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*ORIGINAL RESEARCH ARTICLE*

# Does the presence of high levels of free $Sn^{2+}$ in PYP kit cause *in vivo* red blood cell radiolabeling and high blood pool radioactivity?

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**ABSTRACT**

**Introduction:** Technetium-99m-pyrophosphate scintigraphy (TPS) is a highly effective method for diagnosing transthyretin cardiac amyloidosis (ATTR-CA). It may eliminate the need for endomyocardial biopsy. However, the detection of radioactivity in the blood pool (BP) during these scans can make it difficult to interpret planar images. This often leads to the necessity for longer and repeated scanning sessions.

**Methods:** We conducted a prospective study involving unique patients who underwent TPS. The initial group of 58 patients used a pyrophosphate (PYP) kit labeled with 30 mCi (PYP-30) of radioactivity, while the subsequent group had a kit labeled with 90 mCi (PYP-90), although the administrated dose remained constant. This study aimed to assess how varying radioactivity levels in the kits affected BP activity visualization.

**Results:** Univariable analysis showed creatinine level of the patients and added radioactivity amount in the PYP kits affected BP activity ($p<0.05$), while gender, age, and BMI did not ($p>0.05$). Multivariable analysis confirmed creatinine ($OR<0.001$) and added radioactivity ($OR=0.005$) significantly influenced BP visualization. Patients with creatinine<1.5 showed a significant difference in BP activity between two groups (PYP-30 and PYP-90), but those with creatinine ≥1.5 did not.

**Conclusion:** These findings emphasize the significance of considering the levels of radioactivity in PYP kits, which may be linked to the concentration of free $Sn^{2+}$ in the prepared kit, as well as the patient's kidney function. These factors are essential for optimizing imaging quality and improving diagnostic accuracy in cases of cardiac amyloidosis.

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## INTRODUCTION

Systemic amyloidosis is a disorder that results from deposits of amyloid fibrils in various organs, including the heart, vasculature, nervous system, gastrointestinal tract, kidneys, skin, and lungs. The type of amyloid fibrils determines the pathophysiology, clinical presentation, genetics, prognosis, and treatment of the disorder and includes ATTR-CA, amyloid light chain (AL), and inflammatory amyloidosis [1-3]. Both initial symptoms and cardiac imaging findings tend to be nonspecific, and the disorder is not commonly recognized until it has reached an advanced stage. Historically, the treatment of ATTR-CA was limited to supportive care and heart and liver transplantation in highly selected patients. In 2019, Tafamidis became the first ATTR-CA stabilizer to be approved by the U.S. Food and Drug Administration (FDA) after the drug was shown to reduce mortality in patients with the disease [4-6].

In recent years, there has been a significant shift away from invasive biopsy diagnosis towards a non-invasive method using bone-seeking radiopharmaceuticals like technetium Tc-99m-pyrophosphate ([$^{99m}$Tc]Tc-PYP), which is highly specific for diagnosing ATTR-CA [7, 8]. TPS has a high diagnostic accuracy for ATTR-CA, with a positive predictive value of 100% when serum and urine studies for AL are negative [7, 9]. Cardiac scintigraphy using planar scanning can be prone to false positive results due to blood pooling or bone artifacts. In original TPS protocol, 1-hour planar imaging were routinely used. It is estimated that in about less than 10% of the scans, additional imaging was conducted at 2 or 3 hours to assess for BP washout. With the increasing recognition that planar scans can be subject to false positive results particularly with 1-hour imaging, SPECT scanning was systematically added to the scanning protocol for positive planar scans starting in 2017 [4]. Planar imaging of myocardial uptake using TPS is a rapid and simple technique that allows for visual interpretation and quantification of the degree of uptake by comparing heart to lung ratio or to rib uptake. However, the presence of radioactivity in the BP during a PYP scan can lead to false positive diagnoses [4, 10]. The presence of radioactivity in the BP during planar PYP imaging can lead to the need for repeated scans in subsequent time frames. This repeated imaging increases both the overall cost of the procedure, and the radiation dose delivered to the patient during the subsequent SPECT/CT acquisition. Therefore, minimizing the radioactivity in the blood pool could significantly improve the accuracy of PYP scans for detecting ATTR-CA and potentially shorten the overall imaging process [6].

In this study, we aimed to evaluate the impact of radiopharmaceutical preparation on BP radioactivity by adding different amounts of Na[$^{99m}$Tc]$TcO_4$ to the PYP kit during the radiopharmaceutical preparation procedure. For this study, two doses of 30 and 90 mCi of Na[$^{99m}$Tc]$TcO_4$ were added to the PYP kits in two different time intervals of six months each, and patients were scanned, and the frequency of BP radioactivity presence was compared in those patients' images.

## METHODS

### *Study design*

We conducted a prospective study of all unique patients who underwent a [$^{99m}$Tc]Tc-PYP thorax planar scan followed by SPECT and CT scans to diagnose suspected ATTR-CA at our center between May 2023 and June 2024. This study was carried out in compliance with the Helsinki Declaration and approval from the Review Board of Iran University of Medical Sciences (Ethics Code: IR.IUMS.FMD.REC.1402.535). Patients who had taken heparin on the day of the scan were excluded from the study. For each study subject, the routine examination was carried out to record comprehensive clinical data accordingly. The only difference was the radioactivity concentration in the PYP kit preparation in two groups of patients. For the initial cohort of 58 patients, a PYP kit radiolabeled with 30 millicuries (mCi) of radioactivity was utilized. However, for the subsequent 58 patients, a kit radiolabeled with a higher 90mCi radioactivity amount was employed. Despite this difference in kit preparation, the actual administered amount of radiopharmaceutical was kept constant between the two groups of patients (Figure 1). This methodology was designed to investigate the effect of the amount of radioactivity added to the kit and the frequency of BP activity visualization in patients' planar images.

### *Image data acquisitions*

Each PYP kit was radiolabeled with approximately 30 or 90 mCi of technetium pertechnetate based on the radiolabelling method specified by the manufacturer, Pars Isotope Company. The [$^{99}$Mo]/[$^{99m}$Tc] generators used for radiolabeling the PYP kits in this study was milked every 24 hours. It was specifically set to label all kits at 9 AM, utilizing the radioactivity

obtained from the generator at 8 AM. This approach was implemented to eliminate any potential confounding factors related to the timing of the labeling process. The radiochemical purity of the radiolabeled kits was assessed by instant thin layer chromatography and found to be 90-95% in all preparation. Each study participant was administered an intravenous injection of approximately 740 megabecquerels (MBq) or 20 mCi of [$^{99m}$Tc]Tc-PYP radiopharmaceutical.

Following the intravenous administration, planar imaging was performed at 1-hour post-injection. Anterior lateral views were acquired for 10 minutes each using a dual-head SPECT camera. Immediately after the planar imaging, a SPECT scan was acquired with the patient in the thorax position on the same dual-head SPECT camera. The SPECT acquisition protocol was optimized for imaging the thoracic region to assess myocardial [$^{99m}$Tc]Tc-PYP uptake. The SPECT camera used in this study was equipped with a low-energy high-resolution collimator and a 9.53 mm thick NaI(Tl) scintillation crystal. With the patient's heart positioned in the center of the field of view, planar images were acquired until a total of 750,000 counts were reached. The planar images were obtained using a 256 × 256 matrix and a 1.46 zoom factor. Following the completion of the SPECT acquisition, a low-dose free-breathing CT scan was performed on a dedicated SPECT/CT system for attenuation correction of SPECT images.

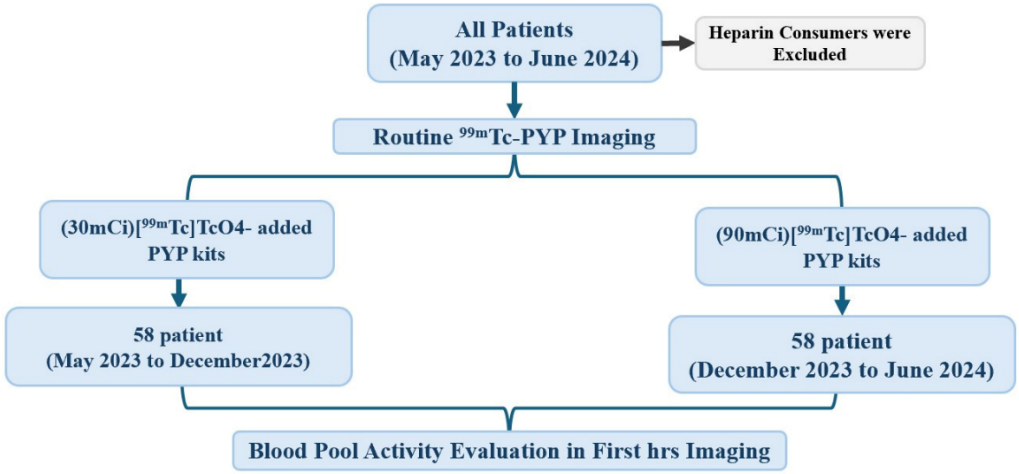


**Figure 1.** The chart depicts the workflow of the study that was carried out

### *Imaging interpretation*

The anterior planar images, rotating projection images, and reconstructed SPECT images were carefully evaluated by two consensus nuclear cardiology readers using SPECT/CT fusion software to analyze myocardial uptake of [$^{99m}$Tc]Tc-PYP in standard cardiac imaging planes. This analysis included common orientations such as short-axis, vertical long-axis, and horizontal long-axis views. To semi-quantify the myocardial uptake, circular regions of interest (ROIs) were delineated over the heart on the planar images. To account for background activity, the heart ROIs were mirrored over the contralateral chest wall. To quantify the myocardial [$^{99m}$Tc]Tc-PYP uptake, the total and mean counts were measured within the circular ROIs drawn over the heart and the mirrored ROIs on the contralateral chest wall. The heart-to-contralateral (H/CL) ratio was calculated by dividing the mean counts per pixel in the heart ROI by the mean counts per pixel in the mirrored contralateral ROI. An H/CL ratio ≥ 1.5 at 1-hour post-injection was classified as positive for ATTR-CA, while a ratio < 1.5 was considered negative for ATTR-CA [11, 12].

Tomographic reconstruction was conducted using standard techniques akin to those employed in myocardial perfusion SPECT imaging. This involved iterative reconstruction algorithms to create tomographic images from the acquired projection data. In instances where planar images indicated no or unclear myocardial uptake of [$^{99m}$Tc]Tc-PYP, regions of interest (ROIs) for reconstruction were placed in standard locations on the anterior view. The presence of diffuse or focal visualization of the left ventricular walls in the reconstructed SPECT images was interpreted as indicative of myocardial PYP uptake [9]. When

tracer uptake in myocardial segments was not evident on tomographic images, the counts observed on planar images were attributed to the blood pool. Activity in the blood pool was identified by analyzing the SPECT/CT images (Figure 2). All planar and SPECT images for this study were interpreted by two independent and experienced nuclear medicine physicians, with any discrepancies resolved by a third expert.

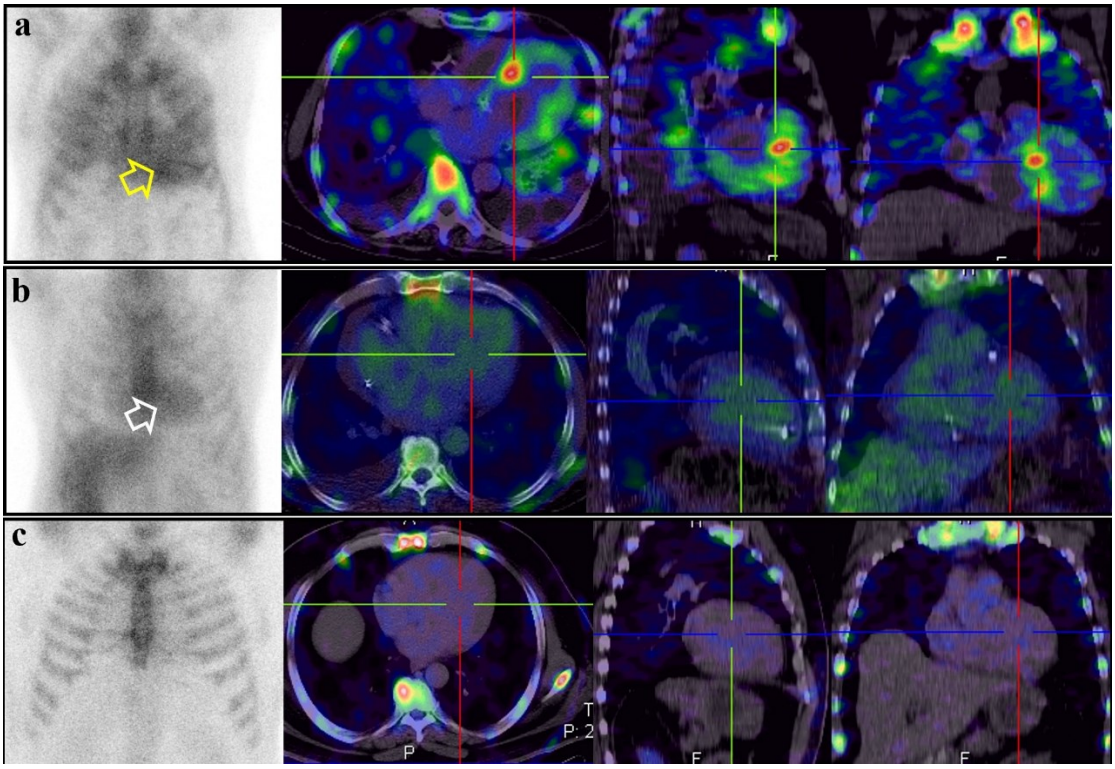


**Figure 2.** Myocardial radioactivity observed in three patients in [$^{99m}$Tc]Tc-PYP scans: (a) An 86-year-old male with positive ATTR-CA shows myocardial activity (yellow arrow) on planar imaging and SPECT/CT(PYP-90). (b) A 69-year-old male with negative ATTR-CA demonstrates activity localized to the blood pool (white arrow) (PYP-30). (c) A 45-year-old male shows no myocardial or blood pool activity on planar imaging and SPECT/CT (PYP-90)

### *Statistical analysis*

All datasets were analyzed using IBM SPSS Statistics version 27. Continuous variables were reported as mean ± standard deviation (SD), while categorical variables were expressed as frequencies and percentages. The Pearson Chi-square test was used to compare categorical subgroups, and risk estimation was performed to determine the Odds Ratio for subgroup evaluations. Both univariable and multivariable binary logistic regression analyses were conducted, with two-tailed p-values reported, considering $p < 0.05$ as statistically significant.

## RESULTS

In this prospective study, 116 patients were enrolled, consisting of 68 men (58.6%) and 48 women (41.4%), with a mean age of 71.33±11.60 years (ranging from 34 to 100). The patients were divided into two groups. The first group included 58 patients who were referred to the nuclear medicine center for imaging and received a radiopharmaceutical labeled with 30 mCi of technetium pertechnetate. The second group also comprised 58 patients who were administered a PYP kit with an additional radioactivity of 90 mCi (Figure 3).

The baseline characteristics of all enrolled patients are summarized in Table 1. No statistically significant differences were found between the two groups regarding age, gender, Body mass index (BMI), and creatinine levels, with all P values exceeding 0.05. Among the 58 patients who received 20 mCi of [$^{99m}$Tc]Tc-PYP, radiolabeled with 30 mCi of Na[$^{99m}$Tc]$TcO_4$, 37(63.8%) exhibited blood pool radioactivity in their images taken one hour post-injection, compared to 22(37.9%) in the other group. Statistical analysis indicated that the addition of a lower radioactivity (30 mCi instead of 90 mCi) to the PYP kit significantly increased the incidence of visualized BP radioactivity in the one-hour post-injection images (P value = 0.004) (Figure 4).

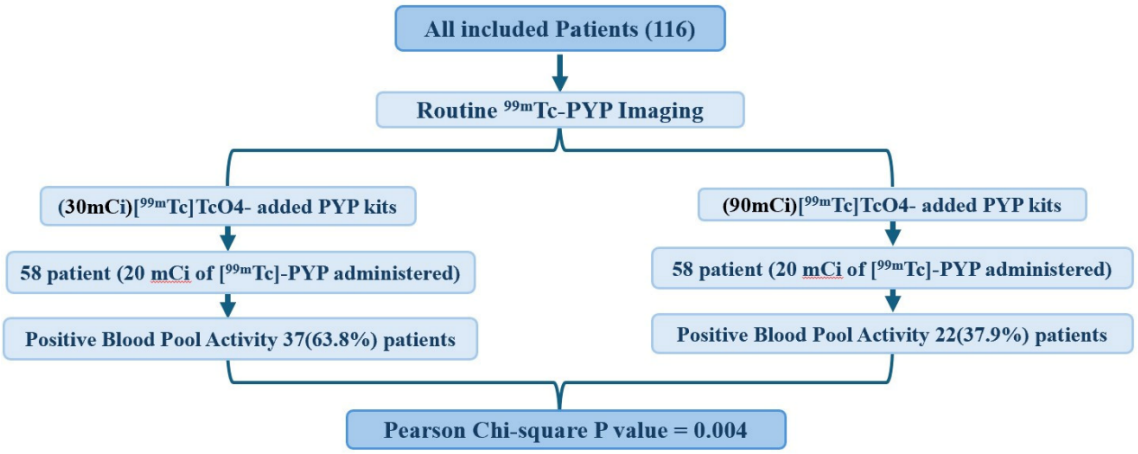


**Figure 3.** The chart illustrates the study cohort and the resulting data from the analysis of patients undergoing TPS for ATTR-CA

**Table 1.** Summary of baseline characteristics of all included patients

| Characteristics | | Group (i) (30 mCi) (n=58) | Group (ii) (90 mCi) (n=58) | P value |
|---|---|---|---|---|
| **Age ± SD** (years) | | 69.50 ± 11.45 | 73.16 ± 11.56 | 0.090 |
| **BMI ± SD** (Kg/m²) | | 29.05 ± 4.54 | 27.70 ± 4.09 | 0.097 |
| **Gender** | Female: n(%) | 25(43.1) | 23(39.7) | 0.706 |
| | Male: n(%) | 33(56.9) | 35(60.3) | |
| **Creatinine Level** | <1.5 n(%) | 33(56.9) | 40(69.0) | 0.178 |
| | ≥1.5 n(%) | 25(43.1) | 18(31) | |

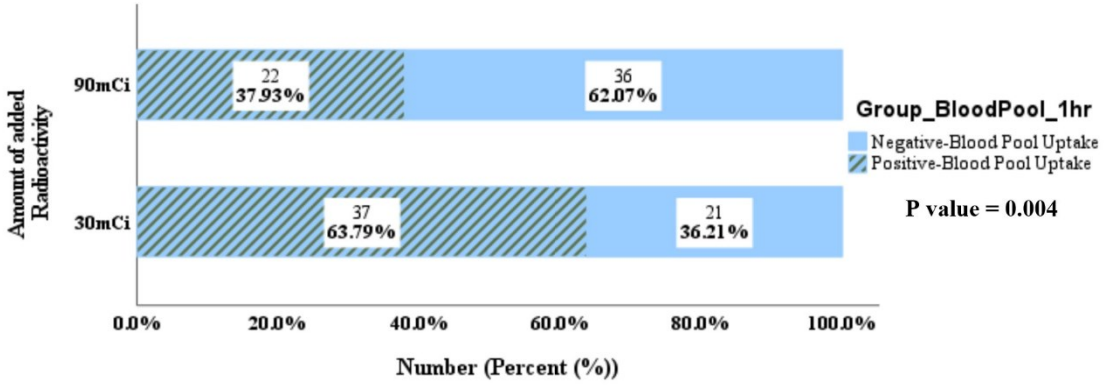


**Figure 4.** Stacked bar chart displaying blood pool radioactivity (positive or negative) in two patient groups scanned with different PYP kits (30 or 90 mCi)

The univariable binary logistic regression analysis indicated that gender, age, and BMI did not significantly influence the incidence of BP radioactivity in the PYP scans (P value > 0.05). However, creatinine levels and the amount of radioactivity added to the PYP kits were found to have significant effects (P value< 0.05) (Table 2). A multivariable logistic regression analysis was conducted using SPSS software to assess the association between positive BP radioactivity and independent variables. The analysis confirmed that gender, age, and BMI still did not significantly affect the visualization of activity in the BP. In contrast, the patients' creatinine level (P value<0.001) and the amount of radioactivity added to the kit (P value=0.005) remained significant factors influencing BP activity

visualization, with this effect not only persisting but also strengthening (Table 3).

The SPSS file was split into two groups based on creatinine levels, and statistical analysis was conducted to assess the presence of blood pool activity between these groups scanned with PYP kits containing different radioactivity levels. As shown in Table 4, patients with creatinine levels above 1.5 mg/dL did not exhibit a significant difference (P value=0.349) in BP activity between the two groups scanned with varying radioactivity kits. However, a significant difference (P value = 0.020) was observed in patients with creatinine levels below 1.5 mg/dL (Figure 5).

**Table 2.** Univariable logistic regression analysis for detecting association between Positive Blood Pool (P-BP) radioactivity and independent variables

| Univariable Binary Logistics Regression | | | | |
|---|---|---|---|---|
| **Variables** | **Blood Pool (+) n(%)** | **Odds Ratio** | | **P value** |
| | | **Base** | **95% Con. interval** | |
| **Gender (Female/Male)** | 27(45.8)/32(54.2) | 1/1.446 | 0.688 - 3.041 | 0.330 |
| **Age** | | 1.005 | 0.974 - 1.037 | 0.740 |
| **BMI** | | 0.990 | 0.910 - 1.077 | 0.817 |
| **Activity in kit (90mCi/30mCi)** | 22(37.3)/37(62.7) | 1/2.883 | 1.357 - 6.126 | **0.006** |
| **Creatinine (<1.5 / ≥1.5)** | 25(42.4) / 34(57.6) | 1/7.253 | 3.010 - 17.478 | **< 0.001** |

**Table 3.** Multivariable logistic regression analysis for detecting association between Positive Blood Pool (P-BP) radioactivity and independent variables

| Multivariable Binary Logistics Regression | | | |
|---|---|---|---|
| **Variables** | **Odds Ratio** | **95% Con. interval** | **P value** |
| **Gender (Female/Male)** | 1.833 | 0.746 - 4.506 | 0.187 |
| **Age** | 1.002 | 0.965 - 1.039 | 0.934 |
| **BMI** | 0.950 | 0.859 - 1.050 | 0.317 |
| **Activity in kit (30mCi/90mCi)** | 3.466 | 1.450 - 8.284 | **0.005** |
| **Creatinine (≥1.5 / <1.5)** | 8.956 | 2.834 - 28.284 | **< 0.001** |

**Table 4.** Results of the Chi-square test for the effect of PYP kit radioactivity (30 vs 90 mCi) on Blood Pool visibility, analysed by creatinine level in two patient groups

| **Characteristics** | | **Group (i) (30 mCi) (n=58)** | | **Group (ii) (90 mCi) (n=58)** | | **P value** |
|---|---|---|---|---|---|---|
| | | **Blood Pool (+) n(%)** | **Blood Pool (-) n(%)** | **Blood Pool (+) n(%)** | **Blood Pool (-) n(%)** | |
| **Creatinine Level** | **<1.5 n(%)** | 17(51.5) | 16(48.5) | 9(22.5) | 31(77.5) | **0.020** |
| | **≥1.5 n(%)** | 21(84) | 4(16) | 13(72.2) | 5(27.8) | 0.349 |

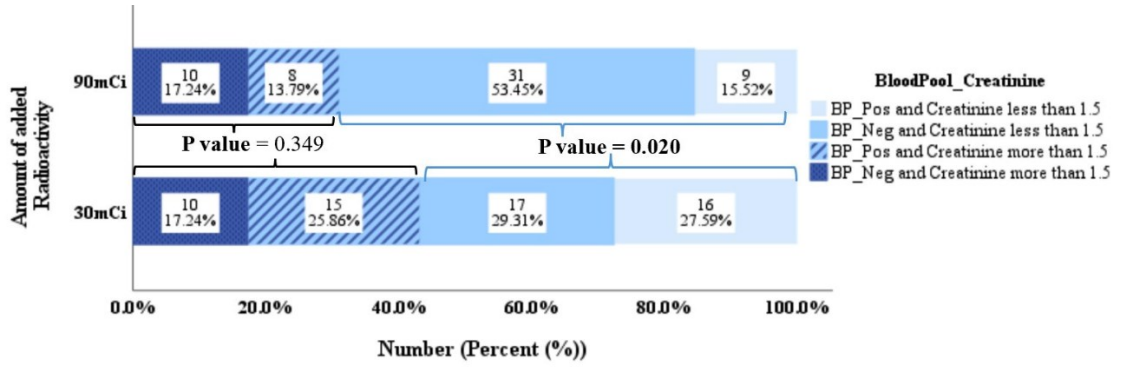


**Figure 5.** Stacked bar chart displaying blood pool radioactivity (positive or negative) in two patient groups scanned with different PYP kits (30 or 90 mCi) within the creatinine subgroup

## DISCUSSION

TPS has emerged as a highly accurate non-invasive method for diagnosing ATTR-CA, obviating the need for endomyocardial biopsy. This imaging technique is now widely utilized in nuclear medicine departments to identify patients with ATTR-CA [12]. SPECT imaging enables the differentiation between the absorption of PYP due to heart failure and the activity in the BP, which can persist for up to three hours after the injection of the radiopharmaceutical [13]. Activity in the BP has been associated with reduced cardiac output in patients with cardiac amyloidosis. However, a systematic review found no significant correlation between BP activity and cardiac performance indices measured by echocardiography [14]. Since the PYP radiopharmaceutical is also used for in vivo radio-labeling of red blood cells, the observed radioactivity in the blood pool during amyloidosis PYP scans may be related to the prepared radiopharmaceutical [15]. This study aimed to determine if the high frequency Blood Pool activity (50.9%) in patients is associated with the amount of radioactivity added to the PYP kit.

Each freeze-dried PYP kit utilized in this study contained 14 mg of Na-PYP·$8H_2O$ (MW=446.06) and 1 mg of $SnCl_2 \cdot 2H_2O$ (MW=225.65). The molar quantity of $SnCl_2$ (n=2.66×10^19) was significantly higher than that of 30mCi Na[$^{99m}$Tc]$TcO_4$ (n=3.45×10^13) or 90mCi (n=10.35×10^13). This high concentration of $SnCl_2$ (3 times higher) may influence in vivo radiolabeling of red blood cells [16], potentially affecting the observed BP radioactivity during scintigraphy performed one hour post-injection. Since Heparin interferes with the radiolabeling of red blood cells [17], patients who had received heparin in the time of imaging were excluded from this study to eliminate its confounding effects. As shown in Table 1, statistical analysis indicated no significant differences between the two main patient groups regarding age (P=0.090), gender (P=0.706), BMI (P=0.097), and creatinine levels (P=0.178) for scans conducted with PYP-30 and PYP-90 kits. This suggests that the patient distribution was appropriate, minimizing the potential confounding effects of these variables in the statistical analyses.

In Table 2, the univariable binary logistic regression analysis shows that creatinine level and the amount of radioactivity added to the PYP kits significantly affect Blood Pool activity visibility. The odds of detecting activity are higher in scans using PYP-30 kits (OR=2.883, 95% CI: 1.357-6.126, P=0.006) compared to those labeled with 90 mCi of Na[$^{99m}$Tc]$TcO_4$. The higher concentration of $Sn^{2+}$ ions remained in PYP-30 kits compared to PYP-90 may enhance in vivo labeling of red blood cells and plasma proteins upon radiopharmaceutical injection, leading to increased BP activity visualization. As illustrated in Figure 3, a greater number and percentage of patients (37, 63.79%) exhibited visible activity in the BP one hour post-injection of the radiopharmaceutical when using PYP-30 kits, compared to PYP-90 kits (22, 37.93%). The results of the statistical analysis support the hypothesis regarding the effect of the amount of radioactivity added to the kit on the visibility of activity in the BP of patients during PYP scans. The univariable binary logistic regression analysis indicated that the odds of detecting blood pool activity are approximately seven times higher in patients with creatinine levels above 1.5 (OR=7.253, 95% CI: 3.010-17.478, P< 0.001) compared to those with levels below 1.5, reflecting impaired kidney function. The radiopharmaceutical [$^{99m}$Tc]Tc-PYP is eliminated from the bloodstream at varying rates, which are affected by bone metabolism and kidney function. When bone metabolism is high and kidney function is normal, clearance occurs rapidly. Conversely, impaired renal function reduces the clearance rate, leading to a longer duration of the radiopharmaceutical's presence in the blood and increased visibility of its activity [18]. Our finding is consistent with previous studies that link impaired kidney function to increased visibility of blood pool activity in cardiac amyloidosis imaging [19].

A multivariable binary logistic regression analysis was conducted to evaluate the combined effects of various variables on BP activity visibility in patients. The results in Table 3 indicate that the influence of the activity level added to the kit and patients' creatinine levels on BP radioactivity has been reinforced. Specifically, the analysis shows that the PYP kit activity level (OR=3.466, 95% CI: 1.450-8.284, P=0.005) and creatinine levels (OR=8.956, 95% CI: 2.834-28.284, P<0.001) significantly affect presence of radioactivity in BP. Patients with PYP-30 kits have 3.5 times higher odds of visible activity compared to those with PYP-90, while those with creatinine levels above 1.5 have about 9 times greater odds of showing BP radioactivity. These findings from the multivariable analysis suggest that both the amount of added radioactivity in the PYP kits and the patient's kidney function are important factors that influence the presence of radioactivity in BP during PYP imaging, and their effects are not confounded by each other. The independent association of these variables with

BP activity visibility underscores their importance in determining the optimal imaging conditions and interpreting PYP scans.

Table 4 presents the statistical analysis results based on creatinine levels. For patients with creatinine below 1.5, Blood Pool activity was visible in 17 of 33 (51.5%) with PYP-30 kits and 9 of 40 (22.5%) with PYP-90 kits, showing a significant difference (p=0.020). In contrast, among patients with creatinine above 1.5, 21 of 25 (84%) with PYP-30 kits exhibited visible activity, compared to 13 of 18 (72.2%) with PYP-90 kits, which was not statistically significant (p=0.349). These results, illustrated in the stacked bar chart (Figure 4), indicate that in patients with normal kidney function (creatinine ≤1.5), applying PYP-30 kits significantly enhance Blood Pool activity visibility compared to PYP-90 kits, an effect not observed in patients with impaired renal function. This suggests that the impact of kidney function on BP visibility outweighs the effect of the added activity during kit preparation, rendering the latter ineffective in cases of impaired kidney function, consistent with the statistical analysis results. The likelihood of detecting BP radioactivity in scans of patients with impaired kidney function is approximately nine times greater than in those with normal kidney function. Additionally, the impact of the added activity in the kit is roughly 3.5 times when using PYP-30 kits. Consequently, the more pronounced influence of kidney function has affected the visibility of radioactivity in BP among patients with reduced kidney function.

## CONCLUSION

Technetium-99m-pyrophosphate scintigraphy demonstrates a high diagnostic accuracy for transthyretin cardiac amyloidosis, effectively eliminating the need for endomyocardial biopsy. However, the presence of radioactivity in the Blood Pool during PYP scans can complicate the interpretation of planar images and require extended and repeated scanning. The research indicates that Blood Pool activity is significantly affected by both the quantity of radioactivity incorporated into the PYP kits and the patient's creatinine levels, which reflect kidney function. Notably, patients using PYP-30 kits (higher free $Sn^{2+}$) showed a greater likelihood of visible blood pool activity compared to PYP-90 kis (lower free $Sn^{2+}$). Moreover, in patients with creatinine levels exceeding 1.5 have a significantly higher chance of observing Blood Pool activity. These results emphasize the importance of considering both the radioactivity levels in PYP kits, which are related to the concentration of free $Sn^{2+}$ in the prepared kit, and the patient's kidney function. Both factors are essential for optimizing imaging conditions and improving diagnostic accuracy in cases of cardiac amyloidosis.

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