

**MEDICAL UNIVERSITY**  
**"PROF. DR. PARASKEV STOYANOV" – VARNA**



**DEPARMENT OF DIAGNOSTICS IMAGING AND RADIOTHERAPY**  
**MEDICAL FACULTY**

**DR. ALBENA DIMITROVA BOTUSHANOVA**

**NUCLEAR-MEDICAL METHODS IN DIAGNOSTIC PARATHYROID  
GLANDS IN PRIMARY AND SECONDARY HYPERPARATHYROIDISM**

**AUTOREFERAT**

In the dissertation work for acquiring the academic degree "Doctor"

**SCIENTIFIC SPECIALTY:**

03.01.28. Medical radiology

**SUPERVISOR**

Assoc. prof. Dr Borislav Chaushev PhD

Varna 2019

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## Varna 2019

The dissertation work was discussed at a meeting of the extended departmental council of the Department of Diagnostics Imaging and Radiotherapy at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna and planned for presentation before the Scientific Jury.

The dissertation work consists of 117 pages, 33 figures and 4 tables. Citations of 104 articles.

The presentation of the dissertation work is going to take place on .....  
from ..... hour

All the materials for the presentation are available in the library of MU- Varna,  
as well as on the official website of the universit

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## **List of used abbreviations**

PHPT – Primary hyperparathyroidism

SHPT – Secondary hyperparathyroidism

US – Ultrasound

MRI – Magnetic Resonance Imaging

SPECT – Single-photon emission computer tomography

PTH – Parathyroid hormone

PTG – Parathyroid gland

TG – Thyroid gland

CT – Computed tomography

RPH – Radiopharmaceutical

SESTAMIBI – Methoxyisobutilisonitrile

CP – Correct positive

FN – False negative

## I.INTRODUCTION

The primary hyperparathyroidism (PHPT) is due to excessive production of parathyroid hormone from one or more abnormal parathyroid glands and causes hypercalcemia. It was considered to be a rare disease in the past, but the incidence of PHPT has changed dramatically over the last thirty years with the introduction of calcium measurement in routine clinical practice, and is now considered to be approximately 42 per 100 000 persons. In most of the cases PHPT undergoes without any clinical signs and is discovered by chance. Women are affected more frequently than men, in a ratio of approximately 3:1. The frequency of PHPT rises with age and reaches 4 cases per 1 000 persons in women after their 60's. At the time of diagnosis, most patients with PHPT do not show classic symptoms of the disease, like osteitis fibrosa cystica, nephrocalcinosis or nephrolithiasis. Symptomatic PHPT is now exception rather than the rule, with more than three-fourths of patients having no symptoms making detected changes of the blood values of calcium, phosphorus and parathyroid hormone (PTH) to be the only reason for diagnosis (1,2). By far, the most common lesion found in patients with PHPT is the solitary parathyroid adenoma, occurring in 85%-90% of patients, while in the rest 10%-15% of patients, primary hyperplasia of the parathyroid glands is present (3). In the past the standard surgical approach for PHPT was the bilateral four-gland parathyroid exploration with the removal of each gland which macroscopically showed changes. While, in most of the patients with PHPT only one parathyroid gland is affected, the above mentioned surgical approach is inappropriate in all cases. Unilateral approach is appealing in cases in which only a single gland is involved (4). These procedures include a unilateral operation in which the gland on the same side that harbors the adenoma is ascertained to be normal. So nowadays, the currently most widely used surgical approach is the minimally invasive parathyroidectomy, which is associated with less postsurgical complications and shortens the time of operation. The objective is to minimize the length of incisions which diminishes postsurgical pain and shortens hospitalization. To be successful this procedure needs to rely on a precise preoperative localization of the abnormal parathyroid glands. That's why preoperative parathyroid imaging gained so large importance. The rationale for locating abnormal parathyroid tissue prior to surgery is that the glands can be notoriously unpredictable in their locations.

There is general consensus that the best imaging procedure identifying abnormal parathyroid glands is the preoperative scintigraphy with  $^{99m}\text{Tc}$  – sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin. It is characterized with high sensitivity and specificity exceeding those of ultrasound imaging, CT or MRI. Combining scintigraphy with the other imaging techniques increases the precision for topic localization. Nuclear medicine methods applying  $^{99m}\text{Tc}$  – sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin can not detect normal parathyroid glands , because of their small sizes.

Secondary hyperparathyroidism (SHPT) is characterized with increased secretion of PTH, usually much higher than in PHPT, as a result of other concomitant diseases which lead to profound and prolonged hypocalcemia. In SHPT more than one parathyroid gland is affected. Due to the underlying disease, the patients are in poor general condition, so the localization of the hyperplastic parathyroid glands, and especially their possible mediastinal localization, is very important for the therapeutic approach.

## **II. THE AIM AND OBJECTIVES**

### **I.THE AIM.**

The aim of the present study is to evaluate diagnostic value of the nuclear-medicine methods for the visualization of abnormal parathyroid glands in patients with PHPT and SHPT

### **II. THE OBJECTIVES:**

1. To evaluate the relationship between visualization of hyperfunctioning parathyroid glands with  $^{99m}\text{Tc}$ -sestamibi and the levels of parathyroid hormone, calcium and phosphate in patients with PHPT and SHPT.
2. To evaluate the relationship between visualization of hyperfunctioning parathyroid glands with  $^{99m}\text{Tc}$ -tetrofosmin and the levels of parathyroid hormone, calcium and phosphate in patients with PHPT and SHPT.
3. To evaluate the relationship between the volumes of the abnormal parathyroid glands determined by US, CT and MRI with scintigraphical findings in patients with PHPT and SHPT.
4. To determine the advantages and disadvantages of dual-phase single isotope scintigraphy with  $^{99m}\text{Tc}$ -sestamibi и  $^{99m}\text{Tc}$ -tetrofosmin in patients with PHPT and SHPT.
5. To determine the advantages and disadvantages of dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetate/ $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -pertechnetate/ $^{99m}\text{Tc}$ -tetrofosmin in patients with PHPT and SHPT.
6. To evaluate the diagnostic value of the combination of the two methods (single isotope dual-phase method and dual-isotope subtractional scintigraphy)
7. To evaluate the role of SPECT techniques (early and late) combined with single isotope dual-phase method and dual-isotope subtractional scintigraphy.
8. To create a diagnostic algorithm and protocol for nuclear medicine evaluation in patients with PHPT and SHPT.

## **II. MATERIALS AND METHODS**

### **1. Participating group of patients**

For the aim of the present study , between years 2004 and 2015, we investigated a group of patients with hyperparathyroidism (according to the widely accepted criteria for the diagnosis of the disease- high levels of PTH and total calcium and hypercalciuria). Two patients dropped out of the study due to their refusal of undergoing nuclear-medicine imaging. 94 patients (78 women and 16 men) fulfilled the inclusion criteria for the participation in the study and they were investigated accordingly to created by us protocols for nuclear medicine imaging in hyperparathyroidism. 78 patients of them were with PHPT (69 women and 9 men ) and 16(9 women and 7 men) were with SHPT (high PTH in patients with end stage kidney disease requiring chronodialysis). All the participating patients were referred from the departments of endocrinology and nephrology at UMHAT "St. George"-Plovdiv, as well as from ambulatory endocrinologists from the region of Plovdiv. All participants had signed "Informed consent for participation in the study" before undergoing predefined by our protocol scintigraphic examinations. Every participant fulfilled all the inclusion criteria and none of the exclusion ones.

### **2. Inclusion and exclusion criteria**

#### **2.1 Inclusion criteria.**

1. Men and women with diagnosed hyperparathyroidism.
2. Signed Informed consent for the participation in the study.

#### **2.2 Exclusion criteria.**

1. Current pregnancy or breast feeding.
2. Intake of any Iodine containing medications or thyroid hormones for the previous month, when subtraction technique with thyroid visualization was performed.

### **3. Characteristic of the involved patients.**

78 women between 31 and 78 years of age (mean age  $56.92 \pm 11.10$  years) and 16 men between 18 and 73 years of age (mean age  $46.00 \pm 14.26$  years) participated in the study

*Concomitant diseases of the thyroid gland.* 18 (23.07%) of women had concomitant thyroid disease. Nontoxic multinodular goiter was found in 16 (21.1%) and Hashimoto thyroiditis in 2 (2.6 %) women. In 3 (18,75%) men nontoxic multinodular goiter was also present. All patients were euthyroid at the day of the examination, documented with normal levels of TSH.

### **4. The place where the study was conducted**

The study was conducted in the department of Nuclear medicine at UMHAT "St. George" -Plovdiv

All the laboratory results were obtained from the Central Clinical Laboratory at UMHAT "ST. George" -Plovdiv. The laboratory results were subjected to a systemic and strict both intralaboratory and external control. The laboratory was certified by The National Board of Certification and by the International Labquality program and possessed the necessary certificates.

who agreed to participate in the study by signing informed consent. The study was approved by the local ethic committee and passed all the necessary steps to be eligible for conduction in the participating hospital.

Exclusion criteria were and.

## **METHODS**

### **1.Clinical methods**

**Case history:** We took a full case history of every participating patient about the duration of the disease, concomitant and thyroid diseases, family history. We paid a special attention about the use of any drugs that could possibly

interfere with the calcium-phosphate homeostasis. All collected data were put in a specially prepared, for the present study, clinical card for each patient .

All of the participating patients fulfill the necessary criteria for having hyperparathyroidism: high level of PTH (above the upper limit of reference range of the laboratory results), high total and ionized calcium, low phosphate in PHPT and high in SHPT. In all patients there were suspicious US data for the presence of an abnormal parathyroid gland.

## **2.Instrumental methods**

### **2.1 Devices**

All planned nuclear-medical examinations of the parathyroid glands between 2004 and 2007 were performed on a Diacam SPECT gamma camera, and between 2009 – 2015 on a dual headed SPECT gamma camera "SYMBIA E DUAL".



Dual headed SPECT gamma camera "SYMBIA E DUAL".

The quality control of Diacam SPECT gamma camera was performed by the producer of the device SIEMENS , according to a predefined procedure. по изготвена от няя програма. The quality control of the dual headed SPECT gamma camera "SYMBIA E DUAL" was performed also by the producer of the device SIEMENS, following a program for a periodic quality control developed by SIEMENS. The current program for a periodic quality control is developed according to the requirements of the Ordinance N30 from 31.10.2005 year concerning the way and order for protection of people from medical radiation

### **2.2. Preparation of the patient**

No preliminary preparation of the patients before performing single isotope dual-phase scintigraphy was necessary.

In subtractional method there were some preliminary conditions to be followed:

- any concomitant use of Levothyroxine or Iodine containing drugs should be stopped minimum 20 days before the examination
- patients needed to be familiarized with the procedure, and especially the need to stay still during the examination.

### **2.3. Radiopharmaceuticals**

We used the following radiopharmaceuticals:  **$^{99m}\text{Tc}$ -sestamibi**,  **$^{99m}\text{Tc}$ -tetrofosmin** and  **$^{99m}\text{Tc}$ -pertechnetate**

#### **$^{99m}\text{Tc}$ -sestamibi**

The limits for intravenously applied radiation in humans should be between 740-1110MBq (20-30mCi). In our study we used Technestan Sestamibi 1mg.

#### **$^{99m}\text{Tc}$ -tetrofosmin**

The limits for intravenously applied radiation in humans should be between 740-1110MBq (20-30mCi). In our study we used MYOVIEWTM for preparation of  $^{99m}\text{Tc}$ -tetrofosmin.

#### **$^{99m}\text{Tc}$ -pertechnetate**

$^{99m}\text{Tc}$ -pertechnetate has a half-life of 6 hours and possesses energy of 140 keV. It is used for visualization of the thyroid gland because it accumulates in a functioning thyroid cells. The obtained image is the subtracted from those received with  $^{99m}\text{Tc}$ -sestamibi and what lefts afterwards presents a potential parathyroid adenoma. Intravenously  $^{99m}\text{Tc}$ -pertechnetate is applied form 74 до 350 MBq ( 2-10 mCi).

#### **Quality control of the radiopharmaceuticals**

Criteria for quality of the radiopharmaceutical is his biodistribution, following the requirements for chemical, radiochemical and radionucleotide purity, as well as the need to be sterile, non-pyrogenic and non-toxic. The last three conditions are guaranteed by the producers of the radiopharmaceuticals, in case the procedure for marking is strictly followed.

## **Control of the radiochemical purity of radiopharmaceuticals**

We performed control of the radiochemical purity of radiopharmaceuticals with quick chromatographic methods (paper and thin layer chromatography) for each delivery and for each batch number of the different kits. Data were recorded in a special diary.

### **2.4.Protocols**

For the aim of this study we used the following protocols.

**1.** For the single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin we used widely described in the literature protocol. The patient was injected, strictly intravenously, with a bolus of 740 MBq  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin. We obtained early (20 min after the injection) and late (120 min after the injection) 10 min images of the neck and thoracic cavity of the patient in a supine position. The detector was in AP position, photopeak 140 keV, width of the window 20%, collimator LEHR, matrix 256x256.

**2.** Dual-isotope  $^{99m}\text{Tc}$ -pertechnetate / $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -pertechnetate / $^{99m}\text{Tc}$ -tetrofosmin scintigraphy we performed following confirmed in the literature protocol. The patient was intravenously injected with 37MBq  $^{99m}\text{Tc}$ -pertechnetate. On the 30-th minute afterwards we recorded 10 minutes high counted images of the thyroid gland of the patient in a supine position. After obtaining the images of the thyroid gland, we injected a intravenous bolus of 555 MBq  $^{99m}\text{Tc}$ - sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin, without changing the position of the patient, staying under the collimator of the gamma-camera. 10-15 minutes after the injection of  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin we obtained a static images of the neck and the chest. We applied subtraction of the late from the early obtained images and recorded areas of increased late fixation of  $^{99m}\text{Tc}$ - sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin. In some patients we obtained also late (120 min) planar images.

**3.** We prepared a protocol for combining the dual-phase method with SPECT technique (single isotope dual-phase method with  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin in combination with early SPECT technique). The patient was injected intravenously with a bolus of 740 MBq  $^{99m}\text{Tc}$ - sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin. We recorded early (20 min after the injection) highly counted 10

minutes images of the neck and chest of the patient in a supine position. Immediately afterwards, while the patient is in the same position we performed SPECT technique for a tomographic registration of the neck and part of the chest. The examination was performed in tomographic mode with low-energy collimator on a circle orbit of  $360^{\circ}$ , "Step and shot" registration, matrix 128x128, 60 projections of 32 sec, at an interval of  $3^{\circ}$  – totally 30 min. Reconstruction of the images with Iterative Reconstruction was done. We registered late, on the 120-th minute, highly counted planar images of the neck and chest. In some patients we performed also late SPECT technique.

4. We prepared a protocol for combining of subtractional method with SPECT technique (dual isotope  $^{99m}\text{Tc}$ -pertechnetate/ $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -pertechnetate / $^{99m}\text{Tc}$ -tetrofosmin method in combination with early SPECT technique). The patient was intravenously injected with 37MBq  $^{99m}\text{Tc}$ -pertechnetate. On the 30-th minute afterwards we recorded 10 minutes highly counted images of the thyroid gland of the patient in a supine position. After obtaining the images of the thyroid gland, we injected a intravenous bolus of 555 MBq  $^{99m}\text{Tc}$ - sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin, without changing the position of the patient, staying under the collimator of the gamma-camera. 10-15 minutes after the injection of  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin we obtained a static images of the neck and the chest. Immediately afterwards we performed an early SPECT technique. The examination was performed in tomographic mode with low-energy collimator on a circle orbit of  $360^{\circ}$ , "Step and shot" registration, matrix 128x128, 60 projections of 32 sec, at an interval of  $3^{\circ}$  – totally 30 min. Reconstruction of the images with Iterative Reconstruction was done. In some patients we obtained also late planar images of the neck and chest.

## 2.5. Ultrasound examination

All the patients had undergone ultrasonographic examination of the neck area with 10 MHZ linear probe and color Doppler on a device Sonoscape. This allowed small lesions in the neck to be visualized and Doppler was used to determine vascularization of the observed lesions, as well as to distinguish between them and the vascular structures. The volume in milliliters of the suspected abnormal parathyroid glands was calculated using the following equation ( $a \times b \times c \times 0.479$ ).

## **2.6. Laboratory**

All the laboratory results were obtained from the Central Clinical Laboratory at UMHAT "ST. George" -Plovdiv. Here only the results concerning the study are described. All other results were archived in separate patients' files.

### **2.6.1 Biochemical results**

#### **- Calcium , Phosphate**

Examined by atom-absorption spectrophotometry. Reference ranges for total calcium 2.12-2.62 mmol/l; ionized calcium - 1.06-1.31 mmol/l; phosphate – 0.77-1.36 mmol/l

### **2.6.2 Hormonal results**

All blood samples were drawn early in the morning, at fasting condition after a 30 minutes of rest. After staying between 30 min and 1 hour, at room temperature, to allow clotting, blood samples were centrifuged. Some of the samples had undergone immediate examination, the other were stored in a freezer  $\leq -20^{\circ}\text{C}$  till the day of examination.

**- Intact (1-84) Parathyroid hormone (iPTH)** – dual immune-radiometric method with two pure polyclonal antibodies, one specific for the N-terminal part of PTH (1-34), and the second specific for (39-84) fragment of PTH was used. Reference ranges for PTH 10-65 pg/ml.

## **3.Statistical analyses**

We used SPSS version 15.0 for Windows for all statistical analyses. The results of analyzed qualitative variables are shown as median value  $\pm$  standard deviation ( $\bar{x} \pm S\bar{x}$ ). The level of statistical significance to reject the null hypothesis  $P < 0.05$  was chosen.

Graphic analyses for presentation of the results was used.

Calculation of sensitivity and specificity was done using the following equations:

$$\text{Sensitivity} = \text{CP} / (\text{CP} + \text{FN}) \times 100$$

$$\text{Specificity} = \text{CN} / (\text{CN} + \text{FP}) \times 100$$

CP – Correct positive; CN – Correct negative; FP – False positive; FN – False negative; PPV – Positive predictive value; NPV – Negative predictive value

## IV.RESULTS AND DISCUSSION

The results of nuclear-medical examinations in the patients with hyperparathyroidism (Fig.1) we divided into:

1. Nuclear-medical examination in patients with radiopharmaceutical  $^{99m}\text{Tc}$ - sestamibi.
2. Nuclear-medical examination in patients with radiopharmaceutical  $^{99m}\text{Tc}$ -tetrofosmin.

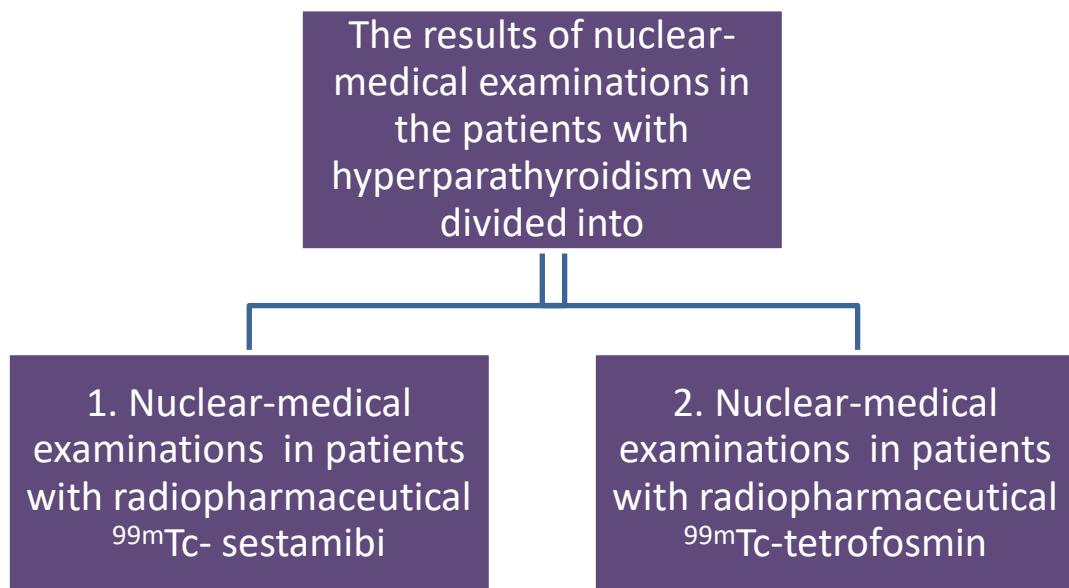


Fig. 1. The results of nuclear-medical examinations in the patients with hyperparathyroidism according to the used radiopharmaceutical

## **1. Nuclear-medical examination in patients with radiopharmaceutical $^{99m}\text{Tc}$ - sestamibi.**

The obtained results from the nuclear – medical examinations with  $^{99m}\text{Tc}$ - sestamibi (Fig.2) we divided in two groups:

- 1.1. In patients with primary hyperparathyroidism (PHPT).
2. 1. In patients with secondary hyperparathyroidism (SHPT).

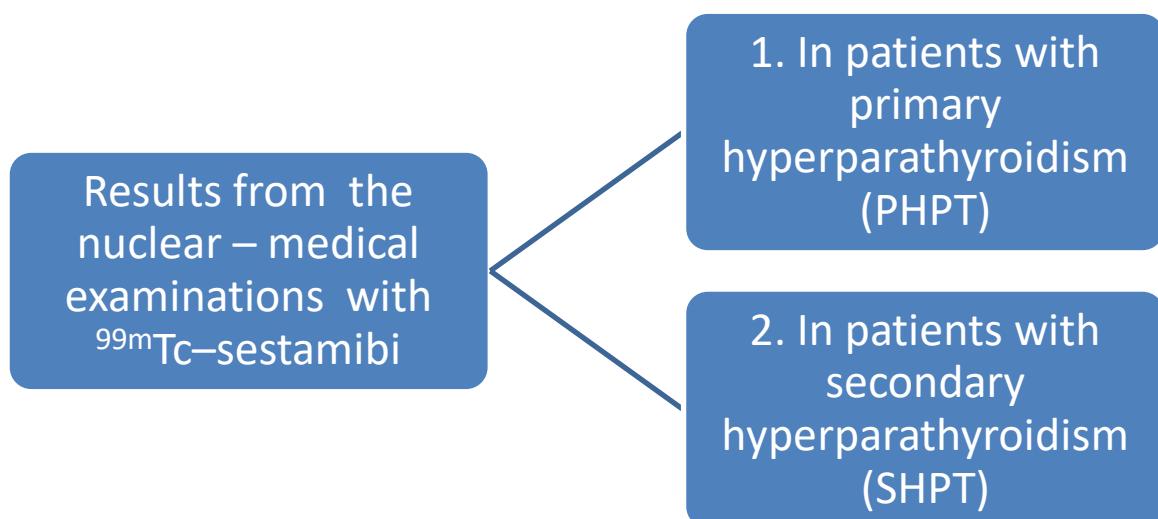


Fig. 2. Results of nuclear-medical examinations with  $^{99m}\text{Tc}$  - sestamibi in patients according to the type of hyperparathyroidism

### **1.1. Results from the nuclear-medical examinations with $^{99m}\text{Tc}$ - sestamibi in patients with primary hyperparathyroidism (PHPT).**

The group of patients with PHPT, who were examined with  $^{99m}\text{Tc}$ - sestamibi included 46 patients (40 women and 6 men). (Fig.3)

The mean age of women was  $56,23 \pm 11,35$  years (from 33 to 78 years), and that of men was  $46,2 \pm 9,60$  years ( from 34 to 59 years).

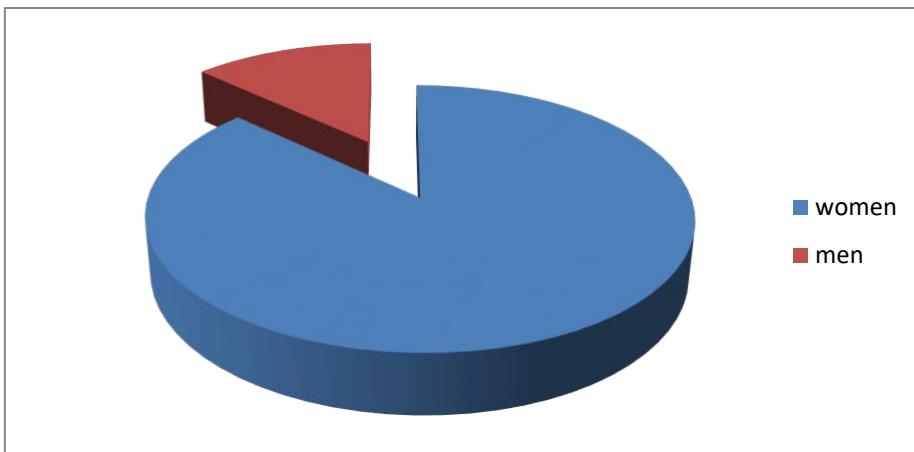


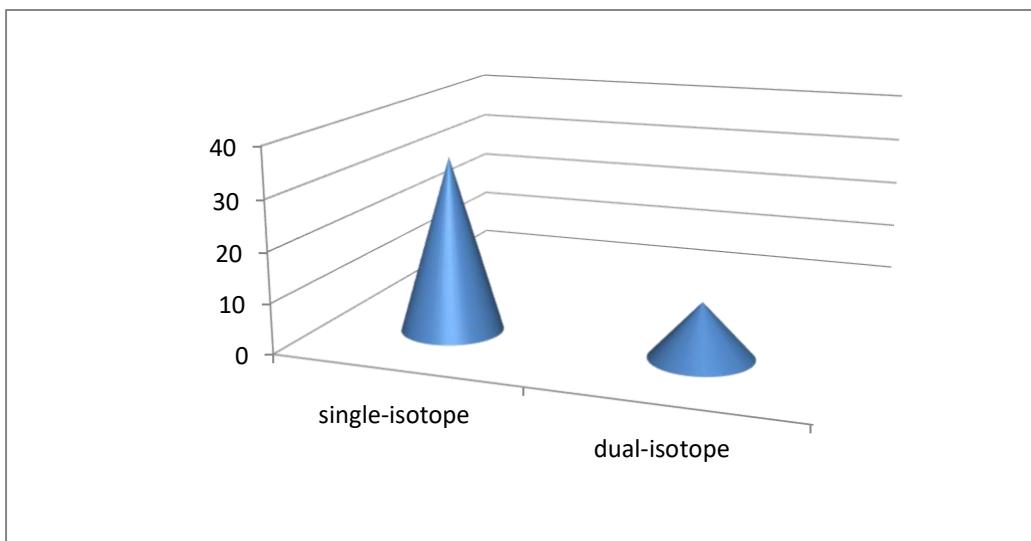
Fig. 3. Distribution of patients with PHPT by sex

During the scintigraphy with  $^{99m}\text{Tc}$ - sestamibi, 7 (15,2%) of patients reported of bitter taste combined with dryness in the mouth, immediately after the injection of the radiopharmaceutical. Our data showed, that this complaints disappeared within 5 minutes, and were connected with the described common side effects in leaflet of the radiopharmaceutical.

Two patients were with MEN -1 syndrome (1 men and 1 women). One men had undergone kidney transplantation. In several patients , there were clinical data for kidney stones with or without end stage kidney disease or bone changes (osteoporosis). In some patients there were no symptoms

We performed single isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi in 35 (76,08%) patients. In 5 cases we combined the above mentioned procedure with SPECT technique. (Fig.4)

In 11 (23,92%) patients we have used dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetat/ $^{99m}\text{Tc}$ -sestamibi, combined with SPECT technique, following the created by us protocol. (Fig.4)



**Fig. 4.** Distribution of patients according to the applied method for examination

In 26(56,52%) cases, out of total 46 patients with PHPT, focuses of residual activity of the radionucleotide were detected. In the rest 20 (43,48%) patients no such areas were visualized in the areas of the neck and chest. (Fig.5)

Combining both methods, we detected 30 abnormal parathyroid glands: 13 right inferior, 1 right superior, 1 intrathyroid in the right lobe, 11 left inferior, 2 intrathyroid in the left lobe and 2 left superior glands.

In 24 patients there US and in 2 MRI data for possible parathyroid adenomas.

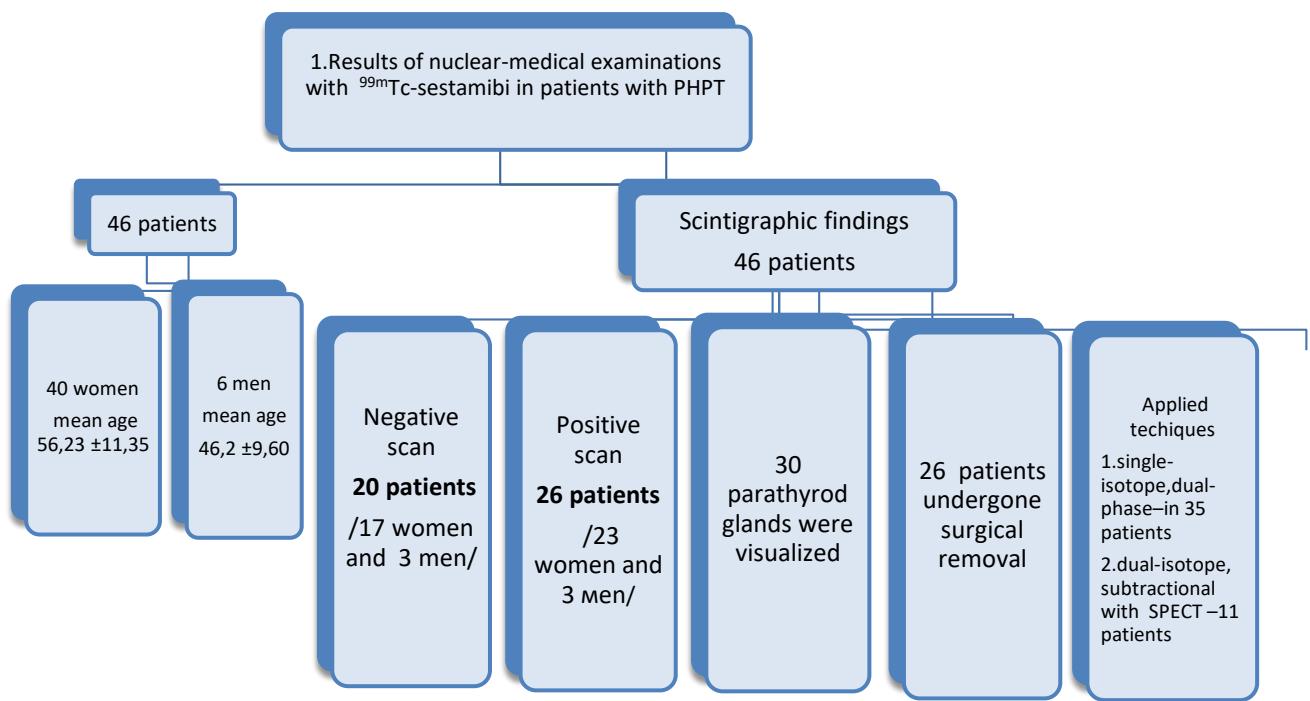


Fig. 5. Results of nuclear-medical examinations with <sup>99m</sup>Tc-sestamibi in patients with PHPT

According to the presence or absence of scintigraph findings we divided the patients into two groups:

**Group A.** Patients with PHPT with positive scans for a suspicious for parathyroid adenoma lesion in the neck or mediastinum.

**Group B.** Patients with PHPT with negative scans for a suspicious for parathyroid adenoma lesion in the neck or mediastinum.

**Group A. Patients with PHPT with positive scans for a suspicious for parathyroid adenoma lesion in the neck or mediastinum**

In 46 patients with PHPT, who underwent scintigraphy with <sup>99m</sup>Tc-sestamibi, positive scans for a suspicious for parathyroid adenoma lesions in the neck or mediastinum were detected in 26 patients (56,52%) – in 20 patients dual-phase method with <sup>99m</sup>Tc-sestamibi was used and in 6 cases subtractional method with <sup>99m</sup>Tc-pertechnetate / <sup>99m</sup>Tc-sestamibi combined with SPECT was performed.

All the 26-th patients with positive scans had undergone surgical removal of the discovered suspicious lesions.

In 35 (out of 46) patients single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi was done and in 11(out of 46) cases dual-isotope subtractional method with  $^{99m}\text{Tc}$ -pertechnetate /  $^{99m}\text{Tc}$ -sestamibi combined with SPECT was performed.

In 35 patients, after performing single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi, positive scans were registered in 20 (57,2%) cases. Negative scans were detected in 15 (42,8%) patients. Using the data from the histologically verified parathyroid adenomas, we determined the sensitivity of the single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi. The sensitivity of this method proved to be 90,90% (20/22), because we have registered 2 patients with false negative results. Those two patients were operated, despite negative scan results, because biochemical changes typical for hyperparathyroidism persisted and parathyroid adenomas were proven by the histological examination.

In 5 (out of 35) patients we performed a combination of single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi with SPECT. In 1 patient early SPECT discovered hyper fixating zone, located at the back of the left lobe, but this zone was not visible on the late phase images of the dual-phase method. This patient was included in the group of patients with positive scans after dual-phase scintigraphy, because surgically was proven to have parathyroid adenoma. This fact can be explained with the rapid washout of the nucleotide, observed in some parathyroid adenomas.

In 11 (out of 46) patients, we performed dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetate/ $^{99m}\text{Tc}$ -sestamibi combined with SPECT. Out of these 11, in 6 cases (54,5%) we found hyper fixating zones after performing the above mentioned combined method, and in all these 6 cases, there were found parathyroid adenomas after operation. Using only subtractional method with  $^{99m}\text{Tc}$ -pertechnetate / $^{99m}\text{Tc}$ -sestamibi, we found 4 patients with positive scans, and 2 cases proved to be false negative. So, the sensitivity of the subtractional method showed to be 66,66% (4/6). Using SPECT in combination, 5 patients were with positive scans and 1 was false negative, which made the sensitivity of the SPECT technique to be 83,33% (5/6). In the false negative patient, a parathyroid adenoma located in the thyroid parenchyma was

discovered, by combining the subtractional method with the late obtained images on dual-phase scintigraphy-120 min.

Our study showed, that SPECT technique increased the sensitivity of the subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetate /  $^{99m}\text{Tc}$ -sestamibi with 16,67 %.

In patients, who showed negative scans after subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetate /  $^{99m}\text{Tc}$ -sestamibi, we obtained late images on 120-th minute. In 1 patient we registered an intrathyroid adenoma. This patient was included in the group of patients with positive scans. We think that combining subtractional method with obtaining late images (120 min) favors detection of parathyroid adenomas located into thyroid parenchyma.

We also looked for a connection between positive scans and the levels of total and ionized calcium, phosphate and PTH. So, we calculated the mean values of this variables for women and men (Tabl.1 and Tabl.2).

**Group B. Patients with PHPT with negative scans for a suspicious for parathyroid adenoma lesion in the neck or mediastinum.**

The total number of patients with negative scan is 20- 17 women (mean age  $56,47 \pm 12,42$  years ) and 3 men ( mean age  $45,66 \pm 12,58$  years).

The mean values of total and ionized calcium, phosphate and PTH for this group are shown in Table 1 and Table2.

Tabl. 1. Mean values of total calcium, ionized calcium, phosphate and PTH in women with PHPT examined with  $^{99m}\text{Tc}$  – sestamibi scintigraphy. The results are shown as mean values  $\pm$  standard error ( $x \pm Sx$ ).

Variable	Positive scan N 23	Negative scan N 17	p
Ca mmo/l	$2.636 \pm 0.0566$	$2.243 \pm 0.176$	<0.001
P mmo/l	$0.96 \pm 0.788$	$0.922 \pm 0.668$	<0.01
Ca++ mmo/l	$1.35 \pm 0.038$	$1.23 \pm 0.779$	<0.001
$\Pi\text{TX}$ pg/ml	$357.21 \pm 73.585$	$132.93 \pm 18.921$	<0.001

Табл. 2. Mean values of total calcium, ionized calcium, phosphate and PTH in men with PHPT examined with  $^{99m}$ Tc – sestamibi scintigraphy. The results are shown as mean values  $\pm$  standard error ( $x \pm Sx$ ).

Variable	Positive scan N 3	Negative scan N 3	p
Ca mmo/l	2.583 $\pm$ 0.161	2.403 $\pm$ 0.111	<0.05
P mmo/l	1.036 $\pm$ 0.297	0.92 $\pm$ 0.017	=0.073
Ca++ mmo/l	1.596 $\pm$ 0.046	0.98 $\pm$ 0.07	<0.001
PTH pg/ml	511.36 $\pm$ 335.824	90.843 $\pm$ 25.036	<0.001

The results presented in table 1, show that in women with PHPT and positive scans, there were statistically significant higher levels of total calcium, ionized calcium, phosphate and PTH in comparison with the group of women with negative scans. The same was also seen in the group of men with positive scans in comparison with those with negative scans, except for phosphate, where such difference was not detected (Tabl.2).

We calculated the mean volume of the detected by US adenomas in patients with positive scans – 1,378 ml $\pm$ 0,485 SE, and in patients with negative scans – 0,227 ml $\pm$ 0,061 SE.

Our results showed, that when compare the mean volumes of the detected by US adenomas in patients with positive and negative scans after  $^{99m}$ Tc-sestamibi scintigraphy, the positive results were related with larger parathyroid glands, and the difference between two groups was statistically significant ( $p<0,05$ ).

$^{99m}$ Tc-sestamibi is composed of lipophilic, positively charged molecules. After being intravenously injected, it is distributed in the body in accordance with the blood supply, passes through the cells membranes by a passive diffusion and accumulates intracellularly into the mitochondria.(25)

Over the years, studying the mechanisms determining the accumulation of  $^{99m}$ Tc-sestamibi, it was discovered, that this radiopharmaceutical accumulates in tissues abundant with mitochondria( O'Donetj M.J. et al., 1989)(26). The cells of the parathyroid adenomas are such type of cells. ( Lee V.S. et al. 1996) (27) It was shown, that in normal parathyroid cells  $^{99m}$ Tc-sestamibi does not accumulate ( Moka D. et al.,2000) (28).

In some circumstances, the accumulation of  $^{99m}\text{Tc}$ - sestamibi in parathyroid cells depends on the metabolic activity of the cells, phases of the cells' cycles, cells; content, weight and size of the adenoma.

In patients with Multiply Endocrine Neoplasia -MEN, when parathyroid glands are engaged, and parathyroid hyperplasia is often observed, according to the literature, only up to 55% of the pathologically changed parathyroid glands can be visualized after scintigraphy. (Piga M.et al.,1996; Pons F.et al.,1997; Лавин Н.,1999).(36,32,29). The same results have been described by J.V. Torregrosa (1999)(37) in patients with secondary hyperparathyroidism.

We have 2 patients with MEN type - 1.

On figure 6 are shown images of the single isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi of a 37 years old woman with diagnosed MEN type - 1: Pituitary microadenoma, hyperinsulinism and undergone surgical removal of a parathyroid adenoma (2003r.). The early phase (20 min.) images showed a diffuse accumulation of the radionucleotide in the area of the thyroid gland. A focus of increased accumulation was registered caudally of the thyroid gland. The late images(120 min.) showed a washout of the nucleotide from the thyroid gland, and persistence of the focus of increased accumulation caudally of the thyroid , seen on the early images. The patient was successfully operated, and pathologic examination proved parathyroid adenoma with a size of 15mm/10mm, made up of chief and oxyphilic cells.

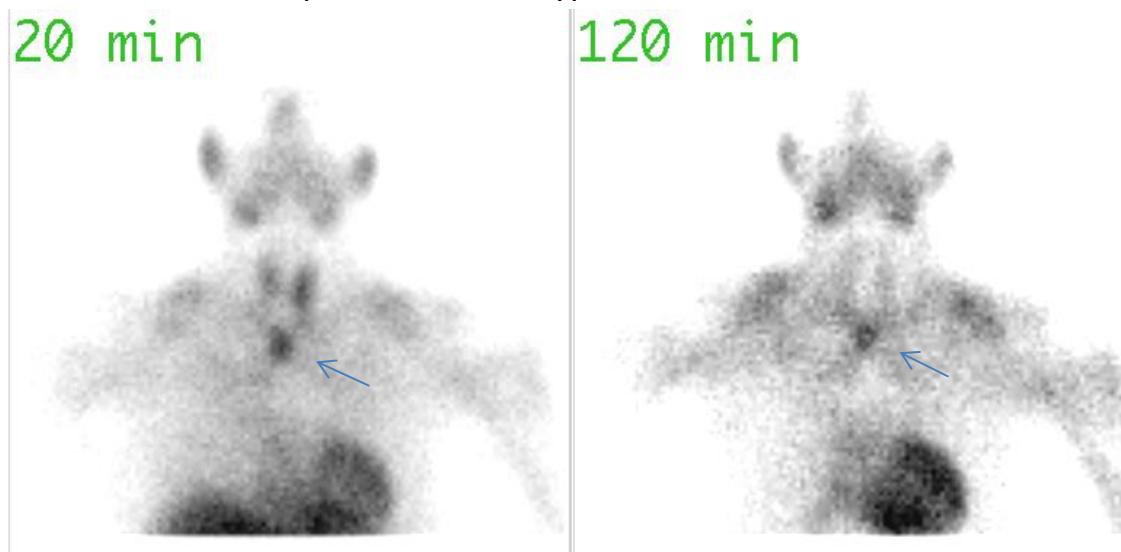


Fig. 6. Early phase images (20 min.) show diffuse accumulation of the radionucleotide in the thyroid gland and a focus of increased accumulation located caudally and medially of the thyroid gland. Late images (120 min.) only this focus of increased accumulation located caudally and medially of the thyroid gland is registered-parathyroid adenoma.

We used **three methods** for discovery and visualization of hyperfunctioning parathyroid glands: single-phase dual-isotope subtractional, dual-phase single-isotope and a combination of the two.

A few studies directly compare subtractional method with , dual-phase single-isotope scintigraphy and the results are contradictory (61,62). The advantages of one over another method are not fully proven, so far.

In our study , we applied different methods for visualization of abnormal parathyroid glands, and tried to determine the advantages and disadvantages of each of them.

### **Single-isotope dual-phase scintigraphy with $^{99m}\text{Tc}$ - sestamibi**

We performed a single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi in 35 patients. Single-isotope method relies on the frequently observed difference of the washout time of  $^{99m}\text{Tc}$ -sestamibi for thyroid gland and hyperfunctioning parathyroid glands. In the latter, the washout period is longer than it is from the thyroid. This method is easily performed, and needs only application of  $^{99m}\text{Tc}$ -sestamibi, followed by obtaining early planar images between 10-15 minutes, and late planar images obtained between 1,5-3 hours after the injection of the radiopharmaceutical.

Figure 7 shows the images of 54 years old woman with PHPT after single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi has been performed. On the late images obtained at 120 minute, a focus of a residual activity , caudally of the right thyroid lobe is seen. The patient was operated, and pathologic examination proved adenoma of the right lower parathyroid gland with a size of 25mm/15mm, made up of chief cells.

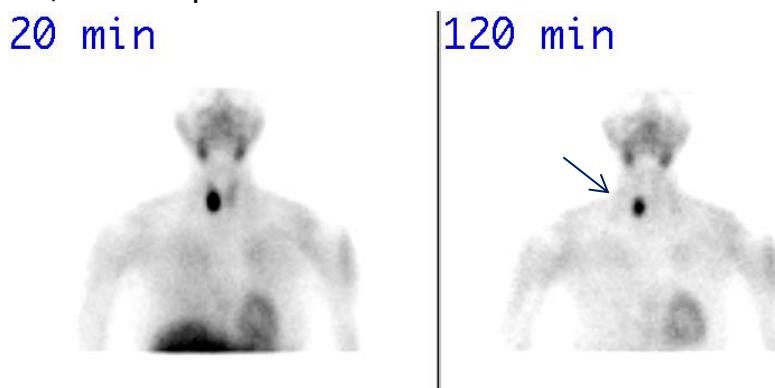


Fig. 7. The late images (120 min.) a focus of a residual activity, caudally of the right thyroid lobe is seen – adenoma of the right lower parathyroid gland.

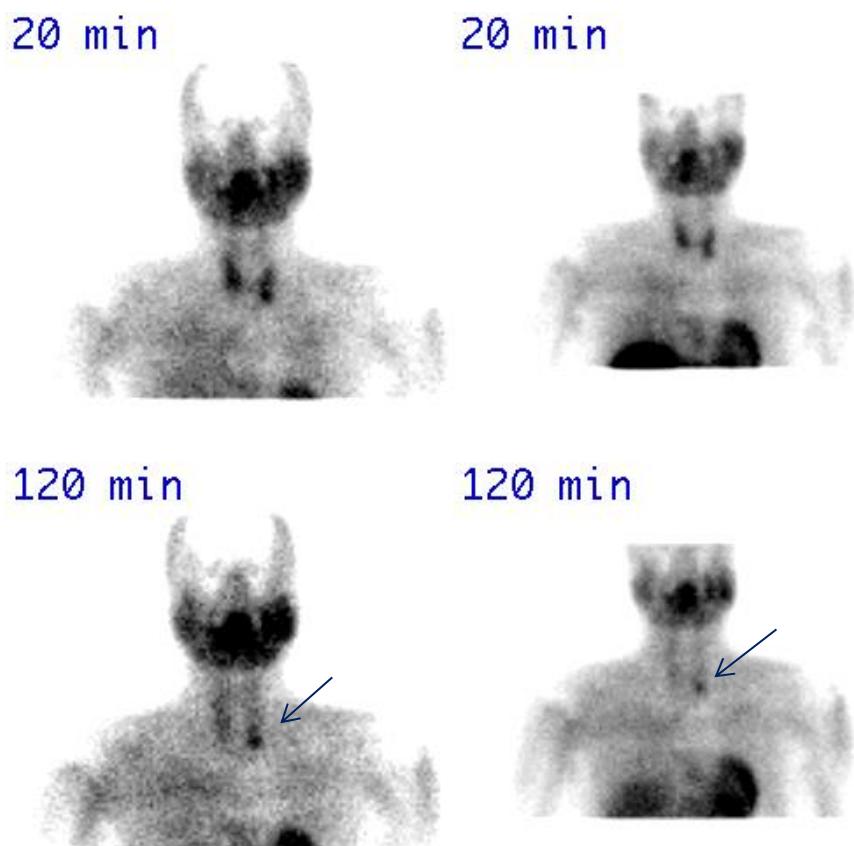
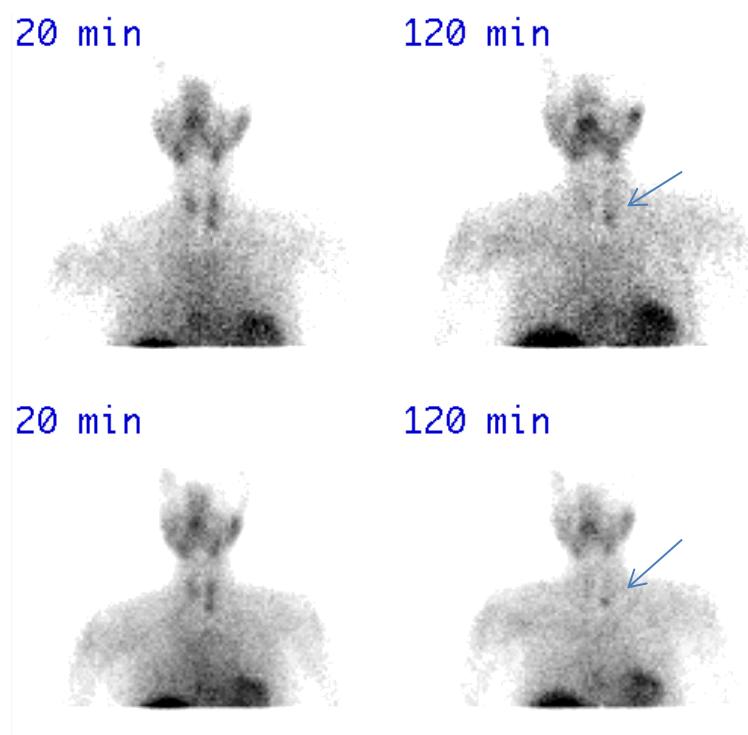


Fig.8. In the early phase images (20 min.) relatively diffuse uptake in the area of the thyroid gland and a focus of increased accumulation of the isotope, caudally of the left thyroid lobe are seen. In the late phase images (120 min.) only a focus of a residual activity, caudally of the left thyroid lobe is visualized-adenoma of the lower left parathyroid gland.

The images of a 42 years old men, after single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi, with total calcium 2,81 mmol/l, ionized calcium 1,48 mmol/l and PTH 232,1 pg/ml are shown on fig.8 . There were data from MRI for a left parathyroid adenoma. In the early phase images (20 min.) relatively diffuse uptake in the area of the thyroid gland and a focus of increased accumulation of the isotope, caudally of the left thyroid lobe are seen. In the late phase images (120 min.) only the focus of a residual activity, caudally of the left thyroid lobe is visualized-adenoma of the lower left parathyroid gland. The finding was suspicious for parathyroid adenoma, which was histologically proven after a successful operation, that the patient had undergone.

The images of a 56 years old woman with PHPT-total calcium 2,61 mmol/l and PTH 582,2 pg/ml , after single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi are shown on fig.9. There were previous data from CT, showing no signs of abnormal parathyroid glands. The late phase images (120 min.) a focus of a residual activity, caudally of the left thyroid lobe is visualized-adenoma of the lower left parathyroid gland. The finding was suspicious for parathyroid adenoma, which was histologically proven after a successful operation, that the patient had undergone.



Фиг. 9. In the early phase images (20 min.) relatively diffuse uptake in the area of the thyroid gland and a focus of increased accumulation of the isotope, caudally of the left thyroid lobe are seen. In the late phase images (120 min.) only the focus of a residual activity, caudally of the left thyroid lobe is visualized-adenoma of the lower left parathyroid gland.

In some cases, the obtained early and late images show no signs of abnormal accumulation of radionucleotide, but when SPECT is applied, than adenomas located at the back of the thyroid become visible.

Figure 10 a and Figure 10 b show images from a single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi in 72 years old woman with total calcium 2,23 mmol/l, PTH 128,0 pg/ml, normal phosphate and ionized calcium 1,24mmol/l . There were US data suspicious parathyroid adenoma caudally of the left thyroid lobe.

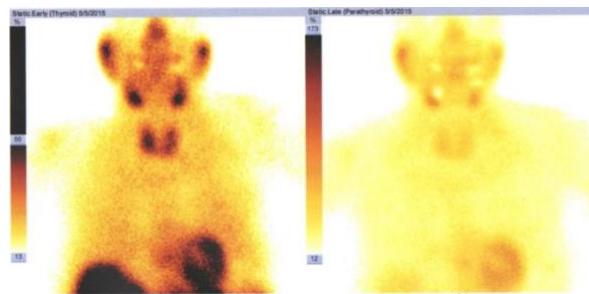


Fig. 10a. Early planar images show diffuse uptake in the thyroid gland. Late planar images showing no sign of focus of a residual activity in the neck or mediastinum.

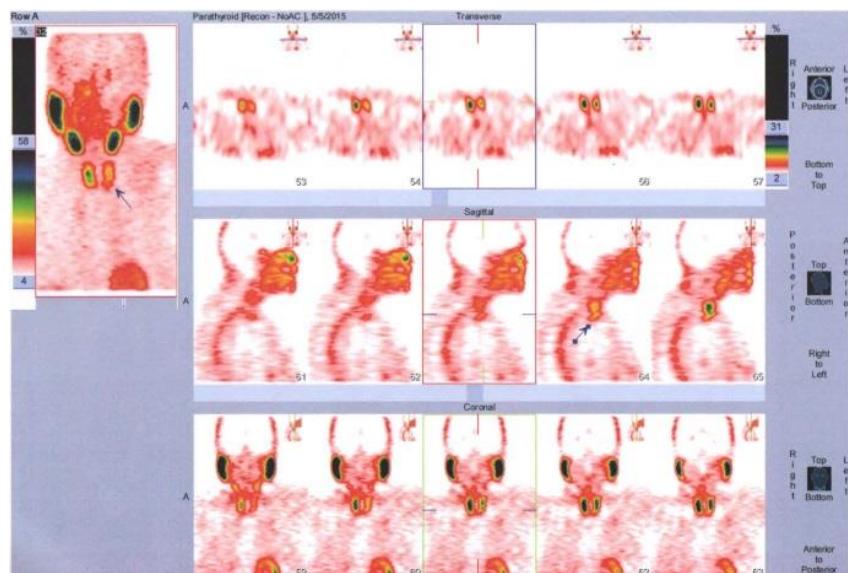


Fig. 10b. SPECT images show an area of hyperfixation, located dorsally and caudally of the left thyroid lobe, suspicious for a parathyroid adenoma.

Our data showed that combination of a single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi with SPECT can be of great help.

During many years in the past, two-dimensional images have been obtained with AP-images, and rarely it was combined with lateral and oblique images(64,65).

SPECT have gained more importance over the recent years, because it gives three-dimensional images, and because of the accumulating data from the literature, that it improves sensitivity for discovering and localizing the

hyperfunctioning parathyroid glands (66,67). The main reason for this is the improved contrast resolution of the SPECT.

Figure 11a and Figure 11 b present combined images obtained from single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi and SPECT in 73 years old woman with PHPT- with total calcium 2,89 mmol/l, PTH 199,7 pg/ml, phosphate 0,97 mmol/l and ionized calcium 1,46 mmol/l. There were US data for a hypoechoic lesion with a size 17,7/6,5/19,7 mm suspicious for parathyroid adenoma below the left thyroid lobe.

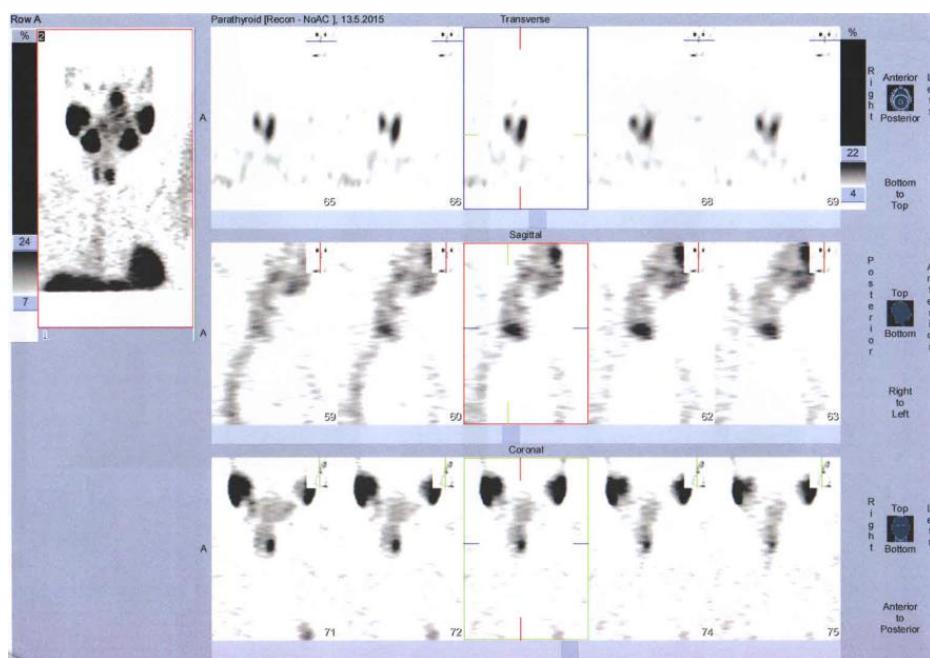


Fig. 11a. Early SPECT images showing an area of hyperfixation, located dorsally and caudally of the left thyroid lobe.

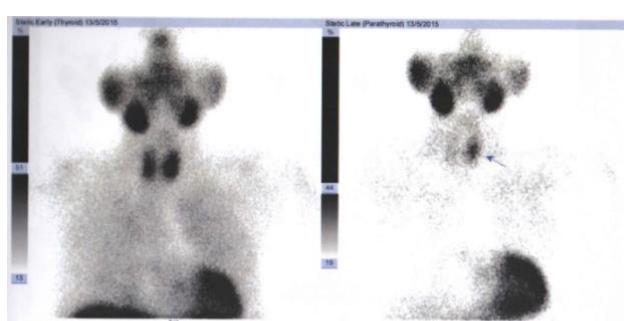


Fig. 11b. Late images (120min.) show a focus of a residual activity, caudally of the left thyroid lobe -adenoma of the left parathyroid gland.

In this case, the planar images showed that the abnormal finding was located below the left thyroid lobe, but combining with SPECT the localization was more precise – dorsally and caudally of the left thyroid lobe.

Figure 12a and Figure 12b present combined images obtained from single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi and early SPECT in 56 years old woman.

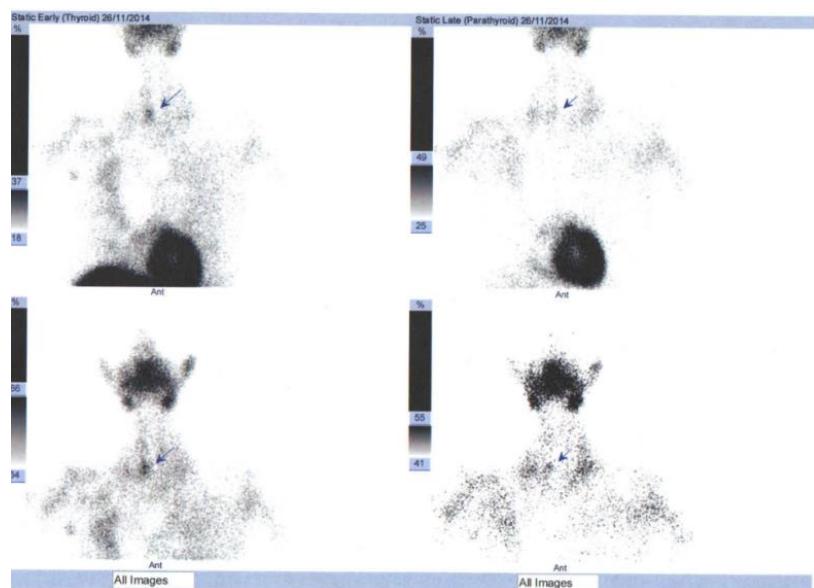


Fig.12a. Early planar images (20 min.) do not detect any accumulation of the radiopharmaceutical in the thyroid gland (the patient had undergone total thyroidectomy). A hyper fixating zone, located in the right part of the neck is seen. The late planar images (120 min.) show a focus of a residual activity in the right part of the neck- right parathyroid gland adenoma.

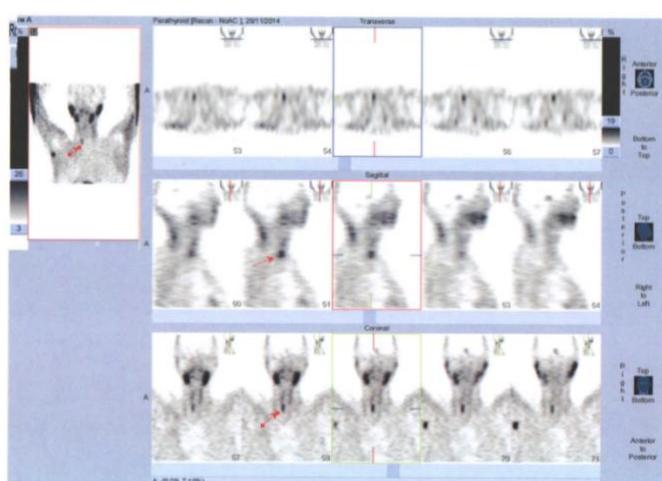


Fig. 12b. Early SPECT images showing an area of hyperfixation located in the right part of the neck.

The patient had been followed up after the operation, and a histologically proven right parathyroid adenoma had been removed. This fact confirms the scintigraphic findings. .

Figure 13a and Figure 13b present combined images obtained from single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi and early SPECT in 62 years old woman with PHPT- with total calcium 3,00 mmol/l, PTH 107,9 pg/ml and phosphate 0,82 mmol/l. There were US data for a hypoechoic lesion with an irregular margins and a size 13,1/6,8/6,2 mm suspicious for parathyroid adenoma below the left thyroid lobe.

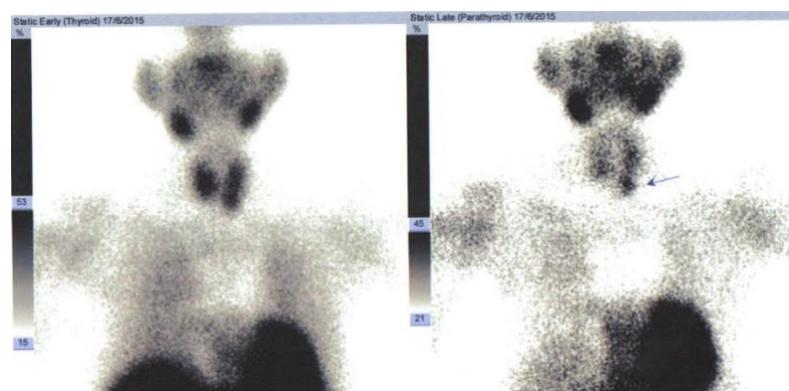


Fig. 13a. Late images (120min.) show a focus of a residual activity, caudally of the left thyroid lobe -adenoma of the left parathyroid gland (see the arrow)

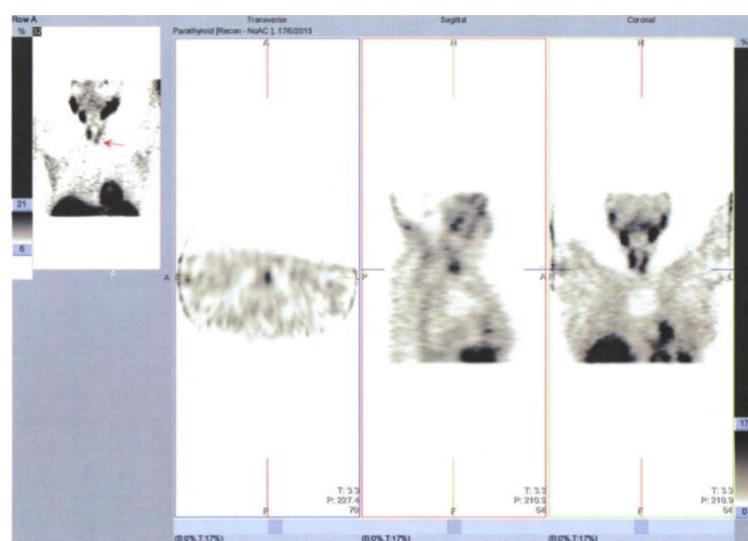


Fig. 13b. Early SPECT images showing an area of hyperfixation, located dorsally and caudally of the left thyroid lobe.

Discussing the results of single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi , we could point out, that the advantages of this method were that it was easy to be performed and no special preparation of the patients was needed. The only required condition was, that  $^{99m}\text{Tc}$ -sestamibi should be injected strictly intravenously like a bolus, and afterwards early planar (20 min.) and late planar (120 min) images should be recorded.

For a disadvantage we could point, that in some cases a focus of accumulation of the radiopharmaceutical was detected on the early images, but on the obtained late planar images this couldn't be seen. This fact can be explained with the early washout of  $^{99m}\text{Tc}$ -sestamibi from some adenomas, for which we have also data from the literature(69,70).

In our study, we performed SPECT immediately after obtaining the early planar images, and in some cases after the late planar images. Our data showed that more informative was performing early SPECT after single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi , because the maximal uptake of the  $^{99m}\text{Tc}$ -sestamibi was soon after the injection and this could help us not to miss hyperfunctioning parathyroid adenomas with a rapid washout of the radiopharmaceutical.

The results showed, that single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi should be combined with an early SPECT, also in cases with poor or no detected accumulation of the radiopharmaceutical. Performing SPECT, did not harm the patient, it is well tolerated and improves the diagnostic sensitivity of this method.

### **Dual-isotope subtractional scintigraphy with $^{99m}\text{Tc}$ - pertehnetat/ $^{99m}\text{Tc}$ sestamibi.**

The rationale that stands beyond dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ - pertehnetat/  $^{99m}\text{Tc}$  sestamibi, is that  $^{99m}\text{Tc}$ -sestamibi is used for visualization of both thyroid gland and hyperfunctioning parathyroid glands and another radiopharmaceutical( $^{123}\text{I}$  or  $^{99m}\text{Tc}$ -pertechnetate) for visualization only the thyroid. Secondly obtained set of images is subtracted digitally from the first set of images. The presence of a residual activity on the subtractional images represents hyperfunctioning parathyroid gland.

Dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ - pertehnetat/  $^{99m}\text{Tc}$  sestamibi in combination with SPECT was performed in 11 patients.

Figure 14a and Figure 14b present combined images obtained from a dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ - pertechnetate/  $^{99m}\text{Tc}$  sestamibi and SPECT in 56 years old woman with PHPT- with total calcium 2.84 mmol/l, PTH 118,0 pg/ml , phosphate 0,91 mmol/l, and ionized calcium 1,35 mmol/l.

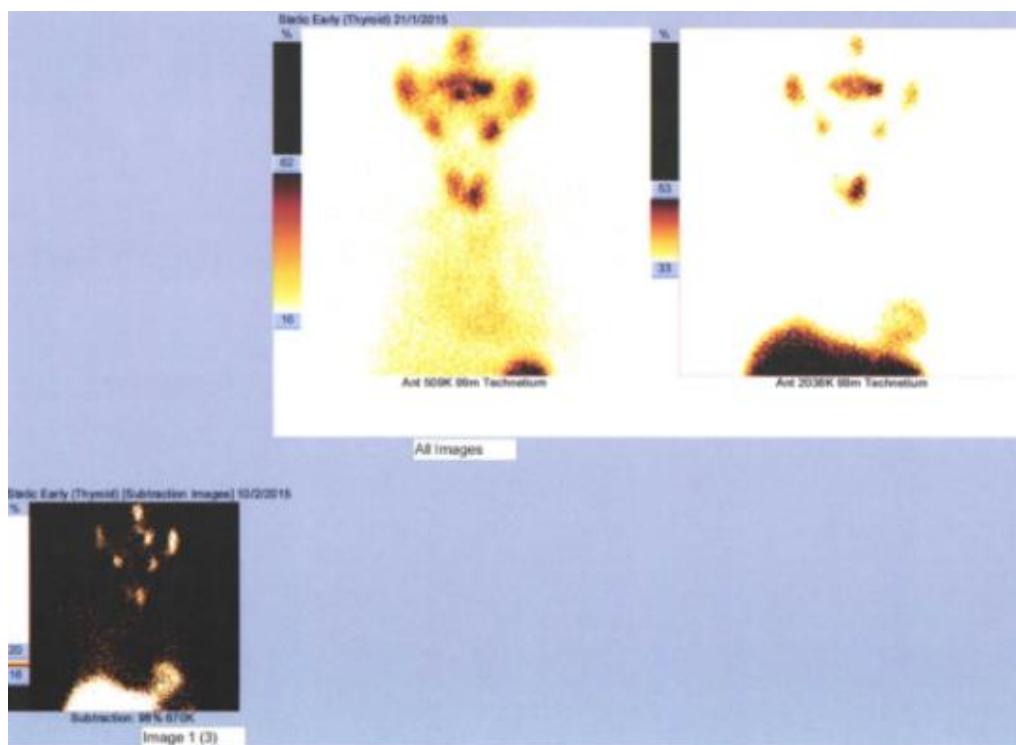


Fig. 14a. Upper row: on the right-Thyroid gland ,on the left Parathyroid gland. Lower row: Subtractional image showing a focus of a residual activity in upper of the left thyroid lobe- adenoma of the left parathyroid gland.

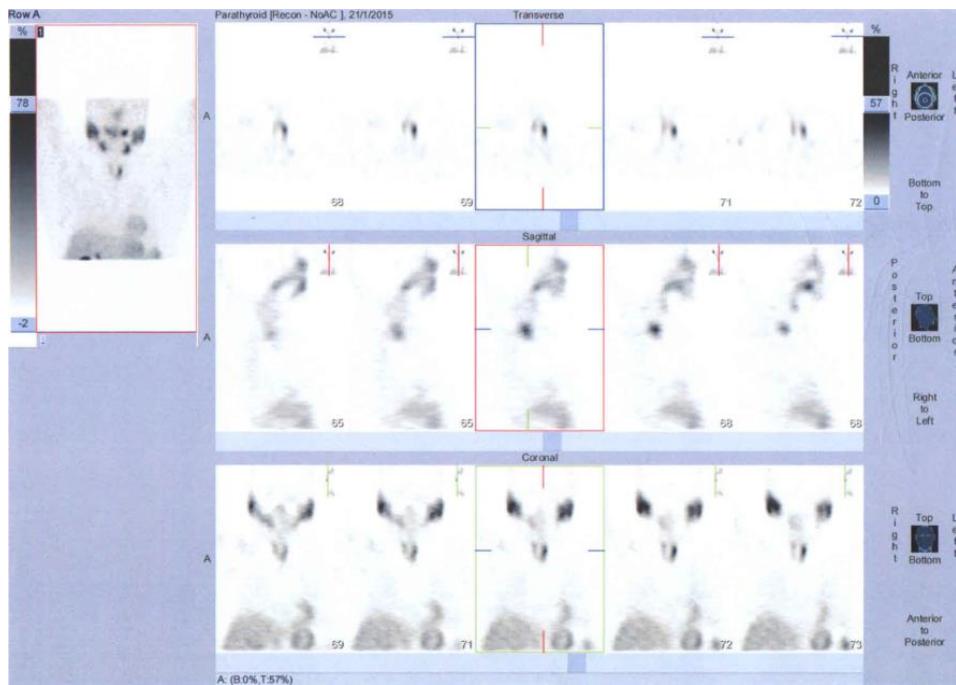


Fig. 14b. Early SPECT images showing an area of hyperfixation, located dorsally and cranially of the left thyroid lobe.

Figure 15a and Figure 15b present combined images obtained from dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ - pertechnetate/  $^{99m}\text{Tc}$  sestamibi and SPECT in 66 years old woman with PHPT- with total calcium 2.90 mmol/l, PTH 993,0 pg/ml, and ionized calcium 0.92 mmol/l.

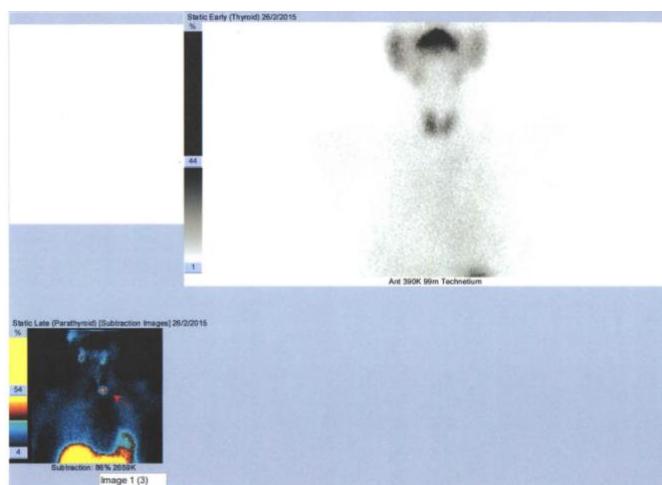


Fig. 15a. Subtractional image showing an adenoma of the left parathyroid gland



Fig. 15b. Early SPECT images showing an area of hyperfixation, located dorsally and caudally of the left thyroid lobe.

The results showed that the subtractional method could be of help, when the patients had undergone surgery of the thyroid, but some thyroid parenchyma is still present. This method is important to detect the presence of more than one abnormal parathyroid gland. The same are also reported by some other authors (54).

Figure 16a, figure 16b and figure 16c present scintigraphic images of a 51 years old man with MEN-type 1syndrom– pheochromocytoma, parathyroid adenoma and prolactinoma. He was referred for parathyroid scintigraphy with the following laboratory results: total calcium 2,37mmol/l, PTH 1180 pg/ml , phosphate 0,7 mmol/l, and ionized calcium 1,8 mmol/l. There were US data for a hypoechoic lesion with a size 22mm/12,9mm suspicious for parathyroid adenoma below the right thyroid lobe, and another nodule below the left thyroid lobe, also suspicious for parathyroid adenoma. The patient haad undergone surgery of the thyroid and parathyroid glands. We performed dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ - pertechnetat/  $^{99m}\text{Tc}$  sestamibi combined with SPECT , and also recorded late images (120 min.) of the neck and chest.

Subtractional images (fig.16a) and early SPECT images(fig.16c) showed two hyperfixating zones, located below the remnants of the both thyroid lobes.

SPECT images showed that the lesion below the right thyroid lobe was located also adjacent to the back of the lobe.

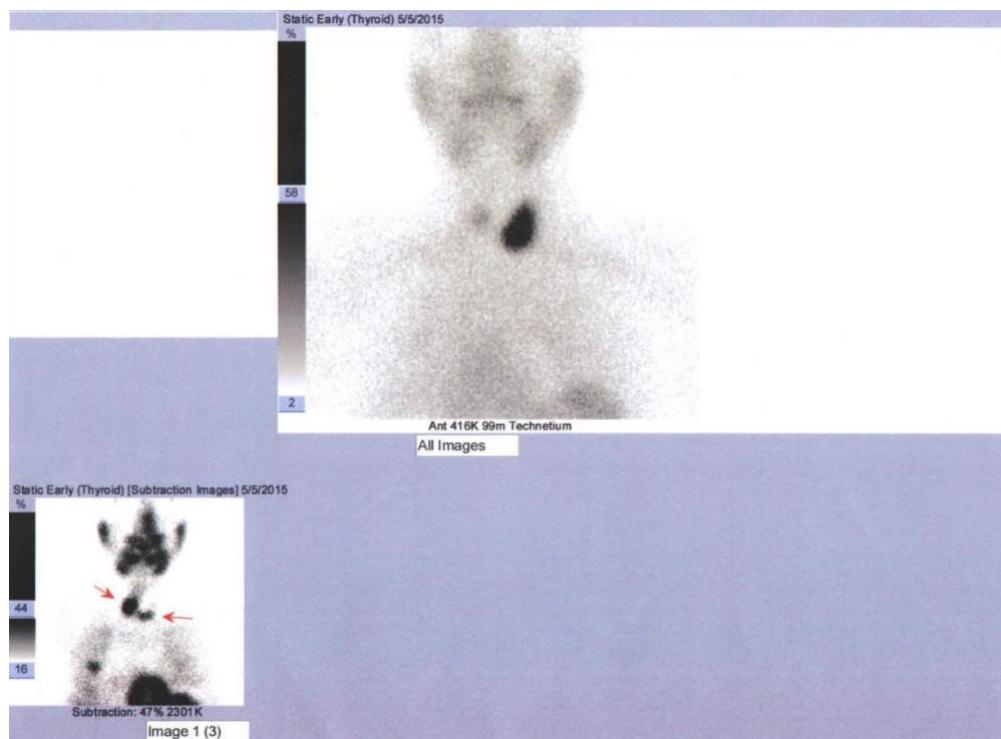


Fig. 16a. Subtractional image showing two hyperfunctioning parathyroid adenomas

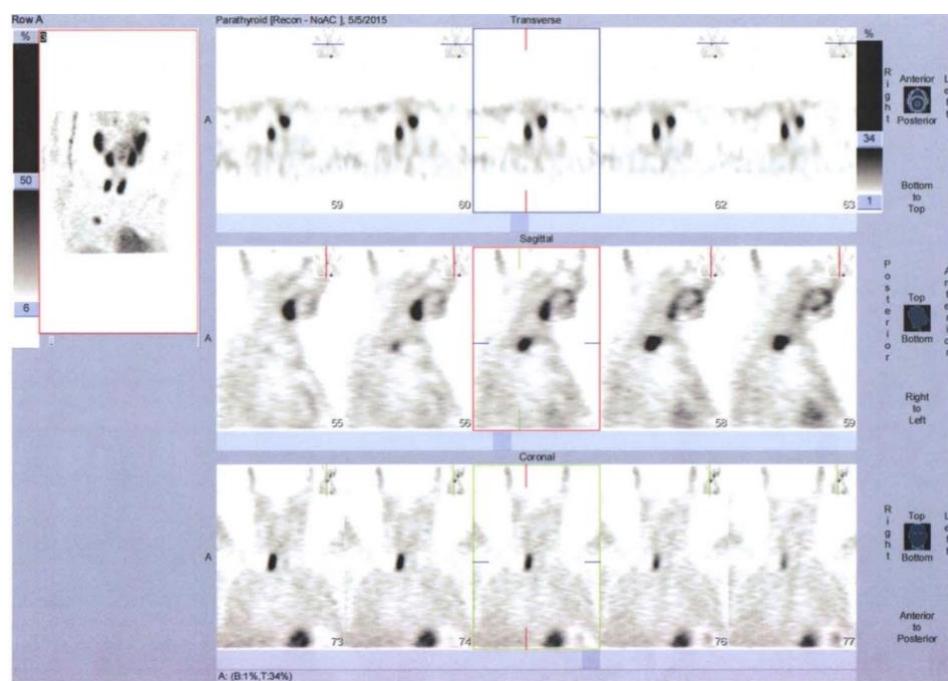


Fig. 16b. SPECT images showing an area of hyperfixation, located dorsally and caudally of the right thyroid lobe

In this patient, we also obtained late on the 120-th minute images of the neck and chest.

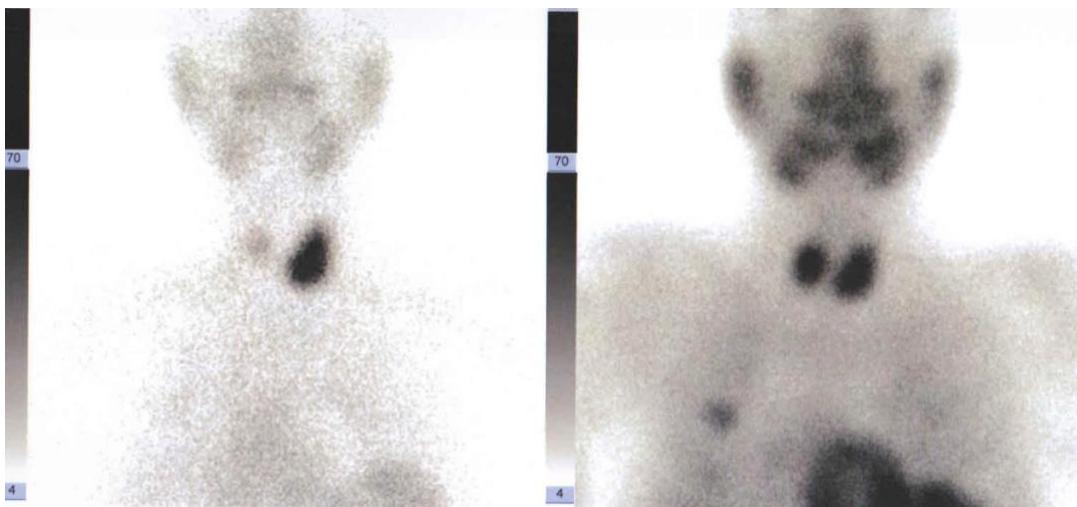


Fig. 16c. Late static images (120 min. ) showing two zones with a residual activity.

In some cases, obtaining late images could also be of help. We combined both methods- dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ - pertechnetate/  $^{99m}\text{Tc}$  sestamibi with SPECT, and also recording late planar images on the 120-th minute (late phase).

Figure 17a and Figure 17b present combined images obtained from a dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ - pertechnetate/  $^{99m}\text{Tc}$  sestamibi and SPECT, and late planar images on the 120-th minute, in 56 years old woman with PHPT- with total calcium 2,77 mmol/l, PTH 90 pg/ml , phosphate 0,89 mmol/l, and ionized calcium 1,29 mmol/l. There were US data for a hypoechoic lesion located in the middle of the left thyroid lobe, suspicious for a parathyroid adenoma.



Fig. 17a. Subtractional image showing no residual activity in the regions of neck and chest.

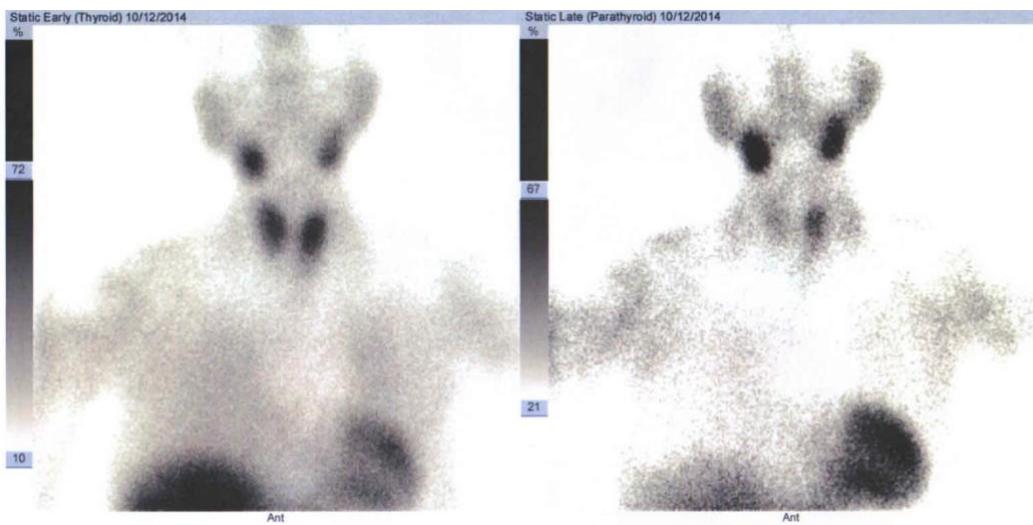


Fig. 17b. Late planar images showing a residual activity in the midddle of the thyroide-left parathyroid adenoma.

Our data showed, that dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetat/  $^{99m}\text{Tc}$  sestamibi, helped abnormal hyperfunctioning parathyroid glands to be visualized after a subtraction had been performed in the early phase. This made performing the late phase inappropriate, and shortened the necessary time for examination with 80-90 minutes. Combining subtractional scintigraphy with SPECT provided us with three dimensional images, improved sensitivity for detecting of abnormal hyperfunctioning parathyroid glands, especially ones which showed early washout of the radiopharmaceutical. The superiority of the SPECT was due to improved contrast resolution.

Our study showed, that SPECT increased sensitivity of the dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetat/  $^{99m}\text{Tc}$  sestamibi with 16,67%.

Dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ - pertechnetat/  $^{99m}\text{Tc}$  sestamibi is very helpful, when examination is performed in patients undergone thyroid surgery, and especially when there are thyroid residues with different shapes and sizes, as well for detection of more than one abnormal parathyroid glands.

Some authors consider, that sensitivity of  $^{99m}\text{Tc}$ -sestamibi SPECT/CT is considerably lower, when there are multiglandular engagement of the parathyroid glands in comparison with uniglandular disease, and this has no connection both with the size and localization of the abnormal gland (104).

Combining subtraction with dual-phase scintigraphy, especially obtaining late images on the 120-th minute, could be of help for discovering intrathyroid

located hyperfunctioning parathyroid adenomas, as is presented by us on figure 17a and 17 b. Some authors consider that this combination is optimal for detecting abnormal parathyroid glands (62).

We can point several disadvantages of the subtractional method, as a result of our study:

- The need two radionucleotides to be injected;
- The need of full collaboration of the patient's side to stand still ;
- The need the patient to be positioned very precisely under the collimator;
- The possible artefacts, after digital subtraction has been performed;;
- The need the patient has been stopped intake of any drugs that can interfere with  $^{99m}\text{Tc}$  intake in the thyroid, for an appropriate period of time.

### **Negative scans in patients with PHPT**

Negative scans in patients with PHPT, could be explained with different reasons. One of them is the size of the parathyroid glands. It is more difficult to detect smaller glands than larger ones.

Trying to explain the reasons for different accumulation of  $^{99m}\text{Tc}$ -sestamibi in parathyroid cells, A.Carpentier and al. (41) discovered the relation between the cells' content of the adenoma and the uptake of the radiopharmaceutical. They stated, that not only the quantity of the mitochondria, but also the quantity of the oxyphilic cells in the adenoma can influence the accumulation of  $^{99m}\text{Tc}$ -sestamibi in the tumors. If the concentration of the oxyphilic cells in the adenoma exceeds 25 %, the accumulation of  $^{99m}\text{Tc}$ -sestamibi is observed in 78 % of cases. Also, if the oxyphilic cells in the adenoma do not contain sufficient quantity of mitochondria, false negative results are possible. ( Benard F. et al.,1995)(42)

Variability of radionucleotide uptake from parathyroid adenomas is another factor, that can lead to false negative results. It is connected with differences of the perfusion, metabolic activity, oxyphilic cells content, expression of P-glicoprotein (multidrug resistance – related protein), as well as cell's cycle. (81,82)

## **1.2. Results of the nuclear-medical examinations with $^{99m}\text{Tc}$ -sestamibi in patients with secondary hyperparathyroidism (SHPT).**

The group of patients with SHPT in whom nuclear-medical examinations with  $^{99m}\text{Tc}$ -sestamibi were performed, included 6 patients -5 women and 1 men. (Fig.18) The mean age of the women was  $44,0 \pm 12,52$  years (from 31 to 63 years). The age of the man was 18 years.

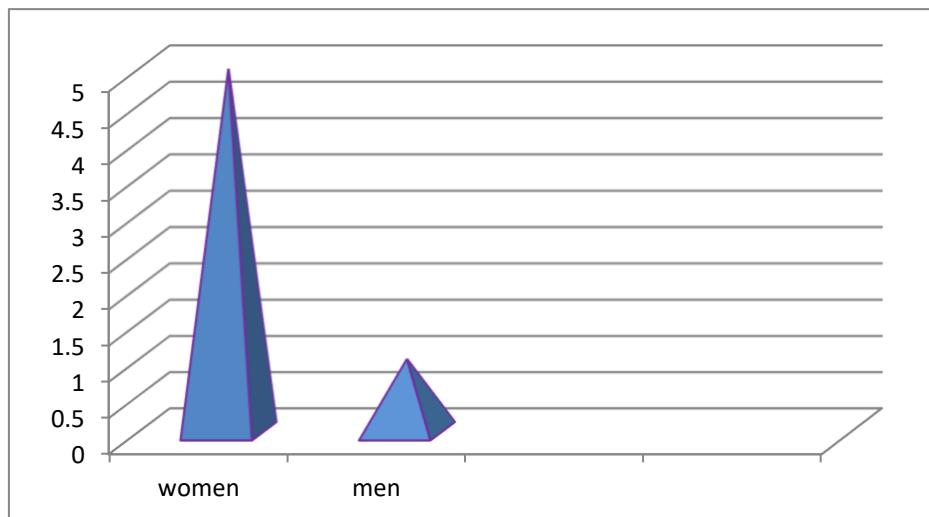


Fig. 18. Distribution of patients with SHPT by sex

All the patients were on a chronodialysis ,for a period of 2 to 5 years ,three times a week. In all patients we used the protocol for single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$  - sestamibi. In 4 patients ( 3 women and 1 men) we registered a focus of residual activity in the area of the neck. The US data were in full concordance with the scintigraphic findings. In 2 patients (women) no suspicious lesions for abnormal parathyroid glands were detected.(Fig.19)

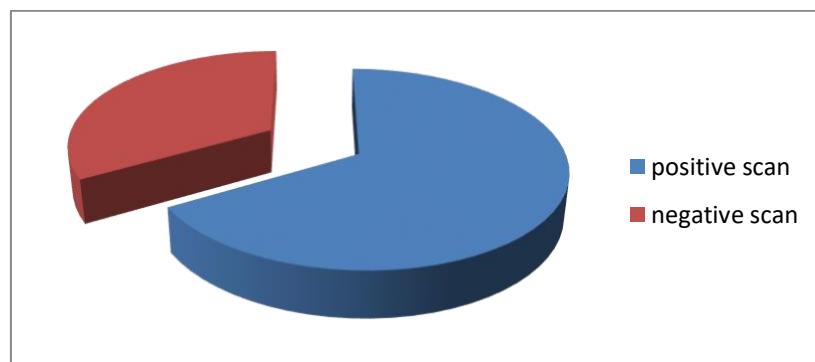


Fig. 19. Distribution of the patients with SHPT by the presence or absence of scintigraphic findings

Figure 20 represents the early and late static images after single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$  – sestamibi of a 31 years old woman on a chronodialysis with normal value of total calcium , PTH-1065pg/ml, and US data for nodule, 12/14/12mm in size, suspicious for a parathyroid adenoma, below the left thyroid lobe.

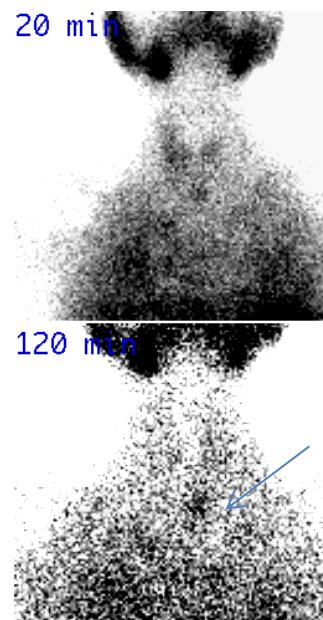


Fig. 20. Late images (120min.) show a focus of a residual activity, caudally of the thyroid gland -adenoma of the left parathyroid gland.

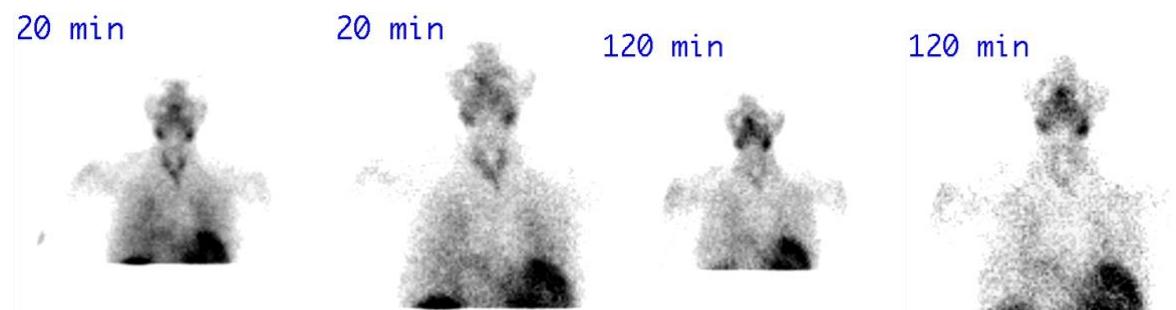


Fig. 21. A 56 years old woman. Early phase (20 min.) diffuse accumulation of nucleotide in the thyroid, an area of accumulation below the thyroid gland is also seen. Late phase (120 min.)

only the extrathyroidal accumulation of the radiopharmaceutical is seen - parathyroid adenoma.

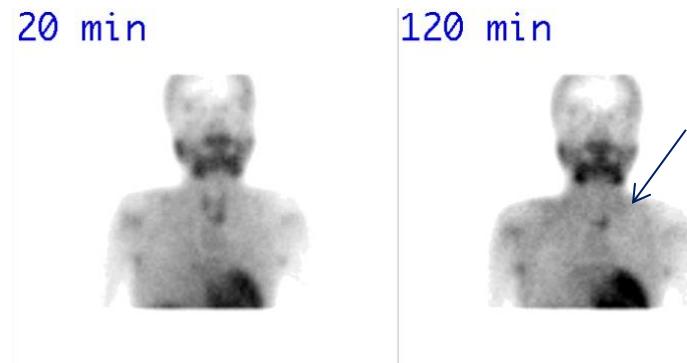


Fig. 22. The late phase -a focus of residual activity is seen - parathyroid adenoma

Figure 22 represents images after single-isotope, dual-phase scintigraphy with  $^{99\text{m}}\text{Tc}$  – sestamibi of a 18 years old man on a chronodialysis with total calcium 2,42 mmol/l, phosphate 2,27 mmol/l, ionized calcium 1,54 mmol/l and PTH 1007,0 pg/ml. The early images (20 min.) registered diffuse accumulation in the area of the thyroid gland with a hyperfixating zone , located caudally of the left lobe. The late images (120 min) a sufficient washout of the nucleotide from the thyroid was observed. Only a focus of residual activity , located below the left lobe was present.

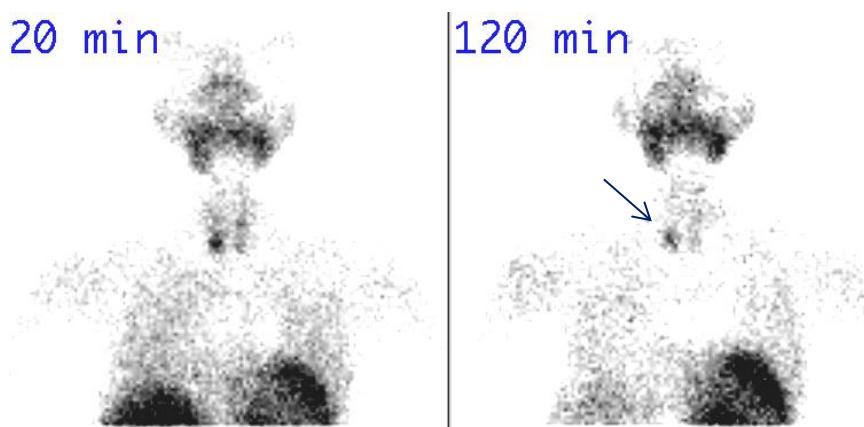


Fig. 23. Dual-phase scintigraphy with  $^{99\text{m}}\text{Tc}$  – sestamibi in a 45 years old woman with SHPT. The late images register only a focus of residual activity below the right lobe- adenoma of the right parathyroid gland.

Although ,without statistical significance, because of the smaller number of the women with SHPT, we calculated the mean values of total calcium, phosphate, ionized calcium and PTH. (Tabl. 3)

In the male patient the laboratory variables were as follows: total calcium 2,42 mmol/l, phosphate 2,27 mmol/l, ionized calcium 1,54 mmol/l and PTH 1007,0 pg/ml.

Table 3. Mean values of total calcium, ionized calcium, phosphate and PTH in women with SHPT examined with  $^{99m}\text{Tc}$  – sestamibi scintigraphy. The results are shown as mean values  $\pm$  standard error ( $x \pm Sx$ ).

Variable	Positive scan N 3	Negative scan N 2	p
Ca mmol/l	2.593 $\pm$ 0.049	2.621 $\pm$ 0.051	=0.062
P mmol/l	1.966 $\pm$ 0.256	1.973 $\pm$ 0.313	=0.12
Ca++ mmol/l	1.44 $\pm$ 0.110	1.54 $\pm$ 0.132	=0.072
PTH pg/ml	1098.366 $\pm$ 68.447	1736,50 $\pm$ 663.5	=0.071

In women with SHPT with positive and negative scans, there was no difference of the above mentioned variables.

Our data showed, that  $^{99m}\text{Tc}$ - sestamibi was accumulated with high intensity in solitary adenomas of the parathyroid glands-in 65 % of patients. In 35 % the scans were negative. Because for subtractional scintigraphy , two radionucleotides should be applied, we consider this to be inappropriate for the patients with SHPT, because of their general condition, and single-isotope method should be used. The results showed that this method helped to discover nodular lesions, but did not show high sensitivity for hyperplastic parathyroid glands.

None of the patients with SHPT, had undergone surgical procedures.

### **Negative scans in patients with SHPT**

False negative results could be due to the fact that oxyphilic cells of the adenoma do not contain enough amount of mitochondria. (42)

It is not known the reason, why not all changed parathyroid glands accumulate the radiopharmaceutical. May be this can be due to a different level of cells' activity and proliferation. There is a correlation between the accumulation of the radionucleotide and the stage of autonomy of the cells of

the adenoma, i.e. the mechanism of calcium suppression of PTH secretion is disturbed. The cells of the parathyroid adenomas have a higher threshold for calcium suppression, or have no threshold at all (Лавин Н., 1999)(29). As a result of this, these cells secrete higher amounts of PTH, have increased metabolism and ability to accumulate  $^{99m}\text{Tc}$ - sestamibi. Hyperplastic parathyroid cells have preserved their functional connection with the body and respond to the metabolic signals for suppression of their function, and respectively have lower rate of metabolism and do not accumulate the radiopharmaceutical.

## **2.Nuclear-medical examinations with radiopharmaceutical $^{99m}\text{Tc}$ -tetrofosmin**

We performed nuclear – medical examinations with radiopharmaceutical  $^{99m}\text{Tc}$ -tetrofosmin in 42 patients (33 women and 9 men).

The obtained results from the nuclear – medical examinations with  $^{99m}\text{Tc}$ -tetrofosmin (Fig.24) we divided in two groups:

1. In patients with primary hyperparathyroidism (PHPT).
2. In patients with secondary hyperparathyroidism (SHPT).

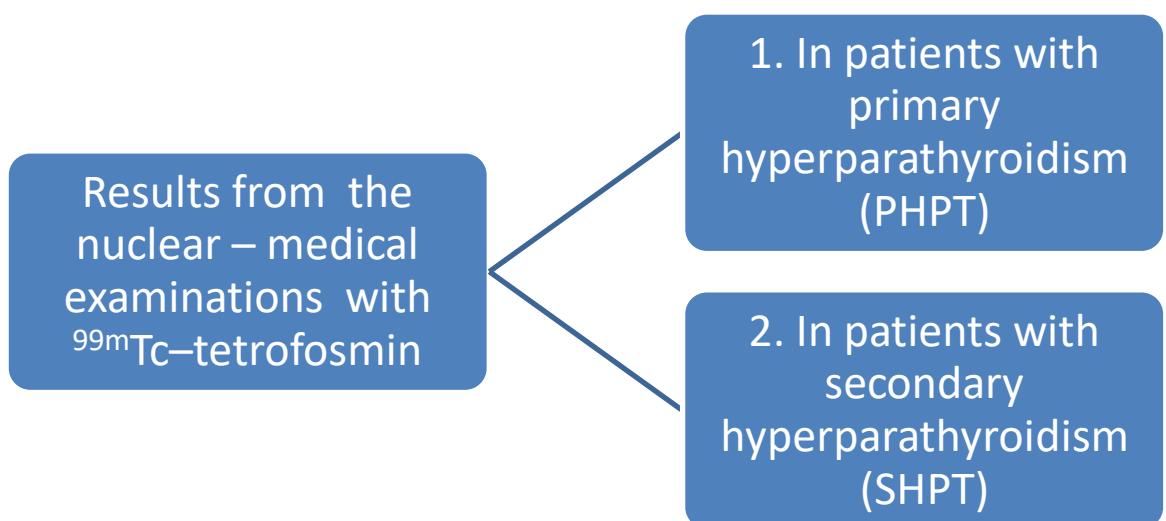


Fig. 24. Results of nuclear-medical examinations with  $^{99m}\text{Tc}$ -tetrofosmin in patients according to the type of hyperparathyroidism

## **2.1. Results of nuclear-medical examinations with $^{99m}\text{Tc}$ -tetrofosmin in patients primary hyperparathyroidism (PHPT)**

We performed nuclear – medical examinations with radiopharmaceutical  $^{99m}\text{Tc}$ -tetrofosmin in 42 patients (33 women and 9 men. The mean age of the women was  $58,15 \pm 10,90$  years (from 37 to 78 years), and that of the men was  $54,0 \pm 8,71$  years (from 44 to 60 years).

One female patient was with MEN syndrome type 1, 5 patients (female) were with a case history of kidney stones, 6 patients (female) were with osteoporosis diagnosed with DXA , 2 patients (female) were with total thyroidectomy due to benign thyroid nodules. One patient (female) was with US data for thyroid nodules at the time of examination and in all the rest the disease had no symptoms.

We used a protocol for single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin combined with SPECT in 26 (81,3%) patients.

In 6 (18,7%) subjects we applied dual-isotope subtractional method with  $^{99m}\text{Tc}$ -pertechnetate/ $^{99m}\text{Tc}$ -tetrofosmin, combined with SPECT according to the protocol.

From total 32 patients with PHPT, we detected suspicious for parathyroid adenoma lesions in 15 (46,8%) cases. In the rest 17 (53,2%) patients there were no such lesions in the areas of the neck and mediastinum. (Fig.25)

Totally, we have managed to visualize 15 hyperfunctioning parathyroid adenomas, and they were located as follows – 5 right inferior, 8 left inferior, 1 intrathyroid and 1 in the mediastinum.

In 22 patients there were previous US, and in 4 CT data for nodules suspicious to be abnormal parathyroid glands.

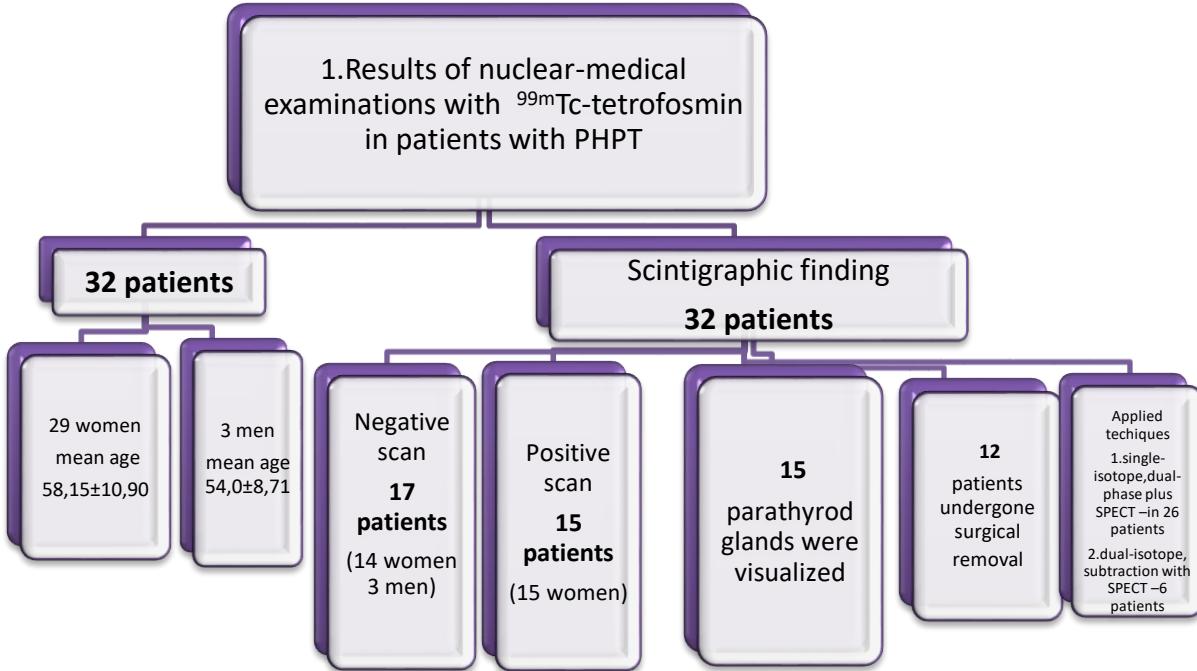


Fig. 25. Results of nuclear-medical examinations with  $^{99m}\text{Tc}$ -tetrofosmin in patients with primary hyperparathyroidism

According to the presence or absence of scintigraph findings we devided the patients into two groups:

**A.** Patients with PHPT and scintigraphic data for zones of a residual activity in the areas of the neck and mediastinum, suspicious for parathyroid adenoma.

**B.** Patients with PHPT and no scintigraphic data for zones of a residual activity in the areas of the neck and mediastinum, suspicious for parathyroid adenoma.

**Group A. Patients with PHPT and scintigraphic data for zones of a residual activity in the areas of the neck and mediastinum, suspicious for parathyroid adenoma.**

From total 32 patients with PHPT, we detected suspicious for parathyroid adenoma lesions in 15 (46,8%) cases . In 26 cases we performed single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin combined with SPECT and in 6 dual-isotope subtractional method with  $^{99m}\text{Tc}$ -perstehnetat/ $^{99m}\text{Tc}$ -tetrofosmin, combined with SPECT. Negative scans were detected in 17 (53,2%) patients.

With the single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin combined with SPECT , we registered zones of hyper fixation 9 (34,61%) out of 26 patients. In all of them, the follow up showed that there were parathyroid

adenomas post surgically. When we analyzes the results only from single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin without SPECT, we succeeded to receive positive scans only in 6 cases, while 3 cases proved to be with false negative results. We determined the sensitivity of this dual phase scintigraphy to be 66,66% (6/9). When we combined the results with early SPECT, we received in this group positive scans in 8 patients, while only 1 proved to remain false negative. The sensitivity of the combination of both methods was 88,88% (8/9). The last patients was with false negative result, because he had an intrathyroid located parathyroid adenoma, which was not visualized with SPECT.

The result showed that SPECT increased diagnostic sensitivity of the single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin with 22,22%.

According to the number of the histologically proven parathyroid adenomas , we determined the sensitivity of the single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin. The sensitivity showed to be 69,23% (9/13), because apart from those 9 cases with positive scans, there were also 4 patients with false negative results, who were operated and parathyroid adenomas were proven.

In 6 (18,7%) subjects we applied dual-isotope subtractional method with  $^{99m}\text{Tc}$ -pertechnetat/ $^{99m}\text{Tc}$ -tetrofosmin, combined with SPECT according to the created by us protocol. Positive scans were registered in all 6 patients. All 6, had undergone surgery, and parathyroid adenomas were established. Only with subtractional method with  $^{99m}\text{Tc}$ -pertechnetat/ $^{99m}\text{Tc}$ -tetrofosmin, we received 5 positive scans , and 1 was false negative. So the sensitivity proved to be 83,33% (5/6). While with SPECT we received 6 positive scans and the sensitivity was 100% (6/6).

SPECT increased diagnostic sensitivity of the subtractional method with  $^{99m}\text{Tc}$ -pertechnetat/ $^{99m}\text{Tc}$ -tetrofosmin with 16,67%.

The patients were followed up. Twelve of them were operated. The patient with a suspicious mediastinally located parathyroid adenoma refused operation. Due to the advanced age and comorbidity , 2 patients considered to be too risky for surgery.

In 1 patient we registered parathyroid adenoma in the mediastinum, supported by the CT data. The patient refuses to be operated. Fig. 26.

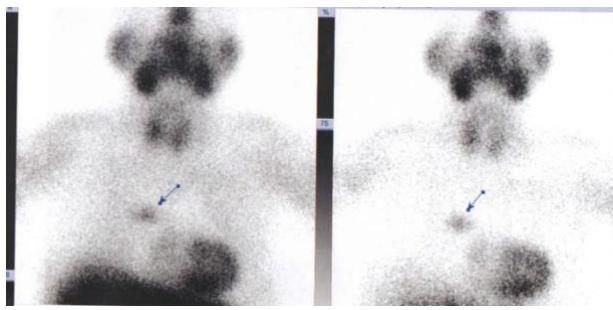


Fig. 26. A 65 years old woman with total calcium 2,95 mmol/l, ionised calcium 1,53 mmol/l, phosphate 0,82 mmol/l and PTH 235,3 pg/ml. On the early and late planar images a hyper fixating zone, located centrally in the mediastinum is seen. This cscan is a highly suspicious for parathyroid adenoma in the medastinum (see the arrow).

$^{99m}$ Tc-tetrofosmin is another myocardial perfusion agent, also used for parathyroid scintigraphy, but there are limited data in the literature for its use.

$^{99m}$ Tc-tetrofosmin has some similarities with  $^{99m}$ Tc-sestamibi, but its mechanism of accumulation in the cells is different. Apart  $^{99m}$ Tc-sestamibi, which accumulation depends on mitochondria's membrane potential, retention of  $^{99m}$ Tc-tetrofosmin depends mainly on cell's membrane potential. (49)

Our data pointed out that  $^{99m}$ Tc-tetrofosmin, showed slower wash out from the thyroid on the late planar images (120 min.) in some cases. This needed to obtain additional later than on 120-th min planar images- between 150-160 minute. The results showed that this slower wash out made  $^{99m}$ Tc-tetrofosmin to be unsuitable for performing single-isotope, dual-phase scintigraphy. There some facts in the literature, which also confirm our.(49)

To avoid misleading for the interpretation of the results, prolongation of the time for which the radiopharmaceutical is kept in the thyroid adenomas, always an US should be performed, especially in iodine deficient areas. (52)

While, analyzing the results of the scintigraphic examinations with  $^{99m}$ Tc-tetrofosmin, we have noticed that early images on the 15-th min were with better quality than the late ones on the 120-th min. (Fig. 27).

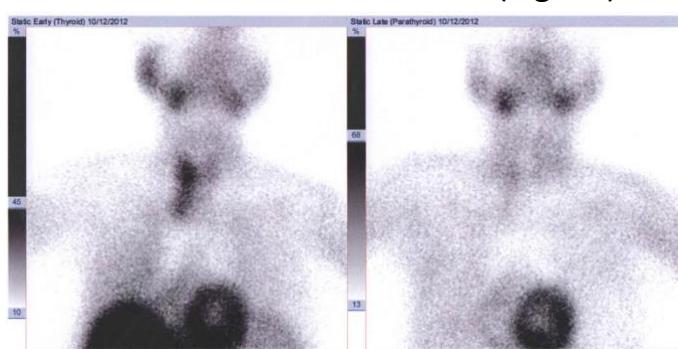


Fig. 27. Early planar images (20 min.) are with better quality.

Our data showed, that early SPECT gave opportunity to visualize adenomas, that were not seen on the late p, which probably was due to rapid wash out of the radiopharmaceutical from some adenomas, as well as to the small sizes of the adenomas.

Figure 28a and Figure 28b. represent images after single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin of a 50 years old woman with PHPT with total calcium 2,7 mmol/l, phosphate 0.7 mmol/l, ionized calcium 1,37 mmol/l and PTH 119.3 pg/ml. The early images (20 min.) registered diffuse accumulation in the area of the thyroid gland with a hyperfixating zone , located caudally of the right lobe. On the late images (120 min) no such zone was seen. The patient was operated, and a parathyroid adenoma 10mm/15 mm was removed.

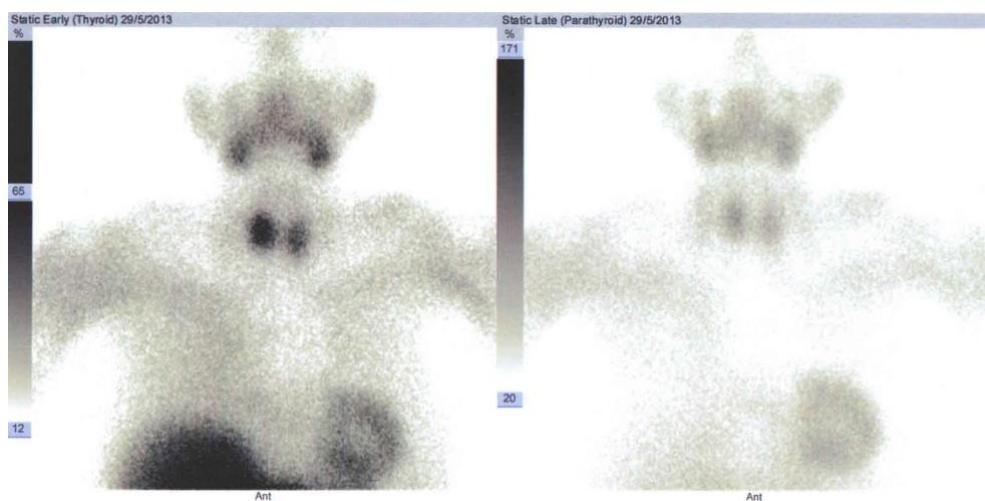


Fig. 28a. Late planar images (120 min.) - negative scan

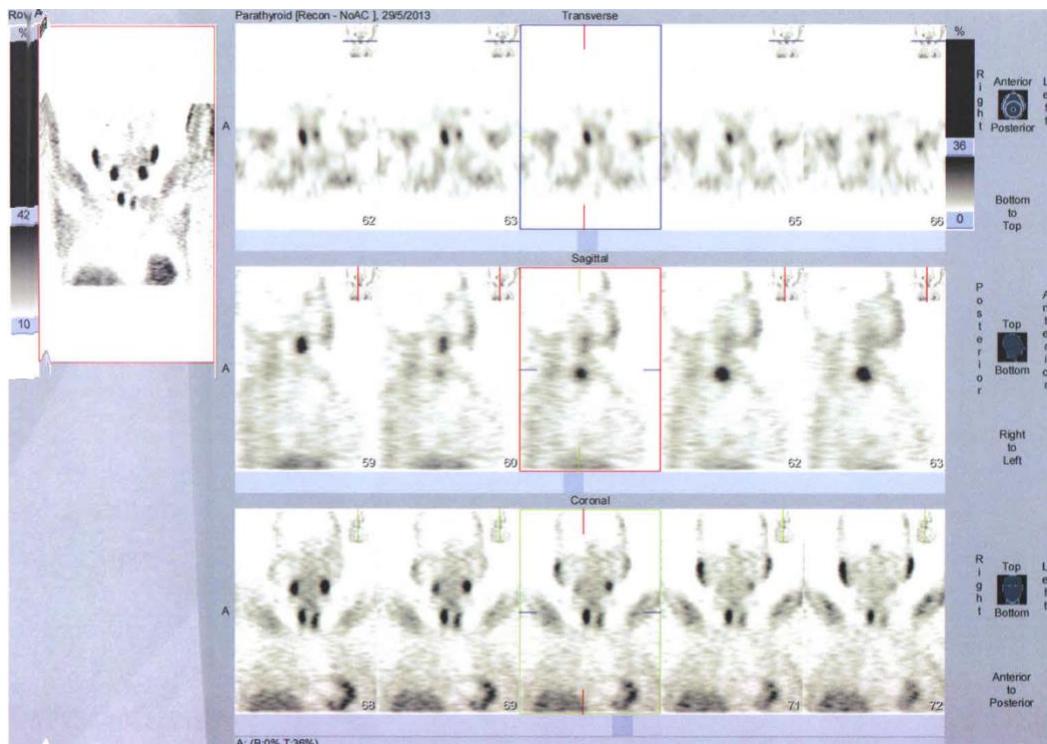


Fig. 28b. SPECT images – a hyperfixating zone located dorsally of the lower right lobe seen – adenoma of right parathyroid gland.

In patients examined with single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin combined with early SPECT, the latter helps adenomas with rapid wash out of the radiopharmaceutical to be detected, and also improves topical localization of the abnormal parathyroid glands to be done and can be of help for future operation.

Figure 29a and Figure 29b and Figure 29 c present images obtained from single-isotope, dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin combined with early SPECT in a 54 years old woman with PHPT.

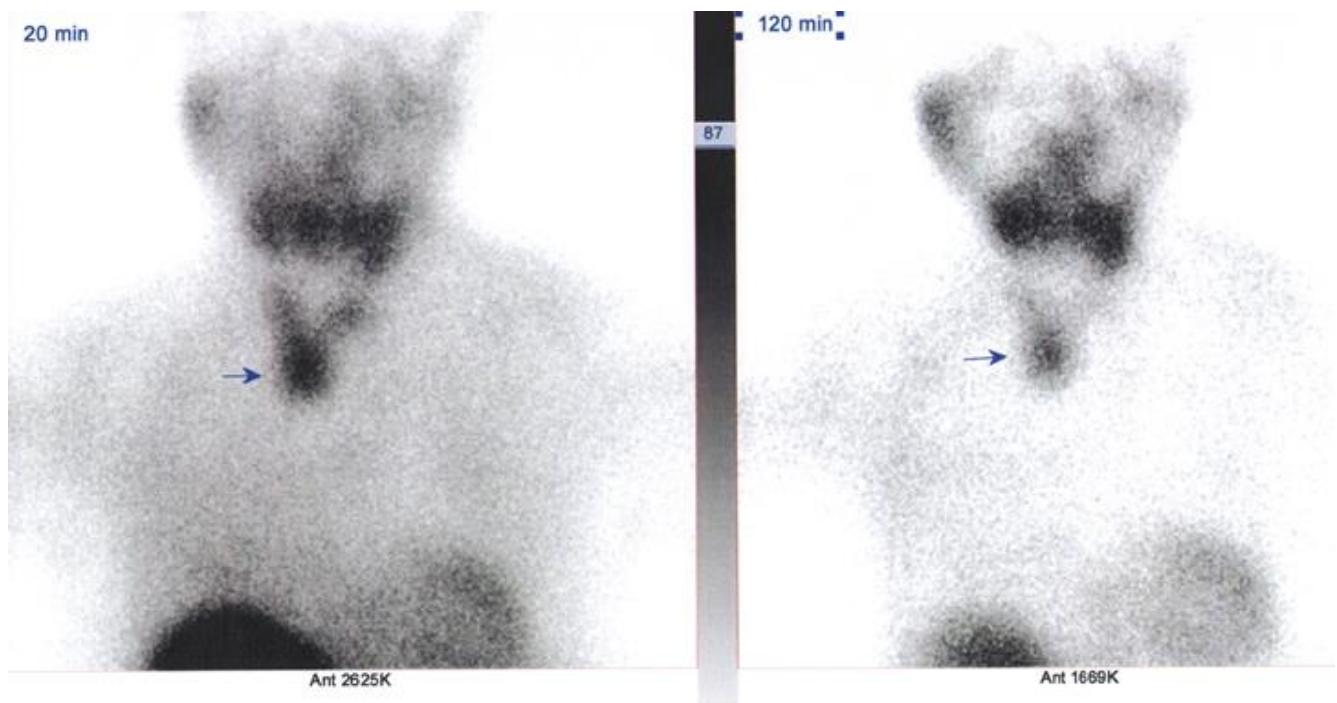


Fig. 29a.– early phase (20 min.)

Fig. 29b. – late phase (120 min.)

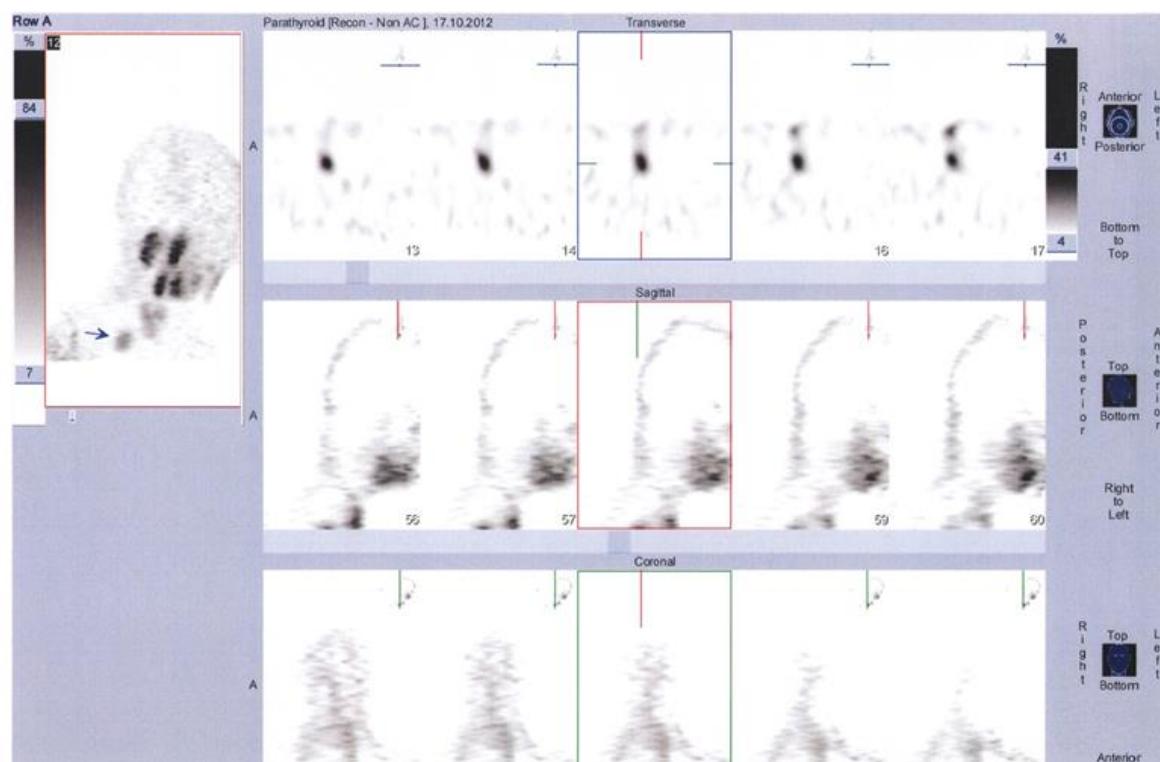


Fig. 29c. – SPECT images

On figure 29a (early phase) thyroid gland and a separate hyperfixating zone located caudally of both thyroid lobes are seen. Figure 29b (late phase) shows a relatively complete wash out of the radiopharmaceutical from the thyroid, only the caudally located zone of hyperfixation is seen. This is suspicious for ectopic parathyroid adenoma. Early SPECT images show (fig.29c) the above zone is located dorsally and caudally of the thyroid. Post surgically it was proven that this exactly was an ectopic parathyroid adenoma.

#### **Dual-isotope subtractional method with $^{99m}\text{Tc}$ -pertechnetate / $^{99m}\text{Tc}$ -tetrofosmin combined with early SPECT.**

We performed dual-isotope subtractional method with  $^{99m}\text{Tc}$ -pertechnetate /  $^{99m}\text{Tc}$ -tetrofosmin combined with early SPECT in 6 patients.

Figure 30a and Figure 30b represent images of dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetate /  $^{99m}\text{Tc}$ -tetrofosmin combined with early SPECT in 43 years old woman with PHPT.



Fig. 30a. The upper row images of the thyroid gland on the right and of the parathyroid gland on the left are presented. The lower row shows subtractional images representing adenoma of left parathyroid gland.

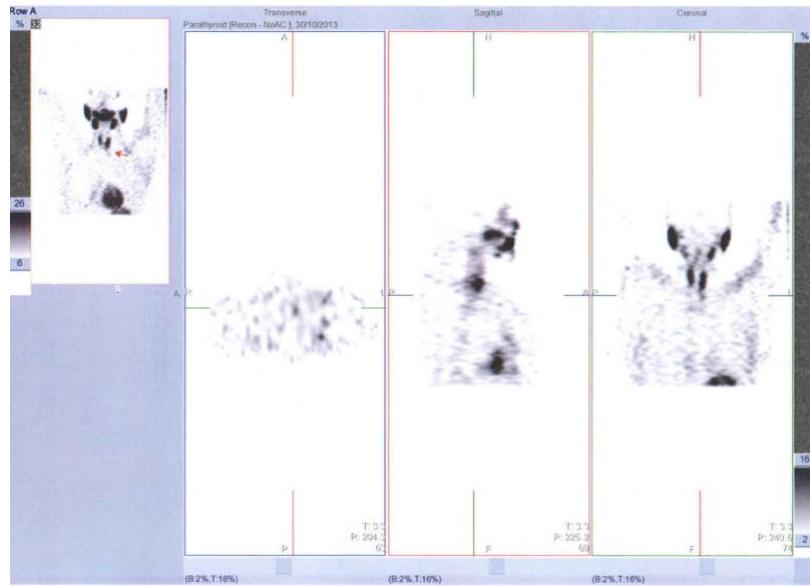


Fig. 30b. Early SPECT images showing an area of hyper fixation, located caudally of the left thyroid lobe.

Our results showed that  $^{99m}\text{Tc}$ -tetrofosmin was not suitable for single-isotope dual-phase scintigraphy, because of the slow wash out of the radiopharmaceutical from the thyroid gland. It should be combined with SDPECT, and when this was done the sensitivity was increased with 22,22%. Early images on the 20-th minute, were with better quality than the late ones obtained on the 120-th minute. We think, that early SPECT had some advantages, considering the early wash out of the radiopharmaceutical from some adenomas, as well as slow wash out from the thyroid gland, i.e.in comparison  $^{99m}\text{Tc}$ -sestamibi ,  $^{99m}\text{Tc}$ -tetrofosmin did not show different rate of elimination from the thyroid and parathyroid glands. (89)

We determined the sensitivity of 83,33% the dual-isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetate / $^{99m}\text{Tc}$ -tetrofosmin, alone, and when combined with SPECT, the sensitivity was increased with 16,67% up to 100 %.

As disadvantages of the method we consider:

- The need two radionucleotides to be injected;
- The need of full collaboration of the patient's side to stand still ;
- The need the patient to be positioned very precisely under the collimator;
- The possible artefacts, after digital subtraction has been performed;;
- The need the patient has been stopped intake of any drugs that can interfere with  $^{99m}\text{Tc}$  intake in the thyroid, for an appropriate period of time.

Our results showed, that with the use of different methods of examination with  $^{99m}\text{Tc}$ -tetrofosmin predominantly adenomas engaging lower parathyroid glands on both sides could be detected.

**Group B. Patients with PHPT and no scintigraphic data for zones of a residual activity in the areas of the neck and mediastinum, suspicious for parathyroid adenoma**

Total number of patients with negative scans was 17- 14 women and 3 men.

The mean values of total and ionized calcium, phosphate and PTH for this group are shown in Table 4.

Table. 4. Mean values of total calcium, ionized calcium, phosphate and PTH in women with PHPT examined with  $^{99m}\text{Tc}$ -tetrofosmin scintigraphy. The results are shown as mean values  $\pm$  standard error ( $x \pm Sx$ ).

Variable	Positive scan N 15	Negative scan N 14	p
Ca mmo/l	2.898 $\pm$ 0.070	2.665 $\pm$ 0.073	<0.05
P mmo/l	0.75 $\pm$ 0.080	0.83 $\pm$ 0.137	=0.076
Ca++ mmo/l	1.603 $\pm$ 0.054	1,388 $\pm$ 0.777	<0.05
PTH pg/ml	745.4 $\pm$ 140.335	215.849 $\pm$ 38.80	<0.01

The results presented in table 4, show that in women with PHPT and positive scans, there were statistically significant higher levels of total calcium, ionized calcium, and PTH in comparison with the group of women with negative scans. Such difference for phosphate was not detected.

We calculated the mean volume of the detected by US adenomas in patients with positive scans – 1,032ml $\pm$ 0,396 SE, and in patients with negative scans – 0,44ml $\pm$ 0,202 SE .

Our results showed, that when compare the mean volumes of the detected by US adenomas in patients with positive and negative scans after  $^{99m}\text{Tc}$ - tetrofosmin scintigraphy, the positive results were related with larger parathyroid glands, and the difference between two groups was statistically significant ( $p<0,05$ ).

The small number of men with negative scans, did not allow statistical analyses of the laboratory variables to be performed.

Men with negative scans:

Total calcium - 2,665  $\pm$  0,20 mmo/l

Phosphate –  $0,83 \pm 0,23$  mmol/l  
Ionised calcium –  $2,58$  mmol/l  
PTH -  $94,8 \pm 32,80$  pg/ml

The precise mechanism of the selective  $^{99m}\text{Tc}$ -tetrofosmin uptake in the abnormal parathyroid glands is still debated. Higher mitochondrial activity is considered to be of great importance in patients with PHPT.

Several reasons for negative scans can be pointed out.

**a) biochemical factors**

- **Total calcium levels** – higher preoperative calcium levels are more frequently seen in patients with positive scans.
- **Parathyroid hormone levels.**

A significant correlation between radiopharmaceutical uptake and preoperative levels of PTH is observed. As higher PTH is, as higher is the possibility for positive scans to be obtained.

- **Vitamin D levels.**

Patients with vitamin D deficiency are more likely to have positive scans.

Suboptimal levels of vitamin D, can stimulate the growth of the parathyroid adenomas by an independent from the hypocalcemia and deficit of 1,25-dihydroxyvitamin D, and can change the set-point of calcium suppressive effect upon PTH secretion. (99).

- **Calcium-channel blockers**

The use of calcium-channel blockers can influence the uptake of the radiopharmaceutical in the parathyroid cells and to diminish the sensitivity of the method.

**b) biological factors**

- **Size.** Although considered to be very important, it is not the only determining the results factor.

– **Cell content of the parathyroid adenoma.** Because oxyphilic cells contain more mitochondria, they uptake technetium isotopes to a larger extent.

- **P glycoprotein and MDR gene products**

Uptake of  $^{99m}\text{Tc}$ -sestamibi and  $^{99m}\text{Tc}$ -tetrofosmin in the cells of the parathyroid adenomas depends on the activity of the P glycoprotein coded by MDR gene, which is functioning as an ATP dependent efflux pump, protecting

against accumulation of lipophilic cationic radiopharmaceuticals, including,  $^{99m}\text{Tc}$ -tetrofosmin (100).

The expression of P glycoprotein in the parathyroid adenomas appears to be important factor determining radiopharmaceutical uptake

In one study 71% (10 out of 14) of adenomas with strong P glycoprotein membrane activity have shown negative scans, 70% (45 out of 64) with negative P glycoprotein expression ( $p=0.006$ ) have shown positive scans. (101)

– **Engagement of more than one parathyroid gland**

It showed to be difficult to visualize more than one hyperfunctioning parathyroid glands by performing scintigraphy. The possible explanation of this, includes preferential accumulation of the radiopharmaceutical in one dominating parathyroid gland.

**Technical factors**

- a) evolution of the parathyroid scintigraphy
- 6) imaging techniques

## **2.2. Results of $^{99m}\text{Tc}$ -tetrofosmin nuclear-medical examination in patients with secondary hyperparathyroidism (SHPT)**

The group of patients with SHPT, in which nuclear-medical examination was conducted with  $^{99m}\text{Tc}$ -tetrofosmin, included a total of 10 patients.

Of these, 4 female patients and 6 male patients. The mean age of women was  $54.5 \pm 2.12$ . The mean age of men was  $53.4 \pm 12.93$ .

All patients were on chronodialysis 3 times a week for a period of 2 to 5 years. A protocol for a single-isotope two-phase method with  $^{99m}\text{Tc}$ -tetrofosmin combined with SPECT technique was used for all patients. In 4 patients (3 female and 1 male) areas suspected of parathyroid adenomas were registered. (Fig. 31)

There is no data for patients who underwent an operative intervention.

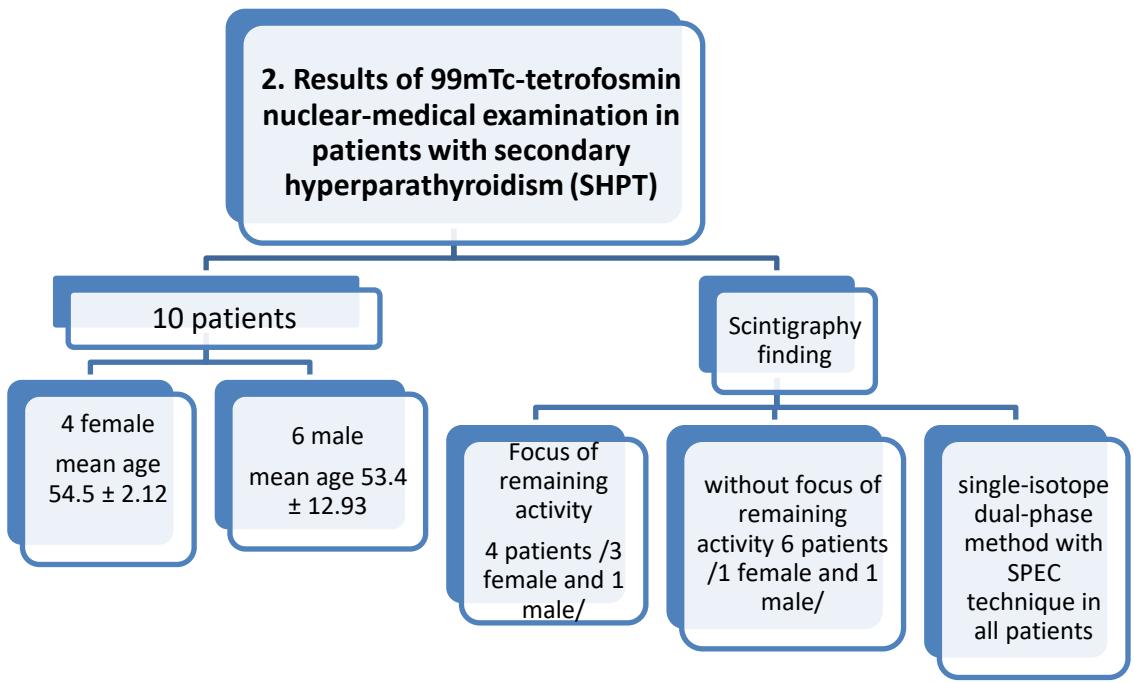
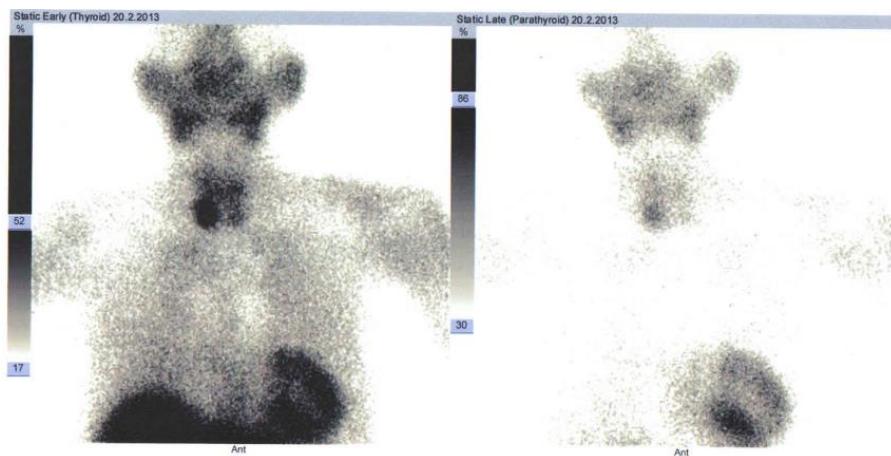


Fig. 31. Results of 99mTc-tetrofosmin nuclear-medical examination in patients with SHPT

On Fig. 32a and Fig. 32b are presented images of the single-isotope dual-phase <sup>99m</sup>Tc-tetrofosmin combined with SPECT technique in a 56 years old female patient with a diagnosed SHPT.



20 min.

120 min.

Fig. 32a. On the late planar images (120 min) a zone of residual activity is visualized in the caudal part of right thyroid lobe – adenoma of the right parathyroid gland.

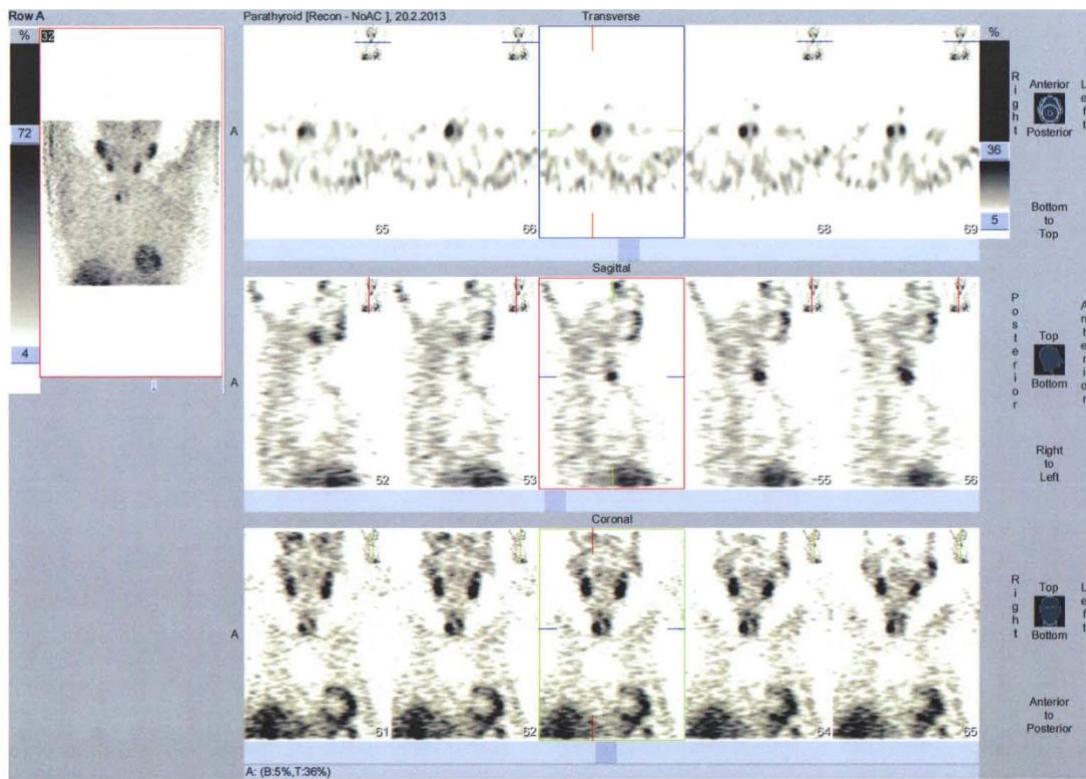


Fig. 32b. On the early SPECT images a hyperfixating zone in the caudal part of the right thyroid lobe is visualized

In 3 patients, areas suspicious for adenoma of the parathyroid glands were visualized only on the early SPECT images and did not register on the late planar images (120 min). This may be associated with faster wash out of the radiopharmaceutical in some adenomas.

On Fig. 33a. and Fig. 33b. we present the images of  $^{99m}\text{Tc}$ -tetrofosmin single-isotope dual-phase procedure combined with SPECT technique in a 37 years old male with diagnosed SHPT.

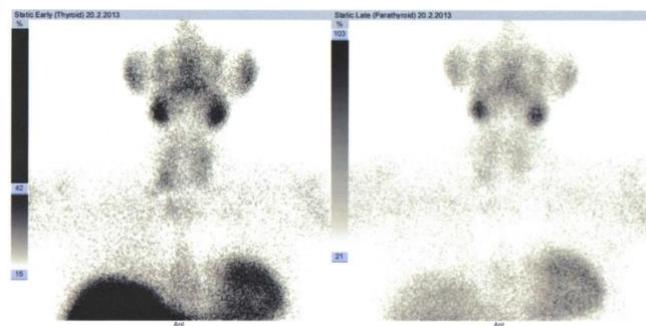


Fig. 33a Late phase (120 min) - no focus of residual activity is registered

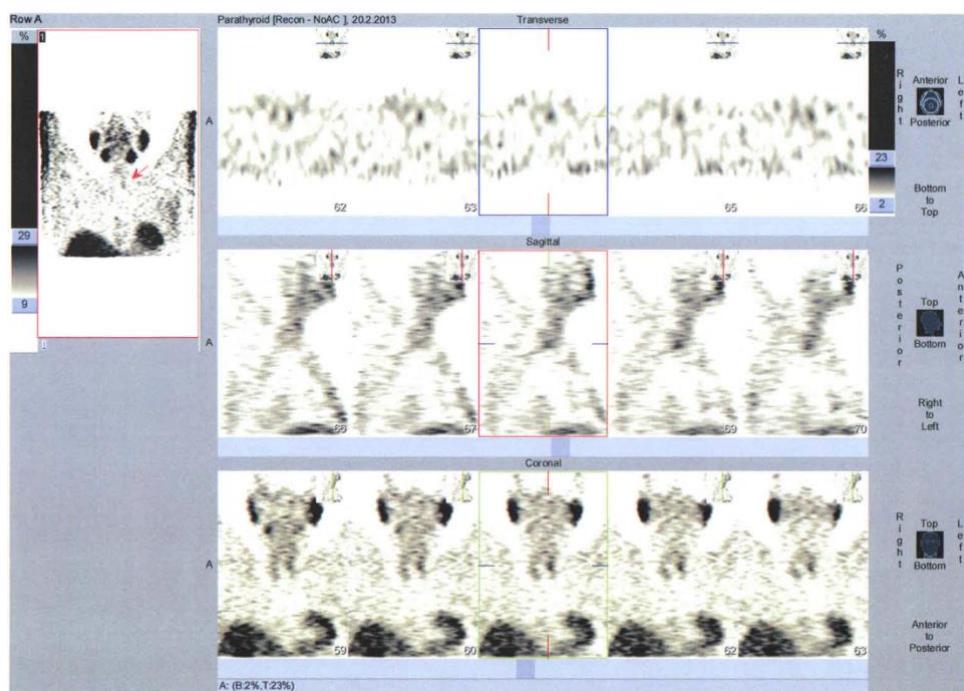


Fig. 33b. Early SPECT images – a hyperfixating zone is registered in the caudal part of the left thyroid lobe, suspicious for parathyroid adenoma

The small number of patients does not allow for a reliable statistical evaluation of the parameters of the calcium-phosphorus metabolism.

We determined the mean values of the following parameters - total serum calcium, phosphorus and PTH. In female patients a positive scan:

serum calcium -  $2.735 \pm 0.26$  mmol/l

phosphorus -  $3,11$  mmol/l

PTH -  $1791.0 \pm 185.26$  pg/m

A negative scan was registered in 6 patients (1 female and 5 males).

Negative scans are most likely due to difficulties in the topical diagnosis in visualization of tumors of the parathyroid glands of small size. Smaller parathyroid glands are less likely to be detected than larger ones.

The variability in the uptake of the radiomarker in the parathyroid adenomas is another factor leading to false negative scintigraphic results and is associated with differences in perfusion, metabolic activity, number of oxyphilic cells, P-glycoprotein expression and multidrug resistance-related protein expression and the different phases of the cell cycle.

Hyperplastic parathyroid glands usually show faster wash out in comparison to adenomas, which makes them more difficult to detect.

Negative scintigraphic scans may be associated with the possibility of suppression of the accumulation of  $^{99m}\text{Tc}$ -tetrofosmin in the parathyroid cells as a result of Calcitriol intake.

The use of calcium channel blockers may affect the accumulation of  $^{99m}\text{Tc}$ -sestamibi by parathyroid cells and reduce the sensitivity of the method. Friedman et al. Found that negative scans are twice as likely in patients taking calcium antagonists than those who do not take these medications. (OR2, 88.95% CI, 1.03-8.10, p 0.045) (103)

The summarized results indicate that scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin does not show a high sensitivity in identifying hyperplastic parathyroid glands.

# **V.DIAGNOSTIC ALGORITHM AND PROTOCOLS FOR EVALUATION OF ABNORMAL PARATHYROID GLANDS IN PATIENTS WITH PHPT AND SHPT**

## **1.ALGORHYTHM**

Our results show that it should include:

- a) on the first place: clinical and laboratory data for the presence of PHPT and SHPT
- b) on the second place : ultrasonographic examination
- c) and on the third place scintigraphy with  $^{99m}\text{Tc}$ - sestamibi or  $^{99m}\text{Tc}$ - tetrofosmin. In patients with PHPT, we recommend either single isotope dual-phase scintigraphy or combination of subtractional technique and SPECT. In SHPT we prefer single isotope dual-phase scintigraphy combined with SPECT.

## **2.PROTOCOLS**

1. We prepared a protocol for combination of dual-phase scintigraphy with SPECT. (single-isotope, dual-phase method with  $^{99m}\text{Tc}$ -sestamibi or with  $^{99m}\text{Tc}$ - tetrofosmin in combination with early SPECT). We injected an intravenous bolus of 740 MBq  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin. We recorded early (20 min after the injection) high counted images for 10 min of the neck and chest of the patient in a supine position. Immediately, afterwards in the same positioning of the patient, we performed SPECT-tomographic registration of the neck and upper part of the thorax. The examination was performed with a low-energy collimator on a circle orbit  $360^0$ , "Step and shot" registration , matrix 128x128, 60 projections 32 sec each.,at an interval of  $3^0$  – for 30 mi. The images were digitally reconstructed with Iterative Reconstruction. On the 120-th minute we obtained late planar images of the neck and chest.

2. We prepared a protocol for combining of subtractional method with SPECT technique (dual isotope  $^{99m}\text{Tc}$ -pertechnetate/ $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -pertechnetate / $^{99m}\text{Tc}$ -tetrofosmin method in combination with early SPECT technique). The patient was intravenously injected with 37MBq  $^{99m}\text{Tc}$ -pertechnetate. On the 30-th minute afterwards we recorded 10 minutes highly counted images of the thyroid gland of the patient in a supine position. After obtaining the images of the thyroid gland, we injected a intravenous bolus of 555 MBq  $^{99m}\text{Tc}$ - sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin, without changing the position of the patient, staying under the collimator of the gamma-camera. 10-15 minutes after the injection of  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin we obtained a static images of the neck and the chest. Immediately afterwards we performed an early SPECT technique. The examination was performed in tomographic mode with low-energy collimator on a circle orbit of  $360^0$ , "Step and shot" registration, matrix 128x128, 60 projections of 32 sec, at an interval of  $3^0$  – totally 30 min. Reconstruction of the images with Interative Reconstruction was done. In some patients we obtained also late planar images of the neck and chest.

## VI.CONCLUSIONS

1. Single isotope dual- phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin is easily performed for visualization of hyperfunctioning parathyroid adenomas in patients with PHPT and SHPT, because no preliminary preparation of the patients is needed. Our study reveals that sensitivity of this method is high if it is performed with  $^{99m}\text{Tc}$ -sestamibi (90,90%), rather than with  $^{99m}\text{Tc}$ -tetrofosmin (69,23%).
2. Disadvantage of the single isotope dual- phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi or  $^{99m}\text{Tc}$ -tetrofosmin is that hyperfunctioning parathyroid adenomas with rapid washout of the radionucleotide could be missed. This makes its combination with early SPECT necessary. Single use of single isotope dual- phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin in patients with PHPT and SHPT is with less significance because of the poor quality of the obtained images and later washout of the nucleotide from the thyroid gland.
3. SPECT is a reliable additional method combined with single- isotope and subtractional methods for visualization of hyperfunctioning adenomas in patients with PHPT and SHPT. It doesn't cause additional and unnecessary exposure of the patients to the gamma-rays and can increase the sensitivity of the method. Our data showed that early SPECT is with greater importance, as it increased sensitivity from 88.88% to 100%. Its use increased diagnostic sensitivity of the single isotope dual-phase scintigraphy with 22.22% (in the group with  $^{99m}\text{Tc}$ -tetrofosmin), while combined with subtractional methods increased sensitivity with 16.67% in both radionucleotides.
4. Dual isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -perthennetat/ $^{99m}\text{Tc}$  sestamibi or  $^{99m}\text{Tc}$ -perthennetat/ $^{99m}\text{Tc}$ -tetrofosmin allows visualization of

abnormal parathyroid glands after subtraction is performed—even in the early phase of obtaining images, which allows to shorten the investigation time to 80–90 minutes. It is helpful in postsurgical follow up and when more than one abnormal gland is present.

5. Disadvantages of the subtractional scintigraphy with  $^{99m}\text{Tc}$ -pertechnetate/ $^{99m}\text{Tc}$  sestamibi or  $^{99m}\text{Tc}$ -pertechnetate/ $^{99m}\text{Tc}$ -tetrofosmin are: necessity of applying of two radionucleotides, the need of full collaboration of the investigated patients, very precise positioning of the patients in this dual phase method, the probability of the presence of artefacts in the obtained images.
6. In patients with SHPT the use of single isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -sestamibi and  $^{99m}\text{Tc}$ -tetrofosmin combined with SPECT is of greater significance, while subtraction after using two consecutive radionucleotides requires preliminary measures to be taken.
7. In women with PHPT with positive scintigraphy with  $^{99m}\text{Tc}$ -sestamibi a statistically significant higher levels of total calcium, ionized calcium, phosphate and PTH were established in comparison with women with negative scan. The same statistically significant differences for the levels of total calcium, ionized calcium and PTH, but not for phosphate were found in men with PHPT and positive scans.
8. In patients with PHPT with positive scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin a statistically significant higher levels of total calcium, ionized calcium, and PTH but not for phosphate were found in comparison with those with negative scans.
9. In patients with SHPT with positive and negative scans with  $^{99m}\text{Tc}$ -sestamibi no differences of the levels of total calcium, ionized calcium, phosphate and PTH were found.

10. Positive scans with  $^{99m}\text{Tc}$ -sestamibi и  $^{99m}\text{Tc}$ -tetrofosmin were found with higher prevalence in patients with higher US determined volumes of the abnormal parathyroid glands and the difference is statistically significant( $p<0,05$ ).
11. Postsurgical follow up of the patients with positive scans shows that single isotope and subtractional methods, as well as applying SPECT are important for minimalization of the surgical procedures with faster postsurgical recovery of the patients and with less side effects.

## VII.CONTRIBUTIONS

1. The results of the nuclear -medical examinations in a comparatively large group of patients with PHPT and SHPT are summarized.
2. The advantages and disadvantages of single-isotope dual-phase scintigraphy and dual isotope subtractional scintigraphy with  $^{99m}\text{Tc}$ -sestamibi and  $^{99m}\text{Tc}$ -tetrofosmin in patients with PHPT and SHPT are pointed out from a practical point of view.
3. The role of SPECT for improving diagnostic sensitivity of single-isotope dual-phase scintigraphy and dual isotope subtractional scintigraphy is underlined.
4. A correlation between the parameters of calcium-phosphate homeostasis in patients with PHPT and SHPT is studied.
5. The diagnostic value of single-isotope dual-phase scintigraphy and dual isotope subtractional scintigraphy combined with SPECT for a precise localization of abnormal parathyroid glands is shown.
6. The risks for false negative results are analyzed.
7. Protocols for nuclear-medical examination of the parathyroid glands are created.
8. Protocols for a combination of single-isotope dual-phase scintigraphy and dual isotope subtractional scintigraphy with SPECT are created.
9. An algorithm for examination of the parathyroid glands is prepared.

## VIII.PUBLICATIONS

1.**Albena D.Botushanova, Nikolay P.Botushanov, Marianna P.Yaneva** Nuclear medicine methods for evaluation of abnormal parathyroid glands in patients with primary and secondary hyperparathyroidism. *Folia Medica* 2017, Vol.59:4;396-403

2.**Ботушанова, Албена Д., Ботушанов, Николай П.** Сравнение на сцинтиграфски образи с различни радиофармацевтици –  $^{99m}\text{Tc}$  MIBI и  $^{99m}\text{Tc}$  Tetrofosmin при един и същ пациент с първичен хиперпаратиреоидизъм. *Endocrinologia* 2017,vol. XXII :2; 98-102

3.**Албена Ботушанова, Марианна Янева, Николай Ботушанов** Двойно изотопна субтракционна методика с  $^{99m}\text{Tc}$ -pertehnetat/  $^{99m}\text{Tc}$ -setamibi за визуализация на хиперфункциониращи парашитовидни жлези при пациенти с първичен хиперхаратиреоидизъм. Научни трудове на Съюза на учените в България –Пловдив, серия Г.Медицина, фармация и дентална медицина 2016г. т. XVIII,206-212.

4. **Албена Ботушанова, Марианна Янева, Николай Ботушанов** Едноизотопна двуфазова сцинтиграфия с  $^{99m}\text{Tc}$ -setamibi и  $^{99m}\text{Tc}$ -tetrofosmin съчетан със SPECT техника при пациенти с вторичен хиперпаратиреоидизъм. Научни трудове на Съюза на учените в България –Пловдив, серия Г.Медицина, фармация и дентална медицина 2016 т. XVIII, 199-205.

### Публикувани резюмета от участие в международни научни форуми

5.M.Yaneva,**A.Botushanova** The role of combined single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin with SPECT technique in minimal invasive parathyreoidectomy EANN Congres 2015-Hamburg

## **Участие в национални научни форуми**

6. **Albena D.Botushanova, Marianna P.Yaneva , Nikolay P.Botushanov**  
Combined metod for diagnostics of abnormal parathyroid glands combining single-isotope dual-phase scintigraphy with  $^{99m}\text{Tc}$ -tetrofosmin with SPECT technique. Folia Medica 2015;Suppl.1,103-104.

**7. А.Ботушанова, М.Янева, Н.Ботушанов** Едноизотопна двуфазова сцинтиграфия с  $^{99m}\text{Tc}$ -MIBI и  $^{99m}\text{Tc}$ -Tetrofosmin съчетан със SPECT техника при пациенти с вторичен хиперпаратиреоидизъм. XVI Конгрес на БАР Suppl.2015,86-87.