EXPANDED MATERIALS AND METHODS

Study Design and Data Collection

This prospective study included three different cohorts (total *n*=45) (Table 1). The first cohort (*n*=15) included healthy subjects imaged with a standard-dose (372±17 MBq) of ^{18}F-FDG on the uEXPLORER total-body PET scanner (United Imaging Healthcare, Shanghai, China) at UC Davis (Sacramento, CA, USA) at 1.5h, 3h and 12h p.i., to determine the feasibility of an extended time window for imaging of the vessel wall. The second cohort (*n*=15) included healthy subjects imaged with an ultra-low dose (19.6±1.7 MBq) of ^{18}F-FDG using the same scanner at 1.5h and 3h p.i., to determine the potential for disease screening and repeat imaging with minimal radiation to the patient. Exclusion criteria were: Any known acute infection, history of metastatic or locally invasive cancer within prior 5 years, chemotherapy within last 5 years, radiation therapy within last 3 months, major surgery within last 6 months, diabetes, or currently pregnant or breast-feeding. The third cohort (*n*=15) included an gender-matched patient group imaged with a standard-dose (307±12 MBq) of ^{18}F-FDG using a conventional PET scanner (Figure 1) with a 22 cm axial field of view (Siemens Biograph mCT flow, Siemens Healthineers, Erlangen, Germany) at Hannover Medical School, for clinical evaluation of pulmonary nodules/lung tumors. Exclusion criteria were: History of vasculitis, cardiac disease or cardiovascular event, concurrent or preceding oncologic therapy. Common cardiovascular risk factors including hypertension, hypercholesterolemia, diabetes, smoking habits, and prior vascular events defined as myocardial infarction or cerebrovascular insult were documented for each patient (*17*) along with potentially relevant medication (*18*), as available from medical records (*n*=15 for standard PET, *n*=11 for standard dose TB PET, *n*=12 for ultra-low dose TB PET). The study protocol complied with the Declaration of Helsinki and the institutional review boards at UC Davis (IRB #1341792) and Hannover Medical

School (No. 8774_BO_S_2019) approved this study. All patients at UC Davis and Hannover Medical School provided written informed consent.

PET Image Acquisition and Reconstruction

Total-body PET. Ultra low-dose total-body CT scans (5 mAs; 140 kVp; tube current modulation on; effective dose ~1 mSv) or low-dose total-body CT scans (50 mAs; 140 kVp; tube current modulation on; effective dose ~10 mSv) were acquired for attenuation and scatter correction. The CT matrix size was 512 x 512 x 828 with 0.977 x 0.977 x 2.344 mm voxels. A static PET scan of the entire body without bed motion was obtained for 20 minutes starting 90 minutes, 180 minutes and 720 minutes (standard dose only) post injection. The administered dose of ^{18}F-FDG was 372±17 MBq for the standard dose cohort (*n*=15) and 19.6±1.7 MBq for the low-dose cohort (*n*=15). Blood glucose was <160 mg/dL in all subjects prior to injection. Studies were reconstructed from list-mode data with vendor-provided software using an iterative algorithm (20 subsets, 4 iterations) incorporating time-of-flight (TOF) information but no point-spread function (PSF) correction. The reconstruction matrix was 256 x 256 x 828 with isotropic voxels of 2.344 mm. Studies were corrected for attenuation, scatter, randoms and deadtime. No smoothing was applied to the reconstructed images.

Conventional PET. Low-dose whole-body CT (25 Ref mAs; 120 kV; CareDose4D; 5 mm slice thickness; pitch, 1.4) was performed. Using continuous bed motion to cover the entire body, a static PET scan was obtained at 60-90 min after administration of 307±12 MBq (range, 291 to 329 of ^{18}F-FDG). Blood glucose was <120 mg/dL in all patients prior to injection. Attenuation corrected studies were reconstructed using Ultra HD®, an iterative algorithm combined with TOF

and PSF information (Siemens Healthcare; 2 iterations, 21 subsets, matrix 200; zoom 1.0; Gaussian filter of 5.0 mm).

Image Analysis

All transaxial PET, CT and fused PET/CT images were analyzed using a dedicated workstation (syngo.via; V50B; Siemens Healthcare, Erlangen, Germany). For segment-based analysis, major arteries were subdivided as follows: right and left common carotid arteries, ascending aorta, aortic arch, descending thoracic aorta, abdominal aorta, right and left common iliac as well as right and left superficial femoral arteries (*19*).

Vessel wall FDG signal. Semiquantitative analysis was performed by obtaining the average maximum standardized uptake value (SUV_{max}), measured in 10 separate transaxial slices beginning at the most proximal part of the vessel using circular regions-of interest (ROIs) in each arterial segment, and then averaged, yielding a whole-vessel uptake (*20*). Arterial wall uptake for each patient was defined as the averaged whole-vessel uptake of all 10 analyzed arterial segments, yielding a measure for assessment of global vascular activity, for comparison with other surrogate markers of CVD (*21*). Aortic wall uptake was separately defined as the averaged whole-vessel uptake of analyzed aortic segments per patient. Note that the aortic wall represents a thicker target structure less prone to the partial volume effect than smaller arteries. Blood-pool SUV ($SUV_{blood-pool}$) was calculated as mean of 3 ROIs of fixed size (10 mm; SUV_{mean}) placed in the mid lumen of the superior vena cava. For the calculation of the arterial TBR, the SUV_{max} of each arterial uptake measurement was divided by the $SUV_{blood-pool}$ (*19*).

Multi-organ crosstalk. To characterize systemic interactions between arterial wall signal and activity of hematopoietic and lymphoid organs (*10*), spleen signal was obtained by the average SUV_{mean} of three separate measurements using circular ROIs of fixed size (2 cm diameter). Bone marrow signal was the average SUV_{mean} of three separate measurements in lumbar vertebrae using circular ROIs (2 cm diameter), carefully avoiding the endplates. For lymph node signal, the SUV_{max} of three mediastinal lymph nodes was measured, and then averaged.

PET image noise. The coefficient of variation (CoV) of normal liver parenchyma was used for assessment of image noise (*22*). A ROI of 3 cm in diameter was placed in the right liver lobe, in a parenchymal area of homogeneous uptake. The CoV was then calculated as the ratio of the standard deviation to the mean SUV.

Calcified Plaque. The number of separate high-density mural areas (attenuation > 130 Hounsfield units) in the wall of the studied arteries were recorded (*17*). Patients were divided into those with and without discernible calcified plaque. The calcified lesion thickness was defined as the maximum calcification diameter measured in the intimo-adventitial direction, and the maximum circumferential extent of calcifications was scored, as described previously (*19*).

Statistical Analyses

Categorical variables were presented with absolute and relative frequencies. Continuous variables were expressed as mean ± standard deviation (SD) and range. Normal distribution of data was verified using the Kolmogorov-Smirnov test. A one-way analysis of variance (ANOVA) with Šídák`s multiple comparisons test, with a single pooled variance, was used for comparison of the PET signal between TB PET and conventional PET groups at 1.5h. Repeated-measures ANOVA with the Geisser-Greenhouse correction and Šídák`s multiple comparisons test was used for

comparison of PET signal at different time points within the standard dose TB PET cohort. A paired t test was used for comparison of PET signal at different time points within the ultra-low dose TB PET cohort. Pearson's correlation coefficients were computed to characterize the association between vessel wall and organ signal. Pearson's correlation coefficients and Bland-Altman plots were used to compare vessel wall signal between different time points within the same cohort. Statistical significance was established for *P*-values <0.05. Analysis was performed using GraphPad Prism® (V9.0 for Windows; Graphpad Software, San Diego, CA, USA).

REFERENCES

10. van der Valk FM, Kuijk C, Verweij SL, et al. Increased haematopoietic activity in patients with atherosclerosis. *Eur Heart J.* 2017;38:425-432.

17. Derlin T, Wisotzki C, Richter U, et al. In vivo imaging of mineral deposition in carotid plaque using ^{18}F-sodium fluoride PET/CT: correlation with atherogenic risk factors. *J Nucl Med.* 2011;52:362-368.

18. Subramanian S, Emami H, Vucic E, et al. High-dose atorvastatin reduces periodontal inflammation: a novel pleiotropic effect of statins. *J Am Coll Cardiol.* 2013;62:2382-2391.

19. Weiberg D, Thackeray JT, Daum G, et al. Clinical molecular imaging of chemokine receptor CXCR4 expression in atherosclerotic plaque using ^{68}Ga-Pentixafor PET: correlation with cardiovascular risk factors and calcified plaque burden. *J Nucl Med.* 2018;59:266-272.

20. Derlin T, Koenecke C, Schultze-Florey C, et al. CD19-targeted immunotherapy attenuates vessel wall inflammation. *JACC Cardiovasc Imaging.* 2021;14:1864-1866.

21. Bucerius J, Hyafil F, Verberne HJ, et al. Position paper of the Cardiovascular Committee of the European Association of Nuclear Medicine (EANM) on PET imaging of atherosclerosis. *Eur J Nucl Med Mol Imaging.* 2016;43:780-792.

22. Yan J, Schaefferkoette J, Conti M, et al. A method to assess image quality for Low-dose PET: analysis of SNR, CNR, bias and image noise. *Cancer Imaging.* 2016;16:26.

Supplemental Table 1 Conventional PET cohort: Prevalence, distribution, and extent of calcification in the studied vasculature.

	Right common carotid artery	Left common carotid artery	Ascending aorta	Aortic arch	Descending thoracic aorta	Abdominal aorta	Right common iliac artery	Left common iliac artery	Right superficial femoral artery	Left superficial femoral artery	Total
No. (%) of patients with calcification sites	4 (27%)	5 (33%)	5 (33%)	12 (80%)	7 (47%)	11 (73%)	12 (80%)	11 (73%)	7 (47%)	6 (40%)	13 (87%)
No. (%) of calcification sites	21 (4%)	18 (3%)	32 (6%)	58 (11%)	81 (15%)	151 (28%)	70 (13%)	64 (12%)	16 (3%)	21 (4%)	532 (100%)
No. of calcification sites per segment											
Mean ± SD	1.4±3.2	1.2±2.4	2.1±3.7	3.9±3.7	5.4±7.4	10.1±9.3	4.7±3.6	4.3±3.8	1.1±1.5	1.4±3.6	3.5±2.8
Range	0 to 11	0 to 8	0 to 12	0 to 11	0 to 23	0 to 27	0 to 10	0 to 11	0 to 4	0 to 14	0 to 27
Calcification score for lesions											
Mean ± SD	1.5±0.6	1.2±0.4	1.6±0.5	1.6±0.7	1.3±0.5	2.6±1.3	2.1±0.7	2.3±0.8	1.0±0	1.5±0.8	1.7±0.5
Range	1 to 2	1 to 2	1 to 2	1 to 3	1 to 2	1 to 4	1 to 3	1 to 4	1 to 1	1 to 3	1 to 4
Calcified lesion thickness (mm)											
Mean±SD	4.5±2.0	2.4±1.0	4.0±1.6	4.5±1.9	3.5±1.1	4.0±1.4	4.0±1.4	4.2±1.0	2.8±0.7	2.8±1.3	3.7±0.8
Range	2.6 to 7.0	1.3 to 3.6	2.6 to 6.8	1.8 to 6.9	1.8 to 4.8	2.0 to 6.8	1.3 to 6.0	2.9 to 5.7	1.8 to 3.8	1.1 to 4.9	1.1 to 7.0

SD – standard deviation

Supplemental Table 2 Standard-dose total-body PET cohort: Prevalence, distribution, and extent of calcification.

	Right common carotid artery	Left common carotid artery	Ascending aorta	Aortic arch	Descending thoracic aorta	Abdominal aorta	Right common iliac artery	Left common iliac artery	Right superficial femoral artery	Left superficial femoral artery	Total
No. (%) of patients with calcification sites	2 (13%)	3 (20%)	1 (7%)	6 (40%)	2 (13%)	6 (40%)	5 (33%)	4 (27%)	2 (13%)	3 (20%)	7 (47%)
No. (%) of calcification sites	3 (2%)	4 (3%)	4 (3%)	19 (14%)	8 (6%)	26 (19%)	10 (7%)	9 (7%)	27 (20%)	26 (19%)	136 (100%)
No. of calcification sites per segment											
Mean ± SD	0.2±0.6	0.3±0.6	0.3±1.0	1.3±2.0	0.5±1.8	1.7±3.2	0.7±1.1	0.6±1.4	1.8±6.7	1.7±5.9	0.9±0.7
Range	0 to 2	0 to 2	0 to 4	0 to 6	0 to 7	0 to 12	0 to 3	0 to 5	0 to 26	0 to 23	0 to 26
Calcification score for lesions											
Mean ± SD	2.5±2.1	2.0±1.0	1	1±0	1±0	1.3±0.8	1.4±0.9	1±0	2.5±2.1	2.0±1.7	1.6±0.6
Range	1 to 4	1 to 3	n.a.	1 to 1	1 to 1	1 to 3	1 to 3	1 to 1	1 to 4	1 to 4	1 to 4
Calcified lesion thickness (mm)											
Mean±SD	4.3±2.6	3.9±0.4	1.7	3.0±1.2	4.4±1.1	4.4±1.3	3.2±1.8	3.2±1.3	3.2±1.0	3.2±1.0	3.4±0.8
Range	2.4 to 6.1	3.5 to 4.2	n.a.	2.0 to 5.3	3.6 to 5.1	2.7 to 6.6	1.0 to 4.9	2.0 to 4.6	2.5 to 3.9	2.4 to 4.3	1.0 to 6.6

SD – standard deviation

Supplemental Table 3 ULD total-body PET cohort: Prevalence, distribution, and extent of calcification in the studied vasculature.

	Right common carotid artery	Left common carotid artery	Ascending aorta	Aortic arch	Descending thoracic aorta	Abdominal aorta	Right common iliac artery	Left common iliac artery	Right superficial femoral artery	Left superficial femoral artery	Total
No. (%) of patients with calcification sites	0 (0%)	1 (7%)	0 (0%)	2 (13%)	1 (7%)	5 (33%)	0 (0%)	1 (7%)	3 (20%)	2 (13%)	6 (40%)
No. (%) of calcification sites	0	1 (2%)	0	3 (5%)	1 (2%)	17 (31%)	0	3 (5%)	12 (22%)	18 (33%)	55 (100%)
No. of calcification sites per segment											
Mean ± SD	-	0.1±0.3	-	0.2±0.6	0.1±0.3	1.1±2.2	-	0.2±0.8	0.8±2.6	1.2±4.1	0.4±0.5
Range	-	0 to 1	-	0 to 2	0 to 1	0 to 8	-	0 to 3	0 to 10	0 to 16	0 to 16
Calcification score for lesions											
Mean ± SD	-	1	-	1.0±0	1	1.2±0.4	-	1	1.7±1.2	2.0±1.4	1.3±0.4
Range	-	n.a.	-	1 to 1	n.a.	1 to 2	-	n.a.	1 to 3	1 to 3	1 to 3
Calcified lesion thickness (mm)											
Mean±SD	-	2.3	-	2.2±1.7	2.0	4.0±1.1	-	3.2	2.7±0.8	2.9±0.4	2.8±1.0
Range	-	n.a.	-	1.0 to 3.4	n.a.	2.0 to 4.9	-	n.a.	1.8 to 3.4	2.6 to 3.2	1.0 to 4.9

SD – standard deviation; ULD – ultra-low dose

Supplemental Table 4 Aortic wall signal, whole-body arterial wall signal and image noise in conventional and total-body PET cohorts.

	Conventional PET	Total-body PET							
		Standard-dose					Ultra-low-dose		
		1.5h p.i.	3h p.i.	*P*	12h p.i.	*P*	1.5h p.i.	3h p.i.	*P*
Aortic wall signal (SUV_{max})									
Mean±SD	2.71±0.37	2.68±0.45^{ns}	2.66±0.42^{ns}	0.8295	4.58±1.09****	**<0.0001**	3.82±0.93**	4.15±1.17**	**<0.0001**
Range	2.00 to 3.23	2.03 to 3.45	2.13 to 3.54		2.71 to 5.96		1.62 to 4.89	1.72 to 5.62	
Aortic wall signal (TBR)									
Mean±SD	1.66±0.30	1.72±0.18^{ns}	2.74±0.40****	**<0.0001**	16.67±5.58****	**<0.0001**	2.70±0.68^{ns}	5.81±1.39****	**<0.0001**
Range	1.27 to 2.32	1.48 to 2.16	2.24 to 3.49		7.90 to 23.65		1.46 to 3.59	3.97 to 8.44	
Arterial wall signal (SUV_{max})									
Mean±SD	2.26±0.31	2.37±0.40^{ns}	2.20±0.36^{ns}	0.0538	3.53±0.79****	**<0.0001**	3.11±0.77**	3.24±0.85*	**0.0447**
Range	1.65 to 2.85	1.61 to 3.09	1.72 to 2.99		2.05 to 4.72		1.29 to 4.37	1.29 to 4.24	
Arterial wall signal (TBR)									
Mean±SD	1.38±0.28	1.51±0.17^{ns}	2.25±0.33****	**<0.0001**	13.3±4.45****	**<0.0001**	2.16±0.53^{ns}	4.51±0.95****	**<0.0001**
Range	1.09 to 2.10	1.28 to 1.84	1.78 to 2.96		6.21 to 19.69		1.20 to 2.89	3.26 to 6.50	
Coefficient of variation (CoV)									
Mean±SD	14.68±3.36	9.55±2.18^{ns}	13.29±3.02****	**<0.0001**	71.72±20.30****	**<0.0001**	33.98±7.45****	49.99±9.99****	**<0.0001**
Range	9.95 to 22.71	6.49 to 14.11	8.62 to 20.87		44.35 to 126.7		25.12 to 47.59	32.74 to 62.80	

p.i. – post injection; SD – standard deviation; SUV – standardized uptake value; TBR – target-to-background ratio

*P values were calculated using an one-way analysis of variance (ANOVA) with Šídák`s multiple comparisons test for comparison of the PET signal between TB PET and conventional PET groups at 1.5h. A repeated-measures (RM) one-way ANOVA with Šídák`s multiple comparisons test was used for comparison of PET signal at different time points within standard dose TB PET cohort. A paired t test was used for comparison of PET signal at different time points within ultra-low dose TB PET cohort. Exact P values shown in the table represent results of RM one-way ANOVA and paired t-test. P value: ns – not significant; * – <0.05; ** – <0.01; *** – <0.001;**** – <0.0001*

Supplemental Table 5 Activity of lymphoid and hematopoietic organs in conventional and total-body PET cohorts.

	Conventional PET	Total-body PET							
			Standard-dose				Ultra-low-dose		
		1.5h p.i.	3h p.i.		12h p.i.		1.5h p.i.	3h p.i.	
Spleen signal (SUV_{mean})									
Mean±SD	2.08±0.33	1.97±0.32^{ns}	1.87±0.33****	**<0.0001**	1.70±0.29****	**<0.0001**	1.77±0.34^{ns}	1.58±0.34*	**0.0143**
Range	1.61 to 2.65	1.36 to 2.48	1.25 to 2.37		1.19 to 2.02		0.92 to 2.15	0.83 to 2.11	
Bone marrow signal (SUV_{mean})									
Mean±SD	2.09±0.41	2.39±0.50^{ns}	2.78±0.61****	**<0.0001**	3.03±0.97**	**0.0017**	2.21±0.54^{ns}	2.48±0.57**	**0.0014**
Range	1.25 to 2.67	1.75 to 3.23	2.02 to 3.83		1.93 to 5.24		1.11 to 2.89	1.18 to 3.624	
Lymph node signal (SUV_{max})									
Mean±SD	1.71±0.25	2.13±0.42^{ns}	1.99±0.57^{ns}	0.1304	3.27±0.91****	**<0.0001**	2.51±0.59*	2.86±1.08^{ns}	0.1550
Range	1.29 to 2.31	1.41 to 2.78	1.10 to 3.23		1.89 to 5.12		1.44 to 3.47	1.11 to 4.69	

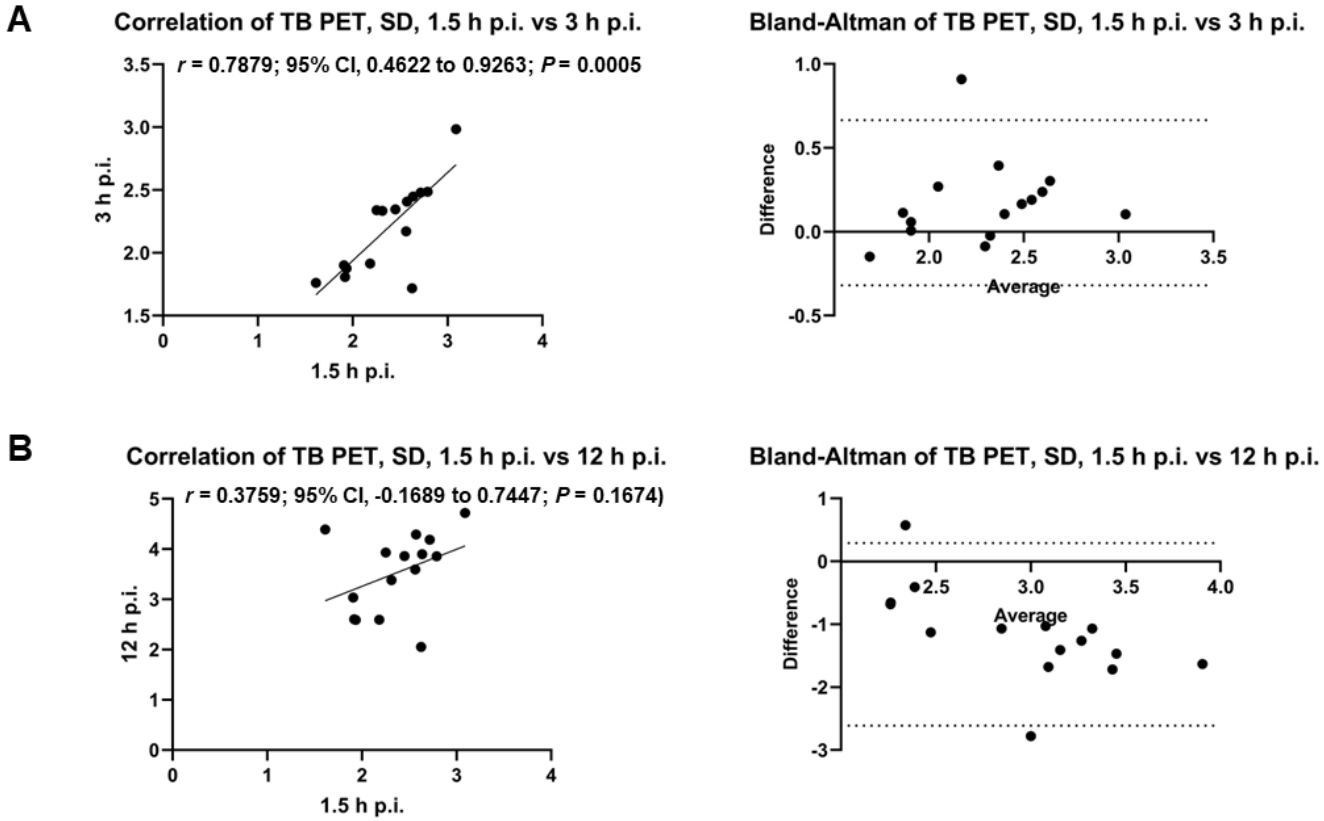
p.i. – post injection; SD – standard deviation; SUV – standardized uptake value

*P values were calculated using an one-way analysis of variance (ANOVA) with Šídák`s multiple comparisons test for comparison of the PET signal between TB PET and conventional PET groups at 1.5h. A repeated-measures (RM) one-way ANOVA with Šídák`s multiple comparisons test was used for comparison of PET signal at different time points within standard dose TB PET cohort. A paired t test was used for comparison of PET signal at different time points within ultra-low dose TB PET cohort. Exact P values shown in the table represent results of RM one-way ANOVA and paired t-test. P value: ns – not significant; * – <0.05; ** – <0.01; *** – <0.001;**** – <0.0001*

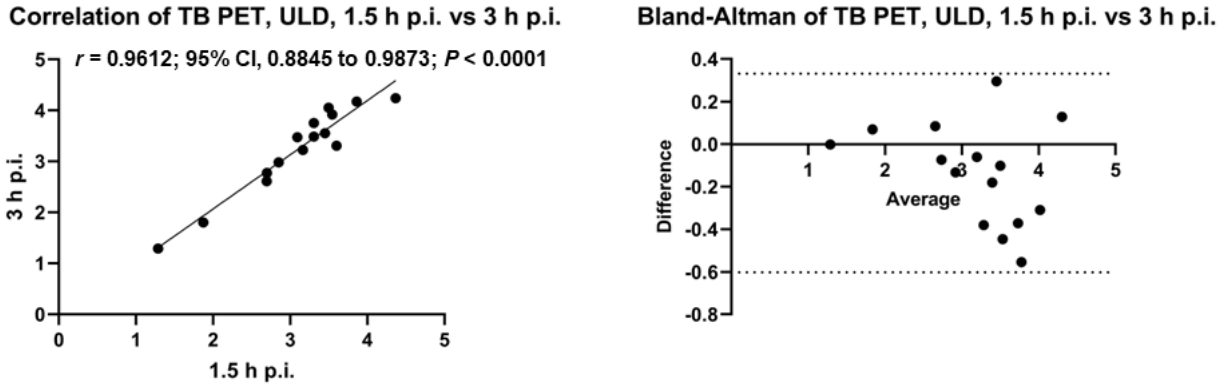
Supplemental Table 6 Quality of Assessment of Multi-Organ Systemic Interactions as Determined by PET Imaging

Arterial wall signal vs	**Spleen signal (SUV_{mean})**			**Bone marrow signal (SUV_{mean})**			**Lymph node signal (SUV_{max})**		
	r	*95% CI*	*P*	*r*	*95% CI*	*P*	*r*	*95% CI*	*P*
Conventional PET	0.65	0.20 to 0.87	**0.009**	0.31	-0.24 to 0.71	0.2657	0.37	-0.18 to 0.74	0.1779
Standard-dose TB PET 1.5h p.i.	0.79	0.46 to 0.93	**0.0005**	0.39	-0.16 to 0.75	0.1551	0.67	0.25 to 0.88	**0.0058**
Standard-dose TB PET 3h p.i.	0.71	0.31 to 0.90	**0.0029**	0.25	-0.31 to 0.67	0.3789	0.58	0.10 to 0.84	**0.0228**
Standard-dose TB PET 12h p.i.	0.52	0.01 to 0.82	**0.0465**	0.23	-0.33 to 0.66	0.4181	0.67	0.25 to 0.88	**0.0058**
Ultra-low-dose TB PET 1.5h p.i.	0.67	0.23 to 0.88	**0.0068**	0.25	-0.30 to 0.68	0.3610	0.74	0.36 to 0.91	**0.0017**
Ultra-low-dose TB PET 3h p.i.	0.80	0.48 to 0.93	**0.0004**	0.16	-0.39 to 0.62	0.5809	0.76	0.41 to 0.92	**0.0010**

CI – confidence interval; PET – positron emission tomography; p.i. – post injection; SUV – standardized uptake value; TB – total-body



Supplemental Figure 1 *SD TB PET.* Correlation plot (r = 0.7879; 95% CI, 0.4622 to 0.9263; P = 0.0005) and Bland-Altman plot (bias, 0.1733 ± 0.2513; limits of agreement, -0.3192 to 0.6658) comparing SUVs of the arterial vasculature at 1.5 h p.i. vs 3 h p.i. (A). Correlation plot (r = 0.3759; 95% CI, -0.1689 to 0.7447; P = 0.1674) and Bland-Altman plot (bias, -1.160 ± 0.7404; limits of agreement, -2.612 to 0.2907) comparing SUVs of the arterial vasculature at 1.5 h p.i. and 12 h p.i. (B).



Supplemental Figure 2 *ULD TB PET.* Correlation plot (r = 0.9612; 95% CI, 0.8845 to 0.9873; P < 0.0001) and Bland-Altman plot (bias, -0.1356 ± 0.2383; limits of agreement, -0.6027 to 0.3314) comparing SUVs of the arterial vasculature at 1.5 h p.i. vs 3 h p.i..