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CASE REPORT

^{68}Ga-nitroimidazole PET/CT imaging of hypoxia in tuberculosis: A case series

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Abstract

Tuberculosis (TB) lesions in humans have been proven to be severely hypoxic with hypoxia leading to latency and dormancy of disease. Dormant TB lesions become less susceptible to standard TB treatment regimens with varying responses to treatment but may have increased susceptibility to nitroimidazole drugs. This in turn implies that positron emission tomography / computed tomography (PET/CT) imaging with radiolabelled nitroimidazoles may identify patients who will benefit from treatment with antimicrobial agents that are active against anaerobic bacteria. This case series aims to highlight the hypoxic uptake and retention of a novel ^{68}Ga-labelled hypoxia-seeking agent in TB lesions at different time points during anti-TB therapy using PET/CT imaging. Patients with confirmed TB underwent whole-body PET/CT after administration of a ^{68}Ga-nitroimidazole derivative at baseline and follow-up. Images were analysed both qualitatively and semi-quantitatively. Hypoxic uptake and change in uptake over time were analysed using lesion-to-muscle ratio (LMR) and lesion-to-blood ratio (LBR). ^{68}Ga-nitroimidazole avid lesions were demonstrated most frequently in the upper lobes of the lung. Low-grade hypoxic uptake was visualised in areas of consolidation, cavitation, nodules and lymph nodes. From baseline to follow-up imaging, the LMR increased with persistent hypoxic load despite morphologic improvement. This case series highlights the dynamic hypoxic microenvironment in TB lesions. From these initial data, it appears that ^{68}Ga-nitroimidazole is a promising candidate for monitoring hypoxic load in patients diagnosed with TB. Such imaging could identify patients who would benefit from individualised therapy targeting other mechanisms in the TB microenvironment with the intention to predict or improve treatment response.

Introduction

Tuberculosis (TB) continues to be one of the leading causes of death worldwide[1] and is considered a 'successful' pathogen due to its ability to become dormant in response to host immune pressures.[2] Imaging plays a vital role in diagnosis, monitoring response to therapy and detecting residual disease in TB. With renewed interest in host-directed therapies (HDTs) to supplement existing therapies,[3] there is an increasing trend in using positron emission tomography/computed tomography (PET/CT) to identify the active disease and monitor treatment response.[4,5] Current treatment options for TB are threatened by the length and complexity of the treatment, as well as treatment-limiting toxicity, high cost, non-compliance, drug interactions and emerging drug resistance.[6,7] Emphasis has shifted toward HDT where the individual's response to the disease is used to refine treatment by targeting host factors rather than pathogen components.[7–9] TB infection is viewed as a continuous spectrum of immune responses, which evolves and adapts to stressors in the host.[10] One such stressor is

hypoxia. TB lesions contain a dynamic hypoxic microenvironment, and hypoxia contributes to disease progression, as bacilli adapt to lack of oxygen and low nutrient concentration.[11,12] Hypoxia is associated with reduced susceptibility to standard drug regimens but increased susceptibility to nitroimidazoles.[13,14] Nitroimidazoles with both aerobic and anaerobic activity, now in clinical trials, may increase the sterilising potency of future treatment regimens.[15,16] This poses the question as to whether radiolabelled nitroimidazoles could be used to target hypoxic areas in TB,[14] in order to determine whether patients would benefit from augmenting current TB treatment regimens with nitroimidazoles. The potential of hypoxic imaging using radiolabelled nitroimidazoles as a method of risk stratification should be explored. The purpose of this case series is to propose the potential for ^{68}Ga-1,4,7-triazacyclononane-1,4,7-tris [methyl(2-car-boxyethyl)phosphinic acid] (TRAP)-nitroimidazole (^{68}Ga-nitroimidazole) to be utilised for hypoxic imaging within TB lesions. Visualising the hypoxic burden in TB could assist with clinical decision-making as to whether the patient may benefit from augmenting the standard TB therapy regime with anaerobic antimicrobial agents.

Methods

Four patients diagnosed with TB were recruited from the local TB clinic. The in-house SnO2-based ^{68}Ge/^{68}Ga generator [iThemba Labs, South Africa] was used to label the ^{68}Ga-nitroimidazoles (the product is not labelled for the use under discussion, and the product is still investigational). All patients underwent PET/CT imaging on a Siemens Biograph 40 [Siemens, Germany] PET/CT camera. Patients had a baseline and follow-up scan. PET/CT images were acquired from the skull vertex to the base of the pelvis in three-dimensional mode with a 4-min emission scan over 7–9 PET bed positions. Images were processed using the Syngo.Via (Siemens Medical Solution, IL, USA) processing software. Circular regions of interest (ROIs), 10 mm in size, were drawn within pathologic areas as identified on the CT images to obtain the mean standardised uptake value (SUV). Identical ROIs were also drawn in the sternocleidomastoid muscle and the left ventricle to calculate the SUV lesion-to-muscle ratio (LMR) and the SUV lesion-to-blood ratio (LBR), respectively. Regions were classified as hypoxic if the LMR was greater than 1.0 and the LBR was greater than 0.5 as described in other studies.[17–20] The semi-quantitative measurements including standardised uptake values (SUV) and ratios (SUV_{mean}, SUV_{max}, LMR_{mean}, LMR_{max}, LBR_{mean} and LBR_{max}) were compared between the baseline and follow-up images using a Wilcoxon signed-rank test. The sternocleidomastoid muscle was used for the LMR, and the left ventricle was used as the blood surrogate region to calculate the LBR.[21] The study was approved by the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (564/2018).

Results

The hypoxic uptake of ^{68}Ga-nitroimidazole is presented in four cases who underwent a baseline and follow-up PET/CT.

Case 1

A 60-year-old newly diagnosed TB patient presented with fever, coughing, weight loss, lethargy and chest pain. The patient also reported suffering from intermittent emesis and was admitted to the hospital with urinary incontinence and suspected vesicoureteric fistula. TB polymerase chain reaction (TB-PCR) test was positive showing antibiotic sensitivity to rifampicin. Sputum culture did not show growth after 43 days. On CT, fibrocavitatory apical disease was noted bilaterally with a thick wall cavity on the right side. Bilateral upper and middle lobe tree in bud opacification was also noted. The patient underwent ^{68}Ga-nitroimidazole PET/CT 2 days after initiating treatment. Imaging was performed at 90 min after administration of 162.8 megabecquerels (MBq). Uptake was noted in the apical regions of both lungs. Figure 1 illustrates the hypoxic uptake in the right apical lung cavity. Unfortunately, the patient was lost to follow-up for imaging; however, a negative auramine stain was noted 9 months after treatment was initiated.

Case 2

A 52-year-old male newly diagnosed TB patient presented with the following clinical symptoms: coughing with haemoptysis, weight loss, fever, lethargy, chest pain and constipation. The TB-PCR test was positive showing antibiotic sensitivity to rifampicin. The patient was imaged 12 days after initiating TB therapy and again at 69 days for follow-up. The patient was still experiencing intermittent coughing episodes, but all other previously reported symptoms had resolved. Auramine stains at 7, 11 and 23 weeks were negative, and considering the improvement in clinical symptoms, the patient was classified as treatment success. Baseline imaging was performed 110 min after injection of 173.53 MBq, while follow-up imaging was done after 121 min post-administration of 135.79 MBq ^{68}Ga-nitroimidazole. On the baseline scan, uptake was noted in a large cavity in

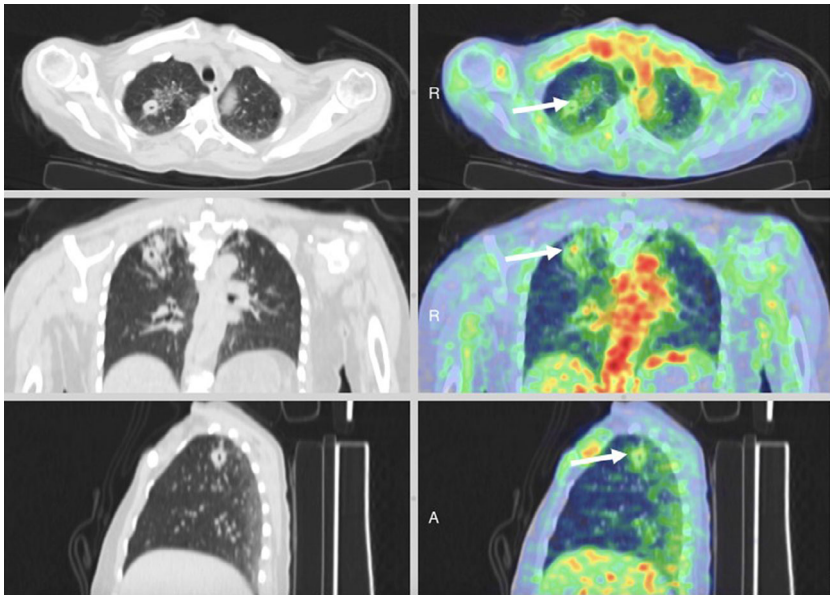

Figure 1. Hypoxic uptake in the cavitatory lesion of the right apical region two days post-therapy initiation.

the right upper lobe and the anterior segment of the left upper lobe. Pretracheal lymph nodes also showed hypoxic uptake. Upon follow-up imaging, ROIs placed in similar regions to the baseline imaging showed persistent and increased hypoxic uptake (LMR) compared to the baseline imaging (Fig. 2).

Case 3

This 32-year-old female presented with coughing, weight loss, intermittent fever and lethargy. Baseline imaging (119.14 MBq at 98 min post-injection) was performed at 15 days after initiating TB therapy. Follow-up imaging (103.6 MBq, 92 min post-injection) was performed 85 days after commencing treatment. At follow-up, the patient only reported still feeling lethargic, but all other initial symptoms had resolved. The TB-PCR result was positive with rifampicin and isoniazid sensitivity. Acid-fast bacilli (AFB) were observed with culture positivity after 8 days. Follow-up auramine stains at seven and 11 weeks were negative. Specimen culture and auramine stain at 23 weeks were negative and showed no growth after 42 days. Thus, the patient was classified as treatment success. Figure 3 illustrates the cavities in bilateral upper lobes at baseline (Fig. 3A) and follow-up (Fig. 3B). ROIs were placed in the walls / consolidation around the

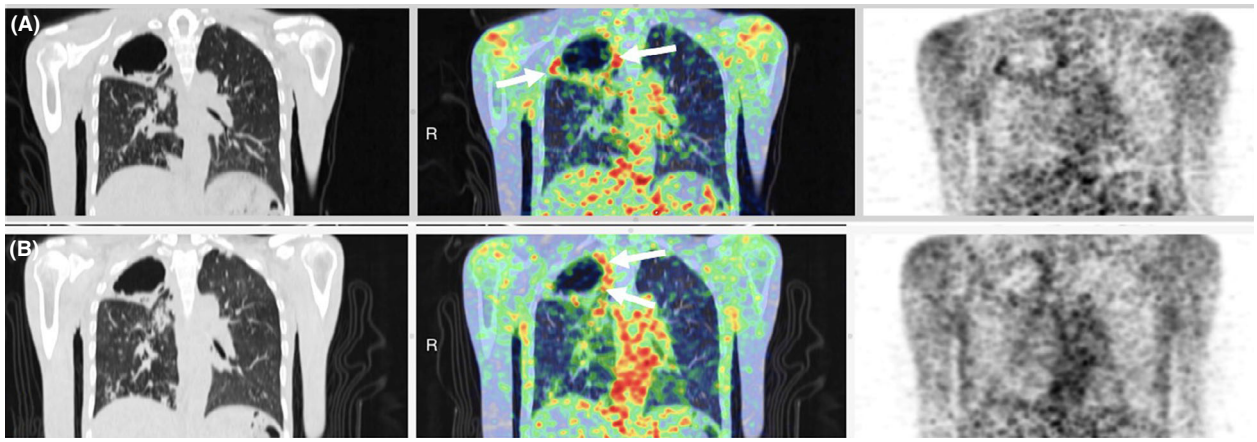

Figure 2. Persistent hypoxic uptake demonstrated in the cavity walls of the right lung at: (A) baseline (12 days after initiating TB therapy) and (B) follow-up (69 days post-TB therapy initiation).

cavities and also in a nodule in the left lower lobe posteriorly. Again, there was an increase in hypoxic uptake on the follow-up scan as compared to the baseline imaging. A nodule in the left posterior lobe demonstrated lower uptake compared to the areas measured around the cavities.

Case 4

The patient had previously been diagnosed with TB on two occasions, 9 and 2 years prior, and had completed 6 months of TB therapy on both occasions. TB-PCR results were negative, and no auramine stain results were available. The patient reported coughing with haemoptysis and weight loss. Due to the COVID-19 pandemic, the patient's baseline imaging had to be postponed. Baseline imaging was performed 89 min after administration of 55,5 MBq ^{68}Ga-nitroimidazole (Fig. 4A), 112 days after initiating TB therapy. A follow-up ^{68}Ga-nitroimidazole PET/CT was then performed 3 months later (88 min post-injection) (Fig. 4B). On the baseline scan, areas of cavitation and consolidation noted on the CT were measured, as well as pretracheal and mediastinal lymph nodes that demonstrated ^{68}Ga-nitroimidazole avidity. On the follow-up ^{68}Ga-nitroimidazole scan, ROIs were placed in similar regions depending on morphologic changes. Some regions showed increased uptake on the follow-up imaging with others including the lymph nodes showing less tracer avidity.

Imaging data

Table 1 summarises the regions demonstrating hypoxic uptake at baseline and follow-up for all cases.

There were no significant differences in the semi-quantitative parameters between the baseline and follow-

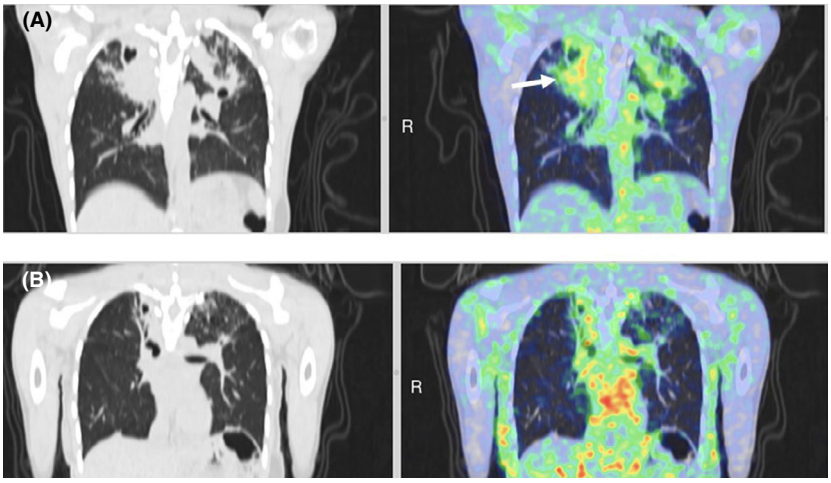

Figure 3. Improved morphologic features indicating good response to TB therapy but persistent hypoxic uptake demonstrated between baseline (A) and follow-up (B).

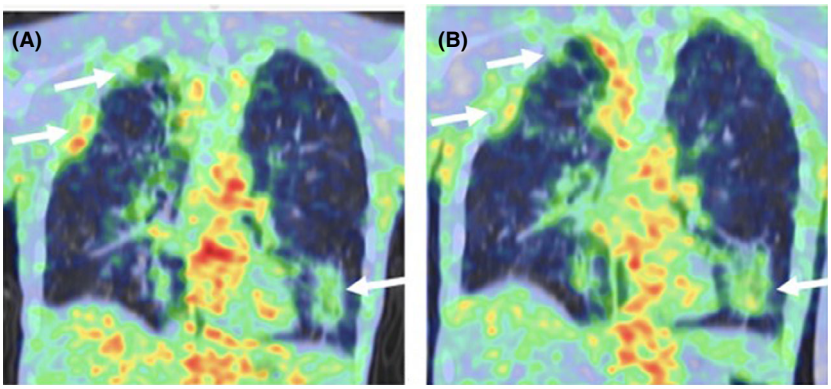

Figure 4. Changes in areas of uptake on ^{68}Ga-nitroimidazole PET/CT at: (A) baseline at 89 min post-injection – 3,5 months. (B) follow-up at 88 min post-injection – 6 months.

Table 1. Hypoxic uptake of ^{68}Ga-nitroimidazole at baseline and follow-up.

Case	Anatomic location	CT appearance	Baseline		Follow-up	
			LMR_{mean}	LBR_{mean}	LMR_{mean}	LBR_{mean}
1	Right upper lobe apical	Cavitation	2.52	0.57	Lost to follow-up	
	Left lobe apical	Fibrosis	3.10	0.70		
2	Right upper lobe	Cavitation				
	Superior		1.82	0.91	2.00	0.55
	Anterior inferior		2.36	1.18	3.33	0.91
	Posterior inferior		2.09	1.05	3.83	1.05
	Left upper lobe	Cavitation				
	Anterior segment		1.36	0.68	1.83	0.50
	Right pretracheal	Enlarged lymph node	1.64	0.82	2.17	0.59
3	Right upper lobe	Consolidation with cavitation	1.44	0.93	2.88	1.32
	Left upper lobe	Consolidation with cavitation	1.33	0.86	2.47	1.14
	Left posterior	Nodules	1.03	0.66	1.06	0.49
4[a]	Right upper lobe cavity wall apical	Cavitation	1.28	0.50	1.33	0.62
	Right upper lobe anterior segment	Consolidation with cavitation	1.16	0.41	0.67	0.31
	Right lower lobe medial basal	Consolidation	1.78	0.63	0.97	0.45
	Left lower lobe basal	Consolidation	1.56	0.55	1.70	0.78
	Right paratracheal nodes	Enlarged lymph nodes	2.38	0.84	2.17	1.00
	Mediastinal nodes	Enlarged lymph nodes	3.00	1.05	2.10	0.97

[a]Baseline imaging delayed.

up imaging. The results confirm persistent but variable hypoxic uptake in the TB lesions that were measured.

Discussion

The strength of this case series lies in the novel application of an in-house-labelled hypoxia-seeking PET/CT radiopharmaceutical in TB. However, the low-grade uptake demonstrated in the small, diverse sample limits the generalisability of the findings. Nonetheless, the potential of hypoxic PET/CT in TB is an area requiring further investigation.

Hypoxia and imaging TB

Hypoxia, characteristic of granulomas in TB infection,[10,11] incites a range of adaptive or pathologic biological consequences as the body tries to meet the cellular metabolic demands.[22–24] Granulomas render the bacteria tolerant to most of the antibiotics as a result of poor drug penetration into necrotic lesions.[3,25] As such, cavitatory disease is associated with higher bacillary load and a worse clinical outcome.[26] Granuloma formation depends on both the pathogen and host factors within the lung and is therefore a good target to consider for HDT.[27,28] Changes in the host cell microenvironment and metabolic processes under hypoxic conditions can be detected and monitored using PET/CT imaging. The most effective use of SUV_{max} and other metabolic metrics is comparing the SUV_{max} of an identified lesion over time to assess disease activity in response to therapy.[29] This notion is also applicable to hypoxic PET/CT imaging.

From this study, it is clear that the hypoxia as quantified by the LMR in the cavity walls of this small series of patients supports the notion that cavities in TB granulomas are hypoxic.[11,22] Therefore, being able to identify patients with hypoxic lesions at an early stage in the treatment plan using ^{68}Ga-nitroimidazoles could assist with designing HDTs to reduce treatment time and improve treatment outcomes. The aim of translational TB research is to identify improved diagnostics, treatments or vaccines to transform the management of TB.[30] Therefore, many authors advocate for HDTs and a shift away from limitations of current TB therapies.[3,7,8,27]

Host-directed therapies

HDTs in TB have been gaining interest since adjunctive options for treatment can lead to an increased antibiotic susceptibility and evasion of drug resistance.[25,27,28] HDTs may utilise varying strategies such as activating immune mechanisms, targeting virulence or stress factors or improving tissue destruction to synergistically augment current therapeutic approaches.[27,28] The purpose of the next generation of hypoxia-activated prodrugs would be to reprogramme the TB lesion to overcome hypoxia or to modulate oxygen levels.[28]

Although the focus of new drug development particularly for nitroimidazoles is towards drug-resistant TB, perhaps (we propose) one should consider these drugs as part of drug-susceptible TB regimens dependent on the hypoxic load. Although this is purely theorised from the uptake of radiolabelled ^{68}Ga-nitroimidazole, preliminary results from clinical trials investigating drug combinations containing nitroimidazole derivates are promising.[16] Thus, PET/CT imaging of hypoxic load with ^{68}Ga-nitroimidazole could inform clinicians as to which patients would benefit from new combination therapies to align with the shift towards HDT. HDTs have proven that they require minimal doses and shorter treatment duration. Of relevance is that they may be used in combination with existing drugs to enhance their overall bactericidal effect.[7,25,28]

Conclusion

The potential of ^{68}Ga-nitroimidazole hypoxic PET/CT to inform HDT in TB has been presented in this case series. Our data support the dynamic hypoxic TB microenvironment between and within patients. All measured lesions demonstrated low-grade ^{68}Ga-nitroimidazole uptake. From baseline to follow-up imaging, the LMR on the ^{68}Ga-nitroimidazole PET/CT increased, which alludes to the effectiveness of TB drugs against aerobic bacteria. Of concern was the persistence in hypoxic load over the course of treatment despite morphologic improvement. If one considers the dynamic nature of hypoxia in TB lesions, we should be targeting aerobic and anaerobic bacteria simultaneously during TB therapy. Furthermore, hypoxic areas lead to latent disease with the probability of reactivation increasing. Therefore, being able to identify patients with hypoxic lesions at an early stage in the treatment plan using ^{68}Ga-nitroimidazoles could assist with designing HDTs to reduce treatment time and improve treatment outcomes. Predicting treatment outcomes using hypoxic PET/CT in TB requires further investigation as does the potential application in other infectious diseases where hypoxia has a role in pathogenesis.

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Conflict of interest

The authors have no conflicts of interest to declare.

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