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The Sensitivity Of ^{99m}Tc-Ciprofloxacin (Infecton) Scintigraphy Imaging To Detect Infection Lesions Induced By Staphylococcus Aureus Susceptible Or Resistant To Ciprofloxacin Antibiotic In Foot's Rat

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Abstract: *The main propose of this approach is to evaluate the efficiency of ^{99m}Tc-Ciprofloxacin (Infecton) imaging to detect infection sites induced in foot's rat by staphylococcus aureus susceptible to ciprofloxacin in comparison with S aureus resistant to drug. S aureus bacteria have been isolated from the patients admitted in Imam Khomeini hospital Ahvaz Khuzestan Iran. According to the table of Clinical and Laboratory Standards Institute (CLSI) the sensitivity or resistant of bacteria to ciprofloxacin was determined. Susceptible or resistant bacteria to ciprofloxacin selected and separated. A total number of forty six adult, Male NMRI rats were chosen. The animals were divided into two groups. Infection lesion in foot's rat was induced by S aureus susceptible or resistant to ciprofloxacin respectively. The 37 MBq (1 mCi) ^{99m}Tc-Infecton administrated intravenously to each animal. Qualitative and quantitative studies have been performed. Infection sites induced by S aureus susceptible to quinolone could be observed and visualized by ^{99m}Tc-Infecton scintigraphy imaging. Qualitative and quantitative assessment indicated that radiotracer has not accumulated at the infection foci induced by S aureus resistant to ciprofloxacin antibiotic agent. Outcome of our assessment indicated that ^{99m}Tc-Ciproflixacin scintigraphy imaging study does not have any value to detect infection, if the bacteria involve in pathogenesis of induced infection lesions are resistant to ciprofloxacin. This study could demonstrate successfully that ^{99m}Tc-Ciproflixacin scintigraphy imaging study can be suggested as an alternative method for investigation of sensitivity or resistant S aureus to ciprofloxacin antibiotic in clinical practice.*

Key words: *Ciprofloxacin, Infecton, Staphylococcus aureus, Scintigraphy imaging, ^{99m}Tc-Ciprofloxacin,*

I. Introduction

The preferentially detection of infection from sterile inflammation is one of the most problems in medicine. Several modalities have been suggested to find the solution of this dilemma. The available imaging techniques such as Plain Radiography, Ultrasonography, Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) have high sensitive but are not specific for infection especially in early phases, when anatomic structures have not been distorted. Radioisotope scintigraphy imaging may be considered as part of the diagnostic procedures to detect infection. The radioisotope 67 gallium is the most primitive radionuclide for this purpose but it has several disadvantages like long physical half-life, multiple energy gamma ionizing radiation causing high radiation absorbed doses, is not available as generator and high sensitive for both infection and non-infectious inflammation (1,2) . The radio labeled leukocytes have been recommended as a gold standard for the scintigraphy imaging to visualize infection from sterile inflammation lesions in nuclear medicine (3). In order to prepare the label leukocyte, the blood must be taken from the patient and, the leukocyte separated, labeled and finally reinjected to the patient. This technique is time-consuming and has potentially the risk of contamination or transmission of blood-borne pathogens to patient or technician (4). In addition to above mentioned factors, the process of labeling of leukocytes with radioisotope requires specialize facilities. The broadly spectrum antibiotic agents have been introduced as a radiopharmaceutical dosage forms for the discrimination diagnosis of infection lesions (5, 6). These molecules can localize in the infectious sites. They are frequently taken up, accumulated and metabolized by microorganisms. Several broadly antibiotic agents have investigated in this regard. Fluoroquinolone group is one of the broadly antibiotics that they have assessed for

preferentially detection infection from sterile inflammation sites. Ciprofloxacin is a synthetic fluoroquinolone derivative with bactericidal activity against a wide range of gram-negative and gram –positive bacteria with a wide distribution in the entire body. Recently, 99m technetium ciprofloxacin (^{99m}Tc-Ciprofloxacin), known as infecton, has been developed as a radiotracer for scintigraphy imaging for distinction between infection and sterile inflammation (7-9). The ^{99m}Tc-Ciprofloxacin radiopharmaceutical (Infecton) combines the advantages of 99m technetium labeling and the broad spectrum bacterial localizing capability due to attach and inhibition of bacterial DNA gyrase. The experience in literature reported that the specificity of scintigraphy imaging with ^{99m}Tc-Ciprofloxacin radiotracer for the localization of bacterial infection exceeds 90 % (10, 11). Staphylococcus aureus (S aureus) is one the most microorganisms involve in the pathogenesis of infection in clinical practice. Fluoroquinolone agents particularly ciprofloxacin have been examined for the treatment of infection caused by S aureus. Initial investigations have demonstrated that the resistance rate of S aureus to ciprofloxacin was quite low (12-14). Increase in the misuse and misappropriate prescriptions of ciprofloxacin which are now leading to emerge resistant pathogens like staphylococcus species. According to literature, reports have revealed a very high incidence of S aureus was found to be resistant (15-18). This study was conducted to evaluate the sensitivity of ^{99m}Tc-Ciprofloxacin scintigraphy imaging to detect infection foci in the rats induced by S aureus resistant to ciprofloxacin in comparison to S aureus sensitive to ciprofloxacin.

II. Materials and Method

All chemical materials have been purchased from Merck, Fluka and Sigma. The chemicals and solvents were of the highest purity and analytical grade and used without further purification. The freeze-dried kits of Ciprofloxacin (Infecton) and ^{99}Mo/^{99m}Tc generator have been provided by Radioisotope Division of Atomic Energy Organization of Iran (AEOI).

2.1. Bacteria Samples

The sample wound swabs were taken from patients admitted to the infection department of teaching center of Imam Khomeini hospital, Ahvaz, Khuzestan, Iran. The specimens were transported in sterile, leak-proof container to the department of microbiology of Ahvaz Jundishapur University of medical Sciences. The isolates were inoculated on blood agar and incubated overnight at 35 ° C aerobically. Gram-positive cocci occurring in pairs, short chains or clusters, that they were Catalase-positive, Coagulase-positive by test tube and DNase-positive on agar were identified as S.aureus and selected. The disc diffusion method was used in order to determine the susceptibility of S aureus to ciprofloxacin antibiotic. By using a sterile-tip applicator, touch the surface of one to four morphologically identical, isolated colonies. Immerse the applicator into a tube containing Mueller Hinton broth. Rub the applicator against the wall of the tube slightly to release a small amount of growth into the liquid. Cap the tube and mix the cells using a vortex to form a suspension, being careful not to form froth or bubbles in the suspension when mixing the cells. The broth was incubated at 35 °C, and then the turbidity was adjusted to a number 0.5 McFarland turbidity standard .A sterile cotton swab on a wooden stick was dipped into the broth. Excess inoculum was removed by rotating the swab against the wall of the tube above the fluid level.

The Mueller-Hinton agar plates were streaked in three dimensions. During 15 min after the surfaces of the agars were inoculated. Ciprofloxacin (5µg) disks were applied. The plates were inoculated at 35 °C for 24 hours. The diameter of each zone of inhibition was measured to the nearest millimeter through the underside of the plate by using a caliper. The isolates were considered to be resistant or susceptible to ciprofloxacin when the zone diameter of inhibition around a ciprofloxacin (5µg) disk was less than or equal to 15 mm ,when zone diameters of inhibition were greater than or equal to 21 mm ,the isolates were considered to be sensitive. The table of Clinical and Laboratory Standards Institute (CLSI) has been used in order to determine the resistance or susceptibility of S aureus to ciprofloxacin. Then the isolates were inoculated in normal saline. The turbidity was adjusted to a number 0.5 McFarland (each milliliter of 0.5 McFarland contains 1.5×10^{8}microorganisms). Half milliliter of inoculums has been injected to each foot's rat. To make sure about the survival of S aureus bacteria 0.1 milliliter of the above mentioned inoculums was inoculated on blood agar. The antibiogram experiment has been repeated three times.

2.2. Labeling of Ciprofloxacin by ^{99m}Tc

Technetium-99m as sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from an in-house ^{99}Mo/^{99m}Tc generator using 0.9% saline. Commercial infecton kits (AEOI, Tehran, Iran) was used and the labeling and quality control procedures were performed according to the manufacturer's instructions.

2.3. Animal study

The rats with average weight 160±20 gr were obtained from research center and experimental animal house of Ahvaz Jundishapur University of medical Sciences. This approach was approved by the ethics committee of Ahvaz Jundishapur University of medical Sciences. All the ethical issues were considered based on the Ahvaz Medical University Ethical Protocols (AMUP) on animal experiments. A total number of forty six adult, male NMRI rats were acclimated to conditions for one week before the experiment. These rats were kept in individually wire-bottom stainless steel cages in an air-conditioned room at 24±1°C with a 12 hours light-dark cycle and were fed with standard pellet diet and had free access to water. They were randomly assigned into qualitative and quantitative experiments.

2.4. Qualitative study

Twelve rats have been divided equally into two distinctive groups. In group A, susceptible S aureus bacteria to ciprofloxacin and in the other group (group B) resistant bacteria to ciprofloxacin antibiotic have been inoculated to each right leg of rat respectively. Animals were returned to their cages. Scintigraphy imaging studies have been performed after 4, 12, 24,48,72,96, 120 and 156 hours after inoculation of bacteria suspension. In all studies, each rat was placed in the restrainer apparatus and the (37MBq) ^{99m}Tc-Ciprofloxacin was administered intravenously by contra lateral tail vein. Two hours after injection of radiotracer sample the rat was anesthetized with diethyl ether and fixed on the board for scintigraphy imaging. For all studies a single-headed camera (E-Cam, Ziemens USA) employing a low –energy high resolution was used. Acquisition parameters for Infecton scintigraphy imaging were as follows: matrix size 256×256, zoom factor ×3, anterior and posterior views for 5 min and energy window 140 keV. Anterior and posterior static images were acquired using a large field of view gamma camera peaked to 140 keV with a 15% window and a low –energy all –purpose collimator for 500kilo counts per image. The gamma camera was positioned to image the affected part and contra lateral healthy site. The rats were killed after radioisotope imaging study. The experiment to addition above process continued for two other times. The experiment carried out each time like to above mentioned process. All the scintigraphy images were interpreted by three nuclear physicians independently and their final opinion was achieved by consensus. The observers were unaware the infection lesions induced by resistant or susceptible bacteria to ciprofloxacin antibiotic.

2.5. Quantitative study

The quantitative study has been performed. A total ten rats were chosen and divided into two groups. Each group contained five rats .In group A, susceptible ciprofloxacin S .aureus bacteria and in the other group resistant ciprofloxacin S. aureus bacteria inoculated into each right foot's rat respectively .The injured area was irrigated with normal saline. The rats were returned to their cages. After 48 hours, each subject has put in the restrainer device and the 37 MBq ^{99m}Tc-Ciprofloxacin radiotracer injected intravenous due to contra lateral tail vein. Then the animals were returned back to their cages and the experiment was continued for another two hours. The rats were sacrificed by diethyl ether .The organs of interest (infected foot, uninfected foot, heart, brain, liver, kidneys, gastro intestinal and lungs) were removed and weighted .The relative activity of each mentioned organs to the interest organs were measured (Fig 1).

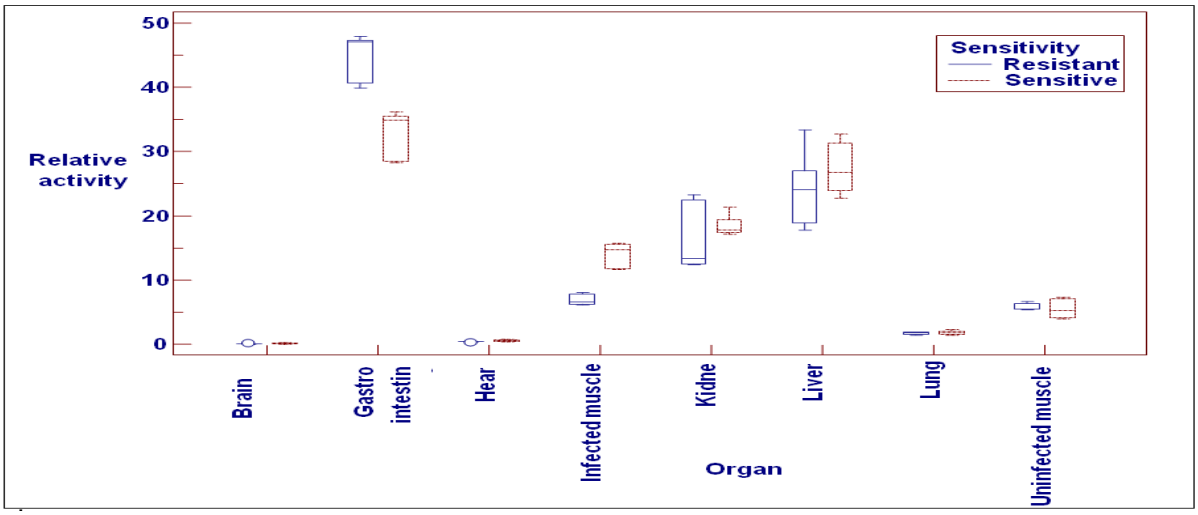

Figure1. Box plot of relative detected activity of ciprofloxacin in different organs of rats infected with sensitive and resistant bacteria. (The rectangle shows the interquartile range. The line inside the rectangle and the whiskers represent median and minimum and maximum of the data, respectively)

2.6. Statistical Analysis

Comparisons of the measured activity between different organs were done by Friedman's ANOVA for related samples followed by Dunn test. Difference of distribution of the measured activity in each of the organs between sensitive and non-sensitive organisms was tested using Mann-Whitney test. Statistical significance was considered at $p<0.05$.

III. RESULTS

The yield of ^{99m}Tc-Ciprofloxacin radiotracer samples were approximately 78%. Microorganisms have been inoculated to right leg of rats in all studies. The right foot of rats has been chosen to avoid misinterpretation of scintigraphy imaging. The radioisotope scanning has been performed after S aureus susceptible or resistant to ciprofloxacin inoculated to the right leg of rats. The scintigraphy imaging studies have been performed 4, 12, 24, 48, 72, 96, 120 and 168 hours after inoculation of bacteria. The 37MBq ^{99m}Tc-Ciprofloxacin radiopharmaceutical has been administrated intravenously due to contra lateral vein tail to each rat near about 2 hours before scintigraphy imaging. The scintigraphy imaging study with ^{99m}Tc-Ciprofloxacin has not demonstrated the infection sites induced by S aureus 4 and 12 hours after inoculation of microorganisms. The erratic uptake of radiotracer has been observed 24 hours after inoculation of bacteria. Therefore, the images were not clear and appear for interpretations which have been obtained during this time. These images have only shown a little uptake or erratic accumulation of radiotracer at the infection sites. The qualities of images were clear and apparent 48 hours after inoculation bacteria. The quality of images has not changed over time significantly. The result of this approach indicated that this time is suitable for scintigraphy imaging for detection of infection lesions induced by S aureus susceptible to ciprofloxacin antibiotic agent. Only the septic lesions induced by S aureus susceptible to ciprofloxacin antibiotic agent could be visualized by radiotracer imaging. The infection sites induced by S aureus resistant to ciprofloxacin antibiotic have no observed in all scintigraphy imaging studies by ^{99m}Tc-Ciprofloxacin radiotracer (Fig 2).

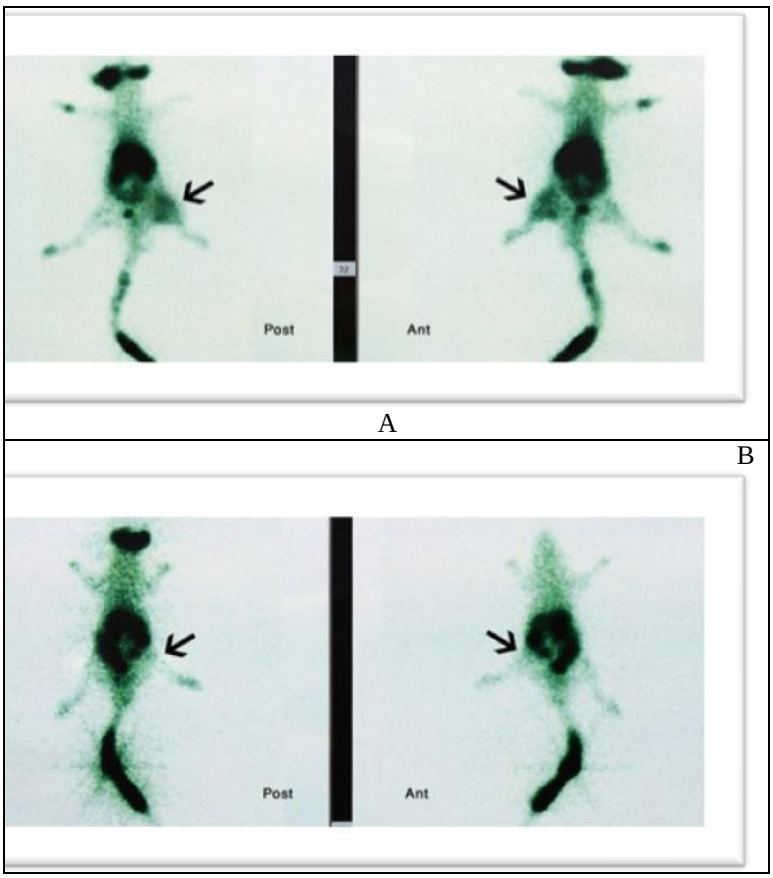

Figure2. Scintigraphy imaging study has performed two hours after the 37 MBq ^{99m}Tc-Ciprofloxacin injected intravenously due to contra lateral tail vein. The scintigraphy imaging study has been performed with ^{99m} Tc-Ciprofloxacin radiotracer 168 hours after inoculation of S aureus A: susceptible to ciprofloxacin B: resistant to ciprofloxacin respectively. The images were shown posterior (Post) and anterior (Ant) views of infected foots.

The infection site induced due to S aureus resistant to ciprofloxacin antibiotic has not visualized by radiotracer. The quantitative study has performed for further investigation. As it has shown in figure 1, the assessment has indicated that minimum and maximum measured relative activities were seen in the brain and gastrointestinal tract of the animals (Mean ranks of 1 and 7.8, respectively). The measured absorption in the brain was significantly different with those of infected foot, kidney, liver and gastrointestinal ($p<0.007$). Also, the observed difference between the measured activity in heart could be considered statistically significant with kidney, liver and gastrointestinal ($p<0.004$). Difference of lung distribution of ^{99m}Tc-Ciprofloxacin with those of liver and gastrointestinal were also statistically significant ($p<0.007$). Similarly, greater activity was measured in gastrointestinal of the animals in comparison with uninfected foot ($p=0.015$).When similar comparisons were made only in animals infected with sensitive bacteria, minimum and maximum of activity were seen in the same organs, i.e. brain and gastrointestinal. However, the significant difference ^{99m}Tc-Ciprofloxacin organ distribution were seen between brain with kidney, liver and gastrointestinal ($p<0.035$). The observed differences between ^{99m}Tc-Ciprofloxacin distribution in heart could be also considered significant with those of liver and gastrointestinal ($p<0.014$).The same was true for the difference of ^{99m}Tc-Ciprofloxacin brain distribution with other organs in animals infected with resistant bacteria. However, the distribution of ^{99m}Tc-Ciprofloxacin could be only considered statistically less than that of gastrointestinal ($p<0.003$). The difference of measured absorption in lung and gastrointestinal was also statistically significant ($p=0.035$).Results of comparison of organs indicated that distribution of ^{99m}Tc-Ciprofloxacin between sensitive and resistant strains of bacteria revealed that the only significant differences were observed for infected foot and gastrointestinal ($p=0.008$).According to the result has been obtained from this approach, sensitivity and positive predictive value (PPV: proportion of subjects with infection that were diagnosed correctly) of ^{99m}Tc-Ciprofloxacin radiotracer to detect induced infectious lesions in the foot's rat caused by S aureus susceptible to ciprofloxacin were 100 % . ^{99m}Tc-Ciprofloxacin scintigraphy imaging could not show infection foci induced by S aureus resistant to this antibiotic agent.

IV. Discussion

Ciprofloxacin is a potent broad-spectrum antibiotic .This molecule is active against most of the gram-positive and gram-negative microorganisms. Ciprofloxacin like the other fluoroquinolone antibiotic agents is incorporated by bacteria and binds selectively to the DNA gyrase enzyme. The therapeutic characteristic of the drug has been exploited for diagnostic use. Recently ciprofloxacin has been developed as a freeze-dried radiopharmaceutical kit (Infecton) for scintigraphy radioisotope imaging for visualizing infection foci. Infecton kit contains 2 mg of ciprofloxacin. Therefore, the dose of ciprofloxacin for scintigraphy imaging has compared to therapeutic dose for eradication and treatment of infection (500mg to 2gr per day) is very low Infecton scintigraphy imaging has been offered several advantages over the other techniques.

The ^{99m}Tc-Ciprofloxacin complex dose not bind to dead bacteria so that sterile abscesses are not shown the uptake of radiotracer .The labeling of ciprofloxacin by ^{99m}Tc can provide images with good quality and a shorter investigation time in comparison to ^{67}Ga-Citrate radioisotope imaging. The preparation of ^{99m}Tc-Ciprofloxacin complex is simple and cheap and it does not involve blood manipulation with the associated risk of blood-borne infections. Infecton scintigraphy imaging is not dependent to the absolute white blood cell count and for this reason can be used in patients with neutopenia. There is an absence of bone marrow uptake when Infecton scintigraphy imaging is used. Briton et al, performed this radioisotope scanning and reported specificity over 90 % for ^{99m}Tc-Ciprofloxacin scintigraphy imaging to detect infectious lesions (10). The dose of ciprofloxacin is used for imaging in comparison to therapeutic dose for treatment and eradication infections like S aureus is very low. Ciprofloxacin acts as a carrier and carries 99m technetium radioisotope at the site of infection .It has provided to perform radioisotope scintigraphy imaging to detect infection foci. In our approach, ^{99m}Tc-Ciprofloxacin scintigraphy imaging could not visualize the infectious lesions induced due to S aureus resistant to ciprofloxacin. Therefore, ^{99m}Tc-Ciprofloxacin scintigraphy imaging does not have any value to visualize the localization of infection if S aureus resistant to ciprofloxacin involve in pathogenesis of infection. The exact mechanism resistant of bacteria to ciprofloxacin has not been elucidated completely. Several mechanisms have been suggested for resistant of microorganisms to fluoroquinolone antibiotics. Resistance to fluoroquinolone is associated with spontaneous mutations in gene that encode for the quinolone target protein, DNA gyrase. In addition to the above suggestion, the resistance may be associated with an increase in drug efflux or a decrease in outer membrane permeability affecting drug influx (19-22). The data have obtained from this investigation indicated that only mutation in gene that encode for DNA gyrase is not itself to explain the mechanism of resistance of S aureus bacteria population to quinolone antibiotic. Multifactor models must be considered for resistance of S aureus to ciprofloxacin. ^{99m}Tc-Ciprofloxacin scintigraphy imaging can be introduced for assessment of susceptibility or resistance of S aureus bacteria to ciprofloxacin as an alternative method for antibiogram assay in clinical practice. Patient suspicious with S aureus susceptible to ciprofloxacin demonstrate

uptake of radiotracer at the site of infection. When S aureus induced infection become resistant to ciprofloxacin during antibiotic therapy or bacteria involve in the pathogenesis of infection are resistant to quinolone. The radiotracer cannot accumulate with sufficient amount at the infection foci. Therefore, the site of infection is not observed in radioisotope imaging with ^{99m}Tc-Infecton. The study has examined on the samples of bacteria, which they have been isolated from the patients .It has provided opportunity to evaluate clinical efficacy and efficiency of ^{99m}Tc-Ciprofloxacin scintigraphy imaging to visualize the sites of infection caused by S aureus resistant or sensitive to fluoroquinolone antibiotics. This matter could be considered as a positive point of our investigation.

V. Conclusion

It is necessary to consider the susceptibility of bacteria induced infection to ciprofloxacin antibiotic when ^{99m}Tc-Ciprofloxacin scintigraphy imaging is performed to preferentially detection of infection lesions. ^{99m}Tc-Ciprofloxacin scintigraphy imaging study may be recommended as a new developed modality to evaluate the sensitivity or resistance of S aureus caused infection to quinolone antibiotic agents.

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Abbreviations

Bq: Becquerel; MO: Molybdenum; PPV: positive predictive value TcO4: Pertechnetate; Tc: Technetium; Competing interests. The authors declare that they have no competing interests.

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