

## DIAGNOSTIC ACCURACY OF 99 MTC LABELLED UBIQUICIDIN (29-41) SPECT/CT FOR DIAGNOSIS OF OSTEOMYELITIS IN DIABETIC FOOT

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### ABSTRACT

**Objective:** To determine diagnostic accuracy of 99mTc labelled Ubiquicidin (29-41) SPECT/CT for detection of osteomyelitis in diabetic foot patients by taking bone biopsy as gold standard.

**Study Design:** Cross-sectional validation study.

**Place and Duration of Study:** Nuclear Medical Centre, Armed Forces Institute of Pathology, from Apr 2017 to Mar 2018.

**Methodology:** Study assessed 122 patients of both genders, aged between 30-80 years (mean age=55.3 years), presenting with diabetic foot ulcers having suspicion of osteomyelitis, by 99mTc-Ubiquicidin (29-41) SPECT/CT followed by bone biopsy (histopathology and culture) taken as gold standard.

**Results:** Among 122 patients [94 male (77%) and 28 female (23%)], osteomyelitis was histopathologically confirmed in 113 patients. 107 out of these patients were positive for osteomyelitis on 99mTc-UBI (29-41) SPECT/CT (true positives) while 6 were false negative. Out of 9 patients declared negative for osteomyelitis on histopathology and culture, 8 were negative on 99mTc-UBI (29-41) SPECT/CT as well (true negative) while only 1 case came out to be positive (false positive). Thus, the 99mTc-UBI (29-41) scan showed 94.6% sensitivity, 88.89% specificity, 99% positive predictive value, 57% negative predictive value with overall 94.2% diagnostic accuracy.

**Conclusion:** 99mTc labelled Ubiquicidin (29-41) SPECT/CT scan can precisely localize infective focus, in diabetic foot osteomyelitis, with simultaneous discrimination between bone and soft tissues.

**Keywords:** Diabetic foot, Infection imaging, Osteomyelitis, 99mTc-UBI (29-41) SPECT/CT.

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### INTRODUCTION

Diabetes mellitus (DM) is chronic incapacitating disease and fast growing epidemic. Diabetic foot is one of the major complication of DM, associated with ulcerations and infections, characterized by rapid progress and compromised healing. In addition to substantial psychological effect for the patient, it has enormous economic burden for both patients and the society<sup>1</sup>. A diabetic patient has got a 12-25% lifetime probability of evolving foot ulcers<sup>2</sup>. More than 20% of diabetic feet necessitate life and limb saving amputation<sup>3</sup>.

Diabetic foot ulcers are entry points for microbes leading to debilitating osteomyelitis. Osteomyelitis, at most of the times, can become challenging especially discriminating it from non-infectious osteoarthropathy. Clinical examination and imaging like plain X-ray, ultrasonography, CT and MRI can be adequate, but their sensitivity is of limited use when they lack specificity for origin of infection. Therefore, bone biopsy followed by histopathology and culture remains the gold standard for diagnosing osteomyelitis<sup>4</sup>.

Various radiolabelled mediators and procedures including 99mTc-MDP 3-phase bone scan, radiolabelled leucocytes or anti-granulocyte antibodies, 99mTc labeled liposomes, chemotactic peptides, cytokines, interleukins, platelet factor-4, and monoclonal antibodies have already been assessed posing varying results. However, none could precisely distinguish between sterile inflammation and infection. 18F-FDG PET and PET/CT utility in diagnosis of osteomyelitis in diabetic foot is not yet clearly defined. Therefore, discovering an ideal radio-pharmaceutical and procedure for precise and prompt diagnosis of osteomyelitis in diabetic foot is yet awaited<sup>5</sup>.

Ubiquicidin (29-41) [UBI] is a synthetic antimicrobial peptide existing in various animals including humans. Originally, it was sequestered from mouse macrophage cells. Later, same UBI was sequestered from human airway epithelial cells. It is extremely homologous to S30 protein purified from rat liver<sup>6</sup>. It is cost effective, carries low radiation burden, easy labeling procedure and high labeling efficiency. 99mTc-labelled peptides may enable the monitoring of the efficacy of antimicrobial therapy<sup>6</sup>. UBI (29-41) attaches to microbial cell membrane present at infection site<sup>7</sup>. They concentrate at infection sites, not in disinfected

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inflammatory lesions, because of chemical interaction between their cationic peptide portion and anionic surface of microbes<sup>8</sup>. Effective dose equivalent is  $4.29 \times 10^{-3}$  mSv/MBq<sup>9</sup> and no significant hostile effects or drug toxicity has been witnessed in animal or humans<sup>9</sup>.

Rationale of this study was to validate radiopharmaceutical (UBI) as well as technique (SPECT/CT) in our setup and incorporate them in routine workup of patients with diabetic foot in order to avoid bone biopsy which is invasive and painful.

## METHODOLOGY

This cross sectional validation study was carried out at Nuclear Medical Centre, Armed Forces Institute of Pathology Rawalpindi from April 2017 to March 2018 after approval from Hospital ethics committee. Through non-probability consecutive sampling, 122 diabetic individuals of both genders and varying ages, having foot ulcers and clinical suspicion of osteomyelitis were enrolled after written informed consent. Patients with history of foot fractures and gout were excluded. Additionally patients having known hypersensitivity to any component of 99mTc-UBI (29-41) and pregnant or lactating females were also excluded.

Patients were briefed about the procedure and written informed consent was obtained. In first phase, each patient underwent 99mTc-UBI (29-41) SPECT/CT scan followed by bone biopsy and culture of the specimen. UBI (29-41) kits supplied by PINSTECH Islamabad, were labeled with 99mTc ensuring all QC protocols and labeling efficiency of >95%. Each patient was given 370 MBq (10 mCi) 99mTc-UBI (29-41) injected I/V in supine position with concurrent dynamic image acquisition was done at 10 seconds per frame for total 60 seconds in 64 x 64 matrix size. Static equilibrium blood pool images were acquired for 2 minutes immediately after dynamic study in 256 x 256 matrix. 1000 kcnt Planar imaging of suspected lesion in anterior and posterior projections was done 30 minutes post injection followed by SPECT/CT tomograms using dual head Gamma Camera Symbia Truepoint with SPECT/CT (SIEMENS) furnished with high-resolution low-energy collimators and a low dose T6 X-ray tube. SPECT images were done at 64 projections over non-circular 360 degree orbit followed by 70kVa CT Data processing was done using Syngo MI Application 2009 AE-soft Software. Tomographic reconstruction was done using filtered back projection. Images were read by two experienced nuclear medicine physicians and

interpreted according to the visual assessment criteria given in table-I.

Each scan was followed by bone biopsy, its histopathology and culture for bacterial growth. Infection was established by presence of necrotic tissue, types of cellular infiltration and culture of microbes.

Data was analyzed using SPSS-21. Mean and standard deviation were calculated for quantitative variables like age. Frequency and percentages were calculated for qualitative variables like gender, positive or negative results of 99mTc-UBI SPECT/CT. A 2 x 2 table was drawn to calculate sensitivity, specificity, PPV, NPV and overall diagnostic accuracy with 95% confidence interval (table-II).

**Table-I: Visual assessment criteria.**

		Visual Score	
99mTc-UBI SPECT/CT	Positive	3	Intense tracer uptake (equivalent to kidney)
		2	Intense tracer uptake (equal to liver)
	Negative	1	Intense tracer uptake (less than liver)
		0	No or minimal uptake (equivalent to soft tissue)

**Table-II: Data presentation in 2 x 2 table.**

		Bone Biopsy		
		Positive	Negative	Total
99mTc-UBI SPECT/CT	Positive	107	1	108
	Negative	6	8	114
Total		113	9	122

## RESULTS

Out of 122 patients, 94 male (77%) and 28 females (23%), were evaluated. Their ages ranged from 30-80 years with mean  $\pm$  SD of  $56.67 \pm 12.59$  years, shown in table-III. Osteomyelitis was diagnosed on histopathology in 113 (92.6%) patients. However, on 99mTc-UBI (29-41) SPECT/CT, out of these 113 individuals, 107 (94.6%) were declared positive for osteomyelitis (true positives) while 6 were found to be negative (false negatives). Among 9 patients who were negative for osteomyelitis on histopathology, 8 (88.9%) were negative on 99mTc-UBI (29-41) SPECT/CT as well and only 1 (11.1%) came out to be positive (false negative). The results are summed up in table-III. All the 9 cases who were negative for osteomyelitis follow up was done which showed adequate healing of foot ulcers. Out of 8 true negative cases, 4 (50%) had soft tissue infection. Based on these calculations, the 99mTc-UBI (29-41) scan showed 94.69% sensitivity, 88.89% specificity, 99.07% positive predictive value, 57.14% negative pre-

dictive value with 94.26% diagnostic accuracy (table-III).

**Table-III: Demographic distribution.**

Age (Years)	Frequency	Percentage
30-40	18	14.8
41-50	19	15.6
51-60	32	26.2
61-70	39	32.0
71-80	14	11.5
Total	122	100.0

**DISCUSSION**

Around 20% patients of diabetic foot infection (DFI) have osteomyelitis and over 60% of patients with severe foot infections pose underlying osteomyelitis in some stage of disease. About 44-68% of diabetic patients hospitalized for a foot infection have bone infection<sup>10</sup>. This increases the likelihood of surgical intervention, amputation or an extended treatment with antibiotics which necessitates prompt diagnosis<sup>11</sup>.

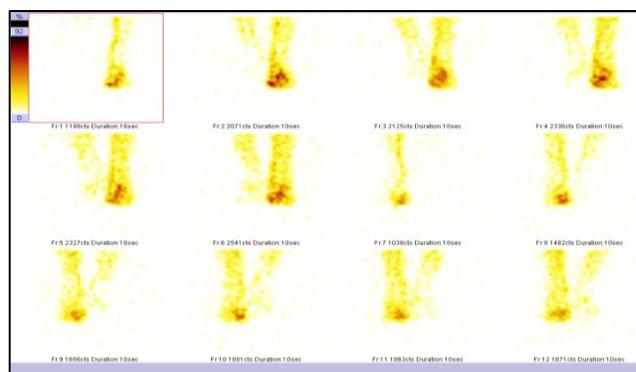
Histopathology and culture is “Gold Standard” for osteomyelitis. However, it is invasive and may not always give correct result as the biopsy can miss actual lesion. Cultures can be negative in case patient is taking antibiotics and wrongly positive if the specimen gets contaminated. Also, bone biopsy being invasive may cause osteomyelitis or necrosis of the bone<sup>12</sup>.

Plain X-ray is typically first step to assess foot infection but sensitivity is reported to be 43-75% and specificity 75-83%. MRI is considered accurate diagnostic test for the diabetic foot osteomyelitis; however, its sensitivity ranges from 77-100%, specificity is limited due to other causes of marrow edema present in Charcot’s osteo-neuropathy and a number of other conditions<sup>13</sup>.

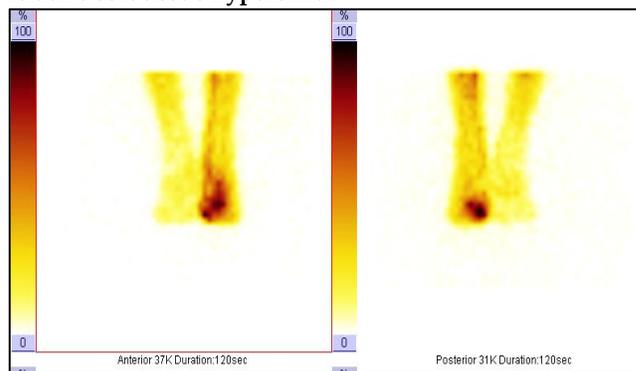
Three-phase bone scan was vastly employed nuclear technique for musculoskeletal infections including diabetic foot osteomyelitis. It is unfortunately non specific and cannot differentiate between osteomyelitis, Charcot osteo-neuropathy, trauma or other conditions affecting bones. Meta-analysis of 3-phase bone scintigraphy, for diabetic foot infection by utilizing planar imaging or SPECT, shows around 90% sensitivity but <50% specificity<sup>13</sup>.

Labeled leucocytes have been extensively studied with 72-100% reported sensitivity and 67-100% reported specificities<sup>13-14</sup>. This technique is laborious, time consuming and technically demanding. Its sensitivity in neutropenia and chronic infection is also questionable<sup>15</sup>. Complementary marrow imaging may be req-

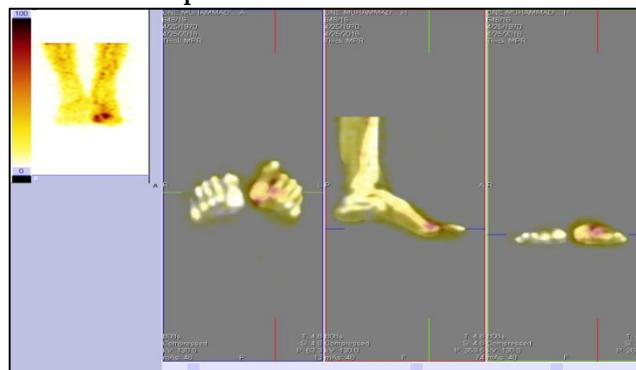
uired if neuropathic joints are also present, increasing the dose, time and cost of the procedure. In vivo labeling of leucocytes with antibodies (99mTc BW 250/183, 99mTc-fanolesomab) or antibody fragments (99mTc Sulesomab) have also been used in the diabetic foot infection imaging with varying sensitivities and specificities and comparable accuracies with that of in vitro labeled leucocytes<sup>16</sup>.



**Figure-1: One minute dynamic flow images of lower legs demonstrating hyperemia in left fore foot along with relative soft tissue hyperemia.**



**Figure II: Equilibrium blood pool images demonstrating increased tracer pool in fore foot.**



**Figure-3: SPECT/CT images showing increased tracer uptake in 2nd metatarsal head along with adjacent soft tissue uptake.**

Out of all labeled antibiotics, only  $^{99m}\text{Tc}$  Ciprofloxacin (Infecton) was investigated for identification of diabetic foot infection and established less precise than labeled leukocyte imaging for diabetic foot osteomyelitis<sup>14</sup>. Ceftizoxime was labeled with  $^{99m}\text{Tc}$  and employed in diabetic foot infection, results demonstrated excellent sensitivity and specificity<sup>15</sup>.

PET/CT with  $^{18}\text{F}$ -FDG is emerging as significant instrument for the diagnosis of various infections<sup>17</sup>. However, its role in diabetic foot infection is still undefined. Diagnostic accuracies are wide-ranging from 54-94%<sup>17</sup>. The major limitations of PET/CT include its reduced specificity as FDG also accumulates in tumors. Moreover it is an expensive modality and has limited availability, especially in developing countries like Pakistan.

$^{99m}\text{Tc}$ -UBI (29-41) has offered substantial outcomes in characterizing infection from inflammation in several investigational studies. Akhtar *et al* studied 18 patients alleged of bone, soft tissue or prosthesis infection with help of  $^{99m}\text{Tc}$ -UBI (29-41) scintigraphy. Their results displayed 100% sensitivity, 80% specificity, 92.9% positive predictive value, 100% negative predictive value and overall 94.4% diagnostic accuracy<sup>18</sup>. Assadi *et al*, considered  $^{99m}\text{Tc}$ -UBI (29-41) scintigraphy for recognition of osteomyelitis in contrast to  $^{99m}\text{Tc}$ -MDP and magnetic resonance imaging. The results showed 100% accuracy of  $^{99m}\text{Tc}$ -UBI (29-41) scintigraphy<sup>19</sup>. The Melendez-Alafort *et al*, evaluated 6 children, of assumed bone infection, with  $^{99m}\text{Tc}$ -UBI (29-41) scintigraphy. The stated sensitivity, specificity and accuracy were 100%<sup>20</sup>. Gandomkar *et al*, assessed  $^{99m}\text{Tc}$ -UBI (29-41) scintigraphy in 7 subjects of suspected bone or soft-tissue infection and reported 100% sensitivity and specificity **Error! Reference source not found.**<sup>1</sup>. Saeed *et al*, studied a group of 65 diabetic foot patients. Each patient underwent  $^{99m}\text{Tc}$  MDP bone scan and  $^{99m}\text{Tc}$ -UBI (29-41) scintigraphy. Both scans were than visually overlaid to differentiate between soft tissue and bone infection. The sensitivity, specificity and diagnostic accuracy came out to be 5 (100%).

Our study was intended to authenticate role of  $^{99m}\text{Tc}$ -UBI (29-41) SPECT/CT in the diagnosis of osteomyelitis in diabetic foot. Hybrid technology of SPECT/CT was used to see whether the uptake is in the bone or in soft tissue to demonstrate the extent of the infection. The sensitivity came out to be 94.69%, specificity 88.89%, positive predictive value 99.07%,

negative predictive value 57.14% and diagnostic accuracy of 94.26%, respectively.

The specificity in our study did not come out 100% and was reduced because of one false positive result. This patient's scan was positive; however, bone biopsy was inconclusive for infection. This could be due to multiple factors, most important being the "miss target", the proper bone biopsy sample may not be obtained from the site of the actual lesion in the bone.

Bone biopsy may produce false-negative results, either because of patchy involvement by the infection or because of previous antibiotic therapy. Other reasons include low number of micro-organisms in tissue, a prolonged time to transport specimen to laboratory, and may include study limitation like previous antibiotic therapy. In this particular patient, after investigation, it was found that he had been taking antibiotic on and off, though he was off antibiotics at the time of the scan.

Most of the patients in our study were true positive for the osteomyelitis. The possible reason may be that most of the patients in our study had a very strong clinical suspicion and hence the results may have been heavily influenced by the pretest probability of osteomyelitis. All the patients with negative study for osteomyelitis were followed up and were found to have excellent resolution of the ulcers.

Multicenter assessment, in vitro and in vivo, has shown proof that there is very precise role of  $^{99m}\text{Tc}$ -UBI (29-41) to trace infection earlier in the progress of disease. The sensitivity, specificity and accuracy of  $^{99m}\text{Tc}$ -UBI (29-41) scintigraphy is found to be 100% in diagnosis of osteomyelitis in recent studies. Meta-analysis of pooled data from ten studies done from 2004 to 2010 revealed overall 94.5 % sensitivity, 92.7% specificity and 93.7% accuracy of  $^{99m}\text{Tc}$ -UBI (29-41) scintigraphy in diagnosing various infections including osteomyelitis<sup>22</sup>.

SPECT/CT is a fusion of functional and anatomical images. Studies were conducted with  $^{99m}\text{Tc}$  HMPAO leukocyte SPECT/CT to diagnose osteomyelitis and have demonstrated that it is more accurate in differentiating diabetic foot osteomyelitis and contiguous soft-tissue infection<sup>23</sup>.

Bar-Shalom *et al*, studied patients of infectious disease with SPECT/CT using  $^{67}\text{Ga}$  and  $^{111}\text{In}$ -leucocytes. He reported 48% enhanced detection and localization of infection provided by SPECT/CT<sup>24</sup>. He also

found that clinical worth of SPECT/CT was much greater in case of labeled leucocytes as compared to  $^{67}\text{Ga}$  (63% vs. 36%). Combination of functional and anatomic imaging data is more important when greatly specific, target-oriented tracers are used for imaging.

In this study, SPECT/CT enabled us to localize precisely and provide a higher diagnostic accuracy than conventional scans. It helped to exclude bone involvement which on planar and SPECT images was misread as osteomyelitis. This is particularly significant because the treatment of soft-tissue and bone infections is different. It was also helpful in defining the extent of the infection (soft tissue  $\pm$  osteomyelitis). In final diagnosis, SPECT/CT added significantly in 57.5% of patients. Interestingly, no contributory role of SPECT/CT was observed in case of negative planar scan.

### ACKNOWLEDGMENT

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### CONCLUSION

$^{99\text{mTc}}$ -UBI (29-41) SPECT/CT imaging is a capable imaging modality for early detection of diabetic foot osteomyelitis. It can precisely localize infective focus with simultaneous discrimination between bone and soft tissues.

### CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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