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Effects of Long-term Anesthesia, Blood Sampling, Transportation, and Infection Status on Hearts and Brains in Pigs Inoculated with *Staphylococcus aureus* and Used for Imaging Studies

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Laboratory animals are widely used in imaging studies, including infection, heart, and brain research. Compared with rodents, pigs are especially useful because of their large organ sizes, ability to tolerate long-term anesthesia, and substantial blood volume, which allows repeated blood sampling. These factors are particularly important in positron emission tomography studies of potential new radioactive tracers, because the scans often are prolonged; in addition, kinetic studies involving repeated blood sampling may be performed to establish the optimal scan time. However, protracted studies may affect the cardiovascular system, brain, and other organs. This raises the question of how to monitor and counteract the effects of long-term anesthesia in pigs in a typical experimental setting yet prevent introducing bias into the experiment. To address this question, we investigated the effects of long-term anesthesia (maximum, 18 h), repeated blood sampling (maximum of 20 mL blood per kilogram body weight), and road transportation (as long as 1.5 h between 2 imaging centers) on key variables of lung, heart, and brain function in the context of a well-established pig model of *Staphylococcus aureus* infection. Pulse rate, oxygen saturation, body temperature, arterial pressure of CO_2, and urine production were stable during anesthesia for at least 16 h, whereas blood glucose slowly decreased. Hct and leukocyte count decreased due to repeated blood sampling. During road transportation, blood lactate levels increased 5 fold and arterial pressure of O_2 decreased by 50%. Repeated CT scans, necropsy results, and histopathology findings documented progressive lung changes and acute cardiac necrosis. No lesions indicative of hypoxia were found in brain. The study data show that the typical monitoring parameters do not fully depict the cardiovascular state of pigs during prolonged anesthesia. We recommend streamlining experimental protocols for imaging studies in pigs to avoid organ pathology.

Abbreviations: ^{18}F-FDG, ^{18}F-fluorodeoxyglucose; PET, positron emission tomography

Preclinical imaging studies play an important part in biomedical research, including investigation into bone infection, cardiovascular studies, and brain research.[13,20] Compared with small animals, such as mice and rats, pigs are characterized by large organ size and blood volume and can tolerate long-term anesthesia.[7] Anesthesia is necessary to keep the animal in a stationary position during scans, achieve optimal images. Specifically, when performing CT scans with the purpose of overlaying a positron emission tomography (PET) scan, which takes much longer to perform, it is very important the animal lies still in the scanner.[7] When examining and evaluating new potential radioactive tracers for clinical use, it is important to

follow the metabolism of the tracers and determine the most optimal scan time. This information is acquired by following tracer kinetics and collecting serial arterial blood samples for analysis, a procedure taking from a few minutes to several hours, depending on the radioactive isotope chosen. Pigs are often used in such studies because rodents sometimes cannot meet these requirements.[7] In addition, PET provides functional images that require stable animal physiology during scans.[6,9] Therefore, the question is how to maintain normal physiologic conditions during long-term anesthesia and frequent blood sampling, which are essential for data.

The current study used a well-established porcine model for comparing the suitability of various radioactive tracers for diagnosing osteomyelitis (that is, the clinical tracers ^{18}F-fluorodeoxyglucose [^{18}F-FDG] and ^{111}In-labeled leukocytes with the experimental tracers of ^{15}O-water, ^{11}C-methionine, and ^{68}Ga-citrate)[2,18,19] to evaluate the effects of long-term anesthesia. after cut-down of the right femoral artery, juvenile pigs were inoculated with pathogenic gram-positive, *Staphylococcus aureus*[5,16,17] and scanned 1 wk later at 1 or 2 scanning centers in different geographic locations in Denmark. *S. aureus* was used

in this model because it is the most common cause of osteomyelitis in humans and pigs.[16,17] These existing pig data were used to shed light on laboratory animal science issues without the need to use further pigs, although the pig studies were not originally designed for this purpose. Long-term anesthesia (as long as 18 h) and road transportation (up to 1.5 h between 2 imaging centers) were necessary for some pigs. A maximum of 20 mL blood per kilogram body weight was sampled to perform kinetic studies during as many as 5 dynamic scans.[2,3,18,19,26] This design could result in significant influences on pig physiology. We, therefore, collected relevant data to perform an in-depth analysis to determine these potential influences.

We chose to compare commonly used anesthesia indicators (pulse rate, oxygen saturation, body temperature, urine production, arterial blood gases, blood glucose, plasma lactate, and Hct) together with blood cell counts and a series of primarily lung CT scans during long-term anesthesia with gross and histologic post mortem examinations, especially the heart and brain. The heart was chosen, because the cardiovascular system affects blood flow and therefore the kinetics of radioactive tracers during PET scans. In addition, we examined the heart for necrosis, because we anticipated that acute necrosis could occur as hypoxic injury during procedures. Examination of the brain was of interest because the pig brain has become an important animal model in neuroimaging.[6,15,22,31] Here, we specifically examined the brains to disclose the presence of hypoxic damage, which might be induced by the many in vivo procedures. The pigs were kept anesthetized and monitored with veterinary equipment to which most preclinical laboratories have access. In particular, pigs were anesthetized through infusion of the fast-acting GABA agonist propofol, which is known to have neuroprotective[14] and vasodilatory effects. Pigs were premedicated by using another GABA agonist, midazolam, and the NMDA inhibitor ketamine, which in combination may have a peripheral vasodilatory effect in pigs, an effect that is relatively short-lived. Analgesia was maintained with buprenorphine, which is a partial agonist–antagonist at the μ and κ opioid receptors and may induce a drop in the pulse rate.[24]

We tested 3 hypotheses. First, we expected that values for the anesthesia monitoring parameters and blood gas analyses would change gradually during anesthesia, thus indicating unstable physiology. Second, we expected that if the anesthesia monitoring parameters and blood gas analyses were stable, neither the heart nor brain would be affected. Finally, we anticipated more damage after a prolonged period of anesthesia with frequent blood sampling and road transportation than after a shorter period of anesthesia with less intense blood sampling and no road transportation.

Materials and Methods

Overall design. This study includes data from 18 pigs (nos. I through XVIII) inoculated with *S. aureus* 1 wk before the experiments. Group 1 (*n* = 9; pigs I through IX) were anesthetized for 6 to 7 h during PET–CT scans at the Department of Nuclear Medicine and PET Centre (Aarhus University Hospital, Denmark) and were then transported for 1.5 h to the Department of Clinical Physiology and Nuclear Medicine (Aalborg University Hospital, Denmark), where the pigs were kept anesthetized for additional 7 to 9 h for SPECT–CT and PET–CT imaging (a total maximum of 18 h of anesthesia). Group 2 (*n* = 9; pigs X through XVIII) were anesthetized for a maximum of 14 h, because they underwent imaging at one institution only. Group 2 was divided in 2 subgroups: group 2A (*n* = 5; pigs X through XIV in Aarhus) and group 2B (*n* = 4; pigs XV through XVIII, in Aalborg). During

the scans, blood samples were collected repeatedly. After euthanasia, pigs were necropsied at the Department of Veterinary and Animal Sciences (University of Copenhagen). The study was approved by the Danish Animal Experimentation Board (license no. 2012-15-2934-000123. The following humane endpoints were used: fever (rectal body temperature greater than 39 °C)[11] for more than 24 h; or anorexia or reluctance to drink for more than 24 h; shallow respiration (all of these 3 features are clinical signs of systemic infection); loss of more than 10% body weight (a sign of pain that is intractable to opioid analgesics; and refusal to stand up. Approximately 1 in 3 pigs was euthanized due to humane endpoints.

Animals. Clinically healthy, female, SPF, juvenile Danish Landrace crossbred pigs (*Sus scofa*) were included. Pigs were free of *Sarcoptes scabie* (mange), *Haemotopinus suis* (lice), *Brachyspira hyodysenteriae* (swine dysentery), *Pasteurella multocida* (atrophic rhinitis), *Actinobacillus pleuropneumoniae* serotypes 1 through 10 and 12 (pleuropneumonia), *Mycoplasma hyopneumoniae* (enzootic pneumonia), and porcine reproductive and respiratory syndrome virus according to clinical testing and blood samples from the pig herds. Four pigs (I through IV) had a body weight of approximately 40 kg (approximately 100-d-old pigs), whereas the rest (V through XVIII) had a body weight of approximately 20 kg (approximately 70-d-old pigs). The pigs were purchased from 2 local commercial pig farmers (an Aarhus farmer for pigs I through XIV and an Aalborg farmer for pigs XV through XVIII) and were housed in separate boxes (5 m^2 per pig) to avoid bites to surgical wounds; boxes had solid flooring, were bedded with straw and sawdust, and included enrichment in the form of toys. Pigs had olfactory and visual contact with each other as well as the possibility of communication by grunts. Pigs were fed twice daily with a restricted pellet diet (DIA plus FI, DLG, Copenhagen, Denmark) and had free access to tap water. The environmental temperature and relative humidity were set to be 20 °C and 51%, respectively. The facility had a 12:12 h light cycle (lights on, 0700 to 1900), and the air was exchanged 8 times each hour. Prior to inoculation with *Streptococcus,* pigs were acclimated for 1 wk or more. Pigs were fasted overnight prior to anesthesia.

Inoculation. At 1 wk prior to imaging, the pigs were anesthetized for 0.5 to 1 h and inoculated with *S. aureus* (strain S54F9; isolated from a chronic embolic pulmonary abscess in a pig)[1] at 10^4 to 10^5 cfu/kg, as already described.[5,16,17] Buprenorphine was given (0.015–0.045 mg/kg IM every 8 h [0700, 1600, and 2300) from before inoculation and until euthanasia. NSAID were avoided in the original protocol, because they would affect inflammation. Instead, penicillin (10,000 IU/kg procaine benzylpenicillin; the *S. aureus* strain was fully sensitive to this antibiotic) was administered intramuscularly as a single dose (pigs IV through XVIII) when the infection threatened to spread to internal organs.[5] All intramuscular injections were given in the neck muscles behind the ears, with care to switch between right and left sides and as far apart as possible, to avoid injecting in the same site twice.

Anesthesia and monitoring. On imaging day, pigs were premedicated with approximately 1 mg/kg midazolam IM and 6 mg/kg ketamine IM, and anesthesia was maintained with infusion of 5 to 10 mg/kg IV propofol hourly by use of a syringe pump (Alaris Guardrail Plus, CareFusion, Hampshire, United Kingdom). Pigs were intubated and mechanically ventilated (model 3000, Veterinary Anesthesia Ventilator, Matrx, Pittsfield, MA) with a mixture of oxygen and medical air (1: 2.2) in Aarhus, during road transport, and in Aalborg (except that pigs were manually ventilated with 100% air for about 10 min before and

after road transportation). During road transportation, another ventilator was used (Dräger Oxylog 2000, Drägerwerk AG, Lübeck, Germany). As a starting point, pigs were ventilated 15 times per minute at 8 to 9 mL/kg initially, with slight adjustment later as needed. Anesthesia (pulse rate and oxygen saturation), body temperature, and urine production were monitored continuously by using a veterinary monitor (PM-9000 Vet, Mindray, Chenzhen, China). Saline (1 to 2 L IV) was administered slowly (approximately 35 to 45 drops per minute, depending on the extent of blood sampling) during anesthesia. During sterile surgery, Cortix catheters were placed through cut-down of a carotid artery and jugular vein prior to imaging. Catheters were sutured firmly to the skin and rinsed with 10 mL of sterile saline. Approximately every other hour, an arterial blood sample (1 mL) was withdrawn from a carotid catheter to measure $PaCO_2$, PaO_2, blood glucose, blood lactate, and Hct. The samples typically were analyzed immediately; otherwise they were stored in a refrigerator, as recommended.[27] $paCO_2$, paO_2, blood glucose, blood lactate, and Hct were measured by using a Radiometer ABL (Radiometer, Brønshøj, Denmark).

Venous blood samples (2 mL) from jugular vein catheters were obtained from group 1 for analysis prior to inoculation and before the first scanning. Furthermore, for some of the pigs (IV-IX) venous blood samples were taken the day before scanning and repeatedly during the imaging (after approximately 2, 6, 8, 12, and 18 h of anesthesia). Venous blood samples were stored in a refrigerator, as recommended,[28] before they were sent by mail for analysis (for example, CBC). An automated CBC count including a leukocyte differential count was conducted by using EDTA-stabilized whole blood (Advia 120 analyzer, Bayer Healthcare Diagnostics, Berlin, Germany). During scanning, pigs were placed in dorsal recumbency, as which allowed blood sampling from the jugular and carotid catheters. Pigs were covered with blankets throughout experiments, from preparation and during scanning procedures and road transportation. An electric blanket was available but was unnecessary because none of the pigs dropped in body temperature.

Blood sampling for tracer metabolite analysis. During the dynamic PET scans, approximately 70 mL of arterial blood (approximately double volumes for 40-kg pigs) was sampled for each tracer tested, to establish time–radioactivity curves and for metabolite analysis. Some scans were completed after 10 min and others after 90 min. The total amount of blood sampled was approximately 20 mL per kg for group 1 and 14 mL per kg for group 2. This volume includes the blood sampled for blood gases and CBC counts.

CT scanning. Whole-body CT scans were performed at least 3 times on the imaging day: in the morning prior to PET scanning; once with the SPECT scan; and once in connection with the PET scan. Results from the CT scans focused on the progression of pathologic changes in the lungs and hearts.

Necropsy, histology, and staining. At the end of the anesthesia period, pigs were killed by using an overdose of pentobarbitone (100 mg/kg IV). Necropsy was performed as described previously.[23] Bacterial culturing was performed from at least one characteristic lesion in each of the animals, occasionally from other lesions, and always from lungs. For histologic evaluation, heart and brain samples were fixed in 3.7% neutral buffered formaldehyde for 4 d to 21 mo, dehydrated, embedded in paraffin wax, cut in 3- to 5-μm thick sections, and stained with hematoxylin and eosin by using standard methods. Heart sections were taken from random locations in pigs I through IV; for the remaining pigs, sections were taken from the left ventricular papillary muscle and wall (pigs V through XIV) or the left ventricular papillary muscle areas (pigs XV through XVIII) known to be most sensitive to damage and representative of generalized disease.[29] Heart sections were scored according to the presence of acute focal myocardial necroses as either 0 (no necroses present), + (few necroses), or ++ (marked presence of necroses). Brain sections were obtained from cutting transversally into the dorsal part of the right or left hemispheres, including both gray and white matter (neurons of the cerebral cortex [layers III and V] are very vulnerable to ischemia).[25] In addition to hematoxylin and eosin staining, brain sections were stained with Fluoro–Jade B as described[30] to identify histologic changes, specifically neuronal degeneration or necrosis. Corresponding brain sections from 2 mock (saline) inoculated control pigs from another study and stained with hematoxylin–eosin and Fluoro–Jade B were used for comparison; the pigs were sedated, not anesthetized, in association with euthanasia. The right and left brain hemispheres from a rat showing ischemic neuronal necrosis due to complete unilateral carotid ligation was used as a positive control for Fluoro–Jade B staining.

Statistics. Unless otherwise noted, medians are reported throughout the text. For each time point the different anesthesia monitoring parameters, blood gas parameters, and blood CBC count were compared by using the nonparametric Mann–Whitney U test with Bonferroni correction for multiple comparisons. For scoring of acute heart necrosis, the Mann–Whitney U test (0; +, 1; ++, 2) was used to compare groups, subgroups, and sampling sizes. A P value of less than 0.05 was considered as significant.

Results
Survival, anesthesia length, and response to inoculation. Pig VII died during road transportation after approximately 6 h of anesthesia; all remaining pigs survived throughout the imaging protocol until euthanasia after 8 to 14 h (median, 10 h) for groups 2A and 2B or after 15 to 18 h (median, 17 h) for group 1. Two of the pigs (nos. III and XVI) did not respond to the inoculation. These animals had no clinical signs observed, no specific pathology, and no bacteriological *S. aureus* findings; in addition, pig III had a decrease in total leukocyte count and C-reactive protein at 1 wk after inoculation, whereas pig XVI had a smaller increase in both of these parameters. Consequently, they were used as unaffected controls. The pigs that responded to the inoculation typically developed 2 or 3 osteomyelytic foci, periosseous abscesses, and increased C-reactive protein. Some of the results from PET–CT and SPECT–CT scans relating to osteomyelitis and soft tissue lesions have already been published.[2,3,18,19,26]

Monitoring parameters. Table 1 shows anesthesia monitoring parameters for the pigs. There were no systematic differences in the monitored values between pigs included in the different groups. Pulse rate was stable (73 to 95 bpm), and only a temporary increase (to 103 beats/min) was observed during road transportation for pigs in group 1. Median oxygen saturation was at all-time points above 97%. Body temperature was stable (37.6 to 39.1 °C), and there was ongoing urine production (median, 115 mL/h). No significant differences were found between subgroups 2A and 2B. In the last hours of the experiments, there were difficulties in determining oxygen saturation, suggesting impaired peripheral circulation in the ears.

Arterial blood parameters. Results of arterial blood gas analyses are shown in Table 2. There were no systematic differences between pigs in groups 1 and 2, and no significant differences were found between subgroups 2A and 2B. Although median $PaCO_2$ was fairly stable during anesthesia, a decrease in PaO_2 was observed during transportation (from 25.3 kPa before to

Table 1. Monitoring parameters of the 18 pigs during anesthesia

	Group	Pulse rate (bpm)	O$_2$ saturation (%)	Body tempurature (°C)	Urine (mL)
Normal range		70–90	>92%	38.7–39.8	–
2 h	1	81 (49–117)	99 (93–99)	38.0 (37.0–39.1)	60 (10–150)
	2	73 (52–82)	99 (96–100)	38.1 (36.8–39.6)	60 (30–110)
	2A	75 (66–82)	99 (96–100)	38.1 (36.8–39.6)	60 (30–60)
	2B	57 (52–62)	99 (99–100)	37.9 (37.4–38.3)	105 (100–110)
4 h	1	81 (62–106)	99 (98–100)	38.2 (36.8–39.5)	100 (90–280)
	2	80 (51–97)	99 (98–100)	38.6 (37.2–40.3)	105 (70–300)
	2A	80 (72–97)	98 (98–100)	39.2 (37.2–40.3)	80 (70–300)
	2B	64 (51–86)	99 (98–100)	37.9 (37.4–38.4)	138 (100–170)
6 h	1	92 (59–112)	98 (84–100)	38.1 (37.2–39.7)	215 (110–1100)
	2	78 (48–149)	99 (90–100)	38.7 (37.0–40.0)	180 (110–500)
	2A	82 (76–149)	97 (90–99)	39.3 (38.4–40.0)	170 (110–500)
	2B	70 (48–74)	100 (99–100)	37.4 (37.0–38.6)	190 (140–400)
8 h	1	103 (88–129)[a]	100 (99–100)	38.6 (36.9–41.7)	450 (420–980)
	2	82 (70–90)[a]	99 (98–100)	38.5 (37.0–39.9)	330 (220–500)
	2A	85 (78–90)	99 (98–100)	38.6 (38.6–39.9)	220 (220–500)
	2B	74 (70–82)	99 (98–99)	37.8 (37.0–39.1)	340 (220–500)
10 h	1	95 (60–123)	99 (89–100)	38.5 (36.5–41.1)	840 (420–980)
	2	84 (80–88)	99 (97–100)	38.4 (38.2–40.0)	450 (350–850)
	2A	84 (82–88)	99 (98–100)	38.8 (38.3–40.0)	350 (350–750)
	2B	84 (80–88)	98 (97–99)	38.3 (38.2–39.4)	525 (450–850)
12 h	1	91 (63–103)	99 (91–100)	37.6 (36.7–39.6)	1100 (830–2000)
14 h	1	81 (67–111)	98 (87–100)	38.4 (37.0–40.1)	1200 (1000–2450)
16 h	1	79 (66–113)	98 (93–100)	39.1 (37.5–40.0)	1430 (1240–2700)
18 h	1	88 (68–122)	97 (70–99)	39.0 (37.5–40.3)	2075 (1400–3050)

Values are given as median and range. Road transportation in a car was performed after 6 to 8 h of anesthesia in the first 9 pigs. A few measurements are missing for some pigs.
[a]Significant ($P < 0.05$) difference between values for groups 1 and 2.

14.2 kPa after), and this decreased level never completely normalized. Blood lactate increased from 0.5 mmol/L before to 2.4 mmol/L just after road transportation. During the next 10 h of anesthesia, blood lactate slowly decreased to 1.2 mmol/L before euthanasia after 18 h. Corresponding drops in paO$_2$ and increases in blood lactate were not observed during the same period of time for the pigs that were not transported (group 2). Blood glucose slowly decreased from 5.2 to 3.5 mmol/L just before euthanasia in the long-term group. Hct slowly decreased from 24% in the beginning to 19% after 18 h in group 1, whereas no changes (from 19% to 20%) in Hct were observed for group 2, from which fewer blood samples were taken.

Hematology. Table 3 shows the results of blood cell counts before and after inoculation with *S. aureus* (hematologic data for the pigs I through IV have been presented previously[26] but are included here for comparison). Leukocyte, lymphocyte, and neutrophil counts decreased during day 7, probably due to a dilution effect from repeated blood sampling. The percentage distribution of the different types of leukocytes remained unchanged, except for eosinophils, which were constant during day 7.

PET, CT, necropsy, and histology findings. Results from the CT examinations and necropsy are shown in Table 4; only pathology in the internal organs is described here. Most striking are the many pathologic findings in the lungs: atelectasis of varying degrees was present in 16 of the 18 pigs. A single pig in group 1 (pig I) had atelectasis due to inappropriate ventilation. In the abdomen, the most common finding was increased fluid, which was found in 8 of the pigs that underwent prolonged anesthesia and in 5 of the other pigs (Figure 1). No macroscopic changes were found in the brain. Table 5 shows the histopathologic findings from the heart. The area of heart muscle evaluated typically fitted the size of a normal glass slide but did vary by approximately a factor of 2. Myocardial necroses were by far the most abundant lesions found, with some having concomitant mineralization or inflammatory reaction due to mononuclear or polymorphonuclear cells. The heart scores did not differ between the 2 groups ($P = 0.311$). Furthermore, within the 2 groups, we did not find any difference in heart sampling size. Both of the pigs without infections had marked necrosis (++) in the heart. Figure 2 shows an example of acute cardiac myofiber necrosis with slight peripheral inflammatory reaction

Table 2. Carotid arterial blood gas analyses of the 18 pigs during anesthesia

Time	Group	$paCO_2$ (kPa)	paO_2 (kPa)	Glucose (mmol/L)	Lactate (mmol/L)	Hct (%)
Normal values		5.3–7.2	12–25	>3.5	0.2–1.2	24–36
2 h	1	6.5 (5.4–10.5)	26.1 (10.4–30.3)	5.2 (4.4–6.8)	0.6 (0.2–1.1)	24 (17–35)
	2	6.8 (6.1–7.8)	23.9 (18.8–29.3)	3.1 (2.3–3.9)	0.3 (0.2–0.3)	19 (18–23)
	2A	6.8 (6.1–6.9)	24.4 (18.8–29.3)	3.1 (2.3–3.9)	0.3 (0.2–0.3)	20 (18–23)
	2B	7.8 (7.8–7.8)	20.4 (20.4–20.4)	3.1 (2.3–3.8)	0.3 (0.2–0.3)	19 (19–19)
4 h	1	6.7 (5.0–8.7)	23.1 (5.0–27.2)	4.9 (4.2–6.3)	0.4 (0.2–1.3)	21 (15–23)
	2	6.2 (5.2–8.1)	24.8 (16.9–27.9)	3.0 (2.5–4.4)	0.3 (0.2–2.0)	20 (18–25)
	2A	6.2 (5.9–8.1)	25.7 (16.9–26.1)	3.0 (2.5–4.0)	0.2 (0.2–0.3)	19 (18–22)
	2B	6.4 (5.2–6.9)	23.4 (17.3–27.9)	3.7 (2.9–4.4)	1.9 (1.7–2.0)	22 (18–25)
6 h	1	6.2 (4.5–8.1)	25.3 (18.0–35.2)	5.0 (3.5–5.8)	0.5 (0.2–0.9)	20 (16–30)
	2	6.7 (5.3–13.3)	24.1 (18.0–27.5)	3.8 (3.0–4.4)	1.0 (0.2–2.1)	23 (16–24)
	2A	7.7 (5.3–13.3)	21.8 (18.0–26.5)	3.0 (3.0–4.2)	0.3 (0.2–0.4)	21 (16–23)
	2B	6.3 (5.3–7.0)	24.1 (20.0–27.5)	4.2 (3.3–4.4)	1.6 (1.5–2.1)	24 (18–24)
8 h	1	6.5 (6.1–6.9)	14.2 (7.8–17.8)[a]	4.8 (2.8–8.1)	2.4 (2.3–3.0)[a]	21 (19–22)
	2	6.1 (5.1–11.6)	25.1 (16.6–29.1)[a]	3.2 (1.7–4.4)	0.3 (0.2–1.6)[a]	20 (15–24)
	2A	6.1 (5.5–7.9)	21.9 (16.6–25.5)	2.8 (1.7–4.0)	0.3 (0.2–0.4)	18 (15–22)
	2B	6.1 (5.1–11.6)	26.4 (23.9–29.1)	3.7 (3.3–4.4)	1.5 (1.3–1.6)	22 (20–24)
10 h	1	6.6 (4.7–7.2)	14.9 (10.4–18.0)	3.5 (2.0–5.8)	2.2 (1.7–2.5)	18 (14–19)
12 h	1	5.9 (5.2–6.7)	17.3 (12.1–18.9)	3.6 (2.5–4.6)	1.6 (1.6–2.0)	21 (17–22)
14 h	1	6.3 (4.2–12.7)	15.0 (8.5–18.8)	3.4 (2.4–5.2)	1.3 (1.2–2.2)	18 (14–24)
16 h	1	6.2 (4.7–15.4)	17.7 (7.0–20.3)	3.5 (2.6–4.4)	1.5 (0.9–1.5)	20 (19–25)
18 h	1	6.5 (5.3–18.0)	17.4 (4.5–19.9)	3.5 (2.5–4.9)	1.2 (0.7–3.6)	19 (12–21)

Values are given as median and range. Road transportation in a car was performed after 6 and 8 h of anesthesia in the first 9 pigs. A few measurements are missing for some pigs.
[a]Significant ($P < 0.05$) difference between values for groups 1 and 2.

Table 3. Leukocyte counts ($\times 10^9$/L) before and after inoculation with *S. aureus* into the right femoral artery of the first 9 pigs (nos. I–IX)

	Leukocytes	Lymphocytes	Neutrophils	Eosinophils
Normal range	11.3–22.8	4.6–10.0	3.1–9.6	0.0–0.9
Before inoculation	17.7 (9.0–21.8)	9.8 (5.3–11.2)	5.8 (2.6–9.7)	0.7 (0.2–1.7)
Day 6 after inoculation	18.4 (13.3–21.0)	5.2 (3.7–6.8)#	11.0 (6.6–13.2)[a]	0.4 (0.4–1.0)
Day 7 (Aarhus start)	15.8 (10.3–30.4)	6.6 (3.4–10.2)	7.4 (4.5–20.3)	0.6 (0.4–2.4)
Day 7 (Aarhus end)	12.5 (9.8–15.5)	5.3 (3.3–7.3)	6.0 (4.4–8.8)	0.6 (0.3–0.9)
Day 7 (Aalborg start)	11.8 (10.2–18.4)	5.8 (4.8–7.2)	5.4 (3.5–9.6)	0.8 (0.7–1.2)
Day 7 (Aalborg middle)	9.5 (8.3–16.6)	5.4 (4.5–6.1)	4.3 (3.2–9.0)	0.6 (0.2–0.8)
Day 7 (Aalborg end)	12.3 (8.4–16.1)	5.8 (2.7–6.8)	5.9 (3.9–7.9)	0.6 (0.5–0.6)

Values are given as median and range. For the first 3 pigs (I–III), blood was sampled before inoculation and after 1 wk only.
[a]$P < 0.05$ compared with baseline value

from a single pig. No signs of hypoxia in terms of neuronal degeneration or necrosis were observed in either the hematoxylin–eosin- or Fluoro–Jade B-stained brain sections from any of the 17 tested pigs.

Discussion

Here we describe a challenging animal study involving long-term anesthesia of pigs, which underwent frequent blood sampling, transportation between scanners 120 km apart, and bacterial infection. Not surprisingly this combined scenario affected the pigs. Especially pronounced were effects on the lungs, where atelectasis was seen despite appropriate intubation and ventilation, except for pig I, for which ventilation was suboptimal during road transport. Placement in dorsal recumbency may have contributed to increasing atelectasis and causing hypostatic atelectasis. Furthermore, the procedures may have added to the

Table 4. CT findings, CT progression, gross pathology, and microbiology for the 18 included pigs

	CT	CT progression	Gross pathology	Microbiology
Pig I (1)				
Lungs	Several abscesses, largest 17 mm in diameter Partly consolidated, infiltrative and atelectatic changes in dorsocaudal part of left lung (59 × 47 x 44 mm) and pleural effusions; atelectatic changes in right lung	Yes, during all later scans	Disseminated lung abscesses Bilateral diffuse atelectasis and edema	*S. aureus*
Heart	Normal	No	No	NT
Abdominal cavity	No	Discrete	Increased amount of abdominal fluid with some fibrin flakes	Negative
Pig II (4)				
Lungs	Multiple lung abscesses (largest diameter, 16 mm) Diffuse, consolidated, and infiltrative ground-glass opacifications in dorsocaudal lung areas, especially left lung	No	Disseminated lung abscesses (largest diameter, 1 cm) some had elicited acute focal fibrinous pleuritis; several pinpoint lesions	*S. aureus*
			Bilateral diffuse atelectasis and edema	*S. aureus*
	—		Increased amount of clear fluid in pleural cavity	
Heart	Normal size	No	No	NT
Abdominal cavity	No ascites	Light ascites on late scans	Increased amount of abdominal fluid with some fibrin flakes	Negative
Pig III (6)				
Lungs	Discrete, infiltrative diffuse dorsocaudal changes	No	Bilateral and dorsocaudal located areas of atelectasis; moderate bilateral edema; disseminated acute petechiae	Negative
Heart	Normal size	No	No	NT
Abdominal cavity	No ascites	No	Increased amount of abdominal fluid with some fibrin flakes	Negative
Pig IV (8)				
Lungs	Multiple lung abscesses (largest diameter, 24 mm) and diffuse infiltrative changes	Atelectasis right lung	Disseminated lung abscesses (largest diameter, 2 cm)	*S. aureus*
			Bilateral dorsocaudal atelectasis; moderate bilateral diffuse edema	NT
			100 mL of sanguineous pleural fluid, both right and left thoracic cavities	*E. coli*
Heart	Normal size	No	No	*NT*
Abdominal cavity	No ascites	Minimal ascites on last scan	Increased amount of abdominal fluid with some fibrin flakes	Negative
Pig V (22)				
Lungs	Lung abscesses	No	Atelectasis dorsally and bilaterally.	*S. aureus*
	Diffuse infiltrative changes in dorsocaudal lung areas	Bilateral pleural effusion	Disseminated abscesses and necrosis bilaterally	
Heart	Normal size	Yes	Pale	*NT*
Abdominal cavity	No ascites	Ascites progression on late scans	No	Probably enterobacteria
Pig VI (24)				
Lungs	Infiltrative diffuse dorsocaudal changes, primarily right	Slight	Edema and atelectasis dorsocaudally	Negative
Heart	Normal size	No	No	NT

Table 4. *Continued.*

	CT	CT progression	Gross pathology	Microbiology
Abdominal cavity	No ascites	Ascites	Approximately 0.5 L brownish, slightly cloudy urine from accidental perforation of the urinary bladder	Negative
Pig VII (28)				
Lungs	Infiltrative diffuse dorsocaudal changes	Yes and atelectasis	~25 disseminated abscesses (diameter, 1–5 mm) Dorsal, bilateral atelectasis	*S. aureus*
Heart	Normal size	Increasing	No	NT
Abdominal cavity	No ascites	No	65 mL serohemorrhagic fluid	Coli-like (possibly contamination)
Pig VIII (29)				
Lungs	Light infiltrative diffuse dorsocaudal changes (primarily right)	Slightly, pleural effusion (right)	Pronounced caudodorsal, bilateral atelectasis	Many different colonies (not conclusive)
Heart	Normal size	No	No	NT
Abdominal cavity	No ascites	Ascites	Increased amount of serous fluid	Negative
Pig IX (33)				
Lungs	Very slight changes No sign of pneumonia	No, improvement	Dorsocaudal atelectasis and edema	*S. dysgalactia* (inconclusive)
Heart	Normal size	No	No	NT
Abdominal cavity	No ascites	No	Increased amount of sero-fibrinous fluid	Negative
Pig X (35)				
Lungs	Discrete diffuse infiltrative and atelectatic changes in dorsocaudal lung areas (primarily right)	No	Bilateral dorsal atelectasis	Negative
Heart	No	No	No	NT
Abdominal cavity	NT	NT	No	NT
Pig XI (38)	CT	CT-progression	Gross pathology	Microbiology
Lungs	Discrete diffuse infiltrative changes in dorsocaudal lung areas	Slight progression	Bilateral edema and atelectasis dorsocaudally	Negative
Heart	No	No	No	NT
Abdominal cavity	No	NT	No	NT
Pig XII (39)				
Lungs	Diffuse discrete infiltrative changes in dorsocaudal lung areas	No	Bilateral pulmonary edema and atelectasis Solitary abscess in the left (2 mm) and right (4 mm) lung	Negative
Heart	No	No	No	NT
Abdominal cavity	NT	Distended urinary bladder	Extensive abdominal fluid Acute stasis in the liver	NT
Pig XIII (44)				
Lungs	No	Yes, bilateral infiltrative changes dorsocaudally and atelectasis	Bilateral edema and atelectasis, most pronounced dorsocaudally	Negative
Heart	No	No	No	NT
Abdominal cavity	No	No	No	NT
Pig XIV (46)				
Lungs	No	Yes, dorsocaudal infiltrative changes and atelectasis in primary right lung	Bilateral edema and atelectasis, most pronounced dorsocaudally	Negative
Heart	No	No	No	NT
Abdominal cavity	No	Markedly distended urinary bladder	No	NT

Table 4. *Continued.*

	CT	CT progression	Gross pathology	Microbiology
Pig XV (49)				
Lungs	Marked infiltrative changes in dorsocaudal lung areas	Improved	Bilateral edema and atelectasis Some serous fluid	Negative
Heart	No	No	No	NT
Abdominal cavity	No	Distended urinary bladder	Some serous fluid	NT
Pig XVI (50)				
Lungs	Infiltrative changes in dorsocaudal lung areas, primarily left	Improved left, but increased right side	Bilateral edema and atelectasis Minor accumulation of serous fluid in thorax	Negative
Heart	No	No	No	NT
Abdominal cavity	No	Distended urinary bladder	Minor accumulation of serous fluid	NT
Pig XVII (54)				
Lungs	Discrete infiltrative changes dorsocaudally in left lung	Left normalized discrete infiltrative changes dorso-caudally in right lung	Unilateral, subacute to chronic, cranioventral bronchopneumonia	Negative (test performed in lung tissue outside the bronchopneumic area)
Heart	No	No	No	NT
Abdominal cavity	No	Discretion	Increased amount of serous fluid. Acute infarction in right kidney	NT
Pig XVIII (56)				
Lungs	Discrete infiltrative changes dorsocaudally in both lungs	Yes, slightly	Pronounced dorsal and bilateral atelectasis	Negative
Heart	No	No	No	NT
Abdominal cavity	No	Discrete	Increased amount of serous fluid	NT

NT, not tested.
The Arabic numbers (1–56) indicate a unique numbering system for each pig (see references 2, 3, 5, 18, 19, and 26).
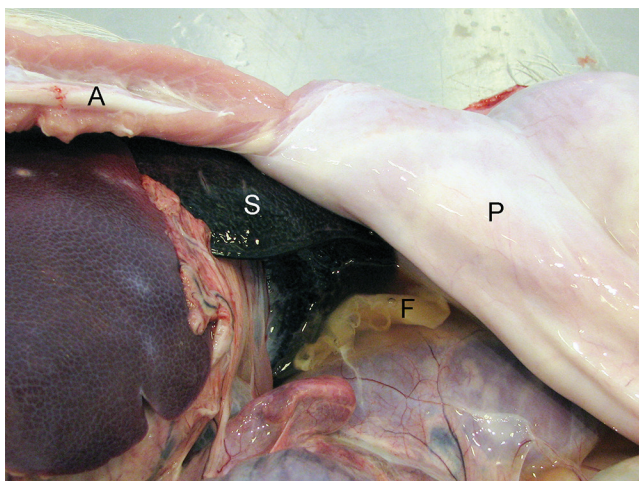

Figure 1. Accumulation of serous fluid admixed with fibrin (F) in the abdominal cavity. Pig I in dorsal recumbency (supine position) with the abdominal wall cut open exposing the parietal peritoneum (P) and the caudal costal arc (A); the spleen (S) is bordering the lateral part of the accumulation.

development of acute necrosis in the heart in both groups 1 and 2, whereas no effects were seen in brain. In humans, complications after prolonged anesthesia are often related to the brain, including discomfort, itching, headaches and postoperative delirium, and cognitive dysfunction—symptoms that, however, hardly leave clear pathologic findings; furthermore, vomiting and urinary problems are commonly seen.[10] Postmortem studies typically are performed only in cases of overt errors in anesthesia; therefore the literature regarding this topic is very sparse. Knowledge of pathologic changes after anesthesia in veterinary patients is similarly sparse; necropsy in those cases is used only as a tool for troubleshooting why the animal died. Necropsy findings and monitoring parameters for the pig that died (VII) did not differ from the others.

The first hypothesis, stating that monitoring parameters and blood gas analyses would gradually change during anesthesia, was partially rejected, given that these remained relatively stable until shortly before euthanasia. This outcome apparently confirms the fact that pigs tolerate anesthesia well,[7,11] however the CT and postmortem studies revealed atelectasis in the lungs and acute necroses in the heart. The CT scans showed progression, which also was found in the 2 noninfected controls. During the many hours of anesthesia, there was a slight decrease in blood glucose, and after approximately 18 h of anesthesia, it was difficult to measure peripheral oxygen saturation—probably due to reduced peripheral circulation in the ears, where the probe was placed; the values might have been different if we had measured oxygen saturation centrally. Continuous measurement of blood glucose should be considered in future studies so that a possible decrease could be counteracted by slow intravenous infusion of glucose. Major changes in monitoring parameters occurred only in connection with road transport and were not due to anesthesia itself. The monitoring parameters that we chose to evaluate are those typically measured on anesthetized pigs,[8,11] except that blood pressure measurements were not performed on our pigs, due to lack of equipment. In a recently published study on the importance of monitoring pigs during PET brain scans, blood pressure did not appear to be significantly corre-

Table 5. Heart histology

Pig	Acute necroses*	Other findings
I	+	Necrosis of the purkinje fibers; edema
II	++	Edema
III	++	Edema
IV	++	Edema
V	++	Two inflammatory foci[b] and slight infiltration subendocardially (Purkinje fibers); edema
VI	++	Necroses more frequent close to the chorda tendineae; edema
VII	++	Peracute necroses
VIII	++	Necroses more frequent close to the chorda tendineae
IX	+	Edema
X	++	Edema
XI	+	Two inflammatory foci[b]; edema
XII	++	Edema
XIII	0	One inflammatory focus[b]; edema
XIV	++	Edema
XV	+	One inflammatory focus[b]; edema
XVI	++	Edema.
XVII	++	One inflammatory focus[b]; necrosis and infiltration subendocardially (Purkinje fibers); edema
XVIII	+	—

[a]The acute cardiac myofiber necroses was semiquantified as 0, no necroses present; +, minimal presence of necroses; and ++, marked presence of necroses; lesions were present with or without mineralization and inflammatory cells.
[b]Occasionally inflammatory reactions were present without evidence of concomitant necrosis, which was interpreted as either inflammation secondary to necrosis, but with the necrotic part being out of focus, or as background pathology.
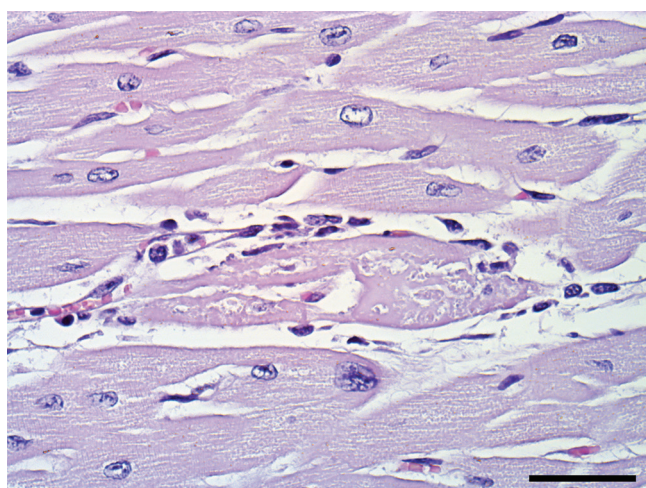

Figure 2. Acute necrosis of myocardial cells with seconday, mainly peripheral, infiltration of inflammatory cells. Pig VIII, HE stain, bar = 70 μm.

lated with blood flow.[8] In addition, CT scan of the thorax and abdomen could be suggested as a complementary monitoring parameter (for example, for edema and atelectasis), given that CT scanners today form an integral part of PET–CT scanners.[4] CT descriptions and the major necropsy findings were consistent (Table 4).

The second hypothesis, stating that as long as anesthesia monitoring parameters and blood gas analyses were stable that neither the heart nor the brain would be affected, was partially rejected, given that the heart was affected in several pigs (mainly acute necrosis) even though the monitoring parameters were stable. This outcome highlights the already mentioned dilemma that the welfare of the pig cannot be guaranteed, even though monitoring yields normal values. In our study, all of the pigs were euthanized during anesthesia, but in recovery studies, such nonidentified pathologic changes in hearts and lungs may exist and affect animal welfare. The necroses are found only when necropsy is performed. The acute nature of the lesions (predominantly acellular) and their variable numbers in the pigs (complete absence in one pig), the apparent lack of coherence with any of the monitoring parameters, and the occurrence of *S. aureus* culture from the lungs (that is, sepsis) or with other parameters could point to lesions precipitated by agonal hypoxia. Indeed, death in humans from accidental CO poisoning, for example, will cause agonal myocardial ischemia and acute necrosis.[12]

The third hypothesis regarding increased damage after prolonged anesthesia, frequent blood sampling, and road transportation only partly could be confirmed. The median heart score was not significantly higher in group 1 than in group 2. Both peracute and acute lesions were observed in the hearts, some of which could be older than 18 h. Given that *S. aureus* is purulent, lesions with mononuclear cells may not be directly causal to the infection. It is our opinion that all these lesions are due to necrosis secondary to the in vivo experimental conditions and not to the infection itself. We cannot exclude some degree of background pathology, but it cannot explain all of the findings. Some of the effects may be due to road transport or to frequent blood collection. Blood lactate increased and paO_2 decreased during road transportation. Furthermore, the only death occurred during the transportation period. Why blood lactate and paO_2 were affected only during transport is unclear, but one explanation is that the pigs were poorly ventilated by using the mobile ventilator and that the lungs, therefore, developed temporary atelectasis. If this situation was the case, the respirator settings could be optimized in the future, but it is uncertain whether this optimization will be sufficient to allow the transport of pigs without any pathophysiologic effects. In addition, the short periods of manual ventilation before and after road transportation may have had an influence, as the air was not enriched with oxygen. Hypostatic atelectasis was not found only in the pigs that were transported; therefore causes in addition to mobile respirator settings must be possible.

Hct and leukocyte counts decreased in the pigs that underwent frequent blood sampling during long-term anesthesia. In general, Hct was slightly lower than stated in the literature (33.9% to 45.9%)[11] and published on some of our own 40-kg pigs (24.4% to 35.9%).[8] Hct remained unchanged in the pigs with less-frequent blood sampling. For both groups, pulse rate was unchanged. The blood loss was replaced with 3 volumes of physiologic sterile saline, and this treatment was sufficient to maintain a constant pulse rate and was supported by continued urine production. However, dilution resulted in a decrease in Hct and blood cell counts. The decreased number of leukocytes may potentially affect the infection status during anesthesia. Furthermore, the decrease in Hct was small, and therefore the physiologic significance probably was limited.

Because this study was based on results from inoculated pigs, the infection may have significantly influenced the results. However, the progression of changes in the 2 uninfected pigs was similar to that in the 16 infected pigs. Neither monitoring parameters nor pathologic findings in the heart, brain, or other internal organs differed between these 2 groups of pigs. This outcome indicates that infection status in itself did not affect how well pigs tolerate long-term anesthesia, blood sampling, and road transport. However, the study population is too small to be able to make a definitive statement in this regard. Notably, no brain hypoxia or any specific pathologic changes were found. This result is substantiated by the fact that the binding of the glucose analog ^{18}F-FDG was similar in all pig brains: the median standardized uptake value during long-term anesthesia was 1.8 (range, 1.5 to 2.6) g/mL compared with 1.9 (1.4 to 3.0) g/mL for the other pigs (data not shown). Likewise, these values are consistent with a previous study in which brain standardized uptake value for ^{18}F-FDG was 1.8 to 3.0 g/mL in young pigs anesthetized with propofol.[21] These values may be due to propofol's neuroprotective effects;[14] therefore hypoxic damage may occur under another type of anesthesia.

Our study has several limitations. First, we chose to reanalyze data from previous pig studies, which prevented our designing the study to better fit testing our hypotheses. Instead, and according to 3R principles, we tried to gain knowledge for refinement without the use of additional animals, in an attempt to follow 2 of these principles. The design makes it difficult to separate the effects of the various factors studied (anesthesia time, blood sampling, and road transportation), and it could have been beneficial to have had control groups. For example, in future studies, we should optimally include healthy pigs that had been inoculated in the femoral artery with saline only and thus would lack any effects due to infection itself. These weaknesses make it more difficult to form conclusions regarding the results. The increases in total leukocyte count and C-reactive protein (data not shown) in pig XVI indicate that the immune system might have been slightly activated after inoculation, but the infection simply failed. However, the increase in C-reactive protein was minor compared with the 52- to 75-fold increases found in pigs with osteomyelitis.[26] For the histologic tissue sections, one disadvantage is that the samples were not all taken from the same areas of the heart and that no precise record regarding the area is available. This situation may represent a potential bias in our study, even though we were unable to find any significant differences within the 2 groups. Furthermore, a major limitation is that the pigs were evaluated in the order listed (from pig I to pig XVIII) rather than in random order. Due to these limitations, we offer no firm conclusions regarding the data, but the study nevertheless points to some of the problems that can be associated with long-term PET scans of pigs.

In summary, given the results of our study, we recommend streamlining demanding experimental protocols for imaging studies in pigs to avoid affecting the physiology of the animals and inducing organ pathology. After the pigs had completed the extensive in vivo procedures, their lungs and hearts—but not their brains—were affected. Common monitoring parameters were not always able to identify the development and progression of subordinate organ pathology. However, CT imaging can be a useful addition to classic monitoring parameters.

References

1. **Aalbæk B, Jensen LK, Jensen HE, Olsen JE, Christensen H**. 2015. Whole-genome sequence of *Staphylococcus aureus* S54F9 isolated from a chronic disseminated porcine lung abscess and used in human infection models. Genome Announc **3:**1–2. https://doi.org/10.1128/genomeA.01207-15.
2. **Afzelius P, Alstrup AKO, Schønheyder HC, Borghammer P, Jensen SB, Bender D, Nielsen OL.** 2016. Utility of 11C-methionine and 11C-donepezil for imaging of Staphylococcus aureus induced osteomyelitis in a juvenile porcine model: comparison to autologous 111In-labelled leukocytes, 99mTc-DPD, and 18F-FDG. Am J Nucl Med Mol Imaging **6:**286–300.
3. **Afzelius P, Nielsen OL, Alstrup AKO, Bender D, Leifsson PS, Jensen SB, Schønheyder HD.** 2016. Bio-distribution of the radionuclides (18)F-FDG, (11)C-methionine, (11)C-PK11195, and (68) Ga-citrate in domestic juvenile pigs and morphological imaging of the tracers in hematogenously disseminated Staphylococcus aureus lesions. Am J Nucl Med Mol Imaging **6:**42–58.
4. **Alstrup AKO.** 2015. Computer tomography is a useful tool in preclinical imaging studies with positron emission tomography. Lab Animal Sci Prof **1:**31–33.
5. **Alstrup AKO, Nielsen KM, Schønheyder HC, Jensen SB, Afzelius P, Leifsson PS, Nielsen OL.** 2015. Refinement of a hematogenous localized osteomyelitis model in pigs. Scand J Lab Anim Sci **42:**1–5.
6. **Alstrup AKO, Smith DF.** 2012. PET neuroimaging in pigs. Scand J Lab Anim Sci **39:**1–21.
7. **Alstrup AKO, Winterdahl M.** 2009. Imaging techniques in large animals. Scand J Lab Anim Sci **36:**1–12.
8. **Alstrup AKO, Zois NE, Simonsen M, Munk OL.** 2018. Monitoring variables affecting positron emission tomography measurements of cerebral blood flow in anaesthetised pigs. Acta Vet Scand **60:**1–7. https://doi.org/10.1186/s13028-018-0369-5.
9. **Balaban RS, Hampshire VA.** 2001. Challenges in small animal noninvasive imaging. ILAR J **42:**248–262. https://doi.org/10.1093/ilar.42.3.248.
10. **Belcher AW, Leung S, Cohen B, Yang D, Mascha EJ, Turan A, Saager L, Ruetzler K.** 2017. Incidence of complications in the post-anesthesia care unit and associated healthcare utilization in patients undergoing non-cardiac surgery requiring neuromuscular blockade 2005-2013: A single center study. J Clin Anesth **43:**33–38. https://doi.org/10.1016/j.jclinane.2017.09.005.
11. **Bollen PJA, Hansen AK, Alstrup AKO.** 2010. The laboratory swine. 2nd ed. Boca Raton (FL): CRC Press.
12. **Edston E.** 1997. Evaluation of agonal artifacts in the myocardium using a combination of histological stains and immunohistochemistry. Am J Forensic Med Pathol **18:**163–167.

13. **Elsinga PM, van Waarde A, Paans AMJ, Dierckx RAJO.** 2012. Trends on the role of PET in drug development. Singapore. World Scientific. https://doi.org/10.1142/7851.

14. **Fan W, Zhu X, Wu L, Wu Z, Li D, Huang F, He H.** 2015. Propofol: an anesteticpossessing neuroprotective effects. Eur Rev Med Pharmacol Sci **19:**1520–1529.

15. **Hansen HD**, **Constantinescu CC, Barret O, Herth MM, Magnussen JH, Lehel S, Dyssegaard A, Colomb J, Billard T, Zimmer L, Tamagnan G, Knudsen GM.** 2019. Evaluation of [18F]2FP3 in pigs and nonhuman primates. J Labelled Comp Radiopharm **62:**34–42. https://doi.org/10.1002/jlcr.3692.

16. **Johansen LK, Koch K, Frees D, Aalbaek B, Nielsen OL, Leifsson PS, Iburg TM, Svalastoga E, Buelund LE, Bjarnsholt T, Høiby N, Jensen H.** 2012. Pathology and biofilm formation in a porcine model of staphylococcal osteomyelitis. J Comp Pathol **147:**343–353. https://doi.org/10.1016/j.jcpa.2012.01.018. PubMed

17. **Johansen LK, Svalastoga EL, Frees D, Aalbaek B, Koch K, Iburg TM, Nielsen OL, Leifsson PS, Jensen HE.** 2013. A new technique for modeling of hematogenous osteomyelitis in pigs: inoculation into femoral artery. J Invest Surg **26:**149–153. https://doi.org/10.3109/08941939.2012.718043.

18. **Jødal L, Jensen SB, Nielsen OL, Afzelius P, Borghammer P, Alstrup AKO, Hansen SB.** 2017. Kinetic modelling of infection tracers [18F]FDG, [68Ga]Ga-Citrate, [11C]methionine, and [11C]donepezil in a porcine osteomyelitis model. Contrast Media Mol Imaging **2017:**1–18. https://doi.org/10.1155/2017/9256858.

19. **Jødal L, Nielsen OL, Afzelius P, Alstrup AKO, Hansen SB.** 2017. Blood perfusion in osteomyelitis studied with [15O] water PET in a juvenile porcine model. EJNMMI Res **7:**1–10. https://doi.org/10.1186/s13550-016-0251-2.

20. **Kiessling F, Pichler BJ, editors.** 2017. Small animal imaging: Basic and practical guide, 1st ed. Berlin: Springer International Publishing.

21. **Lee YA, Kim JI, Lee JW, Cho YJ, Chung HW, Park KK, Han JS.** 2012. Effects of various anesthetic protocols on 18F-flurodeoxyglucose uptake into the brains and hearts of normal miniature pigs (*Sus scrofa domestica*). J Am Assoc Lab Anim Sci **51:**246–252.

22. **Lind NM, Moustgaard A, Jelsing J, Vajta G, Cumming P, Hansen AK.** 2007. The use of pigs in neuroscience: modeling brain disorders. Neurosci Biobehav Rev **31:**728–751. https://doi.org/10.1016/j.neubiorev.2007.02.003.

23. **Madsen LW, Jensen HE.** 2011. Necropsy of the pig, p 83–106. In: Jensen HE, editor. Necropsy a handbook and atlas. Frederiksberg (Denmark): Biofolia.

24. **Martinez EA, Hartsfield SM, Melendez LD, Matthews NS, Slater MR.** 1997. Cardiovascular effects of buprenorphine in anesthetized dogs. Am J Vet Res **58:**1280–1284.

25. **Montine TJ, Anthony DC.** 2011. Toxicological neuropathology in medical practice, p 475–486. In: Bolon B, Butt MT, editors. In: Fundamental neuropathology for pathologists and toxicologist, 1st ed. Hoboken (NJ): Wiley. https://doi.org/10.1002/9780470939956.ch29

26. **Nielsen OL, Afzelius P, Bender D, Schønheyder HC, Leifsson PS, Nielsen KM, Larsen JO, Jensen SB, Alstrup AK.** 2015. Comparison of autologous (111)In-leukocytes, (18)F-FDG, (11)Cmethionine, (11)C-PK11195 and (68)Ga-citrate for diagnostic nuclear imaging in a juvenile porcine haematogenous Staphylococcus aureus osteomyelitis model. Am J Nucl Med Mol Imaging **5:**169–182. PubMed

27. **Olsen AK.** 2003. Effects of storage time and temperature on pH, pCO_2, and pO_2 measurements in porcine blood samples. Scand J Lab Anim Sci **30:**197–201.

28. **Olsen AK, Bladbjerg EM, Jensen AL, Hansen AK.** 2001. Effect of preanalytical handling on haematological variables in minipigs. Lab Anim **35:**147–152. https://doi.org/10.1258/0023677011911516.

29. **Robinson WF, Robinson NA.** 2016. Cardiovascular system, p 1–101.e1. In: Maxie MG, editor. In: Jubb, Kennedy, and Palmer's pathology of domestic animals, vol 3, 6th ed. St Louis (MO): Elsevier. https://doi.org/10.1016/B978-0-7020-5319-1.00012-8

30. **Schmued LC, Hopkins KJ.** 2000. Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. Brain Res **874:**123–130. https://doi.org/10.1016/S0006-8993(00)02513-0.

31. **Villadsen J, Hansen HD, Jørgensen LM, Keller SH, Andersen FL, Petersen IN, Knudsen GM, Svarer C.** 2018. Automatic delineation of brain regions on MRI and PET images from the pig. J Neurosci Methods **294:**51–58. https://doi.org/10.1016/j.jneumeth.2017.11.008.