



Research Article

Synthesis and Quality Control of ⁶⁸Ga-PSMA PET/CT Tracer used in Prostate Cancer Imaging and Comparison with ¹⁸F-Fluorocholine as a Reference Point

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Abstract

The clinical usefulness of choline-based PET/CT tracers (¹¹C-Choline and ¹⁸F-Fluorocholine) in prostate cancer recurrence imaging is well established. However, choline uptake is not increased in a considerable number of patients. In 2012 was proposed the new prostate-specific PET radio ligand PSMA ⁶⁸Ga-labeled, with superior sensibility and specificity than choline-based tracers. This work has as aim to describe the procedures for labeling PSMA ligands and its quality control procedures as a medicinal product and its comparison with ¹⁸F-fluorocholine synthesis and quality control procedures. Both the manual and automated synthesis methods for ⁶⁸Ga-PSMA were taken into account and set of a quality control methods based mainly on chromatographic and spectrometric methods used to determine chemical, radiochemical and radionuclide purity. PSMA synthesis time, dose of radiation to operators and quality control protocols were compared to choline. Finally, some images of PET performed with ¹⁸F-Fluorocholine and ⁶⁸Ga-PSMA in the same patients were showed.

Keywords

Radiopharmacy; Choline; PSMA; PET/CT; Prostate; Cancer; Synthesis; Quality

Introduction

Prostate cancer is one of the more frequent malignancies in developed countries, with high rates of morbidity and mortality [1]. After radical treatment, the rise of serum prostate-specific antigen (PSA) is the most important marker of recurrence. Imaging of recurrence is traditionally based on bone scan, ultrasonography and computed tomography.

In the last years, positron emission imaging (PET) with choline radiolabeled with carbon-11 or fluorine-18 was demonstrated to be able to localize recurrence site in patients with PSA level higher than 2 ng/ml [2]. Recently, a new PET radiopharmaceutical was proposed

for early localization of prostate cancer relapses: prostate-specific membrane antigen ligand ⁶⁸Ga-HBED-CC-PSMA (⁶⁸Ga-PSMA), with diagnostic performance markedly superior to ¹⁸F-fluorocholine PET [3].

Uptake of ⁶⁸Ga-PSMA reflects increased density of N-acetyl-L-aspartyl-L-glutamate peptidase 1 (NAALAD1) [4]. For this reason an increasing number of PET laboratories are switching from choline to PSMA imaging for clinical purposes.

This work has as aim to describe the procedure for labeling PSMA ligands and its quality control as a medicinal product and then to compare results of ⁶⁸Ga-PSMA and ¹⁸F-fluorocholine.

Finally, a pictorially essay of some compared ¹⁸F-fluorocholine and ⁶⁸Ga-PSMA PET images in a series of patients from our site was given.

Materials and Methods

The production of PSMA peptide labeled with ⁶⁸Ga gallium is done with the use of the Eckert-Ziegler pharmacy approved ⁶⁸Ge-⁶⁸Ga generator with and hydrochloric acid 0.1 M as an eluent. The labeling kit contains 30 µg PSMA peptide, 45 mg sodium acetate, 25mg ascorbic acid, ethanol (99,8% and 50%), water for injection, sterile filter 0,22 µm (Millipore), 0,9% NaCl and C18 SPE cartridge (Waters). The Scintomics GRP automated synthesis module is used for automated synthesis and the manual preparation is carried in a shielded hot cell. All radioactivity measurements are proceeding with use of dose calibrator. All materials and methods used during quality control are described in Quality control methods section.

Manual synthesis of ⁶⁸Ga-PSMA

A 10 ml vial containing PSMA peptide, sodium acetate and ascorbic acid (buffer pH like in Eder et al [6]) is heated in a heating block at 80°C. ⁶⁸Ge/⁶⁸Ga generator, connected directly to the synthesis vial, is eluted with 5 ml 0,1M HCl. Heating is continued for at least 10 min. After synthesis, the reaction mixture is pushed through the C18 cartridge (preconditioned with sequence of 2 ml of 99,8% ethanol, 10 ml of water and dried with 10 ml of air) to trap labeled peptide. After trapping of synthesis final product the C18 cartridge is connected to the waste vial and rinsed with 5 ml of water for injection. The final product is eluted from C18 cartridge with 0,5 ml 50% ethanol and 10 ml 0.9% saline to a 30 ml sterile vial through the sterile filter 0,22 µm. At the end of synthesis the activity product, waste vial and C18 cartridge is measured in dose calibrator (Table 1).

Automated synthesis of ⁶⁸Ga-PSMA

The preparation of the synthesis module is done by preparing and connecting the reagents to the appropriate tubing's and by filling the reactor with the 20 µl of PSMA peptide solution in water at the concentration of 1 mg/ml and 2 ml of 1,5 M HEPES buffer solution as it is shown on Figure 1. After the initial check of the Scintomics GRP module software the preparations are complete.

The production process progress in two steps similarly to René Martin et al. [5]. Firstly the ⁶⁸Ga gallium is eluted from the generator with the use of 4ml 0,1M HCl to the V-vial. The second step comes

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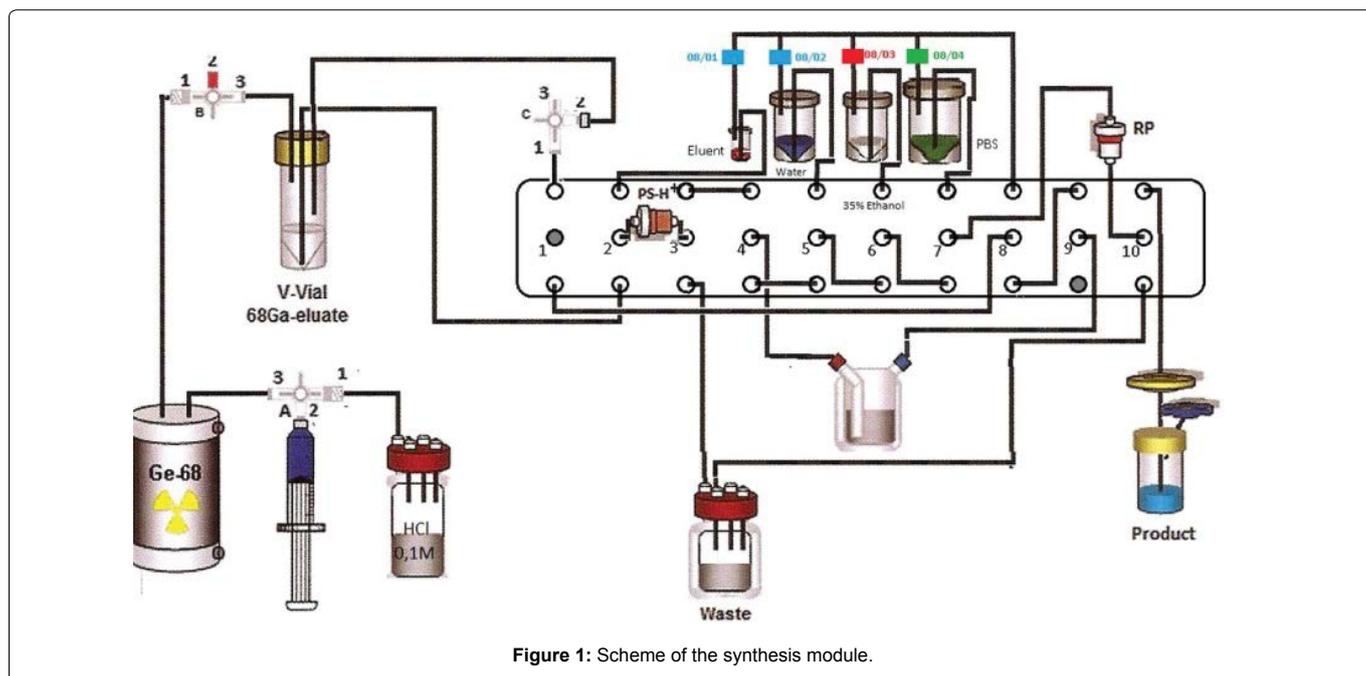


Figure 1: Scheme of the synthesis module.

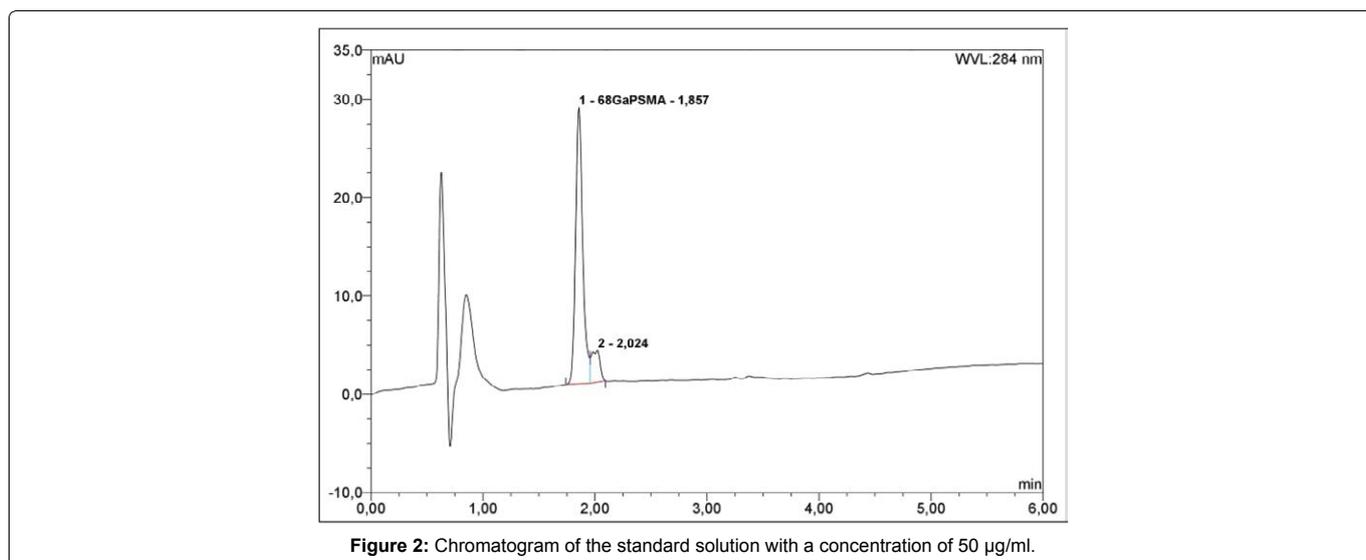


Figure 2: Chromatogram of the standard solution with a concentration of 50 µg/ml.

after the elution, when the operator of the synthesis module starts the labeling process. The whole script runs for approximately 20 minutes and the temperature of heating block is set to 125°C to ensure that the labeling runs in around 100°C. After the start of labeling the ^{68}Ga gallium chloride is transferred to the PSH+ cartridge to remove the excess of the hydrochloric acid and then eluted to the reactor with 2 ml of acidified 5,0 M NaCl. The labeling process takes around 10 minutes and then the purification part of the script begins. First the reaction mixture is sent through the C18 cartridge to the waste bottle. C18 cartridge is preconditioned with 2 ml of 99,8% ethanol and 10 ml of water and dried with air at the end. The ^{68}Ga gallium labeled PSMA peptide is adsorbed by the cartridge and the eluent and HEPES buffer (2-[4-(2-hydroxyethyl) piperazin-1-yl] ethane sulfonic acid) present in the reaction mixture are removed. Then the C18 cartridge is flushed with water to ensure removal of HEPES buffer and other water soluble impurities. When the purification step is complete the

product is removed from the C18 cartridge by flushing it with 2 ml of 35% ethanol solution and transferred through the 0,22 µm Cathivex-Gv (Millipore) filter to the product vial. After the removal of the ^{68}Ga gallium labeled PSMA, the C18 cartridge is further flushed with 20 ml of phosphate buffered saline solution (PBS) to ensure the proper concentration and a pH level of the obtained product.

Quality control methods

Quality control methods are in most based on European Pharmacopoeia (PhEur) monograph describing Gallium (^{68}Ga) Edotreotide for Injection [6], despite liquid chromatography method, which is based on Eder and colleagues [7].

Gamma ray spectroscopy: This examination is about measurement of the energy of the detected gamma quantum. The gamma ray spectroscopy is proceed with use of multichannel analyzer

with 3" x 3" NaI detector. All of the positron emitting isotopes are characterized by energy of the photon, which is emitted during positron annihilation with electron, and it is expected to be 511keV [8]. This test is one of the nuclide identification tests.

Half-life determination: Determination of the half-life is the second test that the purpose is to define the isotope. Half-life is obtained with use of dose calibrator for the activity measurements. Each activity measurement lasts 20 minutes and is repeated every minute. The expected half-life of Gallium-68 is 67.6 minutes [9] and can be calculated using following equation [10]:

$$T_{1/2} = -\ln 2 \left(dt / \ln \left(A_1 / A_0 \right) \right)$$

Where: dt-time difference

A₁-ending activity

A₀-starting activity

These two tests mentioned above uniquely identify the nuclide as Gallium-68.

Determination of residual HEPES buffer: According to PhEur monograph [6] HEPES content is determined by thin-layer chromatography (TLC). It is needed to prepare reference solution of HEPES at concentration of 20 µg/ml. Applicant 5 µl of each, reference and test solution (the test solution is the [⁶⁸Ga]-PSMA final product sample), on TLC silica gel F254 plate with an aluminium backing (3 cm x 8 cm) as a two separate spots and develop on a path over 2/3 of the plate with use of water: acetonitrile (25: 75 V/V) solution as a mobile phase. Expose to iodine vapor for four minutes after drying. Spot corresponding to the test solution should be not more intense than the spot corresponding to the reference solution (less than 20µg/ml of HEPES in test solution).

Residual solvents: The only organic solvent used during [⁶⁸Ga]-PSMA labeling is an ethanol. In Pharmacopoeia there are three methods described to determine the amount of ethanol in a pharmaceutical product: distillation with a vertical condenser (A) and gas chromatography (GC) with a head-space (B) or without head-space (C) [11]. In this study gas chromatography (C) is used in accord with the pharmacopoeial method [12]. Any calibrated analytical GC method adapted to the method mentioned above should be suitable for this analysis. In our apparatus are used the same settings as for FDG analysis i.e. volume of sample injection is 1,0 µl; split ratio 5,0:1 at a total flow of 16,2 ml/min with helium as a carrier gas. Used column is HP-Inn wax (30 m x 0,32 mm; film: polyethylene glycol 0,50 µm) in column heater settled up to 50°C. Detection is carried out on an FID detector (Flame Ionization Detector).

Bacterial endotoxin purity: The bacterial endotoxin test is performed using Endosafe®-PTS™ (Charles River) apparatus and the kinetic chromogenic LAL-test method applied, the same as during [¹¹C]-choline quality control [13].

Radionuclide purity: Gamma spectroscopy of the final product sample also is carried out in the same way as in paper mentioned above [13-18] in a Canberra-Packard gamma spectrometer equipped with a high-purity germanium (HPGe) detector. The spectra acquisition is performed two days after synthesis. Acquisition is proceeded for 180 minutes to obtain high a signal-to-noise ratio. The sample volume should be at least 1 ml. Spectrum is analyzed using Genie 2000 software.

Liquid chromatography: To determine radiochemical and chemical purity of ⁶⁸Ga-PSMA a reversed-Phase HPLC with

Chromolith Performance RP-18e column; 100 x 4.6 mm (Merck) is used. The HPLC system is Thermo Fisher Dionex Ultimate 3000 equipped with on-line degasser, UV and radiometric (Bioscan B-FC-1000) detectors. Measurement is carried out in linear A-B gradient (0 % B to 100 % B) at flow rate 4 ml/min. Solvent A consists of 0,1 % aqueous trifluoroacetic acid (TFA) and solvent B is 0,1 % TFA in acetonitrile [19]. UV absorbance is measured at wavelength 254.0 nm and 284.0 nm. The injection volume is 50 µl. All of obtained chromatograms were compared with natGaPSMA standard solution. To find the best concentration of the standard solution, 1 mg of natGaPSMA-11 (ABX, Radeberg, Germany) was dissolved in 1 ml (1:1 v/v CH₃CN/H₂O) and from this stock the solutions with following concentrations were prepared: 50 µg/ml, 100 µg/ml and 200 µg/ml. Three standard solutions with concentrations of 50 µg/ml (Figure 2); 100 µg/ml (Figure 3) and 200 µg/ml (Figure 4) were analyzed during the study with an UV detector at 284 nm. As it is seen on the recorded chromatograms, the best results were given from solution containing 200 µg/ml of natGaPSMA. Therefore, it was decided to record one more chromatogram with use of the 200 µg/ml standard solution at 254 nm (Figure 5) [7]. The better results were obtained during the measurements at a wavelength of 284 nm and that is why it was decided to measure the ⁶⁸Ga-labeled PSMA-11 peptide at this wavelength.

⁶⁸Ga-labeled PSMA peptide is analyzed with an UV detector at 284 nm and a radiometric detector. During the tests it was found that the concentration of ⁶⁸Ga-PSMA in the sample diluted with a saline is below the detection limit of radiometric detector (synthesis product is diluted with saline to provide isotonicity). Therefore, for the further analysis the undiluted product is used. There are two signals that can be observed in the obtained chromatograms in a ratio of approximately 82%/12%. The more intense signal comes from ⁶⁸Ga-PSMA-11, the second signal comes from its less thermodynamically stable diastereomer and does not affect the test result (Figure 6). These signals can be observed both on the UV detector and the radiometric detector. However, the concentration of ⁶⁸Ga-PSMA-11 in the test sample is too low to obtain a reliable result on the UV detector (Figure 7).

To confirm the identity of the compound, the mixture of 50% of the product sample and 50% of a standard solution of 200 µg/ml were measured (Figure 8).

Results

The activity of the ⁶⁸Ga gallium labeled PSMA peptide depends on the activity eluted from the ⁶⁸Ge-⁶⁸Ga generator. For the 40 mCi of eluate the Scintomics GRP automated synthesis module can produce around 23 - 25 mCi of product.

The activity of the product obtained by automated method of labeling is a little lower than analogical product obtained with the use of manual method because of the more complicated purification (necessity of complete removal of the HEPES buffer). But otherwise it is still comparable to the manual method. On the other hand because of the automatization of the process the human error factor is minimized and the dosages of radiation received by synthesis operators are lower, which means the process is safer.

Short pictorial essay

Patient LJ, 60 yo, prostate cancer treated with prostatectomy in 2013. Biochemical relapse, PET with ¹⁸F-choline on 30th September 2016 with serum PSA 0.79 ng/ml with no pathologic uptake (Figure

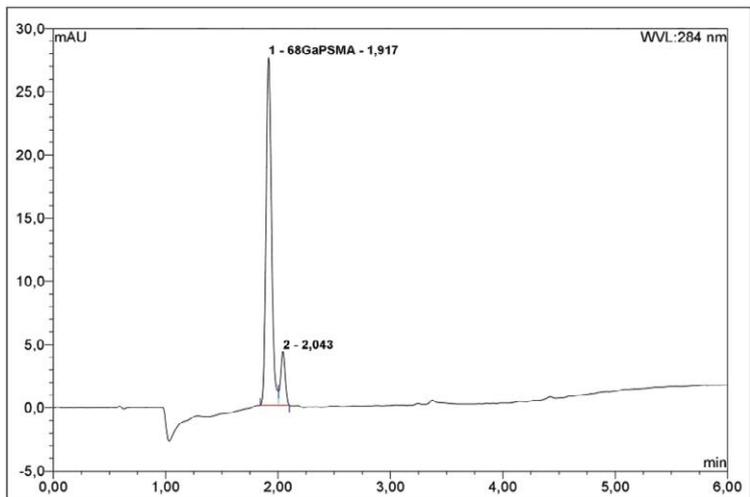


Figure 3: Chromatogram of the standard solution with a concentration of 100 µg/ml.

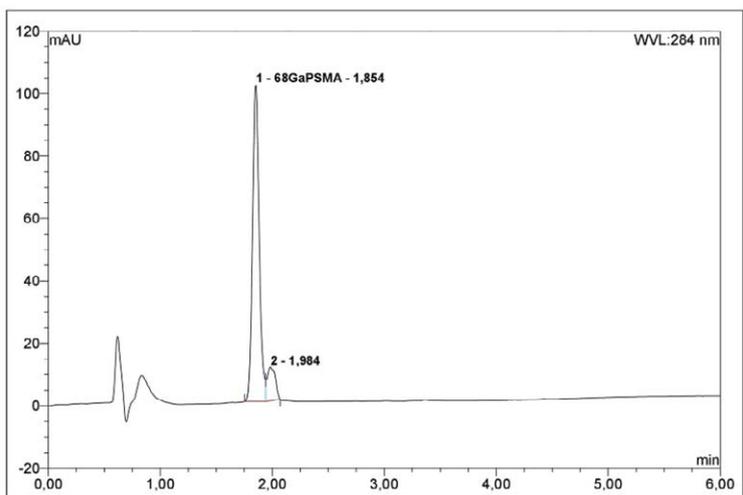


Figure 4: Chromatogram of the standard solution with a concentration of 200 µg/ml.

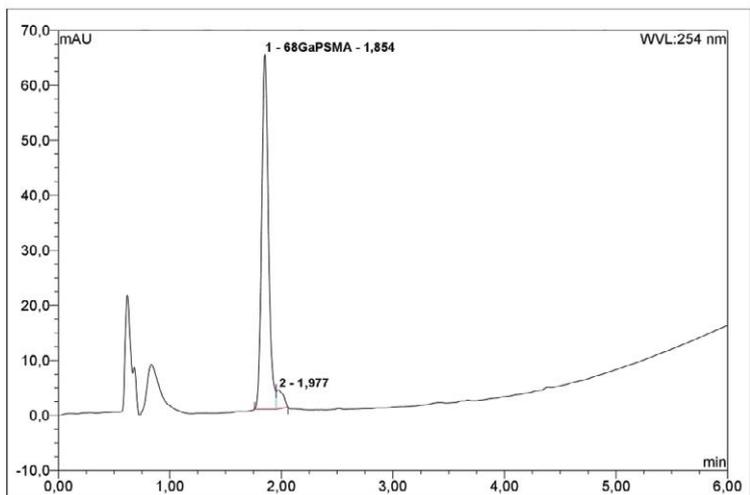


Figure 5: Chromatogram of the standard solution with a concentration of 200 µg/ml at 254 nm.

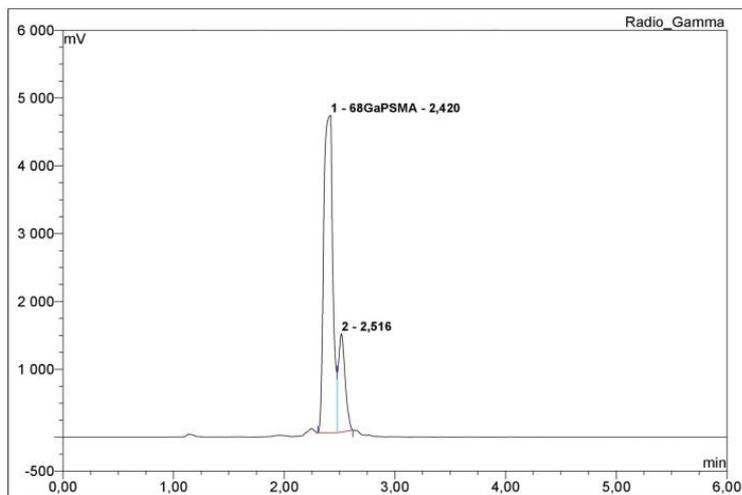


Figure 6: ^{68}Ga -PSMA-11 chromatogram derived from radio detector.

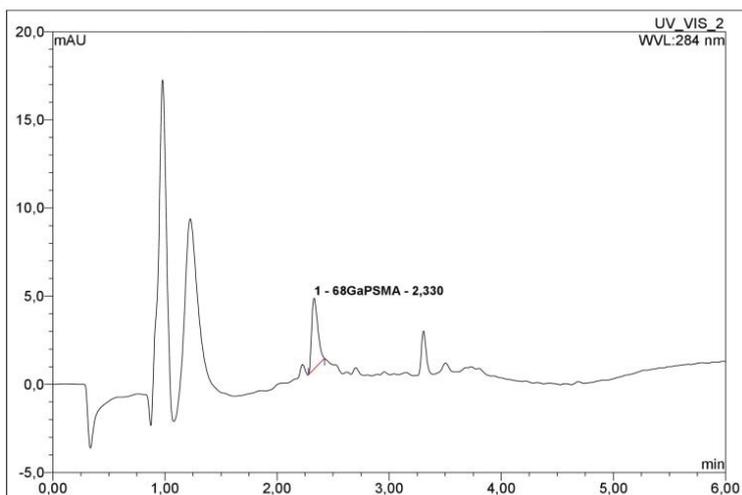


Figure 7: ^{68}Ga -PSMA-11 chromatogram derived from UV detector. The concentration of radiopharmaceutical is too low for analytical purposes.

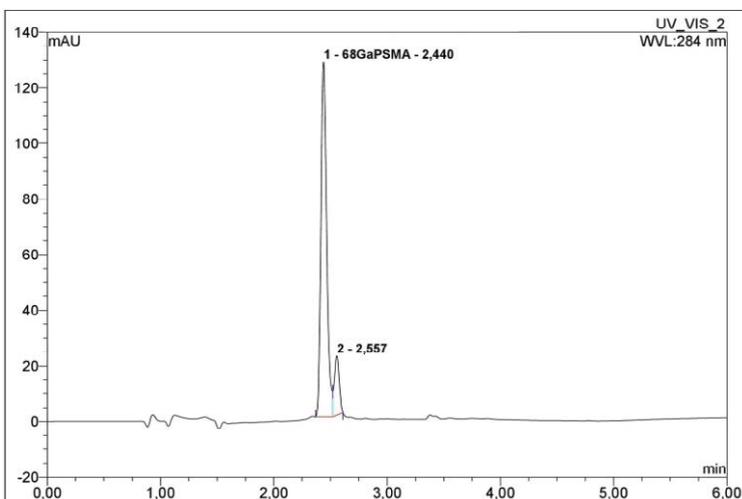


Figure 8: Chromatogram of the mixture of 50% of the product sample and 50% of the standard solution 200 $\mu\text{g}/\text{ml}$.

9). Second PET with ^{68}Ga -PSMA on 26th October 2017 with seric PSA 1,56 ng/ml showed pelvic lymph node with strongly increased PSMA uptake (Figure 10).

Patient KW, 68 yo, prostate cancer treated with radiotherapy in 2014, biochemical relapse during suppressive therapy with PSA 0,94 ng/ml on 29th December 2016. First PET scan 21th April 2017 positive at pelvic lymph node, with no bone uptake (Figures 11 and 12). Due to rising seric PSA to 31, 88 ng/ml a second PET with ^{68}Ga -PSMA was carried out on 15 September, with confirmation of lymph node metastasis and multiple pathologic bone foci not present at choline imaging (Figures 13 and 14).

Conclusion

Synthesis of radiopharmaceuticals: ^{68}Ga -PSMA and ^{18}F -fluorocholine (^{18}F -FCH) used in prostate cancer diagnostic procedures are completely different. In case of ^{18}F -fluorocholine synthesis run in numerous steps with many chemicals compounds such as dibromomethane (DBM) or dimethylaminoethanol (DMAE) which are quite toxic and tough chemical during laboratory work. Some of ^{18}F -FCH synthesis by-products are in gas phase [14], this triggers the need to use radioactive gases anti-contamination systems. The Fluorine-18 used in the ^{18}F -FCH synthesis is

produced in a liquid target of cyclotron [15]. The construction and use of such an accelerator is quite expensive, however the amount of Fluorine-18 isotope produced for a single synthesis, it allows producing a radiolabelled compounds for impressive number of patients (up to 15, using IBA Synthera modules). Such a connection high activity final product and long half-life of Fluorine-18 (110 minutes) allows the ^{18}F -FCH could be transported to the PET diagnostic departments, distant from the place of production [16]. In case of second radiopharmaceutical ^{68}Ga -PSMA, which are used to diagnostic prostate cancer, isotope of Gallium-68 has shorter half-life (68 minutes). This isotope is obtained from $^{68}\text{Ge}/^{68}\text{Ga}$ generator during column elution to receive Gallium-68 chloride. Synthesis of ^{68}Ga -PSMA is a simple process with no complicated steps resulting from reaction mechanism [17]. $^{68}\text{Ge}/^{68}\text{Ga}$ generator can be used for several months, which could be eluted even two hundred times. Short half-life of Gallium-68 allows frequently elution of the generator on the same day, but compare to ^{18}F -FCH, single synthesis of ^{68}Ga -PSMA provide to PET diagnosed at most 3 patients. Compounds which are used during ^{68}Ga -labeled PSMA ligand are easy to operate, and do not require special conditions. Labeling process is characterized by high yield. Finally, product of radiopharmaceutical PSMA is stable and in form of injectable formulation. An important parameter to both radiolabeled compound is time of synthesis.

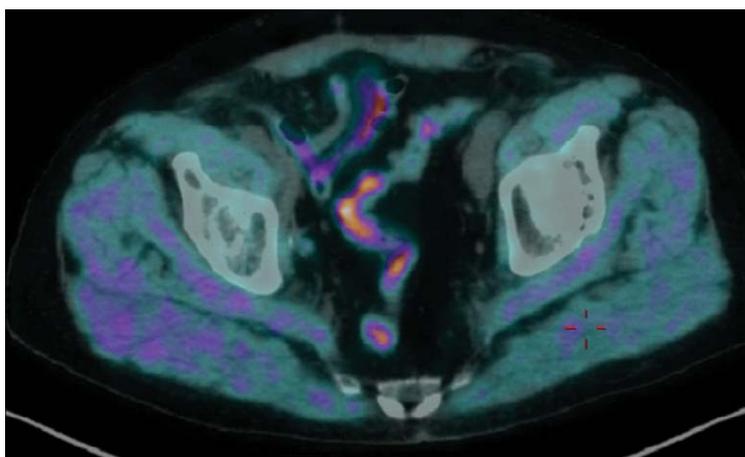


Figure 9: Patient LJ, 60 yo, PET imaging with ^{18}F -choline.

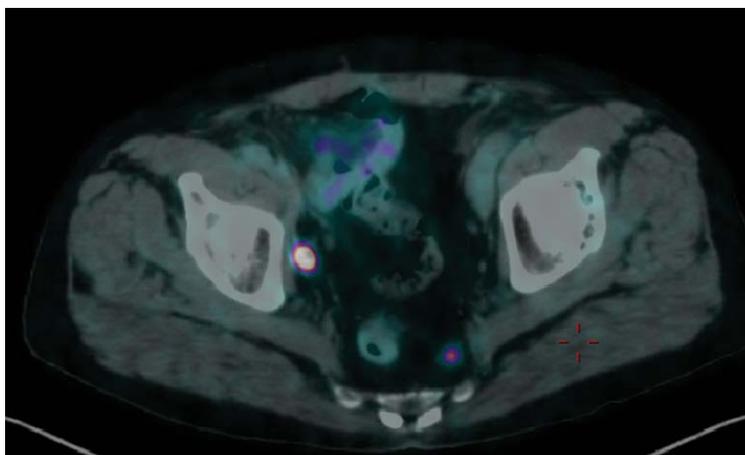


Figure 10: Patient LJ, 60 yo, PET imaging with ^{68}Ga -PSMA-11.

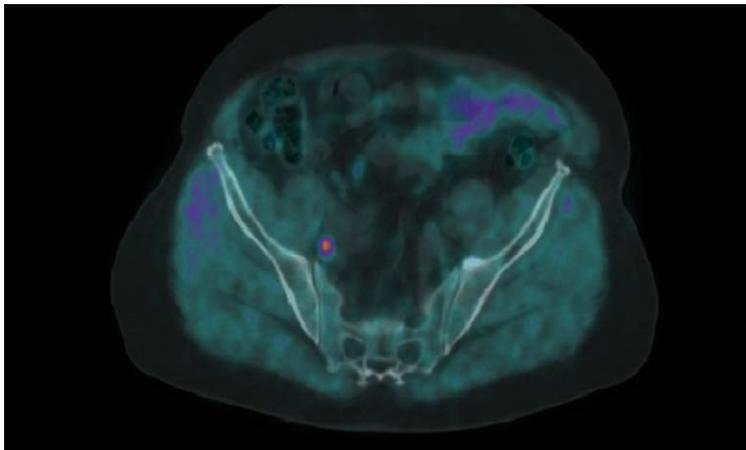


Figure 11: Patient KW, 68 yo, PET imaging with ^{18}F -choline.

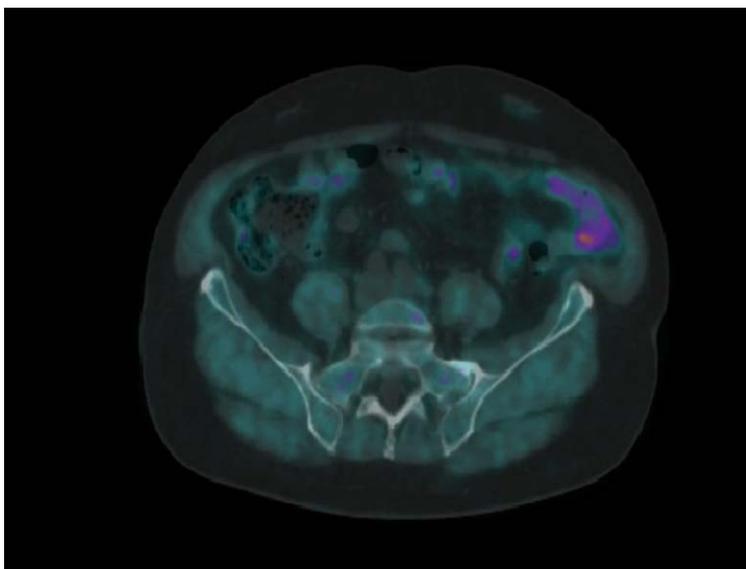


Figure 12: Patient KW, 68 yo, PET imaging with ^{18}F -choline.

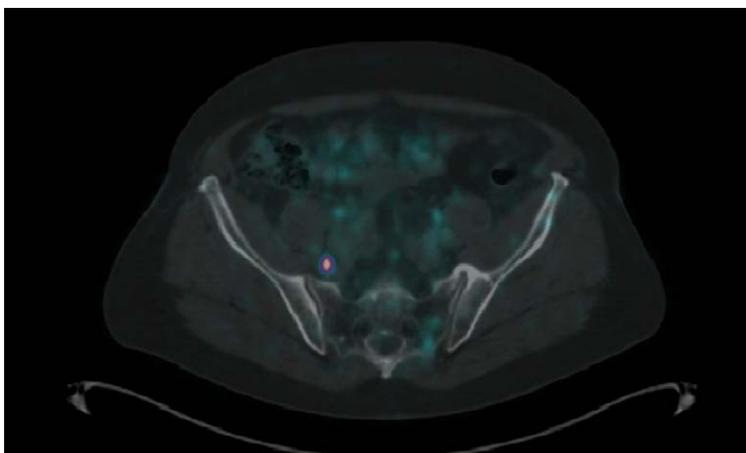


Figure 13: Patient KW, 68 yo, PET imaging with ^{68}Ga -PSMA-11.

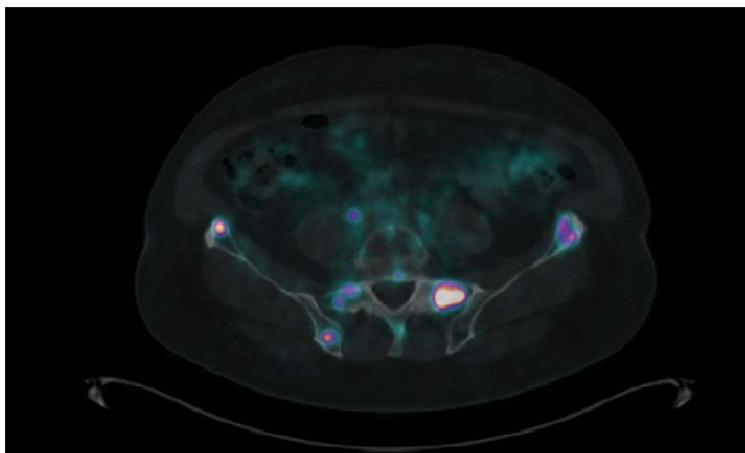


Figure 14: Patient KW, 68 yo, PET imaging with ^{68}Ga -PSMA-11.

Synthesis of ^{18}F -FCH run approximately 90 minutes, compared to ^{68}Ga -PSMA only 15 minutes. Moreover, it is difficult not to mention in view of radiological protection about lower activity of ^{68}Ga -PSMA than ^{18}F -FCH during patient injection [18].

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