myocites and some tumoral cell types (7, 21) have shown that 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:

\[ Tc\text{-MIBI}_{\text{mitochondrial}} = Tc\text{-MIBI}_{\text{cytoplasm}} \times 10^{-(\Delta \phi_{\text{mit}})/60\text{mV}} \]  

(5)

This accumulation can reach 300 times its cytosolic concentration, in the case of rat myocite (8). This final concentration depend both on the plasmatic and mitochondrial membrane potential, like seen if we considere equations 4 and 5:

\[ Tc\text{-MIBI}_{\text{mitochondrial}} = Tc\text{-MIBI}_{\text{extracel}} \times 10^{-(\Delta \phi_{\text{plasma}}+\Delta \phi_{\text{mit}})/60\text{mV}} \]  

(6)

In vitro studies have demonstrated that, between the normal cells, myocites \(^{99}\text{mTc}\) MIBI uptake is the highest. This fact can be explained by their great number of mitochondria, as well as high membranar potential differences (16, 33). Tumoral cells also were found to have a high \(^{99}\text{mTc}\) lipophilic cationic complexes uptake that can be even related with cellular malignant characteristics (20, 24, 25). This is not surprising how at least a 60 mV difference was demonstrated between the mitochondria membrane potentials of neoplastic and normal epithelial cells, possibly explained in relation with Warburg hypothesis for the neoplastic cells mitochondria.

**Intracellular distribution pattern**

It was demonstrated (6) that, into the cell, \(^{99}\text{mTc}\) MIBI rest at 90% concentrated intramitochondrial, the extramitochondrial, cytosolic, fraction being less than 10%.
**99mTc MIBI efflux**

Cellular 99mTc MIBI efflux is, at least partially, realised through an integral membrane protein, MDR1 glycoprotein (multi drug resistance protein). This is coded by the MDR gene and have a 17000 Mr. Being an ATP dependent efflux protein, ATP depletion can increase 99mTc MIBI cellular accumulation from 40% to 90% (7, 21).

b) *Mycobacterium Tuberculosis* has a cell wall of about 20 nm thickness (in electron microscopy). It consist of an inner, electron-dense layer sorrounded by an outer elecron-transparent layer. It contain a basic backbone structure of peptidoglycan, polypeptids and free lipids (*Figure I.5*).

![Figure I.5. The lipids in the MT wall](image)

What is important for the lipophilic radiotracer is that over 60% of the *Mycobacterium Tuberculosis* wall is lipid (complex lipids) and from these, over 50% are mycolic acids which seems to give great fluidity to the wall. The types of the lipids seems to be corelated with the pathogenicity of the bacteria.

Because of the cell wall thickness and high lipid content, the wall is mostly impermeable to hydrophilic molecules and could be permeable to high lipophilic molecules. It could be the case of 99mTc MIBI (18, 30).

**Conclusions:**
1. *Mycobacterium Tuberculosis* proved to have high 99mTc MIBI uptake at 15 minutes in comparison with other kinetics points.
2. This uptake is almost as high as miocytes 60 minutes uptake (known as the highest normal cell uptake).
3. These *in vitro* uptake results could explain the more positives scintigraphic images obtained at 15 minutes, in BK positive patients, in comparison with the delayed images.
4. These data suggest that 99mTc MIBI lung scan for PTB acquisition protocol must include 15 and 60 minutes images.
I.1.2. Membrane fluidity assessment of *Mycobacterium Tuberculosis* wall

**Hypothesis and aims:**
*Mycobacterium Tuberculosis* wall membrane fluidity is one of the factors that could influence $^{99m}$Tc isonitrils MT uptake. The aim of this study is to assess and compare MT wall fluidity with other known cellular membrane fluidity values (red blood cell). No experimental data in the literature were found regarding membrane fluidity on *Mycobacterium Tuberculosis*. This is why no established experimental work protocol could be used like reference.

**Material and method:**
Cells: *Mycobacterium Tuberculosis* in suspension, concentration $10^4$/ml NaCl 9‰ realized from positive *Mycobacterium Tuberculosis* smears.
Method: Static polarization of fluorescence with Diphenylhexatrien (DPH) fluorescent marker.
Device: Spectrofluorimeter PTI.
Work protocol: is presented in the diagram bellow:

![Diagram of the experimental protocol](image)

**Figure 1.6. MT Membrane fluidity assessment work protocol**

Acquisition parameters:
- $\lambda_{\text{excitation}} = 360$ nm
- $\lambda_{\text{emission}} = 430$ nm
Acquisition time interval: 30 seconds
Results and discussions:
Fluorescence intensity results (per $10^4$ MT bacils) are depicted in the next graphs (using like etalon fluorescence intensity assessment for a $10^6$ probe red blood cells).

Figure I.7. MT Membrane fluidity fluorescence intensity results

Conclusions:
1. In vitro data regarding $^{99m}$Tc MIBI cellular kinetics and MT wall fluidity are useful to understand pulmonary scintigraphy in PTB evaluation role as a functional, noninvasive exploration.
2. In vitro studies proved that Mycobacterium Tuberculosis have high $^{99m}$Tc MIBI, 106.47% (compared with the known higher normal uptake cells – the miocytes considered 100%); the highest uptake kinetic point is at 15 minutes radiotracer incubation.
3. The assessment of Mycobacterium Tuberculosis wall fluidity has not brought conclusive results, related, probably to an unsuitable work protocol, on whole cell.
4. These in vitro studies suggest that $^{99m}$Tc MIBI pulmonary scintigraphy could bring functional information regarding the presence and the cellular activity of Mycobacterium Tuberculosis into lesions that, radiologically, can have same tissular density, so, also appearance.
5. $^{99m}$Tc MIBI could be a suitable marker to monitorize PTB patients under treatment.
I.2. In vivo studies
   I.2.1. 99mTc MIBI scintigraphy for PTB evaluation
      I.2.1.1. 99mTc MIBI PTB initial diagnosis evaluation

Hypothesis and aims:
To assess the possible usefulness of 99mTc MIBI for the: 1) scintigraphic diagnostic evaluation of pulmonary tuberculosis (PTB); 2) finding of new sites of PTB (in relation with the radiologic sites).

Material and methods:
Inclusion criteria:
Patients hospitalized in the Pneumology Hospital from Iaşi.
- New cases with PTB diagnostic (clear or suspicion)
- Positive or negative Mycobacterium Tuberculosis smears in microscopy
- Untreated before the scintigraphy
- With evident radiologic lesions
- With other laboratory tests: ESR (erythrocyte sedimentation rate), IDR (tuberculin intradermoreaction, at 2 units PPD), smears for MT in microscopy (mo) and culture (c), blood cell count.

Exclusion criteria: Pregnant women.
Patients related to age and sex (Figure I.8):
We have studied 41 patients diagnosed with active pulmonary tuberculosis, new cases. Sex ratio was women/men = 24/17; mean age: 25 years; 26 positive MT smears, 15 negative MT smears (where the culture results were 12 positive and 3 negative).

Patients sex repartition
(F = feminin, M = masculin)

Patients age repartition

Figure I.8. Distribution of the studied patients related to age and sex

Patients characteristics related to microscopy, culture and radiological features are presented in Figures I.9, I.10, I.11.
For all the patients, the followed characteristics were:
- Smoking status
- Subjective and objective clinical symptoms of the patient
- The epidemiologic context of PTB unset.
Figure I.9. Patients number, related to smears microscopy (mo) and MT culture

Figure I.10. Smears microscopy (mo) and culture results for MT presence: percentage

Figure I.11. The radiographic features of the patients
Work protocol: Scintigraphic images before the beginning at the treatment.
Administered doses: 7.4 MBq (0.2 mCi)/Kg/patient $^{99m}$Tc MIBI dose, i.v., intrabrahial.
Aquisition device: A Gamma camera Siemens-Diacam, single head, parallel - high resolution colimator was used, with a Macintosh computer (ICON system) for image processing, at the Nuclear Medicine Laboratory, ”St. Spiridon” Universitary Hospital (Iasi).
Scintigraphic protocol: Scintigraphic images have been realised as follows: an anterior planar 3 000 000 counts image on the thorax at 15 minutes after radiotracer i.v. administration, followed by a SPECT acquisition and another anterior planar (same number of counts, same region) image at 60 minutes after radiotracer administration; medium energy collimator was used.
Image aquisition protocol (Figure I.12) was established according to personalin vitro data and literature data (17, 18): two anterior planar images, at 15 and 60 minutes after radiotracer i.v. administration and a SPECT acquisition:

Figure I.12. Image aquisition protocol

Aquisition parameters:

a) static planar images:  
- 256 x 256 matrix  
- 3,000 000 counts/image  
- ZOOM 1.78

b) SPECT images:  
- 128 x 128 matrix  
- Images step and shoot, 20 seconds/image, 10° step, 360° rotation.

Image processing:
The obtained scintigraphic images were both qualitatively and quantitively analized; they were also compared with the corespondent radiologic images.

a) Qualitative analysis:
Radiotracer uptake classification:  
- without uptake  
+ low uptake  
++ moderate uptake  
+++ high uptake
b) Quantitative analysis (*Table I.2*): three identical (in dimensions and shape) interest regions were drawn:
- On the pathologic site = ROI$_1$ (representing the pathologic uptake)
- On a normal pulmonary field = ROI$_2$ (representing the normal pulmonary uptake)
- On a myocardial field = ROI$_3$ (representing the higher radiotracer normal tissue uptake)

The quantification has been assessed using three indexes (counts/pixel):
- $I_{\text{MIBI}}(1) =$ lesion/normal
- $I_{\text{MIBI}}(2) =$ lesion/heart
- $I_{\text{MIBI}}(3) =$ normal/heart (to exclude a pathologic pulmonary increased uptake through vascular pulmonary loading, for example in long period smoking persons).

**Table I.2. Qualitative/quantitative correspondence of the indexes**

<table>
<thead>
<tr>
<th>$I_{\text{MIBI}}(1)$:</th>
<th>$I_{\text{MIBI}}(2)$:</th>
<th>$I_{\text{MIBI}}(3)$:</th>
</tr>
</thead>
<tbody>
<tr>
<td>if $I_{\text{MIBI}}(1) &lt; 1$</td>
<td>if $I_{\text{MIBI}}(2) &lt; 0.4$</td>
<td>normal if $0.3 &lt; I_{\text{MIBI}}(3) &lt; 0.45$</td>
</tr>
<tr>
<td>+ if $1 &lt; I_{\text{MIBI}}(1) &lt; 1.5$</td>
<td>+ if $0.4 &lt; I_{\text{MIBI}}(2) &lt; 0.5$</td>
<td></td>
</tr>
<tr>
<td>++ if $1.5 &lt; I_{\text{MIBI}}(1) &lt; 1.8$</td>
<td>++ if $0.5 &lt; I_{\text{MIBI}}(2) &lt; 0.6$</td>
<td></td>
</tr>
<tr>
<td>+++ if $1.8 &lt; I_{\text{MIBI}}(1)$</td>
<td>+++ if $0.6 &lt; I_{\text{MIBI}}(2)$</td>
<td></td>
</tr>
</tbody>
</table>

**Results and discussions:**

*Important results were communicated and published (11, 26, 27, 29, 31, 32, 34).*

Two nuclear physicians have analysed the images and indexes (counts/pixel) $I_{\text{MIBI}}(1)$, $I_{\text{MIBI}}(2)$ and $I_{\text{MIBI}}(3)$ were calculated for each pathologic uptake seen on the lung. The planar findings were completed with the SPECT findings.

For diagnostic evaluation, in all active PTB (BK positive) the images were positives with $I-1$ comprised between 1.3 and 4.2, with differences between 15 minutes and 60 minutes images. For the same patient radiologic sites with a same opacity can have different scintigraphic uptake index values; in order to understand these differences the history of the disease, so the age of the lesion could be related.

From the BK negative patients, 4 had positive images (corresponding to evident radiologic lesions); in two cases no pathologic scintigraphic sites were seen.

New sites: In 9 BK positive patients new sites (radiologic nonevident) have been seen; these were projected: in the pulmonary hilar aria (6 cases), at the trachea bifurcation (1 case); in other pulmonary aria (2 cases); all the times these new sites were more evident (higher uptake) in the 15 minutes scintigrams.

The next table presents the radiologic and the scintigraphic diagramatic aspects for each patient.
### Table. 1.3. Diagramatic presentation of the radiologic and the scintigraphic aspects for each patient

<table>
<thead>
<tr>
<th>Nr Pt.</th>
<th>MT presence</th>
<th>Initial (before treatment)</th>
<th>Scintigraphic uptake degree (qualitatively)</th>
<th>Drog resistance (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Radiographic lesions</td>
<td>I. (initial)</td>
<td>II. (7 days)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scintigraphic positive sites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1</td>
<td>Mo-C+</td>
<td>Low uptake, new sites</td>
<td>No</td>
<td>Stationary</td>
</tr>
<tr>
<td>1.2</td>
<td>Mo+C+</td>
<td>Moderate uptake, new sites</td>
<td>No</td>
<td>Decreased uptake</td>
</tr>
<tr>
<td>1.3</td>
<td>Mo+C+</td>
<td>Low uptake, new site</td>
<td>No</td>
<td>Decreased uptake</td>
</tr>
<tr>
<td>1.4</td>
<td>Mo-C+</td>
<td>Low uptake, new site</td>
<td>No</td>
<td>Stationary</td>
</tr>
<tr>
<td>1.5</td>
<td>Mo+C+</td>
<td>Low uptake, new sites</td>
<td>No</td>
<td>Decreased uptake</td>
</tr>
<tr>
<td>1.6</td>
<td>Mo+C+</td>
<td>Moderate uptake</td>
<td>No</td>
<td>Decreased uptake</td>
</tr>
<tr>
<td>1.7</td>
<td>Mo+C+</td>
<td>Moderate uptake</td>
<td>No</td>
<td>Decreased uptake</td>
</tr>
<tr>
<td>1.8</td>
<td>Mo-C+</td>
<td>Moderate uptake</td>
<td>No</td>
<td>Stationary</td>
</tr>
<tr>
<td>Nr Pt.</td>
<td>MT presence</td>
<td>Initial (before treatment)</td>
<td>Scintigraphic positive sites</td>
<td>Drop resistance (III)</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>---------------------------</td>
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<td>1.9</td>
<td>Mo-C+</td>
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</tr>
<tr>
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<td>Mo+C+</td>
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<td><img src="image4.png" alt="Image" /></td>
<td>No</td>
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<td><img src="image6.png" alt="Image" /></td>
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<td><img src="image8.png" alt="Image" /></td>
<td>No</td>
</tr>
<tr>
<td>2.3</td>
<td>Mo+C+</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td>No</td>
</tr>
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<td>Mo-C+</td>
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<td><img src="image12.png" alt="Image" /></td>
<td>No</td>
</tr>
<tr>
<td>2.5</td>
<td>Mo+C+</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td>Yes (H, R)</td>
</tr>
<tr>
<td>2.6</td>
<td>Mo-C-</td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
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<tr>
<td>Nr. Pt.</td>
<td>MT presence</td>
<td>Initial (before treatment)</td>
<td>Scintigraphic uptake degree (qualitatively)</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>----------------------------</td>
<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Radiographic lesions</td>
<td>Scintigraphic positive sites</td>
<td>I. (initial)</td>
<td>II. (7 days)</td>
</tr>
<tr>
<td>2.7 Mo-C+</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
<td>Moderate uptake</td>
</tr>
<tr>
<td>2.8 Mo+C+ 14 years smoking</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
<td>High uptake</td>
</tr>
<tr>
<td>3.1 Mo-C-</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
<td>High uptake, new sites</td>
</tr>
<tr>
<td>3.2 Mo+C+</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
<td>High uptake</td>
</tr>
<tr>
<td>3.3 Mo-C+</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
<td>Moderate uptake, new sites</td>
</tr>
<tr>
<td>3.4 Mo+C+</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
<td>Moderate uptake, new sites</td>
</tr>
<tr>
<td>3.5 Mo+C+</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
<td>High uptake, new sites</td>
</tr>
<tr>
<td>Nr/Pt.</td>
<td>MT presence</td>
<td>Initial (before treatment)</td>
<td>Scintigraphic uptake degree (qualitatively)</td>
<td></td>
</tr>
<tr>
<td>--------</td>
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<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiographic lesions</td>
<td>Scintigraphic positive sites</td>
<td>Drug resistance (III)</td>
</tr>
<tr>
<td>3.6</td>
<td>Mo+ C+</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
</tr>
<tr>
<td>3.7</td>
<td>Mo+ C+</td>
<td>![Image]</td>
<td>![Image]</td>
<td>No</td>
</tr>
<tr>
<td>3.8</td>
<td>Mo+ C+</td>
<td>![Image]</td>
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<tr>
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<td>![Image]</td>
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<tr>
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<td>Mo+ C+</td>
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</tr>
<tr>
<td>4.4</td>
<td>Mo+ C+</td>
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20 years smoking
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<th>Nr</th>
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<th>MT presence</th>
<th>Initial (before treatment)</th>
<th>Scintigraphic uptake degree (qualitatively)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Radiographic lesions</td>
<td>Scintigraphic positive sites</td>
</tr>
<tr>
<td>4.5</td>
<td>Mo+ C+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>Mo- C+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.7</td>
<td>Mo- C-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>Mo+ C+</td>
<td></td>
<td>Yes (H,R)</td>
<td></td>
</tr>
<tr>
<td>5.2</td>
<td>Mo+ C+</td>
<td></td>
<td>Yes (H,R)</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>Mo+ C+</td>
<td></td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>5.4</td>
<td>Mo+ C+</td>
<td></td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>Mo- C+</td>
<td></td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Nr Pt.</td>
<td>MT presence</td>
<td>Initial (before treatment)</td>
<td>Scintigraphic uptake degree (qualitatively)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Radiographic lesions</td>
<td>Drog resistance (III)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scintigraphic positive sites</td>
<td>I. (initial)</td>
<td>II. (7 days)</td>
</tr>
<tr>
<td>5.6</td>
<td>Mo+ C+</td>
<td><img src="image1" alt="" /></td>
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<td>Moderate uptake</td>
</tr>
<tr>
<td>5.7</td>
<td>Mo+ C+</td>
<td><img src="image2" alt="" /></td>
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<td>High uptake</td>
</tr>
<tr>
<td>5.8</td>
<td>Mo- C+</td>
<td><img src="image3" alt="" /></td>
<td>No</td>
<td>Low uptake</td>
</tr>
</tbody>
</table>

$^{99m}$Tc MIBI pathologic uptake degree

The initial step scintigraphic images were positives in 41 cases with different $^{99m}$Tc MIBI uptake degrees: 20 patients – low uptake (+), 16 patients – moderate uptake (++), 5 patients high uptake (+++). 3 cases with only a positive low uptake site and other more than one radiologic sites with no uptake were considered negatives.

![Pie chart](image)

**Figure I.13. Qualitative analysis of the scintigraphic images**

From the 26 patients with microscopically positive smears, 13 had low scintigraphical uptake of the radiotracer, 10 had moderate uptake, 3 had high uptake. From the 15 patients with microscopically negative smears 7 had low $^{99m}$Tc MIBI uptake, 6 had moderate...
uptake, 2 high uptake. In 3 cases (one with positive smears and two with negative smears), even it was find a low positive site on scintigraphy, there were more than one radiological images with no scintigraphical correspondent, so we considered, finally, these 3 cases to be negatives.

**Figure I.14.** Radiotracer uptake degree for patients with positive (BK+) and negative (BK-) smears in microscopy

**Figure I.15.** $I_{MIBI(1)}$ for different types of lesions: comparison of number of patients with higher initial uptake, higher final uptake and same initial and final uptake

I – infiltrative lesion; C – cavern; R-N – reticulonodular lesion; M/F – miliar/fibrosis; A – lymph nodes; X – new site
Indexes quantification

$\text{I}_{\text{MIBI}}$ (1) had values between 1.02 and 2.16; $\text{I}_{\text{MIBI}}$ (2) between 1.42 and 0.36 being different between 15 minutes (stated as INITIAL) and 60 minutes (stated as FINAL), as represented in the graphs below:

(a) $\text{I}_{\text{MIBI}}(1)$

(b) $\text{I}_{\text{MIBI}}(2)$

(c) $\text{I}_{\text{MIBI}}(3)$

(d) INITIAL
Figure I.16. Indexes quantification: (a) $I_{\text{MIBI}} (1)$; (b) $I_{\text{MIBI}} (2)$; (c) $I_{\text{MIBI}} (3)$; (d) Initial all three indexes; (e) Final all three indexes.

Differences were observed related to the lesion type as presented in the graphs in figures I.15, I.16 and I.17.

Comparison between number of patients with higher initial uptake, higher final uptake and same initial and final uptake was also realized through $I_{\text{MIBI}} (2)$.

As evident in the graphs, the higher uptake at 15 minutes corresponds to the hilus images (considered new sites, with no correspondent on the radiography).

The tomographic SPECT acquisitions were useful to clarify the localizations superposed with the normal uptake projection regions (as the heart).

The sensibility of the $^{99m}$Tc MIBI pulmonary scintigraphy for the PTB diagnostic (in relation with the MT + or – in microscopy) was 96%, the specificity being 86%.
I.2.1.2. $^{99m}$Tc MIBI PTB treatment early evaluation, at 7 days

_Hypothesis and aims:_
To evaluate the early sensibility to the treatment.

_Material and methods:_
Patients
38 patients (from the initial 41 patients) with positive $^{99m}$TcMIBI pulmonary scintigraphies for PTB diagnostic (before treatment), after they have undergone 7 days of treatment.
Method: Work protocol, administered dose, acquisition device, image acquisition protocol, acquisition parameters were the same as in part I.2.1.1, presented above.

![Image processing](image)

\[ p = \left(1 - \frac{\text{black pixels initial image}}{\text{black pixels final image}}\right) \times 100 \]

For this example: \[ p = \left(1 - \frac{310}{1218}\right) \times 100 = 74.54\% \]

Using $^{99m}$Tc MIBI scintigraphy

Image processing:
$I_{\text{MIBI}(1)}$, $I_{\text{MIBI}(2)}$ and $I_{\text{MIBI}(3)}$ were calculated, like presented before.

_Results and discussions:_
Most important results were communicated and published (26, 31).
For the patients reexamined after 7 days treatment, the radiotracer pathologic uptakes were decreased in 26 from 38 cases (in comparison with their first step correspondent uptakes), the mean percentage of pathologic uptake decreasing being 62% (p<0.01), _Figure I.18_.

However, the correspondent radiological images had little modification (improved) that could be considered in only 12 of these 26 cases. In the cases with hilus initial images, the decrease was always more evident on the lung positive sites. The 12 stationary images correspond to 11 radiologic unmodified images and one little modified radiologic image.
The sensibility of $^{99m}$Tc MIBI scintigraphy versus radiography is 96% with a specificity of 56% in our study.
1.2.1.3. $^{99m}$Tc MIBI PTB treatment resistance evaluation at 6 months

**Hypothesis and aims:**
To evaluate the efficiencie of the treatment and the multidrug resistance status of PTB lesions

**Material and methods:**
Patients:
32 patients with initial positive $^{99m}$TcMIBI pulmonary images, reexamined after 6 months of PTB treatment.
Method: Work protocol, administered dose, acquisition device, image acquisition protocol, acquisition parameters and image processing were the same as in part I.2.1.1., presented above.
For these studies, the features of tuberculostatic treatment were registered: drug types, treatment period, patient clinical and laboratory evolution under treatment (with the same laboratory tests set at the second scintigraphic exploration), the sensibility or resistance to the treatment drugs.

**Results and discussions:**
Important results were comunicated and published (11, 23, 30, 34).
At 6 months, from the 32 patients with scintigraphic positive initial images, 29 were cured and had negative $^{99m}$Tc MIBI pulmonary scintigraphies in 27 cases; in 2 cases a diffuse low radiotracer uptake was found. In 3 cases, stationary $^{99m}$Tc MIBI pulmonary uptake at the lesion level was found, compared to the correspondent initial images; in these patients drug resistance to Isoniasid and Rifampicine was considered (figure I.19).

![Figure I.19.](image)

An evident pathologic uptake is seen in the right lung upper lobe correspondig to the radiologic lesion.
$I-1 = 3.8$.
Patient data: men, 22 years, pulmonary tuberculosis, positive BK (2+); MDR+.
The positive predictive value (PPV) of the $^{99m}$Tc MIBI pulmonary scintigraphy for the resistance to the treatment was 0.6.
The two treated patients with still positive scintigraphic images were both long time smockers and this could explain a diffuse radiotracer load of both lungs.

Conclusions:

1) $^{99m}$Tc MIBI is useful for the functional imagistic of pulmonary tuberculosis, both for initial diagnosis and for evolution assessment.

2) $^{99m}$Tc MIBI scan rest positive in the case of multidrug resistance lesions.

3) The time interval and degree of positive images depend on the constitution of the lesion. Images at 15 and 60 minutes have relative distinct significance, earlier images being more evident in high BK positive patients.

4) $^{99m}$Tc MIBI pulmonary scintigraphy can make evidence of new tuberculosis lesions (not evident on X-ray images).

5) $^{99m}$Tc MIBI scintigraphy in pulmonary tuberculosis could be an earlier marker (in comparison with radiologic images) for the efficiency of PTB treatment, the radiotracer uptake decreasing in several days only, also more evident on the 15 minutes images.

6) The tomographic SPECT acquisitions are indicated to clarify the localizations, when superposed with the normal uptake projection regions, for example.

7) In evolution lesions with same appearance on radiologic images can have different appearance on $^{99m}$Tc MIBI scan, meaning that these image types are complementary.

8) These data suggest that the radiotracer uptake elements in the constitution of PTB lesion are different, and *Mycobacterium Tuberculosis* seems to have a great role, which could be understood by correlated *in vivo* and *in vitro* studies. How *Mycobacterium Tuberculosis* demonstrated to have a significant *$^{99m}$Tc MIBI* uptake it could be possible to establish a relation between the scintigraphic image and the infection stage, maybe also the pathogenicity degree of the bacteria.
1.2.2. $^{99m}$Tc Tetrofosmin diagnosis evaluation in PTB

**Hypothesis and aims:**
Radiolabeled isonitrils are useful for the functional evaluation of PTB lesions, even when the structural density (and radiologic image) is unchanged. To evaluate the role of $^{99m}$Tc Tetrofosmin scintigraphy for the scintigraphic functional diagnosis of pulmonary tuberculosis.

**Material and methods:**

Patients - inclusion criteria:
- Patients hospitalized at the Pneumology Hospital from Iași.
- New cases with PTB diagnostic (clear or suspicion)
- Positive or negative *Mycobacterium Tuberculosis* (MT) smears in microscopy
- Untreated before the scintigraphy
- With evident radiologic lesions
- With other laboratory tests: ESR (erythrocyte sedimentation rate), IDR (tuberculin intra-dermo-reaction, at 2 units PPD), smears for MT in microscopy (mo) and culture (c), blood cell count.

Patients - exclusion criteria: Pregnant women.

We have studied 29 patients. Sex ratio was women/men = 11/18; mean age: 43.5 years; 9 positive MT smears, 2 negative MT smears (where the culture result were positive). For all the patients, the followed characteristics were: the smoking status (noted S); the subjective and objective clinical symptoms of the patient; other associated diseases (contemporary or in antecedents); the epidemiologic context of PTB unset.

Work protocol: Scintigraphic images before the beginning of the treatment.

Administered doses: 7.4 MBq (0.2 mCi)/Kg/patient $^{99m}$Tc Tetrofosmin dose, i.v., intrabrahial.

Aquisition device: A Gamma camera Axis, double head, parallel - high resolution collimator was used, at the Nuclear Medicine Laboratory, "St. Spiridon" Universitary Hospital (Iasi).

Image acquisition protocol was established according to literature data (1,4,9,13,14,34,35):
Five anterior and posterior planar images, at 5,10,20,30 and 60 minutes after radiotracer i.v. administration. SPECT acquisition was proposed in case of negative or doubtful planar images.

Acquisition parameters: static planar images with 128 x 128 matrix, 3,000 000 counts/image, ZOOM 1.00.

**Results and discussions:**

*Most important results were communicated and published* (29,31)
Image processing: The obtained scintigraphic images were both qualitatively and quantitatively analyzed; they were also compared with the correspondent radiological images.

a) Qualitative analysis:
Radiotracer uptake classification was coded like follows:

<table>
<thead>
<tr>
<th></th>
<th>without uptake</th>
<th>low uptake</th>
<th>moderate uptake</th>
<th>high uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td></td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

b) Quantitative analysis: three identical interest regions (in dimensions and shape) were drawn:
- On the pathologic site = ROI\(_1\) (representing the pathologic uptake)
- On a normal pulmonary field = ROI\(_2\) (representing the normal pulmonary uptake)
- On a myocardial field = ROI\(_3\) (representing the higher radiotracer normal tissue uptake)

The quantification has been assessed using three indexes (counts/pixel):
- \( I_1 = \frac{I_{\text{Tetrofosmin}}(1)}{I_{\text{Tetrofosmin}}(2)} \) = lesion/normal
- \( I_2 = \frac{I_{\text{Tetrofosmin}}(2)}{I_{\text{Tetrofosmin}}(3)} \) = lesion/heart
- \( I_3 = \frac{I_{\text{Tetrofosmin}}(3)}{I_{\text{Tetrofosmin}}(2)} \) = normal/heart (to exclude a pathologic pulmonary increased uptake through vascular pulmonary loading, for example in long period smoking persons).

So:
- \( I_1 = \frac{D_p}{D_n} \) and \( I_2 = \frac{D_p}{D_m} \) for all images,
- \( D_p = \) counts/pixel density in the selected area of the pathological tissue,
- \( D_n = \) counts/pixel density in the selected normal tissue,
- \( D_m = \) counts/pixel density in the myocardial tissue (considered with maximum uptake degree).

The correspondence between qualitative classes and indexes values used was, at the beginning, the same like the one used in the case of \(^{99m}\text{Tc MIBI}\) uptake, in previous chapter.

**Table I.4. Correspondence between qualitative and quantitative analysis**

<table>
<thead>
<tr>
<th>for ( I_{\text{Tetrofosmin}}(1) )</th>
<th>for ( I_{\text{Tetrofosmin}}(2) )</th>
<th>for ( I_{\text{Tetrofosmin}}(3) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>if ( I_{\text{Tetrofosmin}}(1) &lt; 1 )</td>
<td>if ( I_{\text{Tetrofosmin}}(2) &lt; 0.4 )</td>
<td><strong>normal if</strong> ( 0.3 &lt; I_{\text{Tetrofosmin}}(3) &lt; 0.45 )</td>
</tr>
<tr>
<td>+ if ( 1 &lt; I_{\text{Tetrofosmin}}(1) &lt; 1.5 )</td>
<td>+ if ( 0.4 &lt; I_{\text{Tetrofosmin}}(2) &lt; 0.5 )</td>
<td></td>
</tr>
<tr>
<td>++ if ( 1.5 &lt; I_{\text{Tetrofosmin}}(1) &lt; 1.8 )</td>
<td>++ if ( 0.5 &lt; I_{\text{Tetrofosmin}}(2) &lt; 0.6 )</td>
<td></td>
</tr>
<tr>
<td>+++ if ( 1.8 &lt; I_{\text{Tetrofosmin}}(1) )</td>
<td>+++ if ( 0.6 &lt; I_{\text{Tetrofosmin}}(2) )</td>
<td></td>
</tr>
</tbody>
</table>
Case report:
Patient, m, 32 years
Diagnostic: Infiltrative-cavitary PTB spread to the right, with nodular disseminations controlateral; MT positive in microscopy (BK+)

Figure I.20. Diagrammatic presentation of the radiologic image (a) and two of the scintigraphic images of pt.1: b) anterior view at 20 min, c) posterior view at 20 min; The intensity of the indexes for the lesions is mentioned in figures I.21. and I.22; in c), \(L_1\), with \(I_1\) 2.18 represent an unknown localization (new site)

Figure I.21. Quantitative evaluation of \(^{99m}\)Tc-tetrofosmin uptake degree for the pt. 1 20° anterior view; \(I_1\): mean 1.732637; \(I_2\): mean 0.572069
Figure I.22. $^{99m}$Tc-tetrofosmin uptake kinetic of the scintigraphical pathological sites for the 20 min image: a) for the anterior view, b) for the posterior view

Conclusions:

Important results were communicated and published (31, 34).

$^{99m}$Tc Tetrofosmin lung scintigraphy in PTB evaluation is a noninvasive exploration useful:

1. To confirm the PTB diagnostic both for the MT positive and negative smears (in microscopy) patients.
2. To complete the imagistic diagnostic of PTB, making evidence of new sites not visible on radiology.
3. For the functional investigation of PTB: active PTB lesions have great Tetrofosmin uptake indexes, at different time interval, probable related to their predominant component ($\textit{Mycobacterium Tuberculosis}$ presence, or macrophages).
4. In comparison with the results obtained for the $^{99m}$Tc MIBI uptake in PTB we can say:
   - the uptake of $^{99m}$Tc tetrofosmin in PTB lesions seems to be greater than that of $^{99m}$Tc MIBI since the values of indexes calculated (I1 and I2) are greater for $^{99m}$Tc tetrofosmin (the mean for $^{99m}$Tc MIBI I1 is 1.484 versus 1.95 in the case of $^{99m}$Tc Tetrofosmin I1)
   - the contrast between pathologic uptake and normal uptake is more evident in tetrofosmin images than in $^{99m}$Tc MIBI images
   - the uptake kinetic for tetrofosmin (influx and efflux from the lesion) seems to differ in relation with the $^{99m}$Tc MIBI kinetic.
5. Some results (one patient), show that in some cases of pneumonia it is possible to be founded some accumulation of radiotracer but with very different kinetic (more intense in the first five minutes with washout after). These need to be tested more.
CHAPTER II.

99mTc ISONITRILS FOR EVALUATION OF MDR CHARACTER IN NEOPLASIA

Hypothesis and aims:
Ineffective chemotherapy in some patients with malignancies is most often explained by the MDR (multidrug resistance) status of cancer cells. Multidrug resistance character is a major factor that determines the ineffectiveness of chemotherapy malignancies. This character can be detected and quantified by scintigraphy with 99mTc isonitrils (MIBI, Tetrofosmin) radiopharmaceuticals whose cellular efflux is mediated through the Pgp protein coded by the MDR gene. Pgp protein overexpression, where MDR-positive cells, will cause a rapid efflux and decreased labeled molecule level in the tumor cell. Scintigraphic image will allow evaluation of this efflux by quantifying the degree of radioactivity released from the tumor malignancies. They may, thus, select patients who will not respond to chemotherapy, requiring, possibly, the use of modulators of MDR, which inhibit the MDR protein, allowing both the accumulation of radioactivity (and obtaining a positive image), and the accumulation of the chemotherapeutic to the tumor cells. Numerous in vitro and in vivo studies show that 99mTc-isonitrils transport analysis is an effective and sensitive quantitative measure of the functional expression of the Pgp protein (1-4,10).

Material and methods:
The study was prospective: scintigraphy with radiolabeled molecules was used to assess tumor parameters involved in the effectiveness of cancer therapy. We used molecules labeled with 99mTc by a standardized protocol on a homogenous group of patients established in collaboration with clinical services that diagnose and treat patients with malignancies (Oncology Clinic, St. Spiridon Hospital, and Pneumology Hospital, Iasi).
Evaluation of the results was carried out both qualitative and quantitative, through a technique of scintimetry, using dedicated software. By scintimetry shall be reduced errors arising from the classical physician visual interpretation of scintigraphic images.
The study group was establishing respecting the next inclusion and exclusion criteria:
- Inclusion criteria: patients with malignancies, osteophyle cancers (breast, prostate, lung).
- Exclusion criteria: pregnancy.
The study group consisted of patients hospitalized for diagnosis and treatment in Iasi Pneumology Hospital and Hospital "St. Spiridon" Oncology Clinic. We have studied 19 patients including 7 males and 12 females, aged between 38 and 71 years. The average age was 59 years old. There were all patients with positive diagnosis of lung cancer and breast cancer stages III and IV, without surgery, after the initiation of chemotherapy.
Working protocol: All patients were informed about the exploration performed, giving their written consent. All patients were explored by scintigraphy with the same protocol in the Nuclear Medicine Service of the University Hospital "St. Spiridon" and next examinations:
  • in the primary diagnosis, previous surgical extirpation in order to obtain a reference examination and postoperative treatment orientation;
about 2-3 months after surgical excision, for assessing the presence of tumor recurrence;
during treatment (chemotherapy, radio and hormone therapy), to assess the MDR status of the tumor;
in case of isolated elevated tumor markers.

For all patients were noted: the date of treatment beginning, the followed treatment protocol (chemotherapy, radiotherapy, surgery), the chemotherapy protocol set and laboratory tests (general analysis and a set of specific tests, depending on the type of cancer).

For all patients have been realised a tumor scintigraphy with $^{99m}$Tc Tetrofosmin, at the standard i.v. dose of 7.4 MBq (0.2 mCi) / kg / patient, according to a protocol established in accordance with the literature (1-4). Throughout the investigation the patients were monitored (symptoms, pulse, blood pressure).

**Results and discussions:**

Most important results were communicated and published (31,34).

Qualitative analysis: Scintigraphic images were analyzed, first, in terms of quality. The radiopharmaceutical uptake intensity in the lung lesions and concordance between chest radiographs positive sites and correspondent scintigraphic positive sites were evaluated.

Scintigraphic images were positive in all 19 cases: 9 patients had poor uptake (+), 8 patients moderate uptake (++) and 3 patients intense uptake (+++) of $^{99m}$Tc Tetrofosmin. Of the 19 cases, in 5 patients (24%) total concordance was observed between the two types of explorations - X-rays and scintigraphy. In 3 cases were identified new sites on scintigraphic images, in the lung hilum (possible hilar lymphadenopathy) or at pulmonary level; in 4 patients some lesions evident on radiography were not positive on $^{99m}$Tc Tetrofosmin scintigraphy.

Quantitative analysis

In the second step, scintigraphic images were analyzed in terms of quantity, with indices: $I_{TF}(1)$, $I_{TF}(2)$ and $I_{TF}(3)$. Because the studied patients had no significant anatomical and functional cardiac pathologic modifications, $I_{TF}(3)$ was considered a relatively constant parameter in all patients and therefore was chosen as a reference in interpreting the results.

Using calculated indices, $^{99m}$Tc Tetrofosmin uptake intensity was compared between radiological lesions and scintigraphic images taken at 15 minutes and 60 minutes.

$I_{TF}(1)$ (= pathological / background) was between 1.02 and 2.16 (1.63 ± 0.20). The values were not significantly different for the examination at 15 minutes and 60 minutes.

$I_{TF}(2)$ (= pathological / cord) shows higher values on images taken 15 minutes after administration of the radiopharmaceutical, the differences between the two points (15 and 60 minutes) being so small (~ 0.01). From in vitro and in vivo studies conducted so far, it is known that the myocardium uptake $^{99m}$Tc Tetrofosmin more intense at 60 minutes, which may explain this result. $I_{TF}(2)$ was between 0.36 and 0.78 (0.57 ± 0.19).

In 10 cases SPECT scan was performed, so the lungs can be viewed in 36 incidences, which allowed a more precise localization of the lesion in the lung segments.

![Image](a).

![Image](b).

Figure II.1. Early (a) and late (b) static scintigraphy, anterior and posterior incidences. Early anterior radiotracer uptake rate tumor/controlateral ROI = 1,48/1. Late anterior radiotracer uptake rate tumor/controlateral ROI = 1,41/1. Lung background left/right, early images = 1,16/1; Lung background left/right, late images = 1,09/1

Disease history: The patient declares the onset two months ago, with pain in the left hemithorax, little effort dyspnea, cough with mucopurulent sputum. Is a former smoker, 20 cigarettes / day. Was admitted to the clinic of Pneumology. General clinical examination: There is a general influenced state, asthenic nutrition, with poor representation of conjunctivo-adipose tissue. Laboratory tests: CT scan: mediastinal tumor mass - left upper and middle lung with dimensions of 12/07/12 cm. Mediastinal lymph nodes located pretraheal (maximum 1 cm diameter), vascular (maximum 2 cm diameter). Ventilation disorders at LSS posterior segment level. Pathological examination - Macrocellular Carcinom.
$^{99m}$Tc TETROFOSMIN lung perfusion scintigraphy (figure 23):
Static and SPECT acquisitions make evidence of an heterogenous radiotracer uptake site, more evident in anterior incidence, projected in the left superior lobe, corresponding to the CT evident tumor. The site is evident both in early and late acquisitions, both on planar and SPECT images. Moderate positive image at mediastinal level.

Treatment: chemotherapy, the first cycle CHT (CBDA + VLB).

Tumor response to chemotherapy / radiotherapy depends, also, on the status of intratumoral and peritumoral parameters, such as tumor hypoxia, tumor angiogenesis, tumor apoptosis. For a correct interpretation and understanding of the results, two different categories of factors should be considered:

a) the structure radiotracer ($^{99m}$Tc TETROFOSMIN) and cellular uptake mechanism;
b) the cellular structure of the neoplastic lesion.

a) $^{99m}$Tc TETROFOSMIN (Mioview trade name, produced by Amersham) is 1,2-bisbis (2-ethoxyethyl) fosfinoetan. It is a lipophilic molecule (figure 24) of $^{99m}$Tc isonitrile family, which crosses the cell membranes according to the electrochemical gradient (through the Nernst equation) and in relation to the fluidity of the membrane (3, 10).

Intracytoplasmically, this particular radiopharmaceutical is accumulated into the mitochondria, which explains the $^{99m}$Tc intense TETROFOSMIN accumulation in high mitochondrial activity cells (4, 10). Fibroblasts uptake this radiotracer in a small proportion, considered the lowest among normal cells, as demonstrated in cell culture studies. Necrotic cells do not accumulate the radiotracer (10, 13, 33).

**Figure II.2.** $^{99m}$Tc Tetrofosmin molecule structure

It has been demonstrated that the uptake of $^{99m}$Tc TETROFOSMIN at the cellular level is influenced by molecular factors (degree of lipophily, global charge electric position, arrangement of atoms in the molecule and type of links which determine the shape of the molecule) and cellular factors (plasma membrane potential and mitochondrial report volume / surface, the plasma membrane cellular permeability difference for some ion species).

$^{99m}$Tc-TETROFOSMIN cellular efflux is mediated by an integral protein of plasma membrane, MDR1 P-glycoprotein (Pgp) with Mr 170 kDa, encoded by the MDR gene. Pgp (figure 25) is an active transport system mediating the efflux of chemotherapeutic drugs and $^{99m}$Tc Isonitriles. The protein span 12 times the cell membrane, the intracytoplasmic segment has two binding sites for the substrate (H and R, in Figure 10. A).
Conclusions: The results obtained by $^{99m}$Tc TETROFOSMIN scintigraphy examination of patients with lung cancer demonstrates the usefulness of this non-invasive methods for:

1. The confirmation of positive imaging diagnosis of lung cancer. In all 21 cases lung scintigraphy highlighted positive radiotracers sites.

2. The functional imaging diagnosis of lung cancer. Thus, in three cases were found new fixing $^{99m}$Tc TETROFOSMIN sites (non-evident on the radiograph). It should be noted also that, fibrosis and pleural lesions do not uptake the radiopharmaceutical (present on radiographs). Thus, these two imaging methods for investigating - radiography and scintigraphy - can be considered complementary.

3. Functional investigation of lung cancer. Uptake ROI values of calculated indices varied: ITETROFOSMIN (1) between 1.02 and 2.16 (1.63 ± 0.20) and ITETROFOSMIN (2) between 0.36 and 0.78 (0.57 ± 0.19), in conjunction with the aggressiveness of the lesion (histological structure). $^{99m}$Tc TETROFOSMIN molecule fits most intense in cells with high mitochondrial activity.

4. Assessment of MDR character during chemotherapy: The studied groups of tumors do not present any MDR character.

5. SPECT images provide new data in comparison to planar scintigraphy and enable localization and precise delineation of some lesions of lung cancer.

Final remarks: It must be mentioned that, at the moment of this study, in our country, there have been no other studies of radioisotopic molecular imaging for assessing tumor characteristics (MDR character, angiogenesis, hypoxia, apoptosis or others) so far, this study opening the way for such studies.
CHAPTER III.

**99mTc ISONITRILS FOR THYMOMA DIAGNOSIS EVALUATION**

**Hypothesis and aims:**
Thymic pathology could represent a real challenge for diagnosis and therapeutic choice. 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).

Thymic radiotracers images, as $^{99m}$Tc isonitrils (MIBI, tetrofosmin), $^{111}$In DTPA octreotide scintigraphy, or, recently, $^{18}$F 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 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).

The aims of our study 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 methods:**
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 myastenic 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, 64×64 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 drown 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 ($D_{th}$ - thymic lesion counts density, $D_{l}$ - lung background area counts density):

$$UR = \frac{D_{th}}{D_{l}}$$

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.

Histopathologic morphology assessment of surgical specimen was made with optical microscopy. 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 and discussions:**

Most important results were communicated and published (15, 16).

In **Group 1- Surgery patients (with preoperative scintigraphy)**, 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). The histological characteristics and cytological analysis varied more 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 (Figure 1a). Radiotracer uptake ratio was 3.24, drawing attention to an ectopic thymoma (Figure 1c), identified further by a recall CT investigation (Figure 1b). Surgical specimen (Figure 1e) pathological examination showed a nodular encapsulated tumor having capsular infiltration (Figure 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 hystopathologic diagnosis was B1 thymoma.

In **Group 2- Non-surgery patients (selected for medical therapy)**, 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, with a mean of 1.14 and a standard deviation of 0.05.

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

In **Group 3- Post-surgery patients with recurrent MG**, 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 (UR<1), with a 0.79 mean and a 0.09 standard deviation, 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.

Figure III.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- hystopathologic image showing invasiveness character and lymphocyte rich area; e- surgical resection specimen.

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

Thymoma is constituted by a mix of neoplastic and non-neoplastic cells. 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 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).
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 vivo 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 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 ononitrils, $^{201}$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.

Conclusions:

$^{99m}$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 malignity 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.
CHAPTER IV.
IMAGE PROCESSING STUDIES TO IMPROVE SCINTIGRAPHIC DIAGNOSIS

IV.1. $^{99m}$Tc isonitrils lung scintigraphy image processing studies

**Hypothesis and aims:**
To find a better approach for a quantitatively evaluation of the scintigraphic images and comparison of the in evolution images with the initial images of the patients.

**Material and methods:**
MatLab 6.0 program was applied on $^{99m}$Tc isonitrils scintigraphic acquired images, with the next steps:
- image filtering
- contrast processing
- segmentation
- edge detection.

- Image Filtering and Denoising
A filter in the space domain was used. Modifying the contrast and the original image edges, a simple filtering method (as the average value one) can be disadvantageous.

$$F_{med}(x, y) = \frac{1}{N} \sum_{(i,j) \in V} F(i, j)$$

Where V is the set of neighbors and $N$ is the number of neighbors.

In the spatial domain, the equivalent of high-pass filter in the frequencies space is a mask where contrary signs weights appear.

This means a derivation of the image that highlights the high frequencies corresponding to the gray level changes.

For this reason, in order to make evident the low frequencies we have to inverse the image before and after the filtering.

$$H_s = \frac{1}{(b-2)^2} \begin{bmatrix} 1 & -b & 1 \\ -b & b^2 & -b \\ 1 & -b & 1 \end{bmatrix}, b \in [0,3]$$

In the case of the low pass filter the masks would have weights with the same sign that corresponds to an integration of the image proceeding in fact to the noise elimination. This filter gave the best results.
Another filter that offers good performances is the median filter. This nonlinear filter is applied on a \( W \) window of \( L \) dimension (the value \( L \) is odd, usually \( L=3 \)) and after ordering the pixels in the mask in a raising order, from the vector of \( L^2 \) length thus obtained, the median pixel is extracted.

\[
W = \begin{bmatrix}
86 & 50 & 43 \\
69 & 224 & 120 \\
15 & 75 & 93
\end{bmatrix}
\Rightarrow F(x, y) = 224 \text{ current pixel.}
\]

The vector \( V[9] = \{15, 43, 50, 69, 75, 86, 93, 120, 224\} \), \( F(x, y) = F_{\text{median}}(x, y) = 75 \)

- Contrast Processing. Histogram Equalizing

To increase the contrast the gray scale might be only partially occupied. To increase the quality of such images, a new gray scaling operation is realized. The linear function applied transforms interval of the luminosity values into the maximum interval of these values. The most simple transform function is: \( s^* = a + b s \), where \( s \) is the initial luminosity and \( s^* \) is the final luminosity of a pixel. Inverting the gray scale is a linear transform, too: \( s_{jk}^* = -s_{jk} \).

Nonlinear re-scaling are of the form \( s_{jk}^* = s_{jk}^a \) with \( a = 1/3, 1/2, ..., 2, 3 \).

A histogram method was used, representing, in the case of a 256 gray-level image, the number of pixels that have 0 gray level (black), 1, 2, ..., 255 (white).

The function applied in order to modify the image contrast is the following:

\[
F_k'(x, y) = \frac{2^m - 1}{N^2} \sum_{j=0}^k F_j(x, y)
\]

where \( 2^m \) represents the number of gray levels and \( N^2 \) is the number of pixels from the image.

- Image Segmentation

Segmentation is an operation (a process of pixel classification) that uses special techniques meant to separate an image in regions in order to extract the useful information and to characterize it. The problem is to find an optimal threshold value \( T \), to realize the binary transform of the image:

A system of equation was used:
\[ F_{\text{bin}}(i, j) = \begin{cases} 1 & \text{if } F(i, j) \geq T \\ 0 & \text{if } F(i, j) < T \end{cases} \]

\[ T = \frac{\max_1 + \max_2}{2} \quad \text{or} \quad T = \frac{\max_1 \cdot H[\max_1] + \max_2 \cdot H[\max_2]}{H[\max_1] - H[\max_1]} \]

**Figure IV.1. Example of MatLab processing protocol for a TB $^{99m}$Tc isonitrile scan**

Example of MatLab processing protocol for an image (up), and the correspondent computer implemented program on threshold values established (given below):

**Figure IV.2. Computer implemented program on established threshold values**

**Results and discussions:**

Most important results were communicated, published (18) and are still ongoing to be published. Also, the work on this subject is ongoing in our nuclear medicine laboratory.
Single image processing example:

Two images processing example (for a patient examined initial (before treatment) and after 7 days treatment):

Conclusions:
Matlab protocol to process acquired images can improve the accuracy of the diagnosis. Validation on large image seria is necessary.
IV.2. Computer-aided Diagnosis System to Quantify Metastasis on Bone Scan

There is an increased frequency of bone metastases in several types of cancer. The clinical significance of metastasis bone pathology results from the increased incidence of bone metastases in various cancers, such as prostate or breast cancer (65-75% incidence), myeloma (70-95% incidence) etc. [18]. The impact of this phenomenon is even more important as treatment decisions are strongly influenced by the correct interpretation of the medical images used for diagnosis, in evolution. Metastases Disease (MTSD) bone scan diagnosis can be uncertain and with a certain degree of subjectivity, sometimes. In this respect, several studies have been conducted which revealed that the number of false-negative interpretations is unacceptably high, the diagnosticians performance revealing a sensitivity below 80% [4, 5, 6], sometimes. Therefore, the possibility of improving the diagnosis process of bone metastases is the main reason for this paper. The concept of these systems is to combine the doctor's skills and medical knowledge with the computer's ability to detect lesions.

Our study falls within the international context of increased interest in improving diagnosis on different types of medical images [3]. CAD systems began to be part of routine clinical breast cancer detection on mammograms in several hospitals or institutions where screening is done [7-11]. It has been shown that these systems have significantly improved the performance of physicians in detecting cancer [9-11]. Other CAD systems increased sensitivity for less experienced physicians also in other image types, as in detecting polyps on CT [12] or on interpretation on myocardial perfusion scintigraphy [13]. Therefore, we expect that, in our case, such a system will also bring significant improvements in detection of bone MTS.

**Hypothesis and aims:**

Our goal was to develop a computer-aided diagnosis system (CAD) that is able to evaluate and quantify the presence of bone metastases on whole-body scans using image processing techniques in order to help the nuclear medicine physician in his diagnostic approach.

**Material and methods:**

We worked with a group of 37 paired of whole body scans in evolution, selected from the MTSD patients of the Nuclear Medicine Laboratory archives, “St. Spiridon” Hospital, Iași. Lung, breast and prostate cancers are the leading osteophile cancers. Therefore, the selected patients reflect the spectrum of pathology encountered in routine clinical practice in the aria, having diagnoses of breast, prostate or lung cancers.

The patients were investigated by two whole-body scan, in evolution.

Bone scans were obtained in about 3 hours following an intravenous injection of $^{99m}$Tc-diphosphonate (740 MBq) at a scan speed of 15 cm/min. Whole-body images (anterior and posterior incidence) are 256/1024 matrix sized and were obtained with a dual head Siemens gamma-camera with low energy high resolution parallel collimators. The images were
stored on a dedicated computer. Energy discrimination was made by a 15% symmetric window centered on the 140 keV peak of $^{99m}$Tc. Images obtained from the gamma-camera are saved in DICOM (Digital Imaging and Communications in Medicine) format. DICOM format is generally used for storing medical images and contain, besides the image itself, metadata providing information about the image (size, dimensions, depth, method used, equipment settings) [1].

We used Matlab® software to develop our application. Matlab can recognize, read and process this format.

The method had three steps: preprocessing, processing and postprocessing.

**Preprocessing step**

The images captured by the gamma-camera can have intensity differences, between examinations, determining contrast variations, also. Many of them appears too <dark>, having a low contrast. This depends on a number of factors, like administered radiotracer dose, patient's hydration degree, image's capture time, attenuation degree, etc. Preprocessing phase brings images at about the same contrast level. This is necessary in order to be able to apply the same threshold values in our further processing and to be able to compare the images in evolution. Figure IV.5. shows, in the first position, the original image as it is obtained from the gamma-camera. In the next three positions, we can see the results of three different contrast enhancing techniques [15], of which we chose the latter being the most appropriate.

![Figure IV.5. Illustration showing the original image and the results obtained after applying each of the three Matlab functions for contrast enhancement.](image-url)
It should be noted that, even after applying the function `adaphisteq` once on the original image, the contrast may remain low for some images, requiring to apply the function twice, thrice or more times. Thus, we approximate the contrast of the image by the arithmetic mean of the image intensities and apply the function `adaphisteq` as long as the mean is lower than 14.

```matlab
>> while mean(mean(f)) < 14
    f = adapthisteq(f);
>> end
```

Consequently, we are sure for the same contrast level of all images.

**Processing step**

After the preprocessing step, in which we prepared the image for processing, we started the segmentation phase, at the end of which we identified the areas of interest. We achieved the proper segmentation using the watershed method [2, 19]. For the watershed method, we must think of grayscale images as topological surfaces where pixel values are interpreted as height.

Although the method seems auspicious, the direct application of it was difficult as seen in figure IV.6., in the second position. Further, we appealed to a series of accessory methods that caused the watershed technique to work increasingly better. Thus, we removed the noise in the gradient image, and then we identified internal markers that could help restrain the number of errors.

In addition, we identified the external markers that could help delimitate the basins [14]. Next, the markers were imposed as minima in the gradient image. We applied the watershed method and we obtained a much improved result comparing to the previous one [2].

![Figure IV.6. Direct application of the watershed method](image)

**Figure IV.6. Direct application of the watershed method**

In the gradient image, and then we identified internal markers that could help restrain the number of errors.
However, there are still some problems. Some areas should not be circled, but they are. So, we established more rigorous rules for choosing the internal markers. Finally, another application of the watershed method provides a result that is much closer to what we wanted. These last steps are shown in figure IV.7.

*Figure IV.7. Corrections on the application of the watershed method*

**Postprocessing step**

When the segmentation phase is finished, the nuclear medicine specialist must check to see if everything that has been reported by the computer as different from the normal image could be a metastasis lesion or not. Thus, the diagnostician is able to interfere with the computer's outcome and eliminate areas encircled by the program that are certainly not metastases. Once the final result is obtained, the marked hyperfixing areas are saved in the patient's history. Several descriptive parameters of the lesions are calculated, both global (the ratio between the area occupied by the MTS and the entire skeleton surface, the number of MTS) and local, characterizing each lesion (the gravity center coordinates of the region, area, width, height, maximum intensity pixel value, mean value, standard deviation) for both anterior and posterior incidences. These descriptors were determined to objectify the extent of metastatic disease by mathematical measures. The descriptors are saved in the history of the patient to be able to compare them with later descriptors that will be obtained at subsequent examinations. This permits to objectify patient's evolution.

***Results and discussions:***

*Most important results were communicated and published (20) or are ongoing.*

The application improved the diagnostic process on the data set. This was accomplished by passing through all three phases: preprocessing, processing and post-processing steps.
In the following lines, we illustrate the results obtained for a MTSD case in evolution. We can see in figure IV.8. in the 2$^{nd}$ position, the result obtained by the computer for the first scan, in anterior incidence. For the same figure, in the 3$^{rd}$ position, we can see the final result after the physician removed the areas encircled by the computer but that represent the radiotracer elimination system or certainly known nonmetastatic regions, establishing the final image.

![Figure IV.8. Example of method application on an initial diagnosis MTSD image](image)

The global descriptors calculated for the final image in figure IV.8. were: the ratio between the area occupied by the MTS and the entire skeleton surface – anterior and the total number of MTS – anterior. Further, each hyperfixing area had also some local associated descriptors.

![Figure IV.9. MTSD second image, in evolution](image)
We will exemplify the three colored areas in *figure IV.10*: the 1\textsuperscript{st} hyperfixing area is the one colored in yellow (A1), the 2\textsuperscript{nd} hyperfixing area is the one colored in mauve (A2) and the 3\textsuperscript{rd} is the one colored in red (A3).

*Figure IV.10. In evolution comparison of MTSD, by CAD method*

*Figure IV.10b,* illustrate the results obtained for the same patient (shown in *figure IV.10a*) after 3 years of evolution. We can see in *figure IV.9,* in the 2\textsuperscript{nd} position, the result obtained by the computer for the same patient after 3 years (anterior incidence). For the same figure, in the 3\textsuperscript{rd} position, we can see the final result after the doctor removed the areas encircled by the computer that are certainly not metastases.

Thus, from an examination to another, we can compare the number of bone metastases, the ratio between the area occupied by the MTS and the entire skeleton surface and the local descriptors for each lesion. In our case, 41 metastases were identified at the first examination and 42 at the second (anterior incidence). The ratio between the area occupied by the MTS and the entire skeleton surface is at the first examination $15.2812$ and $16.0114$ three years later (anterior incidence). Also, it can be seen how some lesions have disappeared, some have emerged, some have grown in size and some decreased in size. In our example, the hyperfixing area 2 has an area of 24 at the first examination and an area of 70 at the second.

*Table IV.1.* presents the local and the global descriptors for the three hyperfixing areas highlighted in *figure IV.10,* and the variation of them.

*Weaknesses of the study*

There is a certain risk for the program to provide false-positive or false-negative results. To reduce these risks we plan to replace the entire skeleton processing with the sequential processing of each anatomical area.
**Table IV.1. Global and local descriptors variance for the metastases A1,A2,A3**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Les-</th>
<th>First scan</th>
<th>Second scan</th>
<th>Difference= %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local descriptors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravity center coordinates</td>
<td>A1</td>
<td>142.8750</td>
<td>145.1304</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>155.1250</td>
<td>154.1250</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>175.6154</td>
<td>176.4865</td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td>A1</td>
<td>52</td>
<td>110</td>
<td>+111%</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>24</td>
<td>70</td>
<td>+191%</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>135</td>
<td>200</td>
<td>+48%</td>
</tr>
<tr>
<td>Width</td>
<td>A1</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>5</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>14</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>A1</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Maximum intensity pixel value</td>
<td>A1</td>
<td>213</td>
<td>201</td>
<td>-5,16%</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>89</td>
<td>87</td>
<td>-2,24%</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>215</td>
<td>212</td>
<td>-1,4%</td>
</tr>
<tr>
<td>Mean value ± standard deviation</td>
<td>A1</td>
<td>208.6250 ± 2.8253</td>
<td>200 ± 3.4245</td>
<td>-3.8%</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>84 ± 3.2071</td>
<td>83.2 ± 2.9120</td>
<td>-0.95%</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>209 ± 3.5355</td>
<td>204.4054 ± 3.1925</td>
<td></td>
</tr>
<tr>
<td><strong>Global descriptors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The ratio between the area occupied by the MTS and the entire skeleton surface – anterior</td>
<td></td>
<td>15.28</td>
<td>16.01</td>
<td>+4,8%</td>
</tr>
<tr>
<td>Total number of MTS – anterior</td>
<td></td>
<td>41</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

**Strengths of the study and possibilities for clinical use of this application**

Computer-aided diagnosis increases the accuracy for assessment of bone metastases, which is particularly important in terms of the effectiveness of treatment. Patient’s favorable evolution encourages and confirms the effectiveness of current treatment, while an unfavorable evolution suggests the need for treatment change. Our application helps the doctor to find and characterize the lesions on whole-body scans. It lessens the risk of errors by reducing false negative diagnosis in extreme situations in which some pathological areas (low or heterogeneous hyperfixing) may be omitted. Through
image processing, the computer draws attention to these areas and the diagnostic accuracy can be greatly improved. Therefore, the doctor can apply the program on his patient scintigrams and especially on the scintigrams in evolution from the same patient. So, he can appreciate more accurately, with the computer’s help, if the evolution is favorable or unfavorable. The doctor can assess the patient’s evolution manually using the descriptive parameters provided by the application. In consequence, we believe that this type of CAD6 system is useful for the diagnostician.

Future Directions

There are several directions in which we plan to further improve the application:

- First, we will be interested in advancing from the state where the doctor assesses the patient’s evolution manually via the parameters provided by the program, to the state where the computer can automatically assess the patient’s progress.
- We will introduce new lesion descriptors that will characterize the patient’s progress in more detail: the ratio of the area occupied by growing in intensity metastases, the ratio of the area occupied by metastases that decrease in intensity.
- We will also deal with lowering the risk for false-negative and false-positive results by replacing the entire skeleton processing with the sequential processing of each anatomical area [3] and by introducing the method of successive image subtraction [17].
- Likewise, in a subsequent stage, we plan to verify the program on a statistically significant number of patients through a retrospective study on the Nuclear Medicine Laboratory Archive from the St. Spiridon Hospital, Iasi.

One of the main advantages in using the proposed CAD system lay on the automated quantification by some global and local descriptors. In the presented case, the fact that for the chosen metastases we observed a significant increase of the surface (dublare) of the sites ar putea conduce la concluzia ineficienței totale a tratamentului. However, the fact that the MTS whole surface is only slightly increased, without new sites, and the RF uptake intensity had slightly decreased, indicate the partial efficiency of the treatment, for almost all cellular populations but not for all. A modulation for the chimio – hormone therapy could be useful, in a more personalized approach.

Conclusions:

In this study, we highlighted the importance of quantitative parameters and the importance of quantifying the lesions, as these elements bring more objectivity in the process of scintigraphic image analysis. Therefore, in order to answer in a constructive way to the objectivity problem, we described an automated solution that is falling within the computer-aided diagnosis systems. The presented solution is intended to help the doctor in identifying bone lesions and in calculating quantitative parameters that will ensure objectivity in his diagnostic approach.

As main future directions, we aim to improve the application performance by reducing the risk of false-negative and false-positive results with the help of separate anatomical regions analysis. Similarly, we aim to implement a method to automatically objectivize patients evolutions by subtracting images obtained at different examinations.
CHAPTER V.
The study of $^{99m}$Tc radiolabeled nanoparticles cellular uptake mechanism

Hypothesis and aims:
The main difficulties the drug industry faces today are the poor solubility and the lack of target specificity for specific cellular or molecular targets. Nanoparticles (NP) used as drug carriers are small enough to penetrate cell membranes and avoid the defense mechanisms but at the same time are large enough not to interfere with normal cell processes. Hollow carriers of this type (host-type) can be loaded in different manners (inside or outside) with different types of guest molecules (biomarkers, rugs, bioactive molecules) and can be used in a wide range of applications (i.e. bioassays, ion separation, clinical diagnosis and medicine).

Silica nanoparticles (SNP) are a new and versatile tools in biology and medicine. The main applications for SNP are biomarkers, calibration standards in confocal fluorescence microscopy, drug delivery and targeting systems in clinical research and medicine. The inorganic shell around the active molecules has a controlled size and serves as a protecting shield. Using such an approach, a biomolecule or pharmaceutically active ingredient of interest can be either encapsulated inside the silica nanoparticle or incorporated within the inorganic matrix. Afterwards, the so-doped nanoparticles can be delivered to the desired site in order to study the activity of its load.
SNP provide a great potential of delivery of therapeutic agents into targeted organs or cells. There is also the possibility of creating controlled release drugs.
The benefits for using such advanced systems for drug delivery include a greater therapeutic effectiveness, reduced toxicity, diminished side effects, improved patient compliance and last but not least, a decrease in the therapy costs.
Toxicology data on amorphous silica nanoparticles is scarce. Preliminary studies have been conducted and some of the results suggest that the smaller the size of SNP, the more significant is the bio-toxicity.
In vitro SNP are nontoxic at low dosages but higher doses affect the cell viability.
In vivo studies are contradictory and no clear conclusion has been drawn. Some studies have pointed that SNP administered in high dosage may produce systemic toxicity, pulmonary thrombosis, systemic inflammatory responses and DNA damage. Due to their properties, SNP could be used in many fields in the near future. In order to be used as novel intelligent drug carriers more studies on the effects of SNP should be conducted. The diverse aspects of cellular activities should be carefully evaluated even though the nanoparticles are generally considered as biocompatible at present.
Our studies focuses on the organ distribution and pharmacokinetics of novel SNP (TAW20/AA22, AA115, AA118) in rodents, in order to determine the effects and possible uses as drug carriers.
With the rapid increase of nanoparticle applications, the concerns on the health impacts caused by amorphous silica nanoparticles are also increasing. With this target an acute
toxicology screening of the selected SNP was performed in order to evaluate the safety of these products.

**Material and methods:**

**Animals:** Adult male Swiss mice with an average weight of 20g ± 2g and male Guinea pigs (600 ± 50g). The animals were housed in a temperature controlled room (21°C ± 2°C) with a 12 hours/12 hours light/dark cycle, 4 mice or 1 Guinea pig per cage, and allowed to acclimate for at least 24 hours before use, with free access to food and water.

**Scintigraphic studies protocol**

*Guinea pig catheterization protocol.* The anaesthetized animal is fixed in supine position (*decubitus dorsalis*) with the head in extension. The extension of the head can be done by hand or with a thread which is passed behind the superior incisors and then fixed on the board. The hair of the right ventral region of the neck is cut and shaved. The puncture zone for the iv approach of the jugular vein (in accordance with the consulted literature the right jugular vein is much easier to handle) is located in the right inferior ventral part of the neck, taking the hyoid appendix as a reference, situated in front of the first costal-sternum joint. Here a skin fold is made with a surgical clamp and surgical scissors are used for the excision of a 1 or 2 cm flap. Excision and dilacerations procedures are performed in order to remove the subcutaneous fat and connective tissue or the thymus. Prior to placing the catheter, the jugular vein is placed under tension using the finger tips in the right region of the hyoid appendix until it becomes turgescent. After highlighting the jugular vein, a catheter (24G) is introduced ventro-dorsal, directed towards the skull. After insertion of the catheter, its “wings” are unfolded and attached to the skin of the animal using super glue.

Groups of 4 animals were treated with 1mCi/kg/animal 99mTc-coupled AA115 and AA124 silica nanoparticles (100 nm in size and carrying amino-and hydroxyl- groups on the surface), administered intravenously.

Control groups received 1mCi/kg animal 99mTc.

A dual-head Siemens gamma camera with parallel collimators was used. The following whole body image acquisitions were made:

1. Dynamic image acquisition for 60 seconds (1 image/sec), 64x64 matrix, 1.23 zoom (radio isotopic angio – to point out vascularization)
2. Dynamic image acquisition for 4 minutes (1 image/sec), 64x64 matrix, 1.23 zoom
3. Static planar images (FA/FP), 256x256 matrix, 1.23 zoom (1 million pixels per image) every 15’, duration 2 h. Then every 30’, duration 240 minutes.
4. SPECT acquisition 32 images x 30 sec/image, 64x64 zoom, which was made between 2 static acquisitions after 1 hour.
5. Static planar acquisition centered on the cervical-thoracic region for evidence of thyroid fixation, 10’ after injection, (knowing that the maximum fixation on human thyroid tissue is at 10’ after injection) 256x256 matrix, 3.2 zoom, pinhole collimator and then parallel collimator.
**Bioavailability Studies**

Fluorescent silica nanoparticles (TAW20, AA22) were suspended at a concentration of 7.5 mg / 1ml in PBS (phosphate buffered saline). After ultrasonication, the nanoparticles suspensions were administered on two routes in different groups of mice (2 per time point and route of administration):

- intravenously (via the tail vein of the animals), 0.1ml per mouse and
- orally (intra-gastric administration), 0.2 ml per mouse.

A different group of two mice received a similar volume of PBS as control.

The animals were sacrificed at different time points after intravenous (30 min, 2 h) and intra-gastric administration (1h, 2h, 24h, 48h and 72h) and samples of different organs (brain, liver, lung and bladder) were extracted and prepared for analysis by fluorescence confocal microscopy.

![Diagram of SNP synthesis](image)

*Figure V.1. Schematic presentation of SNPs synthesis*

**Tissue preparation and examination.** Thirty minutes or two hours after intravenous injections, respectively 1h,2 h, 24h,48h and 72h after per os administrations of the nanoparticles marked with the fluorochrome, the adult mice were deeply anesthetized with xylazine and transcardially perfused with saline 0.9% (aprox. 15 ml) followed by fresh 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (aprox. 75 ml).

Thereafter, the whole brain, the heart, the liver, the lung, the kidneys, the spleen, the testis and the bladder were removed and post-fixed overnight in 4% PFA, followed by cryoprotection in 30% sucrose in PBS for at least 72 hours.

Coronal sections of the above mentioned organs were cut using a freezing microtome (CM 1850; Leica Microsystems, Germany). Sections were collected, mounted on slides and
examined in a Laser Scanning Confocal Microscope TCS SPE (Leica Microsystems, Germany), 610 nm.

**General Toxicity Screening**

For acute systemic toxicity screening, groups of mice received TAW20 and AA22 silica nanoparticles (10 mice per dose) in a single intravenous dose (25, 50 and 100 mg/kg b.w.) and were kept under observation for 5 days after dosing. Each animal (Swiss mice, 20+/−2 g) received 0.1 ml solution via intraperitoneal injection. The maximum possible dose administered was defined by limitation due to solubility and administration route limits (20 mg SNP per 1 ml saline). The maximum equivalent SNP administered to each animal was 2 mg or 100 mg/kg body weight.

The animals were carefully observed for death and obvious signs of toxicity, such as convulsions, body weight effects. All mice were necropsied to detect grossly observable evidence of organ and tissue damage or dysfunction.

**Statistics.** Throughout the study data are presented as mean ± SD. ANOVA method followed by Bonferroni post-hoc test was used to identify significant differences between groups (p<0.05). The software used was SPSS 16.0.

**Results and discussions:**

Most important results were communicated and published (13) and are highly cited (9 citations in one year).

A particular challenge for in vivo experiments is localization of nanoparticles following delivery. In this study two different methods of characterization for the bioavailability of silica nanoparticles were used – scintigraphy and fluorescence confocal microscopy.

**Biodistribution of the radiolabelled SNPs by scintigraphy**

It is very difficult to track and detect SNPs in vivo. Although there are some literatures studies about the qualitative in vivo distribution of SNPs, but quantitative distribution findings are still lacking. The investigation of the in vivo destination of SNPs was conducted in this study to evaluate the capabilities of this nanostructure system as a potential carrier for radioisotopes for therapeutic and diagnosis purposes.

To this end, SNPs were labelled with a radioactive tracer (99mTc) in order to monitor their route in vivo and to obtain an accurate biodistribution profile in every investigated organ (thyroid, eyeball right and left, salivary glands right and left, heart, stomach, liver, kidney, bowel, gall bladder, urinary bladder and seminal vesicle). In order to accomplish the radiolabelling and to obtain adequate candidates for it, the SNPs were modified with APTS to introduce amino groups onto the surface and then coupled with 99mTc. These properties make SNPs excellent labelling reagents for bioanalysis and bioimaging techniques (Bagwe et al., 2006).

99mTcO4− is the most commonly used emitting radionuclide in Nuclear Medicine having a convenient half-life of approximately 6 hours, appropriate energy (140 keV) for imaging on a standard gamma camera, less attenuated by soft tissue and widespread availability. Radiolabeling with 99mTc can be performed with or without slight modifications on the original structure of the SNPs. 99mTc pertechnetate is taken up via sodium iodide symporter.
system (NIS), a transmembrane transport system that modulates intracellular transport and accumulation of iodine to some tissues including mainly the thyroid, stomach, salivary glands, and to some extent the small intestine (Kiratli et al., 2009).

A significant difference in biodistribution between $^{99m}$Tc labelled nanoparticles prepared by sodium borohydride method and free $^{99m}$Tc was observed. The $^{99m}$Tc-SNPs exhibited higher distribution in all the investigated organs as compared to free $^{99m}$Tc solution. In the case of free $^{99m}$Tc, a discreet area of fixation corresponding to thyroid area was observed at all times (from 30 to 240 minutes). Also, a diffuse heterogeneous character fixation was quantified in the thoracic and upper abdominal (stomach). Fixation consistent to the bladder region was also present, behaviour that can be explained by the fact that the radiotracer was extensively excreted by urine.

The obtained results show that in the case of $^{99m}$Tc labelled nanoparticles, after 30 min, the radiolabeled nanostructures reached systemic circulation and were captured mainly in the salivary glands, stomach, and kidney in addition to a significant accumulation in the bladder region. This behaviour was also observed at times of 60 min and 240 min after injection, as it was expected, due to the nature of the injected compound.

One hour after injection, it was observed that the $^{99m}$Tc-SNPs accumulated in all investigated organs and animal tissues, except in the thyroid. We also found that, within 4 h after injection, silica nanostructures undergo an increasing accumulation in the stomach at the same value as free $^{99m}$Tc.

The scintigraphic exam has evidenced important percentage of $^{99m}$Tc-SNPs in the eyeballs with an increasing trend in time. This accumulation can be sustained by the nanoparticulate nature combined with a great potential to facilitate the adsorption to capillary walls.

After 4 h, a low percentage of the injected dose could be observed in the liver. This conduct can be explained by the fact that SNPs are inorganic in nature and cannot be metabolized in the body. The fixation corresponding to the urinary bladder also decreases gradually due to the radiotracer’s elimination in the urine (no water was provided to the animal during the experiment).

The SNP radiotracer rapidly appears in the tissues, faster when compared to the $^{99m}$Tc. This could be explained in relation their smaller size which gives them the possibility to a faster transmembrane passage. We know that the transmembrane transport mechanism of $^{99m}$TcO$_4^-$ is meditated by the presence of the NIS, which is well expressed in the stomach and thyroid gland. This active transport system may also mediate the passage of other small ionic compounds, similar to iodide. Our SNP carrying amino groups on their surface are also electrically charged. Due to their small size and ionic charge, it is very likely that a passive transport is involved, according to the electrochemical gradient. In fact, the lack of the thyroid scintigraphic uptake for the SNP sustains the hypothesis of different transmembrane transport mechanisms for the radiotracer. However, more detailed studies regarding the bioaccumulation of SNPs in the liver, spleen, intestines, kidneys, and bladder is warranted to better clarify the adverse effects arising from such an accumulation. These results are consistent with data published by Xie et al. (Xie et al., 2010) in a previous study performed on radiolabelled silica nanoparticles.
These observations are further validated by studying the gamma scintigraphic images of guinea pigs as shown in Figure 7. Scintigraphic imaging studies were performed to detect the retention times of the formulations and to choose the optimum formulations for the further in vivo studies. The dynamic scintigraphic images showing localization of $^{99m}$Tc-SNPs and free $^{99m}$Tc in different organs taken in the first 5 minutes demonstrate the in vivo biodistribution profile in the investigated organs. After 30 min post-tail injection (Figure 8), the nanostructures are rapidly accumulated in the salivary glands, stomach, kidney and bladder region, which is in accordance with the fluorescent biodistribution study. Considering the results encountered in this biodistribution study and scintigraphic imaging, the SNPs showed in vivo behaviour similar to that demonstrated by others on nanostructured systems like as silica nanoparticles or chitosan nanoparticle (Banerjee et al., 2005; Xie et al., 2010). Following the i.v. administration of AA22, at the time points considered, 30 min and 2 h, the SNP did not penetrate the blood brain barrier and did not appear in the heart tissue. Bladder tissue sample were also negative. The rest of the investigated organs (liver, kidney, testis, splein, lung) proved the presence of the AA22. Following the p.o. administration of AA22, at 1 and 2 hours the SNP was present in all the organs investigated except the brain. 24 and 48 hours after the administration, the SNP could still be identified in large quantities in key organs, especially in the liver. After 72 h, the SNP levels decreased in all the organs investigated.

![Figure V.2. $^{99m}$Tc Silica nanoparticles (SNP) planar scintigraphy in guinea pig, showing the early bioavailability of the radiolabeled SNP (5 minutes post injection). SG = salivary glands; H = heart; S+L = projection area of stomach but also liver (explaining the image heterogeneity); B = bladder; RAA = radiotracer administration area; Bk = background.](image)

In synthesis, in the dynamic scintigraphic images took in the first 5’ and followed by static acquisitions under specified protocol, the following were observed:
- Highlighting areas of radiotracer fixation in the area of salivary glands projection, with greater intensity in the SNP use vs. $^{99m}$Tc free use
- Highlighting point of injection in both experiments, better shown in SNP
-Highlighting areas of heterogeneous radiotracer fixation in the thoracic and upper abdominal region. Fixation at this level evolves in time showing that:

In the case of SNP:
- progressive appearance of a fixation increasingly region situated in the middle, which could correspond to the projection of the gallbladder.
- in the first 5’ right predominantly lower thoracic fixation spread on FA images that could correspond to the liver region.
- An area of left predominantly upper abdominal heterogeneous fixation suggestive for fixation at the level of the stomach (wall).
- Late radiotracer accumulation is observed in a lower abdominal region, located in the projection area of the bladder.

In the case of $^{99m}$Tc:
- diffuse heterogeneous character fixation in the thoracic and upper abdominal, suggests intensively the shape of the stomach. The medial fixation region which could correspond to the gallbladder is not shown (it is visible in the case of SNP use).
- There are two symmetrical increasingly fixating regions observed in the upper abdomen situated on both sides of the median line, visible especially in the posterior incidence, which may correspond to renal fixation, more evident in the case of SNP use.
- Fixation corresponding to the bladder is present in this case.
- The fixation in the salivary glands is discreet lower than in previous experiment, between the two glands is a discrete area of fixation that could correspond to thyroid fixation. (both whole-body images and static images centered in the cervical-thoracic region)

Three hours after administration the scintygraphic examination evidenced no presence of the silica nanoparticles in the heart, salivary glands and bladder, supporting the fluorescence microscopy data. At this time the particles were identified only in the stomach.

After 3 hours the remaining uptake corresponds to the stomach projection aria.

The acute toxicity screening identified the dose of 100 mg/kg b.w. as maximum tolerated dose (MTD) for this study, with no significant changes in animal behavior or weight.

The histological examination of main organ tissue (liver, kidney, heart, stomach, intestin) that followed the five days observation period identified no histological changes from the normal tissue characteristics. The MTD was several times higher than the doses administered in the bioavailability studies, profiling a good safety profile for future therapeutical associations of the SNP with drugs.

**Conclusions:**

TAW20/AA22 did not pass the blood brain barrier and was present for more than 72 hours in the animals investigated. No significant signs of toxicity due to the silica nanoparticles administered were noted, proving a good level of safety for the particles tested. Novel drugs require intelligent carriers for improved analgesia and patient compliance and TAW20/AA22 silica nanoparticle is a possible candidate as delivery system for such drugs. The results support the idea of using the AA115, AA124 and AA22 as containers for modular drug delivery systems with promising future in therapeutics.
Part B.

Scientific, professional and academic achievements from the postdoctoral period

I received the title of Doctor of Science in Medicine in February 1997, under the coordination of Professor Valeriu Rusu, with the thesis named: Research on the malignant potential using in vitro and in vivo radioisotope methods, by the diploma No. M series. 000 899 issued by Rector U.M.F. "Gr.T.Popa" Iaşi, 11 February 1997 based on Ministry of Education order no. 3293 Judgment of February 4, 1997 by the National Council for Attesting Titles, Diplomas and Certificates from 23 to 24 January 1997. It was the first PhD both in the biophysics of radiopharmaceuticals and in nuclear medicine, in our country, and, so far there are less than 5 theses in this field.

The area in which this thesis was carried out – the biophysics of radiotracers - is still new even today, as it represent one of the two directions of development of nuclear medicine. All over the world, there are few laboratories where Nuclear medicine is in direct relationship with Biophysics. From this point of view, the Medical School in Iasi follows the francophone model, joining the two areas - one preclinical and the other clinical - particularly useful for understanding the images through the biophysical uptake mechanisms of radiolabeled molecules. In this context, it can be said that some of the conclusions of my thesis, arguing topics even today, were the starting point for my postdoctoral research topics, so there are important references mentioned in this document.

My postdoctoral evolution was conducted at Scientific, Professional and Academic directions, meaning research, university teaching and medical practice. These three levels, defined already during the PhD period, were - and are - certainly interconnected, the teaching and medical practice being in direct relationship with the research part.

B.1. Scientific achievements

My postdoctoral evolution was in continuity with the doctoral period, applying and developing especially some techniques learned under the sign of passion and rigor of the French school, during the PhD period. The essence of this period can be understood, for the most part, under the name: radiotracers biophysics with clinical applications.

One can distinguish several different directions (developed in part A):

1) The study of radiolabeled compounds for the development of new radiopharmaceuticals
2) Development of new imaging diagnostic applications of radiopharmaceuticals if known, based on the study of the mechanism of cellular uptake of these molecules in tissues of different pathological conditions
3) Studies on a number of parameters used in image processing in order to improve the quality of diagnostic imaging.

The most prominent events that I could say that were the backbone of my postdoctoral evolution were the research grants awarded by competition, international and national, obtained as a director. Among these I considered like being most representatives for my postdoctoral professional evolution four scientific grants, that I’ll developed in this presentation, in the ordered that they have arised.
Project Title: “$^{99m}$Tc isonitrils usefulness in pulmonary tuberculosis by in vivo (scintigraphic) and in vitro studies”

Type of the project: International Atomic Energy Agency - IAEA Research Contract (No: 11442/R0 and renewal 11442/R1)

Institute where research is being carried out: Biophysics and Nuclear Medicine Department, Medicine Faculty, University of Medicine and Pharmacy “Gr.T.Popă”, Iasi, Romania

Chief Scientific Investigator: Cipriana Stefanescu

Time period covered: 2000 - 2004

Papers published in relation with the project:


In abstract:

2) C. Stefanescu, V. Rusu, D. Boișteanu, M. Oleniuc, D. Hurjui, M. Costin Comparison between pulmonary tuberculosis scintigraphy with $^{99m}$Tc MIBI and $^{99m}$Tc Tetrofosmin,


7) D. Boişteanu, C. Ştefănescu, V. Rusu, T. Mihaescu, N. Brânză, L’utilité de la scintigraphie au 99m-Tc MIBI dans l’évaluation de la tuberculose pulmonaire Rev Mal Respir, vol. 16, suppl 1, 1999, 185(1S90), ISSN: 0761-8425 IF=0,488

8) D. Holtea (student), A. Savin (student), C. Stefanescu, D. Boisteanu The Importance of Radioisotopic Methods in the Evaluation of Pulmonary Tuberculosis, 13th European Students Conference for Medical Students and Young Doctors, Berlin, oct. 2002- first prize, abstract vol., 239.

This IAEA grant was obtained three years after obtaining the PhD title, after a period of continuous and intensive training. We have identified a direction for research in nuclear medicine that promises to be very useful for the diagnosis of a disease that was becoming more and more threatening for Romania but also for the rest of Eastern Europe: pulmonary tuberculosis.

The essence of this grant was the study of two radiotracers, 99mTc MIBI and 99mTc Myoview, in vitro and in vivo, with the final aim of obtaining diagnostic and evolutive functional imaging complementary to the structural ones in lung tuberculosis. There were two main parts: 99mTc MIBI study and 99mTc Mioview study. The grant was carried out in 4 successive contracts, renewed almost annually, after the IAEA organisms evaluation of the results obtained for each previous period (2000-2004). The results, valuable, were communicated especially to EANM Congress and published in summary in EJNM and in extensor especially in medical-surgical Journal (Rev Med Chir, BDI indexed). Two monographies have been evolved related to the research undergone under these projects.

Final remarks:
If these results have been essentially published in a BDI Journal (RevMedChirSocMedNat Iasi) and not in an other BDI journal or an ISI journal, was only because of a local patriotism, evidence being their citation number (9 for a 2009 published paper, last citation in 2015! - only by abstract); they were and continue to be cited only by the existing summaries in Pubmed and in other international database.
### B.1.2. Project Title: „The study of some radiotracers used in oncology”

<table>
<thead>
<tr>
<th>Type of the project:</th>
<th>Romanian Academy competition Grant, nr.282</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institute where research was carried out:</td>
<td>Biophysics and Nuclear Medicine Department, Medicine Faculty, University of Medicine and Pharmacy “Gr.T.Popă”, Iasi, Romania</td>
</tr>
<tr>
<td>Chief Scientific Investigator:</td>
<td>Cipriana Stefanescu</td>
</tr>
<tr>
<td></td>
<td>- 2008 - Contract nr.144/2008</td>
</tr>
</tbody>
</table>

**Results of this project:**


During 2007 – 2008 years I have coordinated a grant of the Romanian Academy, with two successive contracts. The results were communicated to EANM Congresses, published in summary in EJNM and extensively in the BDI and ISI Journals.

The purpose of the study was to assess the usefulness of 99mTc-labeled radiotracers for tumor image diagnosis and treatment effectiveness evaluation (chemotherapy and/or radiotherapy), in order to become an *in vivo* scintigraphic marker of tumor characteristics, through almost noninvasively techniques. The final aim was to establish protocols for the follow-up by scintigraphy; the results being thought to be useful in optimizing chemo / radio / hormonoterapy, thus improving prognosis in patients with cancers with unfavorable response to therapy. The importance of our study subject was sustained by a number of results published in scientific journals and personal data previously obtained.

I consider that one of the most important results of these two categoria of scientific grants (IAEA and Romanian Academy grants) together with the experience received in the doctoral studies period and the specialization in France is the monography about radiopharmaceuticals, their biophysical cellular uptake mechanisms and their diagnosis applications, and this is why I’ll present it in the next pages.
Nuclear Medicine is in a new period of exceptional evolution, both in terms of equipment and radiopharmaceuticals, but, currently, the development of radiopharmaceuticals occurs at an ferocious rate. However there are few monographs on the subject of radiopharmaceuticals, even in international languages. On the contrary, there are numerous articles that contain results from the research department, starting with the molecular discovery phase or their chemical synthesis, continuing with pharmacology and preclinical studies, followed by the toxicology risks assessment, the galenic step, in which the administration method is determined, followed by the clinical phases I, II and III, and finally the authorization of the new pharmaceutical after quality studies and results validations by experts. Once assimilated in the nuclear medicine practice, the new product will be a definite temptation for the nuclear laboratory studies equipped with an adequate technical infrastructure and staff organized in research teams made out of nuclear medicine doctors collaborating with radiochemists, physio-chemists and physicists.

The monograph shows all the radiopharmaceuticals, starting with the classic radiopharmaceutical, some very commonly used and the most recent ones, that allow obtaining metabolic and molecular images. Recent development, due to advances in genomics, proteomics, physiomics, metabolomics is as fascinating as it is unpredictable. It consists of 6 chapters, starting with chapter I – Some history milestones in nuclear medicine, continuing with physics and biophysics characteristics (chapter II), biophysics mechanisms of fixation and capture (chapter III), quality control (chapter IV) and the use of radiopharmaceuticals in nuclear medicine (chapter VI). The paper is the result of the authors experience in the field of Nuclear Medicine, presented in chapter IV, and, directly, the research conducted in two grants won by selection: $^{99m}$Tc isonitrils usefulness in pulmonary tuberculosis by in vivo and in vitro studies (IAEA) and The study of some radiopharmaceuticals used in oncologie (GAR) – and in the field of medical biophysics that helped us understand and explain the structural bases and fixation mechanisms of the radiotracer in a more explicit manner.

The number of radioisotopes used nowadays in nuclear medicine is relatively small and there has no significant upward trend. Radioisotopes usually obtained in generator (very searched for because of its low cost, obtaining facilities and availability in all nuclear medicine services) are also in relatively small numbers. All in all, the numbers of radiopharmaceuticals are in a continues growth, throw new research and new vector molecules, with more accurate targeting mechanism for a certain tissue / organ that we want to visualize. Radiolabeled molecules are becoming more diverse and numerous, research and introduction of new radiotracers in medical practice representing one of two essential directions in nuclear medicine development (along with the development of detection devices).
Figure B.1. From radionuclide to patient: production algorithm and the use of the radionuclide

To this date, hundreds of RF were studied experimentally in vitro and in vivo. Of these fewer were consecrated in the practice of nuclear medicine, where they would be used by humans for diagnosis and treatment. It can be said that, at present time, there are RF most
commonly used (currently used in nuclear medicine, such as $^{99m}$Tc, $^{99m}$Tc MDP etc.), RF with limited use (some RF used in myocardial scintigraphy, $^{99m}$Tc Teboroxime etc.), specialized RF ($^{131}$I, radiolabeled monoclonal antibodies etc.) and new RF (especially PET RF, with implications for diagnosis and gene therapy).

Firstly, this paper, presents the physical and biophysical characteristics of some radiopharmaceuticals.

II.1. $^{99m}$Technetium and technetium compounds

   II.1.1. $^{99m}$Tc pertechnetate
   II.1.2. Phosphates and phosphonates labeled with $^{99m}$Tc
   II.1.3. $^{99m}$Tc isonitrili
   II.1.4. $^{99m}$Tc human serum albumin
   II.1.5. Macroaggregate and $^{99m}$Tc labeled microspheres
   II.1.6. $^{99m}$Tc sulfocoloid
   II.1.7. $^{99m}$Tc DTPA
   II.1.8. $^{99m}$Tc DMSA
   II.1.9. $^{99m}$Tc MAG3
   II.1.10. $^{99m}$Tc Glucoheptonate
   II.1.11. $^{99m}$Tc HMPAO
   II.1.12. $^{99m}$Tc ECD
   II.1.13. $^{99m}$Tc IDA
   II.1.14. $^{99m}$Tc Technegaz

II.2. Radioactive iodine and iodide compounds

   II.2.1. Iodine radioisotopes
   II.2.2. N-isopropyl-$p^{(123)}$IAMP
   II.2.3. $^{131}$I MIBG

II.3. Other radiopharmaceuticals used in conventional nuclear medicine

   II.3.1. Thallium 201
   II.3.2. Gallium 67
   II.3.3. Indium 111 and labeled analogs of somatostatin
   II.3.4. Radioactive Xenon

II.4. Radioisotopes and radiopharmaceuticals used in PET

   II.4.1. 18-Fluor and radiopharmaceuticals labeled with $^{18}$F
   II.4.2. 11-Carbon
   II.4.3. 11-Nitrogen

II.4.4. 15-Oxygen

   II.4.5. Radiolabeled oligonucleotides
   II.4.6. Nonstandard PET radiopharmaceuticals

II.5. Other radiolabeled molecules

II.6. Radiopharmaceuticals that can be used in therapy

Radiopharmaceuticals available today have sufficient heterogeneous origins and structures. As a result, fixation or location processes at the investigated structures vary and sometimes are poorly known, there for they can not be grouped in a unique and unanimous accepted
general classification. There may be possible a series of classifications, among the most useful is the relationship with a biophysics mechanism for locating the labeled molecule at the structure we want to visualize. In essence, this mechanism can be for cellular capture or fixation on a certain structure (Figure B.2).

Chapter III presents The biophysics mechanisms for fixation or capture of some radiopharmaceuticals, starting with their classification.

- **Classification depending on the group that binds the radioisotope:**
  - Isolated radioisotope (ex.: $^{99m}$Tc, $^{201}$Tl, $^{123}$I)
  - Radioisotope bound to a molecule (ex.: $^{99m}$Tc MDP)
  - Radioisotope bound to a hormone (ex.: $^{111}$In pentetreotid)
  - Radioisotope bound to an antibody (ex.: $^{99m}$Tc IgG$_2$A murin)
  - Radioisotope bound to a colloid particle (ex.: $^{99m}$Tc MAA)

- **Historical classification, depending on the type of emission;**
  - Traditional radiotracers or for “conventional” scintigraphy ($^{201}$Tl, $^{99m}$Tc and labeled molecules with $^{99m}$Tc, iodine radioactive isotopes etc.)
  - PET radiopharmaceuticals: studied ($^{18}$FFDG, $^{11}$C methionine, $^{11}$C thymidine, $^{18}$F fluoroestradiol etc.), in perspective radiopharmaceuticals ($^{18}$F oligonucleotides, aptamers), “non standard” radiopharmaceuticals ($^{64}$Cu, $^{124}$I etc.)

- **Classification by specificity:**
  - Non-specific radiotracers, such as: $^{67}$Ga citrate, $^{99m}$Tc MIBI
  - Specific radiotracers, such as: hormone analogs ( $^{131}$I MIBG, iodo-estradiol), some receptor tracers ( $^{111}$In pentetreotid), monoclonal antibodies.

---

**Figure B.2. Radiopharmaceuticals cellular uptake mechanisms**

![Diagram of cellular uptake mechanisms](image)

- Glucidic metabolism
- Amino acids: $^{18}$N ammonia
- Purines and pyrimidines (DNA components): $^{18}$F 5FU
- Passive transport $^{99m}$Tc isonitrili
- Fatty acids labeled with $^{11}$C
- The resting membrane potential ($V$)
- Nucleus
- Mitochondria
- Fatty acids
- Cell
- Active transport: $^{201}$Tl
- Hormone receptors: $^{111}$In octreotide (somatostatin receptor)

---

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Table B.1. Classification of radiopharmaceutical for diagnosis

<table>
<thead>
<tr>
<th>The nature of the vector molecule</th>
<th>Radioisotope</th>
<th>Radiopharmaceutical and/or utility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Particles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colloids</td>
<td>$^{99m}$Tc, $^{99m}$Tc</td>
<td>Sulfo or phytate colloids, with uptake in the liver, spleen, bone marrow</td>
</tr>
<tr>
<td>Macro aggregate</td>
<td>$^{99m}$Tc, $^{99m}$Tc</td>
<td>Albumine macro aggregate for pulmonary scintigraphy</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>$^{51}$Cr, $^{111}$In, $^{99m}$Tc</td>
<td>Blood pool</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>$^{111}$In, $^{99m}$Tc</td>
<td>Erythrocytes survival</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td>$^{111}$In, $^{99m}$Tc</td>
<td>Infectious site uptake</td>
</tr>
<tr>
<td>Fragile red blood cells</td>
<td>$^{99m}$Tc</td>
<td>Uptake in the trombus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sferocites uptake in the spleen</td>
</tr>
<tr>
<td><strong>Labeled protein</strong></td>
<td>$^{99m}$Tc, $^{111}$In, $^{131}$I</td>
<td>Albumin, immunoglobulin, monoclonal antibody</td>
</tr>
<tr>
<td><strong>Labeled peptide</strong></td>
<td>$^{111}$In, $^{123}$I</td>
<td>Somatostatin analogs</td>
</tr>
<tr>
<td><strong>$^{99m}$Tc complexes</strong></td>
<td>$^{99m}$Tc</td>
<td>HIDA analogs for gallbladder, DTPA, MAG3, DMSA for kidneys</td>
</tr>
<tr>
<td>Essential $^{99m}$Tc anionic</td>
<td></td>
<td>HMPAO for cerebral studies, Labeled leuucocites (inflamations)</td>
</tr>
<tr>
<td>neutral</td>
<td></td>
<td>Isonitrili (miocardium)</td>
</tr>
<tr>
<td>cationic</td>
<td></td>
<td>biphosphonates (bone), PYP (myocardial infarction, blood pool)</td>
</tr>
<tr>
<td>pertechnetate</td>
<td></td>
<td>Pertechnetate (thyroid, Meckel diverticulum), Technegas (lung)</td>
</tr>
<tr>
<td><strong>Other $^{99m}$Tc compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metal ions</td>
<td>$^{201}$Tl, $^{67}$Ga, $^{51}$Cr, $^{111}$In</td>
<td>$^{201}$Tl for myocardial and tumor scintigraphy, $^{67}$Ga for tumor scintigraphy and infectious sites</td>
</tr>
<tr>
<td>Non – metals</td>
<td>$^{131}$I, $^{123}$I, $^{125}$I, $^{133}$Xe</td>
<td>Thyroid scintigraphy, immunoscintigraphy (for radiolabeling monoclonal antibodies) Lung scintigraphy</td>
</tr>
<tr>
<td>Positron emitters</td>
<td>$^{11}$C, $^{13}$N, $^{15}$O, $^{18}$F, $^{68}$Ga</td>
<td>Metabolic analogs, ligands, metabolic intermediates (cellular metabolic studies, vascularization, tissue hypoxia)</td>
</tr>
</tbody>
</table>
- **Classification by mode of production:**
  - Cyclotron (examples: $^{18}\text{F}$, $^{11}\text{C}$, $^{13}\text{N}$, $^{15}\text{O}$)
  - Generator (examples: $^{99m}\text{Tc}$, $^{68}\text{Ga}$, $^{82}\text{Rb}$)
  - Reactor: radioisotopes produced in the reactor are not directly used in nuclear medicine.
- **Classification in relation to the biophysical mechanism for radiopharmaceutical localization (figure B.2.)**
  1. Radiopharmaceuticals cellular uptake mechanisms:
     - passive membrane transport, depending on the electrochemical gradient (eg.: $^{99m}\text{Tc}$ isonitrili)
     - active transport through pumps, ATP dependent (eg.: $^{201}\text{Tl}$)
     - membrane receptor mediated transport (eg.: $^{67}\text{Ga}$)
     - diffusion transport facilitated by a protein carrier (eg.: $^{18}\text{FFDG}$)
  2. Radiopharmaceutical fixation mechanisms at tissue level:
     Some fixation mechanism are physical phenomena (for example the hydroxyapatite crystals absorption phenomena, in case of derived phosphonates labeled with $^{99m}\text{Tc}$ in bone scintigraphy). Other events include tissue accumulation phenomena by pathological disruption of tissue barriers (for example BHE). In some cases tissue accumulation occurs through increased vascularization etc.:
- **Particular classification in some distinct pathologies**, such as tumor scintigraphy
  They are successive presented:
  III.2. $^{99m}\text{TcO}_4^-$: location mechanisms in the biodistribution sites
  III.3. Radiopharmaceuticals uptake mechanisms at thyroid level
  III.4. Radiopharmaceuticals uptake mechanisms at miocyte level
  III.5. Radiopharmaceuticals fixation mechanisms at bone level
  III.6. Radiopharmaceuticals fixation mechanisms at renal level
  III.7. Fixation mechanisms for radiopharmaceuticals used in pulmonary scintigraphy
  III.8. Uptake mechanisms for radiopharmaceuticals used in cerebral scintigraphy
  III.9. Radiopharmaceuticals uptake mechanisms at tumor cell level
  III.10. Radiopharmaceutical uptake mechanisms at infection / inflammation lesions.
A special place lies for radiopharmaceuticals for the thyroid cell, the myocardial cell and the tumor cell.

Chapter IV presents *The experimental study of a radiopharmaceutical cell uptake mechanism, data resulted from personal study conducted during the doctoral and postdoctoral period:*
- In vitro study, $^{99m}\text{Tc}$ MIBI uptake compared to $^{99m}\text{Tc}$ Tetrofosmin and $^{201}\text{Tl}$ at normal and cancer cellular culture level and on *Mycobacterium Tuberculosis* cultures.
- In vivo study, which includes $^{99m}\text{Tc}$ MIBI SPECT scintigraphy for glioms, breast cancer and type I neurofibromatosis

Chapter V presents *The quality control for the radiopharmaceuticals.*

The monograph ends with a chapter on *Radiopharmaceuticals diagnostic utilization in nuclear medicine:*
VI.1. Thyroid and parathyroid scintigraphy
VI.2. Bone scintigraphy
VI.3. Tumor scintigraphy
VI.4. Myocardial perfusion scintigraphy
VI.5. Pulmonary scintigraphy
VI.6. Cerebral scintigraphy
VI.7. Radioisotopes renal exploration
VI.8. Inflammation / infection sites scintigraphy
VI.9. Radioisotopes therapy

A special place is reserved for the location mechanisms for the bio distribution sites for $^{99m}$TcO$_4^-$.

Given how important $^{99m}$Tc is to nuclear medicine, thanks its special characteristics, we will first refer to its bio distribution and location mechanism in the body.

Sodium pertechnetate eluted in the $^{99}$Mo – $^{99m}$Tc generator is, from a chemical point of view, a salt. In solution, it dissociates according to the reaction:

$$\text{Na}^{99m}\text{TcO}_4 \rightarrow \text{Na}^+ + {^{99m}\text{TcO}_4}^-$$

In the body, the $^{99m}$TcO$_4^-$ anion is localized in different tissues, its bio distribution being independent of the Na$^+$ and partially dependent to the patients administration route (orally or intravenously).

**Radiopharmaceuticals uptake mechanisms at thyroid cell level**

*Radioactive Iodide: $^{123}$I / $^{131}$I*

Because iodide is the fundamental element in the way the thyroid works, radioactive iodide ($^{123}$I or $^{131}$I) was the first and the most used radiopharmaceutical for evaluating the function and structure of this gland.

Radioactive iodide is administered orally in the form of sodium iodide ($^{131}$INa or $^{123}$INa). After oral administration, the iodide is absorbed at the intestinal wall. Its maxim blood concentration peaks at three hours after the administration. Approximately 90% of the administered dose is eliminated through the kidneys (half of this being eliminated in the first 24 hours). A small percent is eliminated through sweat and the digestive system.

The thyroid captures the iodide from the bloodstream by an active mechanism. The uptake mechanism was elucidated in 1996 by a group of researchers led by Nancy Carrasci: a glycoprotein made out of 618 amino acids, located in the plasmatic membrane of the tireocit, mediates the active transport of the iodide in to the cell, ATP dependent also known as *iodine pomp* or the *sodium-iodide symport protein* (*Sodium or Natrium Iodide-Symporter, NIS*) because it transports, simultaneously inside the cell, an anion iodide and two sodium cations.

The concentration of iodide in the follicular colloid is 30 – 50 times higher than in the blood and it can reach up to 250 – 350 times higher, in case of maximum iodide uptake.
NIS protein is influenced by TSH, through a process of up-regulation, while iodide moderated doses result in a process of down – regulation. It could also be involved in the uptake of other radiotracers used in thyroid scintigraphy, such as $^{99m}$Tc. Another substance that was assumed to be carried by the NIS symport in a similar manner to iodine, is perchlorate, but later on it was proven that it was a NIS inhibitor and consequently acts not like a substrate but more like a membrane transport blocker.

**Fig.B.4.** Membrane transport system at tireocite level, in which we can observe the natrium-iodide symport protein (NIS), involved in capture of both non-radioactive and radioactive iodide (thyroid scintigraphy)

Figure B.4 and Figure B.5. presents the active mechanism uptake (NIS), which occurs at the basolateral plasmatic membrane level of the tireocit, against an electric gradient (0 – 50mV) and a concentration gradient, intracellular concentration being higher than the extracellular concentration [$^{131}$I]. Iodide transport from the cytoplasm to the follicular lumen is, probably, passive, the electrochemical gradient being favorable for such a transport.

$^{99m}$Tc – pertechnetate ($^{99m}$TcO$_4^-$)

Another radiopharmaceutical with thyroid cell uptake is $^{99m}$Tc – pertechnetate ($^{99m}$TcO$_4^-$). This transport mechanism is not completely understood: whether is acts like an iodide uptake inhibitor, or it is an NIS substrate, like other monovalent anions, its transport being similar, thus, to that of iodide.

This hypothesis that the mechanism uptake for $^{99m}$TcO$_4^-$ is similar to the way in which radioactive iodide is transported, at thyroid cell level, is in relation with a series of factors: - $^{99m}$TcO$_4^-$ has a negative charge like iodide (radioactive or non-radioactive). This would be one of the primary reasons for which the tireocit can not distinguish between the two ions.

This hypothesis is supported by the fact that other monovalent anions are captured by the
tireocit, such as perchlorate ion (ClO$_4^-$) or iodide ion (IO$_3^-$), but it is contradicted by the existence of some monovalent anions that are not captured by the tireocit (such as F$^-$ or Cl$^-$).

![Image]

**Figure B.5.** NIS tireocit transport system at tireocit level. A: immune localization of the NIS protein at the basolateral plasmatic membrane of the tireocit. B: schematic representation of the topology of the NIS protein membrane, based on the data obtained from analysis of secondary structure. C: $^{131}$I transport from extracellular liquid (or plasma) to follicular thyroid lumen

- Another factor to consider is the molecular weight of captured monovalent anions. Examining the captured anion list it can be observed that among halogens, only iodide (I$^-$) and astatine (At$^-$) are captured by the tireocit and from the atomic combination with a single negative charge perchlorate (ClO$_4^-$), iodide (IO$_3^-$) and pertechnetate (TcO$_4^{99m}$). All these anions have two properties in common: all have negative charge and all have a molecular weight ≥ 100. It has been found that anions with molecular weight relatively under 100 have no significant capture (F$^-$, Cl$^-$, Br$^-$ or atom groups such as NO$_3^-$ or HCO$_3^-$).

It seems that for a substance to be captured by the thyroid cell, it has to meet at least two conditions: to present a negative charge and a molecular weight ≥ 100. However as there are numerous atomic combinations that fulfill these conditions, certainly there are other conditions, more likely in relationship with the ionic volume and molecular configuration.

After intravenous administration, $^{99m}$TcO$_4^-$ circulate, partially, bound to plasmatic protein. It rapidly accumulates inside the thyroid, with a concentration of 10/1 against surrounding tissue. It’s captured but not organified in the thyroid cell (like other ionic groups, such as
perchlorate), accumulates intracellularly (which may explain the discordant fixation in case of a nodule that does not uptake radioactive iodide). After it remains inside the tireocit for a while (between 30 and 45 minutes after reaching the maximum concentration at 10 – 15 minutes after the intravenous administration), the pertechnetate ion returns into the bloodstream by passive transport. During this period the intravascular concentration falls, so that the highest concentration of tireocit/blood is reached at about an hour after intravascular administration, after which the intrathyroid concentration decreases proportional with the blood concentration. About 30% of the activity from the intravenous administered $^{99m}$TcO$_4^-$ is excreted in the urine within the first 24 hours, after which the fecal excretion becomes more important. Total excretion (urinary and fecal excretion) of $^{99m}$TcO$_4^-$ is about 50% in 3 days and about 70% in 8 days. Reduced $^{99m}$Tc and $^{99m}$Tc chelated are more rapidly cleared by the kidney, with out accumulation in the thyroid.

**Other radiopharmaceuticals with thyroid tropism**
Other radiopharmaceuticals used some times for viewing thyroid scintigraphy, such as $^{201}$Tl, $^{99m}$Tc isonitrili, $^{18}$F FDG, are captured by the thyroid cell and transformed malignant, through specific tumor cell mechanisms, described in subchapter III.8. The first two are used in the detection of metastatic thyroid cancer, along with $^{131}$I. $^{18}$F FDG has proven to be more useful in follicular thyroid neoplasia staging. Another radiotracer, $^{99m}$Tc DMSA pentavalent, is used in the detection of thyroid medullar neoplasm, primary and metastatic, along with $^{111}$In – pentreotid, which are attached to the somatostatins receptor, that are present in the malignant cells.

**Radiopharmaceuticals uptake mechanisms at miocyte level**
Over time numerous radiopharmaceuticals have been proposed and used in myocardial scintigraphy (Table B.2.), allowing evaluation of different aspects of the myocardium in relation to their location mechanism as you could see in the table below:
A number of radiopharmaceuticals that include positron emitters radioisotopes are used today for viewing the myocardium. These labeled molecule disseminate through the body and their distribution may be monitored by positron emission tomography (PET). In this case, the used radioisotopes are able to mark the molecules or the analog molecules present in the body, that participate in a series of physiological cellular processes, including metabolic pathways. This makes it possible to monitor these processes or the metabolism, in vivo, through a non-invasive method, that revolutionized both nuclear medicine and molecular imaging. This provided that the four basic elements of living matter (C, H, O, N) do not possess radioactive isotopes whose physic properties are adapted for conventional detection scintigraphy. However, in the case of three of this element there are radioisotopes that emit positrons ($^{11}$C, $^{15}$O şi $^{13}$N). Another positron emitter, $^{18}$F, although it is not present in the body, it may mark the glucose molecule, whose metabolism makes it detectable with the help of the PET gamma camera. This, $^{18}$F FDG, is the most used PET radiopharmaceutical in the study of the myocardial metabolism, along with other applications (neoplastic cellular metabolism), the energy required by the myocardium to
contract resulting from glucose metabolism and free fatty acids. The uptake mechanisms for the $^{18}$F FDG at myocardial cellular level is the same as the uptake mechanism from the tumor cell level, which is discussed in detail in this subchapter (III.1), as a result of the particular interests that this radiotracer has in tumor scintigraphy. Inside the miocit the $^{18}$F FDG is stored under the form of FDG – $6^\circ$, as in the tumor cell. The normal adult myocardial uptake is 1 – 4 %. Reports of attachment of the heart to the lungs, blood, liver are: 20: 1, 14: 1 and 10: 1.

**Table B.2. Radiopharmaceuticals used in myocardial scintigraphy**

<table>
<thead>
<tr>
<th>Radiopharmaceutical</th>
<th>Evaluated aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{99m}$Tc-labelled eritrocites $^{11}$C-CO</td>
<td>Miocardial vascularization</td>
</tr>
<tr>
<td>$^{201}$Tl $^{82}$Rb $^{99m}$Tc-MIBI, $^{99m}$Tc Tetrofosmin $^{99m}$Tc-Teboroxim $^{12}$N-NH$_3$ $^{15}$O-H$_2$O $^{99m}$Tc-Pyp $^{111}$In-antimiozin antibodies</td>
<td>Evaluates myocardial perfusion Detection of myocardial infarction / differential diagnosis with myocardial ischemia Presence and location of myocardial infarction Metabolical aspects of the myocardium</td>
</tr>
<tr>
<td>$^{18}$F or $^{123}$I-fatty acids $^{18}$F FDG $^{11}$C acetat $^{125}$T MIBG $^{111}$In labelled thrombocytes $^{99m}$Tc-fibrin antibodies $^{11}$C-MQNB $^{11}$C-CGP12177</td>
<td>Myocardial innervation Muscarinic receptor Adrenergic receptors</td>
</tr>
</tbody>
</table>

After intravenous administration of the radiopharmaceutical, the blood clearance of the $^{18}$F FDG is three exponentially having three components with half times (0,2 – 0,3 min), 11,6 ± 1,1 min şi (88 ± 4 min).

For the study of myocardium metabolism there are also other PET radiopharmaceuticals used. Thus, fatty acids labeled with $^{123}$I (heptadecanoic acid and iodo-p-iodophenylpentadecanoic acid) can be used for myocardial metabolic imaging. Palmitic acid labeled with $^{11}$C and glutamic acid labeled with $^{13}$N are used in PET imaging for myocardial metabolism of the amino acids. $^{11}$C acetate presents a particular interest as a metabolic marker, participating in myocardial oxidative metabolism. Its fixation allows for the evaluation of oxygen consumption by the myocardium and differential diagnosis between viable and necrotic myocardium.
Project title: **Advanced, Cross-Disciplinary & Integrated Medical Imaging for all Europeans through a Network of Regional Clusters and Development Strategies (AMI-4EUROPE)**

**Grant type:** International, FP7-REGIONS Project  
**Project reference:** 265435

**Project Director:** Prof. dr. Cipriana Ştefânescu / Prof. dr. Dragoș Pieptu (P15)


**Coordinator:** Asociacion Matrid Network, Spain

**Parteners:**  
P1 - Asociacion Plataforma de la Salud y el Bienestar Madrid, Spain  
P2 - Asociacion Madrid Plataforma de la Biotecnologia, Spain  
P3 - Investitiiun Forderbank Niedersachsen Madrid, Spain  
P4 - Zentrum fur Biomedizinische Technik und Innovation Hannover, Germany  
P5 - Societa Finanziaria Laziale di Sviluppo Roma, Italy  
P6 - Innovas Eszak Debrecen, Hungary  
P7 - Pharmapolis Klaszter KFT Debrecen, Hungary  
P8 - Agencija za Ekonomski Razvoj Opstine Prijedor, Bosnia and Herzogovina  
P9 - Servicio Madrileno de Salud, Spain  
P10 - Xpertia Soluciones Integrales SL Madrid, Spain  
P11 - Desarrollros Informaticos Abadia Madrid, Spain  
P12 - Andago Ingenieria SL Madrid, Spain  
P13 - Universitas Degli Studi di Roma tor Vergeta, Italy  
P14 - Advanced Computer Systems Roma, Italy  
P15 - Universitatea de Medicină și Farmacie "Gr. T. Popa" Iași, România  
P16 - Spitalul Clinic Județean de Urgență "Sf. Spiridon"Iași, România  
P17 - Romsoft Iași, România  
P18 - Europrojekt Centar Banja Luka, Bosnia and Herzegovina  
P19 - Univerzitet u Banjoj Luci, Bosnia and Herzegovina  
P20 - Zdravstvena Ustanova Opsta Bolnica Prijedor, Bosnia and Herzegovina

**Period:** 01.10.2010 - 30.09.2013

**Project budget:** 64.657,96 euro

**Project Website:** www.ami-4europe.eu *(reference)*

**Important results, papers, distinctions:**  
- IMAGO-MOL cluster setting  
- The Public Award (2014, Gala medica, The College of Physicians  
In the period 2011-2013, I was coordinator of the Romanian partner (P15) in the FP7 grant AMI4Europe. The results of the grant have resulted in establishing the first Romanian medical Cluster, the Cluster IMAGO-MOL, which was awarded with the The PublicAward (2014, Gala medica, The College of Physicians from Romania.

AMI-4EUROPE aimed to acquire a comprehensive insight into the design, implementation and impact of existing medical imaging and associated health related technologies research and innovation support programmes and initiatives in Europe. The scope of this objective was to create the common coordination and knowledge base necessary to enter the identified market niches and then establish appropriate scientific advice and sustainable support mechanisms to assist the European Community and national and regional policy makers to better define a strategy and show the way for future research actions in favor of advanced medical imaging (AMI), regions of knowledge and the European Research Area (ERA) to maximize their socioeconomic impact in support of the health related economy. More specifically, AMI-4EUROPE project science and technology objective was to provide a comprehensive, authoritative, evidence-based, quantitative and qualitative study on the overall state of play in medical imaging in Europe.

AMI-4EUROPE grant had:
- mapped and identify all relevant 'non-scientific' stakeholders in this field
- performed a comprehensive and evidence based socioeconomic strengths, weaknesses, opportunities and threats (SWOT) analysis of the regions
- identified key elements that determined success or failure on achieving targets and objectives
- identified key drivers and opportunities for the development of such initiatives and programmes
- started identifying current barriers to regional economic development and European regional and small and medium sized enterprises (SMEs) increased share and active participation in the health-related economy related to AMI activities
- assessed its impact on the economy and on social and environmental issues
- performed a value chain analysis of the medical imaging sector in Europe
- develop market entry and market expansion strategies
- carried out a benchmarking analysis regarding the impact of medical imaging in the European regions
- identified key regional socioeconomic actors and players best positioned to take advantage of the emerging market opportunities
- identified, understand and capitalise on complementarities among research driven clusters, both emerging and mature, research and technical development (RTD) infrastructure sharing, access to private or public funding schemes, skills and knowledge transfer enhancement, etc
- became able to make more informed business decisions from the insightful and in-depth analysis of the global and European medical imaging market.

Another project objective was to define the AMI-4EUROPE joint action plan (JAP). This JAP was to describe our strategy to drive and boost regional and European economic
development and efficiency through research, technological development, networking, synergy searching, improved transnational cooperation and full exploitation of the SWOT analysis. In addition, the project aimed to start creating 'AMI infrastructure' to support a quick and full development and implementation of AMI for maximum socioeconomic impact.

The project consisted in several work packages (WPs).

WP2, titled 'impact of medical imaging, nanomedicine and biotechnology based healthcare in Europe', aimed to prepare a comprehensive and authoritative, evidence based, quantitative and qualitative study of the overall state of play in medical imaging in Europe. Its deliverables were a value chain analysis of the medical imaging sector in Europe, as well as a current state of play of medical imaging in Europe report.

WP3 realised the analysis on 'non-scientific' stakeholders, programmes, initiatives, policies and regional strategies. Its objective was to get a comprehensive insight into the programmes, regional strategies and initiatives that supported research and innovation in the field of medical imaging.

WP4 focussed on the structure and definition of advanced, cross-disciplinary and integrated medical imaging. Its objective was to gather a complete overview on the involved regions with respect to medical imaging from a scientific point of view. In order to reach this ambitious goal the involved clusters and scientific partners firstly identified the key scientific stakeholders in the targeted regions in order to have a detailed database. A SWOT analysis was also carried out.

WP5 was concerned with the project's JAP. The JAP aimed to describe our strategy to drive and boost regional and European economic development and efficiency through research, technological development, networking, synergy searching, improved transnational cooperation and full exploitation of the SWOT analysis results in the field of advanced, cross-disciplinary and integrated medical imaging, comprising nanomedicine, biotechnologies and information and communications technology (ICT) for health.

WP6 developed the AMI-4EUROPE business plan, including the JAP implementation, synergies and sustainability. The overall aim of the financial plan (FP), the balanced scorecard (BSC) and the business plan (BP) was to deepen and further process the results derived from the JAP. The FP identified, examined and analysed all those funding schemes which were available and applicable for regional authorities. The BSC included the whole set of key performance indicators (KPI) in order to facilitate, integrate and monitor the future implementation of JAP activities. The BP with all its elements and component documented the strategies and tactics for the complete and comprehensive implementation of the AMI-4EUROPE JAP.

WP7 included mentoring activities. It included the mentoring for capacity building in regions with a less developed research profile. It was intended to define, develop and implement all appropriate activities for the setting up of two new regional research driven clusters on AMI, one in the northeast region in Romania and the other in the Republika Srpska in Bosnia and Herzegovina. Its realise: an initial analysis report on northeast region in Romania and the Republica Srpska in Bosnia Herzegovina; a regional SWOT on capacity
building and value chain analysis report and a template of common issues to consider for other regional research driven clusters.

WP8 focussed on dissemination and exploitation activities and included the definition of a communication strategy, a visual identity for AMI-4EUROPE and the definition and implementation of the project website. It realised the project website AMI-4EUROPE integrated four mature research driven clusters with complementary fields of expertise as per the cross-disciplinary and integrated approach taken by the consortium. AMI-4EUROPE was a project willing to develop and conform a truly European cluster on AMI, therefore the consortium was open to integrate and perform similar activities and tasks to the ones just explained targeting northeast region in Romania and the Republika Srpska in Bosnia and Herzegovina with any other interested cluster to be on AMI.

The strategic goal of the project was not just to coordinate and integrate clusters but rather to set up and create infrastructure on the use the new technologies and cross-disciplinary RTD areas available within a clearly defined and market lead approach to allow European socioeconomic development by entering the AMI market through the identified niche. The creation of the initial 'AMI infrastructure' aimed to support all regional and national efforts, stakeholders and interested parties to a quick and full development and implementation of AMI for maximum socioeconomic impact. This would allow:
- delivering more effective investments in R&D at regional level through the definition and implementation of regional strategies based on business needs.
- 'non-scientific' stakeholders, initiatives, programmes, policies and regional strategies that would assist the collaboration between research on medical imaging, nanotechnologies, biotechnologies and ICT for health and the health related economy.
- develop market entry and market expansion strategies by identifying the emerging market categories and geographic markets poised for strong growth.
- promote synergies notably with the structural funds and the competitiveness and innovation programme (CIP).

AMI-4EUROPE also:
- identified key regional socioeconomic actors and players best positioned to take advantage of the emerging market opportunities by developing insight on the prevalent and anticipated competitive landscape
- identified, understand and capitalise on complementarities among research driven clusters, both emerging and mature, RTD infrastructure sharing, access to private or public funding schemes, skills and knowledge transfer enhancement, etc.
- be able to make more informed business decisions from the insightful and in-depth analysis of the global and European medical imaging market and the factors shaping it
- the project would also define and develop a BP in support of the overall AMI strategy thus assuring maximum synergy searching and mid and long term sustainability.

Regarding mentoring of regions with a less developed research profile, all appropriate activities were undertaken for the setting up of, at least, two new regional research-driven clusters on AMI. By the end of AMI-4EUROPE time both clusters should be formally and legally established in their respective regions and countries and in full operation with a
minimum membership as per the 'founding partners' included in this consortium. Where appropriate, delivery of 'guidance' solutions for those regions would be undertaken. In addition, a permanent scheme and roadmap for other research driven clusters to be in Europe would be developed, so that similar international cooperation (INCO) activities could benefit from the outcomes and activities of AMI-4EUROPE. The finality of AMI-4Europe project was to create a number of medical Clusters in different Europe regions and a further collaboration network in the medical field, for the benefit of patient diagnosis and treatment and a better research communication, with economic impact. In Romania, The medical cluster concept does not existed at that moment. The only existant clusters were in the economic field. However, in medicine the <cluster> notion is usually associated with the worst headache ever! So this task allocated especially to the UMF partner was not at all easy. However, it was accomplished, after long discussions and explanations, in September 2012 the IMAGO-MOL Cluster was functional!

So, the North-East Innovative Regional Cluster for Structural and Molecular Imaging (IMAGO-MOL), the only medical cluster from Romania, was established like a non-governmental, non-profit organization that aims to support the growth of scientific competitiveness of its members and the competitiveness of the North East Region in the field of Medical Imaging by developing a framework of cooperation based on diversification and optimization of services in this area.

The cluster was established on 2012, as a result of the project AMI4Europe, by joint decision and contribution of its founding members - University of Medicine and Pharmacy Iasi „Gr. T. Popa”, North East Regional Development Agency, Sf. Spiridon Emergency Clinical Hospital of Iasi, Regional Oncological Institute and Romsoft SRL Iasi, in order to develop a better collaboration in the use of innovative medical imaging and implementing better health care services by establishing a framework to improve efficiency, quality, productivity and visibility of these members. Subsequently, joined the cluster as associated members: Iași County Council, Scientific and Technological Park TEHNOPOLIS, Technical University “Gheorghe Asachi” Iași, Al. I. Cuza University Iași, Emergency Clinical Hospital “Prof. Dr. N. Oblu” and 10 SMEs from the IT and health sector (Phoenix Diagnostic Clinic, Micromedica Medical Center SRL Piatra Neamț, Scan Expert Roman, X Med Center Iași, Optim Diagnostic Botoșani, Tissuegnostics Romania Iași, Centrul Medical Sapientek Buzău, Coramed Suceava, Serv Sistem Iași, Shopfit Online Iași).

The institutional component of the cluster IMAGO-MOL was developed around the scientifical force of the three most performant and with great tradition Universities from Iasi: University of Medicine and Pharmacy "Gr T. Popa" Iasi, "Al. I. Cuza” University and
Technical University "Gheorghe Asachi" Iasi, the medical experience of three great hospitals from Iasi (the biggest Emergency Hospital ("St. Spiridon") from the NE Romania, the Regional Institute of Oncology Iasi (the second biggest oncology institute from Romania), the Neurology Hospital from Iasi. Their scientific force is strengthened by the experience of the Regional Development Agency for North East Romania, the Iasi County Council, the Scientific and Technological Park TEHNOPOLIS and 11 SMEs (some acceding). The number of members joining IMAGO-MOL is rapidly growing, as well as its internal and external collaborations. By its support and collaboration from the beginning with Spain, Italy, Germany, Hungary, Bosnia-Herzegovina AMI 4Europe partners, IMAGO-MOL has quickly evolved, supporting the competitive development of the structural, functional and molecular imaging sector in the entire North-East region. IMAGO-MOL creates the premises for a better understanding and cooperation among the molecular imaging sector on one hand, IT and economic sector on the other hand, bases for collaboration, diversification, optimization and more innovative use of medical imaging so that we all benefit from enhanced efficiency, quality of services, productivity and visibility.

Imago-Mol Cluster gathers under its umbrella promoters of research and development, innovation and education, hospitals, promoters of regional development, SMEs in the fields of ICT and health. IMAGO-MOL Cluster mission is to support the competitiveness development of the structural and functional molecular imaging sector in North East Region.

The scientific objectives of IMAGO-MOL Cluster:

• The cluster aims to initiate and develop medical studies in vivo and in vitro, fundamental and applied. The studies will track the physiological and pathological medical aspects through molecular imaging methods, functional (e.g. scintigraphy) and structural proper resolution (CT, MRI, ultrasound etc.).
• The cluster aims to develop a platform for molecular and structural medical imaging competitive in the context of medical imaging on nationally and internationally level.

Fields of activity:

• Development of a platform in the molecular and structural medical imaging providers/stakeholders in the North East Region, including basic and applied medical studies that will follow the medical aspects of physiological and pathological molecular imaging.
• Research-developing activities for Medical Imaging domain and technologic transfer, with formative impact in universitary and post-universitary education, economic impact (SME development) and practical impact (improving some disease diagnosis and treatment)
• Development of projects in partnership in order to obtain funds necessary for researching activities and technology transfer in the field of medical imaging.

Examples of research themes, that are in study are:

• Retro-prospective structural and functional imaging research for the early diagnosis of the cerebro-medular degenerative modifications and stroke
• Research concerning the synthesis and use, in vitro and in vivo, of new radiotracers for the SPECT and PET-CT /IRM techniques
• Studies in combined structural and functional imaging techniques, in vitro and in vivo, for the diagnosis and evolution following of certain neoplasia.

The setting up of the IMAGO-MOL Cluster was one of the main results of the project Advanced Medical Imaging on the project, interdisciplinary and integrated by creating a network of Regional Clusters and Development Strategies in Europe "(AMI-4Europe) financed by the European Commission through the Seventh Framework Programme–Regions of Knowledge. Project Consortium was made up of the coordinator – Madrid Network Association (Spain) and 21 partners from 6 countries (Spain, Germany, Italy, Hungary, Romania and Bosnia Herzegovina), being the first medical cluster from Romania and the only medical imaging cluster from EU.

The project was implemented during 2010 – 2013, having a total budget of 2.649.565 Euro.

The objective of AMI-4Europe was to define and develop the concept of “next generation” Medical Imaging integrating disciplines such as nanomedicine, pharmacological breakthrough and biotechnologies for healthcare and ICT.

The main activities of the project were the design of medical imaging sector value chain analysis in Europe, socioeconomic and SWOT analysis in the field of Advanced Medical Imaging ; developing a joint action plan in the field of Advanced Medical Imaging at European level; implementation of the first European Medical Imaging platforms in order to create a common framework to promote policies, technologies and research results in the aimed sector, the establishment of two clusters in medical imaging - IMAGO – MOL Cluster Romania and Bosnia Herzegovina RTD Cluster .

The main events in the North East Region organised within the framework of AMI 4 Europe project were the following:

• International Launching Conference of the IMAGO-MOL Cluster, 29-30 October 2012
• National Symposium IMAGO-MOL - Biomedical Imaging in North-East: Past, Present and Future, September 26, 2013

The cluster is the first one in this medical domain in Romania and it carries out its activity in compliance with the legislation in force and the provisions of the Government Ordinance no. 26/2000 concerning the establishment and operation of associations and foundations.

Key data sheet:

➢ 25 September 2012 – setting up the IMAGO-MOL Cluster with social headquartes in University of Medicine and Pharmacie "GR. T. Popa" Iasi
➢ International Launching Conference of IMAGO-MOL Cluster, 29 - 31 October 2012
➢ National Symposium IMAGO-MOL - Biomedical Imaging in North-East: Past, Present and Future, 26th September 2013
➢ Designing the first project of IMAGO-MOL Cluster and applying for financing within Competitiveness Operational Program 2007-2013, Operation Support for integrating enterprises into supply chains and clusters in October 2013 and declared winner in Jan 2014
➢ Nominated as the most innovative cluster accordingly to CLUSTERO Romanian Clusters Competitiveness Analysis, 2013
➢ Launching Conference of IMAGO-MOL Cluster Competitiveness Operational Program project, 11th June 2014
Cluster IMAGO-MOL, partner organization of the International Summer School in Medical Imaging, 4th August 2014, Iasi
Member of CLUSTERO, Romanian Association of Clusters, 2014
Public Prize for Innovation at Gala Medica 2014 organized by the Romanian Association of Physicians, 2014
Horizon 2020 Projects Generating Seminar, November 21, 2014, Piatra-Neamt
Horizon 2020 Projects Generating Seminar, March 3, 2015, Iasi
Cluster Management Course, 4-7th March 2015, Piatra-Neamt
Technologic audits of SMEs members of IMAGO-MOL Cluster, 30 March -3 April 2015
Participation at ITC Zone Fair, Utrecht Netherlands, 18 to 20 March 2015
Promoting the IMAGO-MOL Cluster at the Romania - Belgium Economic Mission, 6-8th May 2015, Brussels

More specifically, AMI-4EUROPE has supported the following actions:
- Set up and bring into operation the AMI-TP Platform
- Create and maintain the AMI Central DataWarehouse
- Create and maintain the AMI BackOffice
- Link with the rest of Projects funded under this FP7-2010-REGIONS-1 call in support of boosting of the health-related economy.
- Establish contacts and information exchange mechanisms with projects, initiatives, European, National and Regional stakeholders and networks aiming at promoting the advanced R&amp;D and innovation activities in the Advanced Medical Imaging sector and, by extension, on the Health-related economy.
- Raise the awareness of the target groups concerning the activities of the AMI-4EUROPE project not only on the initial phase covering the analysis and integration of research agendas of actors in Regional research-driven Clusters and JAP definition but also on the following Mentoring, International Co-operation and definition of measures towards the implementation of the JAP and their sustainability (European AMI Cluster and Lobby).
- Disseminate the good practices and lessons learned and the Advisory Council on AMI recommendations and action plans towards National and European decision and policy makers dealing with AMI and Health-related economy relevant stakeholders.
- Promote synergies among the communication and dissemination actions and to facilitate the dialogue between the future partners of different organizations.

The whole project intended since the beginning to be a source of high value activities to ensure future impact. This is why the JAP (Joint Action Plan) has been completed with real and concrete actions, with a measurable impact in society and Medical Imaging industry. Furthermore, implementation of the AMI-4EUROPE JAP took place within the Multiannual Financial Framework 2014-2020 where the Key Challenge is to stabilize the financial and economic system while taking measures to create economic opportunities. Thus, an additional effort is to be made to assure mid and long-term sustainability and synergy searching to allow for real and complete JAP implementation.

The JAP has been developed and structured combining both, scientific as well as non-scientific issues while having in mind a European-wide dimension to reach and achieve Key
Strategic Objectives, which are organized along the stated priorities within the Horizon 2020 approach. This structure and mission, ensures the impact in European Market and Policies. To create the appropriate environment to ensure results, the key issues facing the development of Advanced Medical Imaging across Europe as well as their related strategies (considered in detail in D3.5 and D4.6) are summarized in two SWOT charts, which are the ground basis to develop the AMI-4EUROPE Joint Action Plan. See AMI-4EUROPE deliverables for detailed information.

Fostering trans-national co-operation between research-driven clusters, improving links between regional authorities, RTD actors and local business communities at a European level for an integrated and cross-disciplinary approach for advanced Medical Imaging will boost regional economic competitiveness in the health-related economies thus creating more jobs, growth and social welfare.

AMI-4EUROPE has set up the ground base to mobilize European health-related economy stakeholders to the creation of an environment in which enterprises can start, grow and thrive, thus supporting the competitiveness and sustainable development in the advanced Medical Imaging market that Europe requires.

Patents, new medical devices, start-ups and spin-offs generating new employment will appear quicker and sounder as a result of this trans-national and integrated approach. The creation of an “AMI infrastructure” to support all regional and national efforts, stakeholders and interested parties to a quick and full development and implementation of AMI will guarantee the maximum socio-economic impact.

AMI-4EUROPE has created and established the appropriate mechanisms and elements to derive policy recommendations to remove barriers and to foster the development of a wider and more solid base of regions active in advanced research and innovation activities in the field of advanced medical imaging.

To close a full circle, the identified Key Performance Indicators (KPIs) can be directly traced back to the primary objectives of the Programme HORIZON 2020 as it is explained in D6.3, where an illustrative graphic chart has been included (including the Market Impact). The aimed impact of AMI-4EUROPE is in line with the objectives of HORIZON 2020.

The AMI-4EUROPE project has been driven by the aim to acquire a comprehensive insight into the design, implementation and impact of existing Medical Imaging and associated Health-related Technologies research and innovation support programmes and initiatives in Europe. The scope of this objective is to create the Common Co-ordination and Knowledge Base necessary to enter the identified market niches and then establish appropriate scientific advice and sustainable support mechanisms to assist European Commission (EC), national and regional policy makers to better define a Strategy and show the way for future research actions in favour of AMI, Regions of Knowledge and the European Research Area (ERA), to maximise their socio-economic impact in support of the health-related economy.
Between 2014-2015 I was scientific coordinator of the IMAGO-MOL Cluster during its first grand (to be seen). The results are being evaluated and published in due course.

The Cluster IMAGO-Mol have implementing starting with 14th May 2013 a first important project, for the Cluster, named: *Institutional building and increasing the visibility of North-East Innovative Regional Cluster for Structural and Molecular Imaging - support framework for increasing the RDI capacity of the members and SMEs competitiveness in Romania*

<table>
<thead>
<tr>
<th>Type of the project:</th>
<th>Grant POSCCE, Cod SMIS 49820, 516,000 euro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institute where research was carried out:</td>
<td>IMAGO-MOL Cluster, at University of Medicine and Pharmacy “Gr.T.Popa”, Iasi, Romania</td>
</tr>
<tr>
<td>Chief Scientific Investigator:</td>
<td>Scientific coordinator (RDI) - Cipriana Stefanescu</td>
</tr>
<tr>
<td>Time period covered:</td>
<td>2014 – 2015 (18 months)</td>
</tr>
</tbody>
</table>

**Papers published in relation:**

- Some other results are in process.

Between 2014-2015 I was scientific coordinator of the IMAGO-MOL Cluster during its first grand (to be seen). The results are being evaluated and published in do course.

The Cluster IMAGO-Mol have implementing starting with 14th May 2013 a first important project, for the Cluster, named: *Institutional building and increasing the visibility of North-East Innovative Regional Cluster for Structural and Molecular Imaging - support framework for increasing the RDI capacity of the members and SMEs competitiveness in the area - IMAGO MOL*, financed within Competiveness Operational Program 2007-2013, Support for integrating enterprises into supply chains or clusters.

The project had a duration of 18 months, his total value being of approx 595,000 euro, out of which the grant represents 516,000 euro.

The overall objective was to increase the competitiveness of medical imaging services providers through intelligent specilization in the biomedical sector.

Research activities aim to achieve research, design and implementation of a unique system of management of medical data (USMED). This system should improve the medical diagnostic and treatment process, increase quality of medical care, improve the dialogue between patient and doctor, decrease the duration and costs of hospitalization and improve the patients quality of life. Other aims of this project were:

- Creating an unique system to store medical data for all the patients enlisted in the health care institution within the cluster.
• Creating an unique library containing all the medical imaging tests of the patients. This library will include automatic connection facilities for different medical imaging devices and facilitate the imaging process which will assist the physician in diagnosing the patient.
• Research and implementation of efficient protocols for transmission, archiving and data storage of medical imaging, compatible with the volume and image quality obtained with the latest generation of imaging devices.
• Development and implementation of research which will enhance the medical care by utilizing the unique system of data management. The research aims to implement modern, online, analysis tools of images and medical data, improving the dialogue between physicians or between physician and patient.
• Improving the imaging diagnostic process obtained thru combination of structural imaging and functional imaging methods, by automatic access by the physician to the complementary imaging medical data.
• Improving surgical treatment of tissue injuries imagistically identified thru structural and functional methods, by automatic access by the surgeon to the previous acquisitioned images, even in the operating room.

It was a complex theme that involved close collaboration between the cluster members from different areas of expertise: physician of different specializations, IT engineers, specialists in signal processing, specialist in gathering and reading medical imaging data.
The system will be developed and implemented to all the partners with medical profile or related to the cluster, following the expansion of the database to include the major medical institutions from the North East Region of Romania. Therefore, the project’s target group is represented by all the patients whom benefit from medical services from this region.

Social-economical impact:
• Reduce hospital costs and patient management
• Diseases can be diagnosed and treated faster and more efficiently
• Reduce stress for both the patients and physicians
• Increase the quality of the diagnostic
• Enhance communication between physician-physician and physician - patient
• It is an unique data storage for all the cluster members, which allows reading the patients medical data anywhere, anytime, 24 hours a day
• Filtering, selection and data processing much faster and more efficiently
• Reduce risk of information loss

The specific objectives were:
• IMAGO-MOL Cluster institutional capacity development aiming to attract new members and to intensify the members cooperation in research, development and innovation activities;
• Increasing the level of training and specific skills of the cluster members in order to increase the innovation of the SMEs;
• Increasing the cluster visibility at national and European level through the implementation of promoting and branding activities;
• Enhancing national and international cooperation of the IMAGO-MOL Cluster and its members in the field of RDI.
• Direct beneficiaries were:
  • Medical imaging equipment users in the region
  • SMEs active in the medical field involved in the development and implementation of the USMED system
  • IT SMEs involved in the development of the USMED system
  • Students, PhD students and residents training in conducting medical imaging in hospitals affiliated to IMAGO-MOL Cluster
• Final beneficiaries were:
  • all patients receiving medical services in North East Region
  • In order to perform successfully the management, promotion, training and consultancy activities, it was founded an IMAGO MOL Cluster Regional Contact Point in the North East Regional Development Agency.

*Context and work hypothesis (Figure B.6.)*
Improving the medical process diagnostic-therapy by using an integrate management system of clinical – para clinical data.
Example: the systems role in enhancing the diagnostic-therapy medical process in thymic-thyroid pathology.
Thyroid pathology is more frequent in the Nord East region of Romania, compared to other regions, firstly because this region is lacking iodide in the drinking water (goiter region) and the close vicinity to the worst nuclear accident that ever existed, the Cernobil accident. In this context, the biggest Endocrinology Clinic in Moldavia from “Sf. Spiridon” Hospital has the highest patient addressability and a high patient turnover, most of them being admitted for one day only.
Diagnosing thyroid pathology includes laboratory tests and, most essentially, structural imaging (echography, sometimes CT, MRI) followed by functional imaging (scintigraphy – nuclear medicine), final diagnostic requiring the correlation of all the medical data, ideally during the same day. To interpret the scintigraphy it is absolutely necessary to know the result from the ultrasound, nevertheless there are situations in which the absents of the ultrasound image made it impossible to interpret correctly a scintigraphy. This problem delays the diagnostic and unnecessarily increases the period of hospitalization and treatment decisions. In addition, diagnostic accuracy increases if the images are compared directly and not only throw the comment wrote at the bottom of the image.
If surgical treatment is required, the patient can not be scheduled for surgery until the surgeon has all the results from all the investigation the patient was put throw and, sometimes, not before the surgeon discusses directly or in some cases even goes to the imagistic laboratory to view directly, on the computer screen, the operating region and/or the tumor formation which must be extirpated.
Advantages, impact of project

An integrated management system of all the patients medical data would allow the physician to access quickly and securely the patient’s primary information and clinical – paraclinical analysis. This would result in obtaining the laboratory and imaging diagnostic in the shortest possible time and therefore reducing the period of hospitalization. In case of surgical treatment it would result in a more efficient procedure. For the surgeon, access to view the structural and functional images acquired previously carried out by the patient, even in the operating room, would be of real help in establishing the operation protocol, making surgical intervention easier, safer, more efficient. By default this should reduce hospitalization, medical care costs and temporary disability and would improve patient prognosis and quality of life.

Figure B.6. Working hypothesis: diagram of an integrated management system of clinical-para clinic data in a thyroid – thymic pathology case; 2-3 = communication terminals; 1 and 9 = primary users; green = expected results

Benefits and impact in terms of fundamental and applied research:
The system will enable fundamental and applied research on several directions, throw collaboration between physicians and IT specialists, such as (general topics):
- correlation studies of acquisition parameters and image processing with tissue and/or serum markers with diagnostic specificity
variability studies of image parameters depending on the individual parameters of the patient
• correspondence and complementary studies on structural and functional imaging
• retro-prospective studies on the assessment of hospitalization period in relation to the usage of the system compared with previous period
• studies on technical operatory efficiency in relation with the usage of the system.

I. First part of the project: Integrated management system of medical data - USMED

Aims and implementation:
Research and development component in the POS CCE project:
Institutional strengthening and North-East Molecular and Structural Imaging Innovative Regional Cluster visible growth represents the support frame for increasing growth capacity of Innovative Development Research members and the competitiveness field of IMM from Romania – IMAGO MOL

USMED project aimed to build an interactive electronic platform which will be used as a storage instrument and medical data analyzer especially in medical imaging originated from various sources. Platform functionality includes data research, systematization, lab work, all in all improving the physician’s activity during the diagnostic and treatment process in different medical conditions.

Project USMED includes two principle components:
• An unique data base which collects all the medical imaging data of a certain patient from all the imagistic sources with which the patient came in contact with at some point
• The “Surgery Assist” application which involves visualizing and transmitting on a TV screen located in the operating room the medical images of the patient; the application accept voice command and can assist the surgeon during the surgery.

The USMED data base is a virtual data base that develops by indexing all the medical images coming from any kind of institutions, hospitals, offices geographically distributed and connected to the USMED system through the Internet. As a research project, the first version of USMED aims to realize a functional prototype capable of integrating the database from systems that meet the DICOM standards (PACS type systems: “Picture Archiving and Communication System”).

The physician computer runs a workstation type application throw which the physician can search and download the medical image files for a certain patient. This files are brought in from the local PACS data base but at the same time they are searched and downloaded and files found in the data bases of all the institutes that are part of the USMED.

All access to the patients medical data is done only after it’s explicit consent. Do this goal there was developed an application throw which the patient can consent for access to the medical data for a certain physician or medical institution. The application has two implementations: one of them is that it can run on the patients mobile phone and the other consists of an web application which can be accessed throw any browser connected to the internet.
General work scheme – System architecture
All the data is stored on a central server which can be accessed from all the geographical regions. The server will contain analysis methods, advanced diagnostic tools, communication tools and data management, like presented in Figure B.7.

![USMED system architecture](image)

Figure B.7. USMED system architecture

Protecting the patients medical data
Regarding the protection of patients personal data, the USMED system is based on the principles of the European Union: Privacy by Design and Privacy by Default. According to this principles the soul owner of the medical image data is the patient. Basically if you wan’t
the images from one PACS system which belongs to an organization to another PACS system belonging to another organization you will need the patients express consent. Early in the relationship with the patient (at the triage or at an appointment) someone with in the organization (hospital, doctor’s office) will request access to the patients medical data from the USMED system throw a web application or a mobile application. The patients consent is based on a notification system interface that has two buttons that allows or denies access for the doctor to the personal data. Once the system receives consent from a patient, it automatically starts copying the patients medical data (in background) from various external systems to the doctor’s PACS system (at the hospital). The USMED system does not modify the workflow of the doctors, authorized personal can still access the patients imagistic medical data throw the hospitals PACS, via CNP or patients name. The USMED system implies the existence of a PACS central hub for integrated access, at hospital level, to the imagistic medical data. In the absence of such a node, you can use PACS open source (DCM4CHEE) to demonstrate the capabilities of the USMED system. Workstation systems allow for local document DICOM transfers between different PACS nodes.

Management organizations, doctors and patients
For periods of development and pilot, not having access to official systems of patient management (like the database from insured patients etc.) organizations and patients management will be made throw an USMED administration portal. The screens below show the interface which allows adding and editing by new organizations and new USMED members. (doctors and patients).

A user may be given the role of a medical doctor in a medical institution, role which allows him to access the medical data from that institution and ask for patients consent to download the medical data from other institutions.

II. Second part of the project: Surgery Assist System
“Surgery Assist” is an application which assists the doctor by providing the images and useful details during surgery.
Functionalities:
● Displaying the medical images downloaded from the data base on a screen in the operating room
● Usage of voice command to manage displayed images
● Complex features of analysis and image segmentation

USMED and Surgery Assist systems implementation in “Sf. Spiridon” Hospital
There are several medical imagistic sources in “Sf. Spiridon” Hospital distributed to various laboratories and clinics, which are not interconnected, each laboratory being equipped with it’s own means of data storage and medical data management (local PACS). The USMED project aims to interconnect all the local PACS by creating a central PACS where the physicians can upload all the imagistic medical data from various local bases. The
central PACS of the hospital can be visible to other USMED system members whom then can retrieve the data only by previous consent from the patient.

In order to implement the USMED system within the hospital it is necessary to follow these steps:

- Obtaining an agreement from the companies that maintain the local PACS system to transfer the data to the hospital's central PACS. This way, doctors who work with medical imagistic files can access the data from the local PACS through a Workstation type application which can then transfer the data to a central PACS. At the moment, the doctors have limited access possibilities to the electronic images, the only possibility being to print and then scan the image to obtain an electronic image.

- Implementing a PACS central system: requires a server type computer on which all the data from all the medical imaging laboratories from the hospital can be stored. This central PACS will represent the interface between the hospital and the other institutions that are part of the USMED system.

- Installation of the “Surgery Assist” application in the operating room: installing the TV receiver in a point of maximum visibility, connecting the doctor’s laptop to it, downloading and visualizing the medical images on the TV. Purchasing a microphone and using it for giving voice commands to the application. By using the “Surgery Assist” application, the time allotted for the surgery can decrease and at the same time surgery results may improve.

**Objectives of USMED and Surgery Assist research theme:**

1. **Create a database of unique medical data for all medical units part of the system**

   A unique database is created by integrating all PACS (Picture Archiving and Communication Systems) available from all hospitals. Medical data is stored in local PACS and the moment a patient comes to a medical unit that is integrated into the system, a request is made into the system to collect all data specific to that patient. All PACS containing data of that patient will send these data to the local PACS where the current doctor can analyze them in order to establish the correct diagnostic.

2. **Implementing data safety mechanisms**

   Medical data belong to the patient and accessing these data by various physicians in the system requires prior patient acceptance. A mobile responsive web platform will allow patients to give their consent so that medical personnel can access their personal medical data for diagnosis purpose.

   Data safety also means implementing a mechanism to prevent confidential data theft by unauthorized persons, or even to prevent information leaks by persons who have access rights in the system. For this purpose, the medical data access authorization requests mechanism is distributed on multiple “key storage” type servers maintained by different administrators. Any data theft attempt needs simultaneous acceptance from multiple administrators, which reduces considerably the risk that such a situation occurs.

   Medical data is also accessed by physicians who work in scientific research. For this purpose the system allows data anonymization. Anonymized medical data will be backed up
on a distinct server where they can be safely accessed by the medical personnel logged into
the system.
Anonymization implies separating medical images from their owner by introducing generic
values in the demographic data fields of the patient:

3. Displaying medical imaging data in the operating room and surgeon assistance
during the surgery act (SurgeryAssist)
The SurgeryAssist application connects to USMED, downloads requested images and
displays them on a screen in the operating room. This application includes the main
functionalities of a DICOM viewer (zoom, annotation, rotate, split, etc.) most of them
through voice command. In this way the surgeon can voice control the image display mode
without pausing the surgery act.

Advantages and impact of USMED are:
An integrated patient data management system allows the physician safe and quick access to
primary patient medical data, collected from all medical institutions in the system. This
would result in a precise imagistic and laboratory diagnostic in the shortest time, reducing to
a minimum hospitalization time.
For surgical treatment it would become more efficient. For the surgeon, having access to all
medical imagery functional and structural priory performed on the patient, it would mean a
decisive help in establishing surgery protocol, making surgical intervention easier, safer, and
overall better. By reducing hospitalization time, costs with medical care are reduced and
disease prognosis is improved, reducing the patient’s temporary work incapacitation.

Final remarks
I am not afraid to state that the work techniques acquired during the training courses in
France in my doctoral period certainly continue to be up-to-date, constituting the starting
point and the “matrix” with which one can study and introduce in the medical practice new
radiotracers, especially useful for the functional imaging, for the early, targeted diagnosis,
therapeutic choice and the study of different diseases in evolution, this being one of the two
essential directions for the development of nuclear medicine, therefore, in equal measure
something to keep the wheel turning for future research in nuclear oncology, nuclear
cardiology – to name just a few of the most important fields of application of nuclear
medicine in which we had and we intend to develop in vitro and in vivo research areas.
Chapter B.2. Professional achievements

After completing the specialization of Endocrinology, I continued with the specialization of Nuclear Medicine (2nd specialization) which included (equaled) the year from 1994 to 1995 accomplished in Nuclear Medicine Service in France (Creteil), as a resident physician of hospitals in Paris by the Grant of College of Medicine in Paris, under the guidance of Professor Meignan and (regreted) Professor Maublant, period that marked, decisive and definitive, my entire postdoctoral professional development. After I obtained the nuclear medicine specialist degree, I began working with integration in the Laboratory of Nuclear Medicine of the Hospital of Saint Spiridon Iasi and, after another four years, the title of full nuclear medicine physician, continuing to work with integration in the same laboratory; from 2009 I took the leading of the laboratory, currently named after the former head, "Laboratory of Nuclear medicine Prof. Dr. Valeriu Rusu". During this period my medical work has developed from the point of view of the understanding the images through the biophysical mechanism of radiopharmaceutical cellular uptake and not simply "reading" the images, without limited understanding the cellular and molecular level, in relation to disease mechanisms. This concept, taken by the French school model, it is possible particularly due to the relationship between biophysics and nuclear medicine in our medical school as well as in the French medical school. This approach enables the development of applications using radiopharmaceuticals, meaning personalized medicine, with clear benefits for the patient. (e.g. isonitriles imaging, exemplified in the first part).

The teaching and medical practice combines for residents training in the specialty as coordinator of the nuclear medicine resident teaching in the University aria. The results obtained in time led to the placement of the laboratory and the center of our university (being, until this year, the only one laboratory of nuclear medicine from NE of the country) in the first three training centers for residents, taking, usually, about 40% of the nuclear medicine residents (another 40% being trained in Cluj, and 20% in Bucharest). In 2015-2016 the number of residents who chose to prepare in Iasi increased, reaching a rate of 70%, with residents to the second specialty and a resident in Nuclear Medicine specialization graduate of the Faculty of Medicine and Pharmacy Iasi in the French seria.

This was possible related to the fact that our laboratory has addressability from the entire northeast region of the country. In these circumstances, seeking maximum efficiency in the use of radioisotopes needed in nuclear imaging, laboratory work was extended in weekend days, to the maximum number of patients and appointments for some months before.

As a crowning of the the work done in the field of nuclear medicine, both in research and clinic, can be mentioned the functions:

- Vice-president of SRMN-IM, from 2014 and today
- President of the Scientific Council of the Cluster IMAGO-MOL, from 2012 and today
- Nuclear Medicine Laboratory Head, from 2009 and today
- Nuclear Medicine Residency coordinator, from 2009 and today
- Nuclear medicine expert for UAIC Iasi.
A number of publications in relation to the radioisotope imaging and applications in different medical fields – results of good collaboration between nuclear medicine and clinical domains, together with a number of published or communicated papers (see the list of papers) are an expression of my professional evolution in this post doctoral period, examples being:


Working in the nuclear medicine laboratory, in vivo techniques, interfere in a positive stimulative way both with students teaching activities, which increased with the introduction of Nuclear medicine as a subject separately in IVth year Medicine, and resident teaching activities. Areas of nuclear medicine research are related to the topics exposed previously.

Chapter B.3. Academic achievements

I believe that the teaching task is extremely important and can combine happily with the research part. As a result, I have worked and continue to work in grants under my coordination with undergraduate and graduate students.

Since the beginning of my teaching, I started from the premises that Biophysics is, on the one hand, one of the few subjects in medical education in close relationship with the fundamental sciences but, on the other hand, a borderline discipline related to fundamental research but with certain applications for medical practice. Into this concept, that we try to develop (with medical proofs) for students, stand my whole teaching and research activity. Immediately after obtaining the PhD title, in the same year, I was promoted to the post of Lecturer in the same discipline, Biophysics and Medical Physics, and later the post of Associate Professor (2002 ..) and than Professor PhD (2008 ). Since 2009, after the retirement of former head of discipline, Prof. Valeriu Rusu, I assumed the position of coordinator of the teaching activity discipline. I coordinated and supported, during this period and currently, lectures and practical works on:
- **Biophysics and Medical Physics**, series Romanian, English, French - for students of the Medicine Faculty and AMG and Nutrition specializations
- **Research methodology** - series Romanian, English, French, Faculty of Medicine
- **Nuclear Medicine** - series Romanian, English, French
- **Medical Physics** - associate professor at the Faculty of Physics, Iasi UAIC
- **Nuclear Medicine** - residents (residency coordinator for the University Center Iasi for this specialty)

- **Doctoral school** - module I coordinator and collaboration to module II
- Postgraduate Applications of Nuclear Medicine - coordinator; contributor to other postgraduate courses in related fields

- Pre and postcongres courses in biophysics, nuclear medicine and endocrinology. Starting with the academic year 2000 – 2001, Nuclear Medicine was introduced as a separate object of study in the fourth year at the Faculty of Medicine. I taught and teach the practice of Nuclear Medicine and works both series with teaching in Romanian as well as those taught in English and French.

My teaching methods were adapted to the evolution existing abroad, focusing increasingly more on formative education, together with the maintained classical methods, through exercises, interactive study themes, free exposure, ppt slides and video projection; evolution was always adapted in relation to the feedback requested at the end of each semester students but also with the students' exam results, they respected each year Gaussian distribution. I planned to teach this preclinical field, **Medical Biophysics**, starting from examples from clinical pathology, valorising fully my first medical specialization, - **Endocrinology** - which allowed me to develop topics of biomembranes biophysics, and the second one - nuclear medicine - which allowed me to develop topics on the physical principle of the technique particularly useful for understanding the medical imaging.

I worked and I work perfectly with the students, interfering the teaching with research, proof being the inclusion of students in a number of research grants that I have coordinated, the coordination of a large number of papers presented by students in some congresses (national and international), some being awarded with prizes; the collaboration continued sometimes in the years ahead faculty and was completed in many cases by the license paper (both at the faculty of Medicine and at the faculty of Physics, sometimes in a double coordination). Sometimes even some BDI published papers arised. The topics addressed in these works have resulted from my concerns on basic research of Biophysics, my work in the laboratory of Nuclear Medicine, or the relation between both of these. I have tried that each licence I coordinated gave rise to an Conference / Congress abstract or an extenso published paper. I strongly believe that students are a force that can lead to excellent collaboration in research.

The goals that I followed in working with students were:
- Motivating the students (which I consider the most important thing), by doubling the biophysical concepts to precise application in medicine area.
- Teaching the subject in the first year, an important thing is initiating a logically mode of thinking, the memorization being necessarily matched by a phenomenological logic.
- Shaping of a number of concepts and notions that, specific to biophysics, are absolutely necessary for understanding the body processes and understanding of disease pathology.
- Initiating the students to research activity through the formation of basic skills in order to approach experimental phenomena, as well as the skills of search and use bibliography.
- Consequently with the introduction of basic medical terms, students growing in rigor of expression and interpretation of results obtained in different measurements, providing precisely and accurately medical language.
- Not least seems very important structuring a moral character of the students, with the purpose of acquiring a maturity of thought to become doctors, intellectuals with integrity.

Work with the resident doctors

I participated, and participate in theoretical and practical training of resident doctors of Nuclear Medicine and resident doctors from other specialties during the internship training of Nuclear Medicine, both presenting bases physical techniques of nuclear medicine, as well as the medical applications of nuclear medicine, especially in vivo.

Participation in and, then, coordinating the publication of teaching material

I published in collaboration with department of biophysics colleagues and also with specialist colleagues in other disciplines, a series of teaching materials, among which:

A direct utility in the evolution of my teaching was the time carried out in France, in the laboratories of Biophysics from the Grange-Blanche Faculty of Medicine, Claude Bernard University, Lyon and Créteil Faculty of Medicine, University Paris XII. Although the extension biophysics in French medical education is considerably larger than in our country, it was useful for me knowing the general knowledge education organization, curricula and the participation in courses and practical works. These internships have meant contact with laboratories where Biophysics and Nuclear Medicine are merged, as in our university at present, assisting and attending hours of lecture and practical, libraries contact with an impressive base of documentation and awareness of its role complementary to the teaching process, work integrated learning mode: education - research - clinical. This experience gained during these internships I tried to use it in my further teaching activities. I practiced the idea of stimulating a certain independence in the activity of students in the laboratory in the sense of initiative, one of the main weaknesses of the students in our country, in my opinion. Also, I tried, for motivated students with appropriate intellectual resources, to increase the weight of formative learning versus the informative learning. Among the achievements at the academic level, I consider to be most important the next:

- CIDMEF Representative of the Faculty of Medicine
- ERASMUS representative of the Faculty of Medicine since 2011
- Faculty Member of the Board since 2011 and currently
- Member of the Curriculum Office since 2011, currently coordinator
- Member of the Professional Commission of the Faculty
- Coordinator of the Biophysics and Medical Physics - Nuclear medicine Department
- Member of the Board for license (every year) since 2008
- Coordinator of the Committee for Physics at University entrance examination (2016)
- Member of the committee of Congressis (Students and Young Physicians Congress)
- Member of the evaluation committee EUA 2012 and 2016
- Fulbright internships evaluator
- Member of the editorial staff of RMC journal since 1990 and currently
- Reviewer for the journal Acta Endocrinologica
- UMF Grigore T Popa internal grants evaluator
- Coordinator in international students exchange programs since 2014
- Invited speaker in different local, national and international scientifical manifestations
- Representative of the University and of IMAGO-MOL Cluster at various conferences, congresses, scientific meetings (Brussels etc.).
- Coordinator of CEMEX PET-MRI laboratory
- Vice president of the Society of SRBPA (until 2014)
- Vice president of the Society of SRMN-IM, from 2014
- Chairman of the Scientific Council of IMAGO-MOL

Finally, I can say that in all didactic and scientific activities I tried to have a moral integrity behavior, to be a positive example for students, both in scientific and human.
Part C.
Directions of development and projects for the following years, scientifically, professionally and academically

Next pages present my future plans regarding my professional, scientific and academic career growth, research opportunities/teaching methods/practical applications and ways in which they I think they could be implemented.

In the future, I would like to further develop ways to combine research with a formative academic and postgraduate education.

C.1. Scientific projects

From a scientifical point of view, together with my colleagues and collaborators, I hope to succeed in:

- Developing the PET-MRI laboratory from CEMEX, by applying for internal and international grants, and in collaboration with my colleagues and others laboratories of the center.
- Developing the IMAGO-MOL Cluster, especially by applying for internal and international grants.
- Developing a study platform for the radiopharmaceuticals, after the model used by INSERM Units in France, which would allow a standard study of new radiolabelled molecules, in collaboration with the members of the IMAGO-MOL cluster, and most of all with the University “Gr.T.Popa” and the Institute for molecular chemistry Petru Poni, with the goal of becoming a pilot study unit for the new radiolabelled molecules produced by various companies.
- Developing the studies on the mechanisms of cellular uptake of radiolabelled molecules, and in particular radiolabelled nanoderivatives, in order to develop new radiopharmaceuticals and pharmacological applications for disease therapy.
- Developing “Prof.dr.Valeriu Rusu” Nuclear Medicine Laboratory by introducing new nuclear medicine techniques and extending their applications. Also, extending the studies on the processing of scintigraphic images and the initiation of new strategies with the purpose of setting up a pilot study unit for the processing software used to analyze scintigraphic images for different medical equipment firms, in the field of nuclear medicine.
- Further developing internal (Petru Poni, USAMV, IRO) and external (study and research medical units in France, England, Belgium, Germany, Moldavia etc.) collaborations facilitating the application for internal and international grants.

All the ideas discussed above will allow the initiation of collaborations with students and residents, doctoral topics, in relation with very new research fields, competitive both internally and internationally.
Examples of specific subjects, focusing on certain pathologies, are presented in the table below:

**Table C.1. Examples of future research subjects**

<table>
<thead>
<tr>
<th>Subject title</th>
<th>Subject objectives</th>
<th>Collaborators</th>
</tr>
</thead>
</table>
| The *in vitro* and *in vivo* study of various biophysical characteristics of radiopharmaceuticals | 1. The study of $^{99m}$Tc isonitrils in different neoplasia  
2. The study of $^{99m}$Tc -labeled vector molecules  
3. The study of nano derivatives marked with $^{99m}$Tc | *Nuclear medicine, pharmacology, biochemistry, hematology, biophysics  
** Petru Poni Institute |
| The analytic study of scintigraphic planar images, SPECT and compared/fused with eco/CT/IRM | 1. The study of parameters used for processing scintigraphic images  
2. The study of physical parameters for fused images | * Nuclear medicine, radiology  
**Univ. Al.I.Cuza, Univ. Tehnica Gh. Asachi, Romsoft SRL |
| The utility of several $^{99m}$Tc radiolabelled molecules, used for the evaluation of different types of tumors | 1. The role of isonitrils in the image-based diagnosis of cancer  
2. The role of isonitrils in the image-based diagnoses and under therapy evaluation of tumors  
3. Radioisotopic investigations in neuroendocrine tumors  
4. The role of scintigraphic imaging in the study of thyroid nodules differential diagnosis | * Surgery, Thoracic Surgery, Endocrinology, Oncology, Neurology, Hematology, Gastroenterology, Dermatology, Radiology, Nuclear medicine |
| The utility of $^{99m}$Tc labeled isonitrils, for the noninvasive evaluation of P-glycoprotein and the multidrug resistant phenotype | 1. The evaluation of tumors before chemotherapy and hormonal therapy  
2. The evaluation of tumors before chemotherapy and hormonal therapy | * Nuclear medicine, Radiology, Endocrinology, Surgery, Thoracic surgery, Oncology, Hematology  
** Universitatea Tehnica Gh. Asachi, Romsoft SRL |
The study of various biological structures through physical and biophysical methods

FTIR (IR spectroscopy) in the structural and dynamic study of various molecules and bio membranes

*Internal medicine, Surgery, Biochemistry, Hematology, Biophysics

**Petru Poni Institute

The study of various normal/vs. pathological structures through physical and biophysical methods

The study of erythrocytes in autoimmune thyroiditis through atomic-force microscopy

Endocrinology, Surgery, Biophysics

**Universitatea Tehnica Gh. Asachi

Functional molecular imaging in neuro-endocrine tumors

Differential diagnosis of NET

Nuclear medicine, Radiology, Endocrinology, Oncology, Surgery

* Internal collaborators: from within UMF “Gr.T.Pop” Iasi
** External collaborators: from the outside of UMF “Gr.T.Pop” Iasi

The list of research subjects can increase progressively as the research furthers. The involved clinics and laboratories being part of the higher education context where, under the careful coordination of doctors and professors, students of local universities and also exchange students from other countries, PhD students, medical interns and Master’s degree students study. Therefore the research activities will have a highly formative impact with the end result being scientific papers to be published in prestigious journals, ISI, indexed in national and international databases, presentations and lectures at prestigious scientific national and international events, graduation and PhD theses. This will increase the visibility both of the University “Grigore T. Pop” and of IMAGO-MOL cluster.

C.1.1. Developing the PET-MRI laboratory from CEMEX, with the help of internal and international grants, and in collaboration with other laboratories of the center

Although biomedical research yielded exceptional results with some impact in medical practice, research regarding cancer remains a major problem. New perspectives are opened by studies of genomics and proteomics and molecular imaging with radioisotopes (PET - Positron Emission Tomography - and SPECT - Single Photon Emission Computer Tomography). Imaging with radioisotopes is practically a noninvasive method (Figure C.1) through which certain tumor processes can be monitored using radiolabeled molecules (radiopharmaceuticals) administered in very small quantities. By measuring the radioactivity released by the target tumor tissue, the uptake degree of the radiotracer at this level can be evaluated, so, it is possible to explore and quantify biological processes involved in radiotracer uptake at the cellular or even molecular level.
Positron or gamma emitting radiolabeled molecule
↓
Specific uptake in the tumor tissue,
in relation to certain malignant phenotypic characteristics
↓
Detection: gamma camera
positive scintigraphic images in relation to the malignant potential

Figure C.1. Principle of new radiolabeled molecules imaging studies

Related to the radiopharmaceutical use, research in nuclear oncology allows the study of cellular processes, molecules and pathways involved in the process of carcinogenesis, the process of invasion and metastasis, as well as those involved in acquiring resistance to treatment. Among the methods that can be used to study the malignant process, the use of radioisotopes can address different stages of the neoplastic development like presented in the image further. The use of radiopharmaceuticals with high affinity and specificity, allowing quantification of molecular processes in tumors, in vivo, in a reproducible way, repeatable in evolution, is undoubtedly the most important direction of the nuclear oncology development, with a decisive impact on diagnosis and cancer therapy. The transport of molecules, such as glucose and some amino acids is increased within neoplastic cells. Assessment of tumor glycolysis is definitely the real breakthrough in the field of nuclear medicine, through the PET technique with $^{18}$F FDG, a radiolabeled glucose analog that is taken up by cells with high glycolysis, thus allowing visualization of cancer cells, regardless of their location in the body. This technique is very useful both for initial diagnostic imaging as well as for the evaluation of the effectiveness of chemo and radiotherapy and studies demonstrated to be dependent on the cancer type and cell malignity. This is why new studies on this topic are welcomed, for other cancers, no yet elucidated.

Changing levels of cyclic nucleotides can influence a number of transport processes, perhaps through the phosphorylation of membrane proteins. Thus, the Na$^+$-K$^+$ pump shows an increased expression and increased activity in malignant cells. The membrane potential differences, mitochondrial and plasma, are increased by comparison with healthy cells of the same type. These changes in the transport processes are involved in modifying cellular accumulation of radiopharmaceuticals such as $^{201}$Tl (potassium analog that enters the cell by Na$^+$-K$^+$ pump) or $^{99m}$Tc isonitrils (monocationic lipophilic molecules, whose influx is dependent on membrane potential differences, increased in large tumor cells). These radiopharmaceuticals will accumulate in large quantities in cancer cells. Worldwide there are studies in molecular oncology imaging for assessing tumor parameters such as tumor proliferation, tumor hypoxia, tumor angiogenesis, tumor apoptosis, metabolism of amino acids, tumor glucose metabolism, gene expression, MDR character. With the development of PET, most of these studies have focused on the use of positron-emitting radiotracers, assessing metabolic pathways, and particularly intratumoral changes of glucose metabolism (about 90% of PET studies), but other metabolisms and cancer cell phenotypic
characteristics wait to be studied. Concentration in this research area is needed in order to detect the best molecular phenotypic characteristics to be radiolabeled and use in early noninvasive image diagnosis, all along the cancerisation and metastasis process (Figure C.2).

Some cancer cell phenotype characteristics (Figure C.3) tempted to be studied using radiolabeled molecules (Figure C.4), some others are not enough studied yet, such as:

- Tumor Hypoxia: the existence of tumor hypoxia can cause resistance to radiotherapy and certain forms of chemotherapy. Determining this parameter allows the selection of patients who may benefit from more aggressive therapies; but traditional methods (the best known is the transdermal electrodes measurement) are invasive and difficult in routine practice. Scintigraphy with $^{99m}$Tc-nitroimidazoles may allow noninvasive assessment of tumor hypoxia. These radiopharmaceuticals are selectively captured and retained in hypoxic tumor
tissues. In normal cells nitro group undergoes reduction to a radical anion. In this intermediate hypoxic cells is further reduced and retained intracellularly, resulting in positive scintigraphic images in pathological hypoxic tumors (6, 11, 16, 17, 44, 60).

**Figure C.3.** Cancer cell general phenotype modifications (Murray K., modified)

**Figure C.4.** Examples of cancer cell phenotype modifications that can be used for imaging
- Angiogenesis: is the process of formation of new vascular capillaries chaotically within the tumor, from pre-existing vascularization. Without angiogenesis, tumor growth would be limited to 1.2 mm, and the metastatic potential, also dependent on angiogenesis, could be reduced. Evaluation and monitoring of features that allow the use of tumor angiogenesis inhibitors would be useful in therapy. It seems that tumor size measurement for assessing response to therapy may be less useful than evaluating the tumor angiogenesis status. There are studies that demonstrate the possibility of evaluating tumor angiogenesis through a series of scintigraphic studies with SPECT and PET radiotracers with a high index of first-pass extraction, by measuring tumor perfusion. Such molecules can be $^{99m}$Tc MIBI or $^{99m}$Tc HMPAO (1, 7, 20, 24, 27).

- Tumor Apoptosis: programmed cell death is a factor conditioning treatment success of the tumor through chemo or radiotherapy. A whole enzyme cascade is participating in this process, a major pathway being caspase activation. In this process phosphatidylserine (PS) is outsourced. Annexin V, an endogenous human protein with a molecular weight of 36000, binds with high affinity to PS. Annexin V can be labeled with radioisotopes for PET and SPECT studies, including $^{99m}$Tc. A number of studies demonstrate that the accumulation of radiolabeled Annexin V correlates well with tumor apoptosis, and thus, enables the assessment of tumor apoptosis by noninvasive scintigraphic studies (8, 15, 16, 35, 65, 66).
More and more, the treatment of different diseases is established only after exhausting the diagnostic imaging possibilities, irreplaceable for the patient, even more so since they are easy to apply. The imaging techniques are complementary, the structural ones (ultrasound, CT, MRI) being completed by the functional ones, especially with radiolabelled molecules, molecular imaging (SPECT and PET), leading toward fusion imaging, SPECT-CT, PET-CT, PET-MRI. But the progress comes at different rates, with research groups in different parts of the world with similar objectives but who work separately. Identifying and facilitating the collaboration between such groups has become a necessity for the evolution of imaging in this nowadays new era in molecular radioisotopic imaging: finding the ideal key molecule to be radiolabeled (Figure C.5).

C.1.2. Developing of research relationship between the members of the IMAGO-MOL cluster and collaborating with the BioRONE cluster and other clusters, national and international

Numerous research subjects exist, retro or prospective, which involve all the partners within the IMAGO-MOL cluster, sometimes in the larger context of scientific partners, context in which the collaboration between the members of the cluster has been ongoing for a long time and has consolidated during this period, through finalized research, through publishing papers in ISI journals and international databases (41-46). A very good communication, essential condition, is realized through students (with the outcome of scientific papers that were presented in student congresses – national or international, oftentimes with awards – like mentioned previously, and/or license papers, which, sometimes constitute the starting point of PhDs), PhD students, resident doctors, together with specialist. Thus, this collaboration has a high formative character for young doctors and researchers. A very dynamic and high-performance field, internally as well as internationally, medical imaging owes its progress in large part to the tight relationship between the users (doctors and patients) and IT specialists; the final product has both medical purposes (improving diagnosis, treatment, outcome, increasing the quality of life of the patient) and reducing the time spent in the hospital including the financial benefit in relation with the promotion of medical and IT research results internally and internationally. In this context, the research subjects which the partners plan to take up are a natural evolution of the preexisting collaboration (the next table includes only a part of these, in reality, being significantly more numerous, basically starting from each of the partners of the cluster), consolidated through the development of the cluster through the system of communication that the members of the cluster intend to implement.

Examples of specific subjects, dedicated to certain pathologies, that can be developed by clusters collaboration are included in the next table; some of them are on-going.
**Table C.2. Future researches by IMAGO-MOL Cluster Collaborations**

<table>
<thead>
<tr>
<th>Subject title</th>
<th>Partners</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em> and <em>in vivo</em> studies of various new radiolabelled molecules, SPECT, PET, nanoparticles (1-4, 9, 12, 14, 33, 38, 39)</td>
<td>*Nuclear medicine, Pharmacology, Biochemistry, Haematology, Biophysics, *<em>Inst. Petru Poni</em></td>
</tr>
<tr>
<td>The analytic study of scintigraphic images, SPECT and fused images in comparison with structural images through the integrated system of clinical-paraclinical data management (7, 8, 15, 20)</td>
<td>* Nuclear medicine, Radiology *<em>Univ. Al.I.Cuza, Univ. Tehnica Gh. Asachi, Romsoft SRL</em></td>
</tr>
<tr>
<td>The usefulness of certain radiotracers for the evaluation of different tumor types – a study in relationship with the integrated system of management of clinical-paraclinical data system USMED (5, 13, 16, 29, 34, 37)</td>
<td>*Surgery, Thoracic surgery, Endocrinology, Oncology, Neurology, Hematology, Gastroenterology, Dermatology, Radiology, Nuclear Medicine</td>
</tr>
<tr>
<td>The use of radiolabelled molecules for the noninvasive evaluation of cancer phenotype characteristics in diagnosis and treatment - study through the integrated system of clinical-paraclinical data management (6, 11, 17, 19, 21, 30, 31, 32)</td>
<td>* Nuclear medicine, Radiology, Endocrinology, Surgery, Thoracic surgery, Oncology, Hematology ** Universitatea Tehnica Gh. Asachi, Romsoft SRL*</td>
</tr>
<tr>
<td>The study of various biological structures through physical and biophysical methods study through the integrated system of clinical-paraclinical data management (40-46)</td>
<td>*Internal medicine, Surgery, Biochemistry, Hematology, Biophysics *<em>Petru Poni institute</em></td>
</tr>
<tr>
<td>Creating an integrated management system of the clinical-paraclinical data in the case of brain tumor pathology (10, 18, 22, 28)</td>
<td>* Prof. Dr. N. Oblu Hospital, Radiology, Neurology, Neurosurgery, Nuclear medicine, Oncology.*</td>
</tr>
<tr>
<td>Implementing an integrated management system of the clinical-paraclinical data in the case of traumatic cranial and maxillo-facial emergencies (23-26)</td>
<td><em>Neurosurgery, OMF, ENT, Ophtalmology, Radiology, Neurology, Psychiatry, Legal medicine</em></td>
</tr>
<tr>
<td>The role of telemedicine in the optimization of the workflow in stroke centers in the NE region of Romania.</td>
<td><em>Radiology, Neurology, UPU, SMURD, Interventional radiology</em></td>
</tr>
<tr>
<td>The contribution of an integrated management system of neuro-endocrine-tumors (27).</td>
<td><em>Neonatology, Radiology, Nuclear medicine, Endocrinology, Biochemistry,</em></td>
</tr>
</tbody>
</table>
The development vision of the cluster essentially represents a general objective, the implementation of which surpasses the coverage of the implementation of the new installed USMED and Surgery Assist systems. This refers to the strategic relevance of the past projects for its larger framework, defining long term high-level objectives, towards which the accomplished projects can contribute. The general objective explains the reason for which these projects are important to the society, concerning the long-term benefits for the final beneficiaries and the important benefits for other groups.

The development must be realized mainly through the institutional consolidation and the increase of the visibility of the Medical Imaging Cluster, IMAGO-MOL, in such a way that it will ensure the increase of the research, development and innovation capacity of the cluster members, centered around the University, becoming a pilot unit for the collaboration between the academic environment and the high-tech services in Romania, with multiple ‘beneficiaries’ (Figure C.6.).

Future fields of activity of IMAGO-MOL Cluster will be:

- R & D activities in the field of medical imaging and related fields and in technology transfer, with formative impact in university and post-university economic impact, through the development of SMEs, and practical impact, in improving the diagnosis and treatment of diseases, the final beneficiary being the patient.
- Medical imaging, the cluster IMAGO-MOL core field of activity, including topics:
- In vivo imaging, structural (ultrasound, CT, MRI, Rx etc.) and functional (conventional scintigraphy and PET) and fused (PET-CT and PET-MRI), in clinical trials or experimental animal model, as well as transfer techniques, fusion and image processing and any other topics derived therefrom.
- In vitro imaging, advanced techniques, including all types of microscopy, optical, electron or derivative techniques (atomic force microscopy, tunneling microscopy, microanalysis, nuclear, etc.) and any other tissue analysis techniques in relationship with them.
- IT techniques, reception and processing of medical data, with the possibility of dedicated software development with role in prevention, epidemiological alert and tracking of the disease evolution in relation to treatment etc.
- All other issues in relation to any field of medical or related imaging that can bring a benefit in addressing the patient, for diagnostic or treatment, direct or indirect, with relations with biomedical research.

- Developing partnerships between clusters, at national and international level, to obtain grant funds needed to finance research and development activities and technology transfer in the field of medical imaging.
- Developing a platform for suppliers / actors in the field of molecular, structural and functional, medical imaging in the Northeast Region, which includes research and medical studies, fundamental and applied, which will address issues of physiological and pathological medical imaging methods.

Future RDI projects of IMAGO-MOL Cluster could be:

- Retro-prospective researches of functional and structural research for early diagnosis of cerebro-spinal degenerative changes and stroke
- Radiotracers uptake mechanisms studies: finding the best molecules to be traced for neoplasia evaluation
- Research on the synthesis and applicability, by in vitro and in vivo studies, of new radiotracers for PET and SPECT techniques and in combination with structural imaging
- Research on the synthesis, structural and functional characteristics, by in vitro and in vivo studies, of new nanoparticles radiotracers and applicability in diagnosis and treatment
- Combined structural and functional imaging studies, both in vitro and in vivo, on the diagnosis and evolution of certain malignancies
- Surveillance and epidemiological alert IT software for the sensitivity profile for bacterial infections, useful in preventing the multidrug development and for finally realization of a geographic "map" distribution of microbial antibiotic sensitivity level
- Involvement in imaging research of telemedicine and ITC sector in the context of the fact that IT softwares have applicability in improving patients healthcare and preventions of certain pathologies
- Developing and improving e-health services with participative approach of the patients (empowering patients in taking part in managing their health state).
- An application for tele-monitoring patients: using wireless sensors to feed online patient health parameters values. This would be a web application that the physician can connect to anytime and anywhere in order to assess the health state evolution of the patient.
- An integrated system to assist communication and tele-monitor severely incapacitated patients. The application implements a two-way communication system with patients who lost their speech ability following a neurological accident. Dialogue is built though technology methods using keywords or pictograms displayed in front of the patient, successively, in order for them to choose the most appropriate one, by using a mechanical control or by eye focalization.

C.1.3. Studies about radiolabeled nanoderivatives with the purpose of discovering new radiopharmaceuticals

Context: Starting from the phenotype changes of the malignant cell, the identification of some molecules in relation with the histological type and malignity which could be labeled with reasonably-priced isotopes could be useful, and they could be useful for the diagnosis imaging of that certain tumor. Nanoderivatives seems to be promising.

Example of research topic:
Structural and functional studies, *in vitro* and *in vivo*, of radiolabelled nanoparticle complexes used as new radiotracers for diagnosis and therapy in nuclear medicine.

The aim of such projects could be to develop new radiopharmaceuticals that can be used for *in vivo* diagnostic imaging and therapy. By means of silica nanoparticles, it is intended to bind vector molecules to radioisotopes. The role of this complex is to direct the radioisotope to the targeted tissue or organ. Steps are presented in *Table C.3*.

Due to the missing accessibles radiotracers to make evidence of estrogen receptors (that could be an usefull phenotype characteristics for cancer imaging, the first radiotracer studied to be developed will be the estrogen-SNPs-\(^{99m}Tc\) complex.

The working team that I collaborate with has an appropriate research experience in the domain (as demonstrated in the first part of this thesis) and is recognized in biophysics and nuclear medicine in Europe. They have been working together for several years, with good results that took the form of papers published in journals included in medical databases or ISI, annual participations at the European Congress of Nuclear Medicine, license thesis finished in the field, the grants won and finished. The obtained results are good so we feel we should further the research in this area.

Medical Imaging has evolved greatly in the last years, and continues to develop, improving diagnosis and therapeutic choice and contributing to the development of personalized medicine. Equally, the same way that it is considered that we live in the era of molecular and nanomedicine, one may say that we are moving towards an era of the nanomolecular diagnosis imaging.
<table>
<thead>
<tr>
<th>Objectives</th>
<th>Activities</th>
<th>Infrastructure</th>
<th>Place</th>
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<tbody>
<tr>
<td>1. Synthesis of silica nanoparticles</td>
<td>1.1. Identification of optimal conditions for obtaining silica nanoparticles with a narrow size distribution</td>
<td>Water Purification System, analytical balance, magnetic stirrer, centrifuge, chemical hood, freeze dryer, calcination oven</td>
<td>Centre of advanced research in bionanoconjugates and biopolymers, Petru Poni, Iasi</td>
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<td></td>
<td>1.2. Optimizing the size of nanoparticles</td>
<td></td>
<td></td>
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<td></td>
<td>1.3. Surface functionalization of silica nanoparticles</td>
<td>Zetasizer, FTIR</td>
<td></td>
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<tr>
<td>2. Characterization of nanoparticles properties</td>
<td>2.1. Size distribution studies of nanoparticles</td>
<td>AFM, SEM, TEM</td>
<td>Centre of advanced research in bionanoconjugates and biopolymers, Petru Poni, Iasi</td>
</tr>
<tr>
<td></td>
<td>2.2. Nano and microscale phase characterization of lyophilized solutions</td>
<td>XRD</td>
<td></td>
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<tr>
<td></td>
<td>2.3. Structural characterization</td>
<td>AFM-Raman, FTIR, UV-VIS</td>
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<td></td>
<td>2.4. Studies on the silica nanoparticles stability in physiological solutions</td>
<td>DLS</td>
<td></td>
</tr>
<tr>
<td>3. Silica nanoparticles coupling with vector molecules (estrogens)</td>
<td>3.1. Studies regarding the efficiency of the coupling process</td>
<td>Zetasizer, FTIR, XPS</td>
<td>Centre of advanced research in bionanoconjugates and biopolymers, Petru Poni, Iasi</td>
</tr>
<tr>
<td></td>
<td>3.2. Stability study for the estrogen-SNPs complex</td>
<td>DLS</td>
<td></td>
</tr>
<tr>
<td>4. 99mTc radio- labelling of the estrogen- SNPs complex</td>
<td>4.1. Radiolabelling of the complexes by using Na99mTcO4</td>
<td>99Mo-99mTc generator, dose calibrator, digital dosimeters with alarm threshold and direct reading</td>
<td>Laboratory of Nuclear Medicine and Radioisotopes, UMF, Iasi</td>
</tr>
<tr>
<td></td>
<td>4.2. Testing the 99mTc radiolabelling efficacy of the new radiotracer</td>
<td>Chromatography connected to a gamma detector</td>
<td></td>
</tr>
<tr>
<td>5. Biodistribution studies of the radiotracer</td>
<td>5.1. In vitro biodistribution study on tissue samples</td>
<td>fluorescence microscope</td>
<td>Laboratory of Nuclear Medicine and Radioisotopes, UMF, Iasi</td>
</tr>
<tr>
<td></td>
<td>5.2. In vivo biodistribution study on animal model by scintigraphy</td>
<td>gamma cameras with dual heads</td>
<td></td>
</tr>
</tbody>
</table>
C.2. Professional projects

The fact that I teach a number of different lectures for both graduate and undergraduate students - first year (Biophysics and medical physics), third year (Scientific research methodology), fourth year (Nuclear medicine), and several others for residents and PhD students, and that I am usually part of the board for the License oral presentation - gives me the opportunity to continue observe the students throughout their studies, and by doing so, to directly participate in their all-over professional evolution. All along the time, I come to the conclusion that involving students in research activities is beneficial for both the student, in the process of professional growth, and the university that receives new and fresh ideas, filled with the energy and enthusiasm of youth.

At teaching level, my plan is to develop, in the Biophysics and medical physics laboratory, together with the members of my team, a section for computer simulations with the purpose of teaching the biophysical processes in the human body. I want, also, to develop the part dedicated for the understanding of physical principles and methods used for the laboratory studies in the biophysics curricula.

I hope to encourage the study of the medical applications for radioisotopes.

I want to further extend the study of the uptake mechanisms of radiopharmaceuticals for students - during their Nuclear medicine modul - as well as for residents. It is my opinion that processing the scintigraphic images, after a clear understanding of the radiopharmaceutical uptake mechanisms, provides the opportunity for a more complex interpretation, that goes beyond the “simple evaluation” by playing a role in the clinical shaping of the future doctor, and in particular, of the nuclear medicine specialist.

We are also in the process of drafting the documents needed to support the introduction of a new Master’s degree study opportunity, consisting of two years and 120 credits, titled: The physical and biophysical basics of imaging, with medical applications. This master’s degree is meant to reduce the deficit of radiology technicians, medical physicists for the field of imagine processing, diagnostics and therapeutics (radiotherapy), dosimetry specialists and technologists, that is currently affecting the medical field in Iasi.

I want to promote formative teaching as much as possible, together with informative teaching. Without a doubt, the medical student has to accumulate as much knowledge as possible (in order to develop one of the two essential skills in the field, the memory), but this information must be used in a wise way, processed and bound by logical connections, and should offer the foundation for a diagnostician who will always think: “there are no illnesses, only ill people” which is, in fact, the equivalent of nowadays Personalized medicine. Therefore, the student must also develop the other skill that any good doctor and researcher should have, logic. The formative way of teaching is very useful in accomplishing this goal. Different ways of teaching, such as gamification and modular study, revolving around the medical application, could maintain or even amplify the working enthusiasm and efficiency of the medical student.
C.3. Academic projects

In my opinion, research development represents the engine for all developments at professional and academic level. A good research subject, material possibilities and a good research team are the essential conditions for a research with good results, citations and recognition both of the individual and of the institution, in our case, of the University. Nowadays, good research topic means both a ‘hot’ subject for diagnosis and/or treatment, useful for an important number of patients but also a research idea with economic impact, through the prism of medical clusters and medical startups, developed all around the world today, so, which need to continue to develop in our region, and I’ll militate for this.

Questions about the real world: medical research topics

Scientific curiosity, patience and perseverance!

Figure C.7. Relation between scientific, professional and academic accomplishments

I will continue the collaboration with the Faculty and University structures. All the results of the previously mentioned scientific activities, in the context of the work groups within the university, will undoubtedly increase the visibility of the university.

Final remarks

Scientific recognition means also professional development and academic recognition. But, for me, the most important recognition comes from the students, residents and PhD students, developing their motivation and enthusiasm for research, which means that they will continue what we start know, so education for research, developing to the young’s scientific curiosity, patience and perseverance for research, that means a promising future for our University and over the boundaries of the University.
REFERENCES

References for Chapters I-II


33. Stefanescu C., Research concerning the cellular malignity potential through in vitro and in vivo radioizotopic methods, PhD Thes., 1996.


References for Chapter III


7. Scagliori E, Evangelista L, Panunzio A, Calabrese F, Nannini N, Polverosi R, Pomerri F. Conflicting or complementary role of computed tomography (CT) and positron emission tomography (PET)/CT in the assessment of thymic cancer and thymoma: our experience and literature review. Thorac Cancer 2015; 6(4): 433-42.


15. Stefanescu C, Rusu V. From the physics and biophysics of radiopharmaceuticals to functional and molecular imaging, Tehnopress Ed., 2008 Iasi.


References for Chapter IV


References for Chapter V


References for Part C


40. Cererea de finantare aprobata de Autoritatea de management POSCCE / Contract de finantare IMAGO-MOL Final.pdf (Documentele se gasesc pe serverul FTP al proiectului).


42. Drobota M, Griersou I, Radu I, Stefanescu C. Modification of protein conformation can be monitored by Fourier Transform Infrared (FTIR) spectroscopy in oncological patients. Rev Roum Chim, 2014; 59(6-7): 509-13. ISSN: 0035-3930. IF: 0, 311


