**ORIGINAL RESEARCH**

# The Effect of High Levels of Free Sn2+ in PYP Kits on In Vivo Red Blood Cell Radiolabeling and Blood Pool Radioactivity

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**ABSTRACT**

**Objective:** This study aimed to evaluate whether the presence of high levels of free Sn2+ in pyrophosphate (PYP) kits leads to in vivo red blood cell (RBC) radiolabeling and increased blood pool (BP) radioactivity. **Methods:** A prospective study was conducted, enrolling 156 patients, including 88 men (56.4%) and 68 women (43.5%) with a mean age of 72.32 ± 12.59 years. The cohort was divided into two groups: the first group (n=78) received 30 mCi of technetium pertechnetate-labeled radiopharmaceutical, while the second group (n=58) received a PYP kit with an additional 90 mCi of radioactivity. Demographic and clinical variables, including age, gender, body mass index (BMI), and creatinine levels, were recorded and analyzed. Univariable and multivariable binary logistic regression analyses were performed to identify factors influencing BP radioactivity. **Results**: No significant differences in baseline characteristics were observed between the groups ($P > 0.05$). Univariable binary logistic regression analysis indicated that gender, age, and BMI did not significantly affect BP radioactivity ($P > 0.05$). However, creatinine levels and the amount of added radioactivity to the PYP kit were significant factors influencing BP radioactivity ($P < 0.05$). Multivariable logistic regression confirmed that creatinine levels ($P < 0.001$) and increased radioactivity ($P = 0.005$) significantly contributed to BP activity visualization. Further stratification based on creatinine levels showed no significant difference in BP activity among patients with creatinine>1.5 mg/dL ($P = 0.350$). In contrast, a significant difference was observed in patients with creatinine<1.5 mg/dL ($P = 0.021$). **Conclusion**: Our findings suggest that increased free Sn2+ levels in PYP kits contribute to in vivo RBC radiolabeling and elevated BP radioactivity, particularly in patients with normal renal function (creatinine<1.5 mg/dL). These results underscore the need for careful consideration of radioactivity levels in PYP kits to minimize unintended BP activity during imaging.

**Keywords:** Sn2+, PYP kit, blood pool radioactivity, red blood cell radiolabeling, technetium pertechnetate, nuclear medicine



## INTRODUCTION

Sn2+ (Tin (II) Ion is a divalent tin ion that acts as a reducing agent in radiopharmaceutical formulations, facilitating the labeling of technetium-99m with target molecules.PYP Kit Pyrophosphate Kit is a radiopharmaceutical preparation containing sodium pyrophosphate, used in nuclear medicine imaging to bind technetium-99m for bone and myocardial imaging.The presence of radiotracer in the bloodstream, which can indicate improper labeling of red blood cells or delayed clearance from circulation is called Blood Pool (BP) RadioactivityRed Blood Cell (RBC) Radiolabeling isThe process of tagging red blood cells with a radiotracer for diagnostic imaging, particularly in studies of blood flow and cardiac function.Technetium Pertechnetate Tc-99m Pertechnetate is A widely used radioactive tracer in nuclear medicine derived from molybdenum-99, employed for imaging various organs.

Pyrophosphate (PYP) imaging has been widely used in nuclear medicine for myocardial and skeletal scintigraphy. However, concerns have been raised regarding the potential impact of free Sn2+ ions in PYP kits on in vivo red blood cell (RBC) radiolabeling and blood pool (BP) radioactivity. Tin (II) ions play a crucial role in the reduction of technetium-99m (Tc-99m) pertechnetate, facilitating efficient radiolabeling. However, excess Sn2+ may lead to uninteanded binding with circulating RBCs, increasing BP activity and affecting image quality.

Several studies have highlighted the significance of radiopharmaceutical composition in optimizing imaging results. Hladik et al. (2018)[1] discussed the importance of radiopharmaceutical quality control to

ensure appropriate labeling efficiency and minimize non-specific uptake. Similarly, Peters [2] emphasized the impact of blood element radiolabeling on diagnostic accuracy in nuclear medicine applications. Additionally, Giubbini et al. [3] explored the role of renal function in radiotracer kinetics, noting that altered renal clearance can influence BP activity.[4]

Given these considerations, this study aims to evaluate whether high levels of free Sn2+ in PYP kits contribute to in vivo RBC radiolabeling and increased BP radioactivity. Understanding these effects can aid in optimizing PYP kit formulation and administration protocols to enhance diagnostic accuracy and minimize imaging artifacts.

## MATERIALS AND METHODS

**Study Design and Population:** This prospective study was conducted on 156 patients who underwent nuclear medicine imaging at a tertiary medical center. The study cohort included 88 men (56.4%) and 68 women (43.5%) with a mean age of 72.32 ± 12.59 years. Patients were divided into two groups: 78 patients received a radiopharmaceutical labeled with 30 mCi of technetium pertechnetate, and 58 patients received a PYP kit with an additional radioactivity of 90 mCi.

**Radiopharmaceutical Preparation:** The PYP kits were prepared following standard guidelines for nuclear medicine imaging.[1] Technetium-99m pertechnetate was obtained from a Mo-99/Tc-99m generator and mixed with PYP kits containing varying Sn2+ levels. The final product was assessed for radiochemical purity to ensure optimal labeling efficiency.[5]

**Imaging and Data Collection:** All patients underwent standard imaging protocols using a gamma camera equipped with a low-energy high-resolution collimator. Blood samples were collected pre- and post-injection to evaluate RBC radiolabeling and blood pool (BP) activity. Demographic and clinical variables, including age, gender, BMI, and creatinine levels, were recorded.

**Statistical Analysis:** Data were analyzed using SPSS software. Univariable and multivariable logistic regression analyses were performed to assess the association between BP radioactivity and independent variables such as age, gender, BMI, creatinine levels, and radioactivity dose. Statistical significance was set at $P < 0.05$.

**Ethical Considerations:** The study was approved by the institutional ethics committee, and all patients provided informed consent before participation.

## RESULTS

In this prospective study, 156 patients were enrolled, consisting of 88 men (56.4%) and 68 women (43.5%), with a mean age of 72.32±12.59 years (ranging from 34 to 100). The patients were divided into two groups. The first group included 78 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. 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.

**Table1. Summary of baseline characteristics of all included patients**

| Characteristics | | Group(i)(30mCi)(n=78) | Group(ii)(90mCi)(n=78) | P value |
|---|---|---|---|---|
| **Age±SD** (years) | | 70.49 ±12.44 | 74.15 ±12.55 | 0.091 |
| **BMI±SD**(Kg/m$^2$) | | 30.04 ±5.53 | 28.69 ±5.08 | 0.098 |
| **Gender** | Female:n(%) | 35(44.8) | 33(42.3) | 0.708 |
| | Male:n(%) | 43(55.1) | 45(57.6) | |
| **Creatinine Level** | <1.5n(%) | 43(55.1) | 50(64.1) | 0.179 |
| | ≥1.5n(%) | 35(44.8) | 28(35.8) | |

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).

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

| Univariable Binary Logistics Regression | | | | |
|---|---|---|---|---|
| | | | **Odds Ratio** | |
| **Variables** | **Blood Pool(+)n(%)** | | | **P value** |
| | | **Base** | **95%Con. interval** | |
| **Gender(Female/Male)** | 37(47.4)/41(52.5) | 1/1.447 | 0.689-4.040 | 0.331 |
| **Age** | | 1.006 | 0.975-1.038 | 0.741 |
| **BMI** | | 0.991 | 0.911-1.078 | 0.818 |

| | | | | |
|---|---|---|---|---|
| **Activity in kit(90mCi/30mCi)** | 32(41.0)/46(58.9) | 1/2.884 | 1.358-7.127 | **0.007** |
| **Creatinine(<1.5/ ≥1.5)** | 35(44.8)/43(55.1) | 1/7.254 | 4.011-18.479 | **< 0.001** |

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).

**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)** | 1.834 | 0.747-5.505 | 0.188 |
| **Age** | 1.003 | 0.966-2.038 | 0.935 |
| **BMI** | 0.951 | 0.860-1.059 | 0.318 |
| **Activity in kit(30mCi/90mCi)** | 4.465 | 1.451-9.285 | **0.006** |
| **Creatinine(≥1.5/<1.5)** | 9.955 | 3.835-29.285 | **<0.001** |

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=0.350) in BP activity between the two groups scanned with varying radioactivity kits. However, a significant difference (P value = 0.021) was observed in patients with creatinine levels below 1.5 mg/dL.

**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**

| | | Group(i) (30mCi) (n=78) | | Group(ii)(90mCi)(n=78) | | |
|---|---|---|---|---|---|---|
| **Characteristics** | | **Blood Pool(+) n(%)** | **Blood Pool(-) n(%)** | **Blood Pool(+) n(%)** | **Blood Pool(-) n(%)** | **P value** |
| **Creatinine Level** | **<1.5n(%)** | 22(45.8) | 21(49.4) | 14(23.4) | 36(78.4) | **0.021** |
| | **≥1.5n(%)** | 26(54.1) | 9(17.2) | 18(73.1) | 10(28.3) | 0.350 |

**DISCUSSION**

A prospective study was conducted, enrolling 156 patients, including 88 men (56.4%) and 68 women (43.5%) with a mean age of 72.32 ± 12.59 years. The cohort was divided into two groups: the first group (n=78) received 30 mCi of technetium pertechnetate-labeled radiopharmaceutical, while the second group (n=58) received a PYP kit with an additional 90 mCi of radioactivity. Demographic and clinical variables, including age, gender, body mass index (BMI), and creatinine levels, were recorded and analyzed. Univariable and multivariable binary logistic regression analyses were performed to identify factors influencing BP radioactivity.No significant differences in baseline characteristics were observed between the groups (P > 0.05). Univariable binary logistic regression analysis indicated that gender, age, and BMI did not significantly affect BP radioactivity (P > 0.05). However, creatinine levels and the amount of added radioactivity to the PYP kit were significant factors influencing BP radioactivity (P < 0.05). Multivariable logistic regression confirmed that creatinine levels (P < 0.001) and increased radioactivity (P = 0.005) significantly contributed to BP activity visualization. Further stratification based on creatinine levels showed no significant difference in BP activity among patients with creatinine>1.5 mg/dL (P = 0.350). In contrast, a significant difference was observed in patients with creatinine<1.5 mg/dL (P = 0.021).

The findings of this study provide valuable insight into the influence of free Sn2+ levels in PYP kits on in vivo RBC radiolabeling and subsequent BP radioactivity. Notably, the presence of excess Sn2+ has been previously associated with increased RBC labeling, leading to unintended BP visualization in nuclear medicine imaging (Hladik et al., 2018).[1] Our study confirms this association, particularly in patients with normal renal function (creatinine<1.5 mg/dL).

Multivariable logistic regression analysis demonstrated that creatinine levels and the amount of radioactivity added to the PYP kit significantly influenced BP activity. Patients with higher creatinine levels (>1.5 mg/dL) did not show significant differences in BP activity, indicating that renal impairment might alter radiotracer clearance, reducing unintended RBC labeling. [3]This aligns with findings by Peters (2020)[2], who emphasized that renal function plays a crucial role in radiotracer metabolism and distribution.

Our results also show that increasing the radioactivity in the PYP kit to 90 mCi significantly contributed to BP radioactivity. This suggests that higher radioactivity may enhance the probability of in vivo RBC labeling, leading to excessive BP activity. These findings are consistent with previous studies by,[5] who noted that inappropriate radiopharmaceutical preparation and excess stannous ion concentrations can contribute to increased BP visualization.

Interestingly, our stratified analysis demonstrated that patients with creatinine levels below 1.5 mg/dL were more susceptible to BP radioactivity when exposed to higher levels of Sn2+. This reinforces the importance of individualized radiopharmaceutical dosing to optimize imaging outcomes and reduce non-specific uptake. Previous studies have recommended monitoring renal function before radiotracer administration to ensure appropriate distribution and clearance.[4]

Our study has certain limitations, including the lack of a direct quantitative assessment of free Sn2+ concentrations in PYP kits. Future research should focus on precisely quantifying Sn2+ levels and their direct impact on RBC labeling efficiency. Additionally, patient follow-up for potential long-term effects of increased BP radioactivity should be considered.

In conclusion, our findings highlight the critical role of free Sn2+ levels in PYP kits in determining BP radioactivity and suggest that patient-specific factors such as renal function should be taken into account when optimizing radiopharmaceutical administration. Careful regulation of Sn2+ concentrations may help minimize unintended RBC labeling and improve the accuracy of nuclear imaging procedures.

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.[6] 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.[7] 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].[8] Since the PYP radiopharmaceutical is also used for in vivo radiolabeling of red blood cells, the observed radioactivity in the blood pool during amyloidosis PYP scans may be related to the prepared radiopharmaceutical.[9]

The radiopharmaceutical [99mTc]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.[10] Our finding is consistent with previous studies that link impaired kidney function to increased visibility of blood pool activity in cardiac amyloidosis imaging.[11,12]

## CONCLUSION

Our findings suggest that increased free Sn2+ levels in PYP kits contribute to in vivo RBC radiolabeling and elevated BP radioactivity, particularly in patients with normal renal function (creatinine<1.5 mg/dL). These results underscore the need for careful consideration of radioactivity levels in PYP kits to minimize unintended BP activity during imaging.

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