

Utility of SPECT and PET to diagnose infection in a porcine osteomyelitis model

Supplementary Table 1. Body weight of pigs at scan, sequence of tracer injection, activity of tracers and time of diagnostic scan

Scan sequence								
Pig 1, body weight 40 Kg	¹¹ C-methionine	¹¹¹ In-leukocytes	¹¹ C-PK11195	¹⁵ O-H ₂ O ^A	^{99m} Tc-Nanocoll	¹¹¹ In-leukocytes	⁶⁸ Ga-citrate	¹⁸ F-FDG
Time of injection	0 min	1 h 45 min	2 h 0 min	4 h 23 min	6 h 30 min	-	10 h 35 min	14 h 16 min
Activity	474 MBq	20.2 MBq	424 MBq	500 MBq	500 MBq	-	173 MBq	299 MBq
Time of scan	1 h 5 min	-	3 h 5 min	Immediately	7 h 15 min	7 h 15 min	12 h 35 min	15 h 50 min
Inj.-scan interval	1 h 5 min	-	1 h 5 min	Immediately	45 min	5 h 30 min	2 h 0 min	1 h 34 min
Pig 2, body weight 40 Kg	¹¹ C-methionine	¹¹¹ In-leukocytes	¹¹ C-PK11195	¹⁵ O-H ₂ O ^A	^{99m} Tc-Nanocoll	¹¹¹ In-leukocytes	⁶⁸ Ga-citrate	¹⁸ F-FDG
Time of injection	0 min	1 h 25 min	2 h 5 min	4 h 8 min	6 h 34 min	- ^B	9 h 16 min	13 h 27 min
Activity	408 MBq	21.0 MBq	414 MBq	1000 MBq	500 MBq	-	113 MBq	389 MBq
Time of scan	1 h 5 min	-	3 h 10 min	Immediately	7 h 39 min	7 h 39 min	11 h 29 min	14 h 34 min
Inj.-scan interval	1 h 5 min	-	1 h 5 min	Immediately	1 h 5 min	6 h 14 min	2 h 13 min	1 h 7 min
Pig 3, body weight 39 Kg	¹¹ C-methionine	¹¹¹ In-leukocytes	¹⁵ O-H ₂ O ^A	¹¹ C-PK11195	^{99m} Tc-Nanocoll	¹¹¹ In-leukocytes	⁶⁸ Ga-citrate	¹⁸ F-FDG
Time of injection	0 min	1 h 24 min	1 h 54 min ^C	2 h 37 min	6 h 21 min	-	9 h 6 min	13 h 24 min
Activity	500 MBq	14.3 MBq	1000 MBq	464 MBq	500 MBq	-	150 MBq	365 MBq
Time of scan	1 h 5 min	-	Immediately	3 h 42 min	7 h 1 min	7 h 1 min	11 h 9 min	14 h 24 min
Inj.-scan interval	1 h 5 min	-	Immediately	1 h 5 min	40 min	5 h 37 min	2 h 3 min	1 h 0 min
Pig 4, body weight 42 Kg	¹⁵ O-H ₂ O ^A	¹¹ C-methionine	¹¹¹ In-leukocytes	¹¹ C-PK11195	^{99m} Tc-Nanocoll	¹¹¹ In-leukocytes	⁶⁸ Ga-citrate	¹⁸ F-FDG
Time of injection	0 min	45 min	1 h 24 min	3 h 03 min	6 h 59 min	-	9 h 22 min	13 h 52 min
Activity	850 MBq	498 MBq	17.8 MBq	263 MBq	500 MBq	-	175 MBq	389 MBq
Time of scan	Immediately	1 h 50 min	-	4 h 8 min	7 h 52 min	7 h 52 min	11 h 22 min	14 h 59 min
Inj.-scan interval	Immediately	1 h 5 min	-	1 h 5 min	53 min	6 h 28 min	2 h 0 min	1 h 7 min

^AResults of ¹⁵O-H₂O scan will be recorded elsewhere. ^B-indicates that the information is irrelevant. ^CA second ¹⁵O-H₂O scan was performed: injection at 2 h 15 min with 1000 MBq.

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Supplementary Table 2. Haematology of pigs before and after inoculation with *S. aureus* into the right femoral artery

Normal Tests values ^A	Pig 1		Pig 2		Pig 3		Pig 4		Pig 1-X ^B		Pig 2-X ^B		Pig 3-X ^B		Pig 4-X ^B		
	-4pi ^C	+5pi ^C	Opi ^C	+7pi	-2pi	+7pi	-3pi	+7pi	-4pi	+5pi	Opi	+4pi	-2pi	+4pi	-3pi	+4pi	
Leukocytes (10 ⁹ /L)	11.3-22.8	19.9	22.5	19.2	30.4	21.6	15.2	NT ^D	21.9	16.5	25.6	17.9	18.1	22.3	NT	13.7	13.0
Lymphocytes (10 ⁹ /L)	4.6-10.0	11.2	9.8	10.9	6.6	10.0	6.8	NT	4.2	9.5	6.6	9.8	7.3	12.5	NT	7.0	7.7
Neutrophils (10 ⁹ /L)	3.1-9.6	3.3	9.5	5.8	20.3	9.7	7.4	NT	16.8	5.1	16.9	6.7	8.7	8.5	NT	5.4	4.0
Eosinophils (10 ⁹ /L)	0.0-0.9	4.4	2.4	1.7	2.1	0.7	0.5	NT	0.4	0.7	1.1	1.0	0.9	0.6	NT	0.8	0.7
Haemoglobin (mmol/L)	6.2-9.4	6.9	7.0	6.7	5.2	6.8	7.5	NT	4.9	7.4	7.3	6.7	5.5	6.8	NT	6.6	6.1
CRP (µg/mL)	< 15.0	1.6	82.6	0.8	59.8	9.9	2.2	0.6	41.3	2.0	62.3	2.0	NT	1.5	44.3	1.0	34.6
Glucose (mmol/L) ^E	3.5-6.6	NT	NT	NT	3.8	NT	3.8	NT	3.1	NT	NT	NT	NT	NT	NT	NT	NT

^ANormal values supplied by the Central Laboratory. For the C-reactive protein (CRP) the normal-value shown here (< 15 µg/mL) corresponds to the highest value recorded in 5 normal pigs presented in two different publications using the same CRP test as employed in the present study [1, 2]. ^BPig 1-X was not scanned because scanners and laboratory facilities were unavailable, and pig, 2-X, 3-X and 4-X were euthanized prior to scanning for ethical reasons (humane endpoints reached). ^C-4pi indicates blood sampling four days prior to inoculation, +5pi indicates sampling five days after inoculation, and Opi indicates sampling immediately before inoculation. ^DNT indicates not tested. ^EBlood glucose was measured at the day of scan (+7pi) in pigs 2, 3 and 4.

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Supplementary Table 3. Alignment of diagnostic CT, diagnostic PET (¹⁸F-FDG, ⁶⁸Ga-citrate, ¹¹C-PK11195 and ¹¹C-methionine), diagnostic SPECT (^{99m}Tc-Nanocoll and ¹¹¹In-leucocytes), gross pathology and microbiology in the pelvic (hind) limbs and pelvic region seven days after inoculation with *S. aureus* in the right femoral artery of pigs 1, 2 and 4; significant lesions were only present in the right side as reported here

PIG 1	CT	PET	SPECT	Gross pathology	Microbiology ^B
Soft tissue related to inoculation site	Subcutaneous located swelling, 5.2 x 5.2 x 2.0 cm	---	--- ^A	Subcutaneous cystic lesion, diameter app. 5 cm	NT ^C
	--- ^A	¹⁸ F-FDG: intramuscular, craniomedial thigh muscle region, 0.9 x 1.1 x 1.2 cm (metabolic measurement)	---	Abscess, craniomedial thigh muscle region, diameter approx. 1 cm	<i>S. aureus</i> (heavy growth), <i>Escherichia coli</i> (few colonies) ^D
Bones	Osteolysis (medulla) and sequestration, femoral head and neck	¹⁸ F-FDG: femoral head and neck	---	Suppurative osteomyelitis, femoral head and neck	NT
	Osteolysis (medulla and cortex) and sequestration, distal physis region femur	¹⁸ F-FDG: distal physis region femur	¹¹¹ In-leukocytes: distal physis region femur	Suppurative osteomyelitis, distal physis region femur	<i>S. aureus</i> (moderate growth)
	Osteolysis (medulla), proximal physis region tibia	¹⁸ F-FDG: proximal physis region tibia	---	---	NT
	Osteolysis (cortex), distal physis region tibia	---	¹¹¹ In-leukocytes: distal physis region tibia	---	NT
	Osteolysis (medulla and cortex) and sequestration, distal physis region metatarsus III	¹⁸ F-FDG: distal physis region metatarsus III ¹¹ C-methionine: distal physis region metatarsus III	¹¹¹ In-leukocytes: distal physis region metatarsus III	Suppurative osteomyelitis and medial suppurative periostitis, distal physis region metatarsus III	NT
Soft tissue peripheral to bones	Edema peripheral to distal femur	---	---	---	NT
	Swelling peripheral to distal metatarsus III	¹⁸ F-FDG: distal metatarsus III ¹¹ C-methionine: distal metatarsus III	¹¹¹ In-leukocytes: distal metatarsus III	Subcutaneous abscess medial to distal metatarsus III, diameter 1.5 cm	<i>S. aureus</i> (heavy growth)
Joints	---	---	---	Serous arthritis knee joint	<i>Aerococcus viridans</i> (heavy growth)
Lymph nodes	Mammary lymph node slightly enlarged	¹¹ C-methionine: mammary lymph node	---	---	NT
	---	---	--- ^E	Enlarged medial iliac lymph nodes	NT
PIG 2	CT	PET	SPECT	Gross pathology	Micobiology ^B
Soft tissue related to inoculation site	--- ^A	---	---	---	NT ^C
Bones	Osteolysis (medulla an cortex), patella	---	¹¹¹ In-leukocytes: cortical osteolytic region of paella ^{99m} Tc-Nanocoll: absence of activity in medulla of patella	Suppurative osteomyelitis, patella	NT

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Soft tissue peripheral to bones	Swelling and edema peripheral to the knee joint	¹⁸ F-FDG: tissue peripheral to patella and bordering patella ⁶⁸ Ga-citrate: tissue peripheral to patella and bordering patella ¹¹ C-methionine: tissue peripheral to patella ¹¹ C-PK11195: tissue peripheral to patella	¹¹¹ In-leukocytes: tissue peripheral and in particular lateral to patella	Cellulitis peripheral to patella, diameter approx. 10 cm	<i>S. aureus</i> (few colonies)
	Edema in the soft tissues of the thigh and in the soft tissues of the lower limb and tarsal joint	---	---	Subcutaneous edema in the lower limb and around the tarsal joint	NT
	Edematous region with gas-bubbles deep in the caudal thigh muscles	---	¹¹¹ In-leukocytes: tissue deep in the caudal thigh muscles	---	NT
Joints	---	---	---	Fibrino-suppurative arthritis knee joint	<i>S. aureus</i> (heavy growth)
Lymph nodes	---	¹⁸ F-FDG: medial iliac lymph node area ^F ⁶⁸ Ga-citrate: medial iliac lymph node area ^F	--- ^E	Enlarged medial iliac lymph nodes	NT
PIG 4 ^G	CT	PET	SPECT	Gross pathology	Microbiology ^B
Soft tissue related to inoculation site	Subcutaneous located swelling, 8.0 x 6.6 x 6.1 cm	¹⁸ F-FDG: sphere-formed subcutaneous accumulation, 4,7 x 6,0 x 6,4 cm (metabolic measurement) ⁶⁸ Ga-citrate: sphere-formed accumulation ¹¹ C-methionine: sphere-formed accumulation	¹¹¹ In-leukocytes: subcutaneous located swelling	Subcutaneous abscess, diameter approx. 8 cm; abscess, craniomedial thigh muscle region, communicating with the subcutaneous abscess by a fibrous stalk, diameter approx. 1 cm	<i>S. aureus</i> (heavy growth), both abscesses
Bones	Osteolysis (medulla and cortex), almost entire metatarsus II	¹⁸ F-FDG: entire metatarsus II ⁶⁸ Ga-citrate: entire metatarsus II ¹¹ C-methionine: entire metatarsus II	¹¹¹ In-leukocytes: entire metatarsus II and associated soft tissue (not possible to distinguish the two tissue components)	Suppurative osteomyelitis and suppurative periostitis, entire metatarsus II	NT
Soft tissue peripheral to bones	Swelling peripheral to metatarsus II	¹⁸ F-FDG: sphere formed accumulation, 2.6 x 4.6 x 5.1 cm (metabolic measurement) ⁶⁸ Ga-citrate: semi-sphere formed accumulation located in the deep part ¹¹ C-methionine: semi-sphere formed accumulation located in the deep part	¹¹¹ In-leukocytes: soft tissue peripheral to metatarsus I	Subcutaneous abscess medial to metatarsus II, diameter 2,0 cm	<i>S. aureus</i> (heavy growth)
Joints	---	---	---	Arthritis joint between metatarsus II and proximal phalanx	NT
Lymph nodes	Mammary lymph node enlarged	¹⁸ F-FDG: mammary lymph node ⁶⁸ Ga-citrate: mammary lymph node ¹¹ C-methionine: mammary lymph node ¹¹ C-PK11195: mammary lymph node	---	---	NT
	Medial iliac lymph node area slightly enlarged ^F	¹⁸ F-FDG: medial iliac lymph node area ^F	--- ^E	Enlarged medial iliac lymph nodes	NT

^A--- indicates absence of signal at any location within the stated tissue compartment. ^BAll *S. aureus* isolates from the pigs were phenotypically indistinguishable from the inoculated strain. ^CNT indicates not tested. ^DDeemed to be a contaminant. ^EOnly mammary lymph nodes scanned, i.e. included in the SPECT bed position. ^FDistinguishing the various anatomical soft tissue structures within the pelvic cavity by CT can be challenging, which is why the scan signal has been assigned to the area of the medial iliac lymph nodes. ^GPig 3 was without infection and is not included in this table.

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References

- [1] Soerensen KE, Nielsen OL, Jensen HE, Leifsson PS and Iburg TM. Pulmonary Lesions Following Intravenous Inoculation with A High Concentration of Staphylococcus Aureus in Pigs. *Journal of Comparative Pathology* 2010; 143: 324-324.
- [2] Leifsson PS, Iburg T, Jensen HE, Agerholm JS, Kjelgaard-Hansen M, Wiinberg B, Heegaard PM, Astrup LB, Olsson AE, Skov MG, Aalbaek B and Nielsen OL. Intravenous inoculation of Staphylococcus aureus in pigs induces severe sepsis as indicated by increased hypercoagulability and hepatic dysfunction. *FEMS Microbiol Lett* 2010; 309: 208-216.