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Medical imaging of pulmonary disease in SARS-CoV-2-exposed non-human primates

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Chest X-ray (CXR), computed tomography (CT), and positron emission tomography–computed tomography (PET-CT) are noninvasive imaging techniques widely used in human and veterinary pulmonary research and medicine. These techniques have recently been applied in studies of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-exposed non-human primates (NHPs) to complement virological assessments with meaningful translational readouts of lung disease. Our review of the literature indicates that medical imaging of SARS-CoV-2-exposed NHPs enables high-resolution qualitative and quantitative characterization of disease otherwise clinically invisible and potentially provides user-independent and unbiased evaluation of medical countermeasures (MCMs). However, we also found high variability in image acquisition and analysis protocols among studies. These findings uncover an urgent need to improve standardization and ensure direct comparability across studies.

The role of medical imaging in the assessment of coronavirus disease 2019 in humans and NHPs

A hallmark of coronavirus disease 2019 (COVID-19) is lower respiratory tract infection and viral-induced pneumonia in most hospitalized patients [1]. In humans, parenchymal lung involvement in COVID-19 is typically evaluated using medical imaging, most commonly CXR (see Glossary) and CT. A spectrum of lung imaging features characteristic of COVID-19 has now been established, including alveolar, interstitial, pleural, and vascular abnormalities. Most found COVID-19 abnormalities are alveolar ground glass opacities (GGOs), often with interlobular and intralobular septal interstitial thickening (crazy-paving pattern) in a bilateral, multilobar, and peripheral distribution [1,2]. In patients, these readouts have been found to uniquely complement real-time reverse transcription qPCR (RT-qPCR) measurements of SARS-CoV-2 loads and to aid the evaluation of COVID-19 progression and severity [3]. However, the host–virus determinants of parenchymal disease and radiographic pulmonary abnormalities in the pathogenesis of COVID-19 remain unclear. Specifically, a clear understanding of the relationship between upper or lower respiratory tract viral loads and pulmonary immunopathology is lacking. These shortcomings arise, at least partially, because collecting serial imaging data that can be rigorously mapped to clinical, virological, and immunological markers is an operational challenge in the clinical setting, frequently limited by the absence of baseline pre-infection lung imaging.

Noninvasive, longitudinal imaging of established animal models of SARS-CoV-2 infection and COVID-19 may allow more effective delineation of the pathogenesis of lung disease in vivo in controlled experimental settings. NHPs are arguably well suited as animal models of COVID-19 progression and severity [3]. However, the host–virus determinants of parenchymal disease and radiographic pulmonary abnormalities in the pathogenesis of COVID-19 remain unclear. Specifically, a clear understanding of the relationship between upper or lower respiratory tract viral loads and pulmonary immunopathology is lacking. These shortcomings arise, at least partially, because collecting serial imaging data that can be rigorously mapped to clinical, virological, and immunological markers is an operational challenge in the clinical setting, frequently limited by the absence of baseline pre-infection lung imaging.

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Highlights

In non-human primates (NHPs), characteristic radiographic abnormalities (best observed by high-resolution imaging), combined with concordant immunological, virological, and lung histopathological findings, mirror mild-to-moderate human disease.

Noninvasive medical imaging can visualize otherwise ‘silent’ disease at high resolution and generates quantifiable measurements that are particularly important in sublethal models, providing an essential complement to standard immunological, virological, and pathological assays, which can only be performed as snapshots (not longitudinally) or may be difficult to perform.

The incorporation of advanced medical imaging tools in NHP studies for coronavirus disease 2019 (COVID-19) research is not without challenges, including high experimental costs due to the limited availability of equipment in high containment environments, frequency of anesthesia events required for imaging, and the number of animals that can practically be imaged at a single time point.
due to their overall close evolutionary relationship to humans and the consequent physiological similarities [4], including elicited host immune responses and systemic distribution of angiotensin-converting enzyme 2 (ACE2), the cellular receptor of SARS-CoV-2 [5]. Some of the typical manifestations of SARS-CoV-2 infection in humans (i.e., asymptomatic, subclinical, infection, or mild disease) are modeled successfully in NHPs (see Clinician’s corner and Box 1) [6,7].

During the COVID-19 pandemic, medical imaging with CXR, CT, and PET-CT has been applied to characterize lung involvement in SARS-CoV-2-exposed NHPs, for which it has been proven a meaningful, noninvasive complement to standard virological assessments. Serial imaging (including pre-exposure baselines) enables longitudinal data collection through frequent examination of each animal and uses a limited number of animals [8,9]. In addition, because the animals are imaged using the same modalities and scanners used to examine patients, readouts of SARS-CoV-2-exposed NHPs are highly translational. However, as is the case with other viral pathogens, imaging of SARS-CoV-2 infection in NHPs has suffered from a lack of standardization across different laboratories, impacting image acquisition, analysis, and, ultimately, reported imaging metrics. Efforts to harmonize imaging approaches in the setting of SARS-CoV-2/COVID-19 modeling are urgently needed.

In this review, we provide a summary of noninvasive imaging studies in NHP COVID-19 models completed thus far. We identify current challenges and future needs in this arena and offer guidance to enhance future imaging studies of SARS-CoV-2 exposure and disease progression in NHPs.

An in-depth look at the role of CXR, CT, and PET-CT in the evaluation of COVID-19

Diagnostic confirmation of SARS-CoV-2 infection/COVID-19 requires virus-specific nucleic acid amplification (e.g., RT-PCR) or antigen detection, and chest imaging is not recommended for pre-exposure baselines) enables longitudinal data collection through frequent examination of each animal and uses a limited number of animals [8,9]. In addition, because the animals are imaged using the same modalities and scanners used to examine patients, readouts of SARS-CoV-2-exposed NHPs are highly translational. However, as is the case with other viral pathogens, imaging of SARS-CoV-2 infection in NHPs has suffered from a lack of standardization across different laboratories, impacting image acquisition, analysis, and, ultimately, reported imaging metrics. Efforts to harmonize imaging approaches in the setting of SARS-CoV-2/COVID-19 modeling are urgently needed.

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**Box 1. Similarities and differences in phenotype of symptomatic SARS-CoV-2 infection in NHPs versus humans**

The aim of experimental animal models is to understand infection and disease to enable effective prevention or treatment. Studies in NHPs have explored the natural history of infection and disease, including viral dynamics, host immune responses, and host pathology. NHP models have been used to evaluate vaccine and therapeutic countermeasures. Evaluation of these results requires an understanding of how well (or not) the NHP models SARS-CoV-2 infection and COVID-19 in humans [38].

The clinical manifestations of SARS-CoV-2 infection observed in current NHP models are variably present but have included fever, weight loss, loss of appetite, pale appearance, dehydration, diarrhea, and nasal discharge. These signs are comparable with the spectrum of clinical symptoms detected in humans with mild disease. In most NHP studies, and similar to humans with mild disease, no clinical differences were consistently observed between males and females or young and old macaques. In addition, severe and critical disease, though reported in a few aged NHPs, has not been observed reliably [34].

In NHPs, viral replication was mainly observed in the respiratory tract (nasal cavity, oropharynx, and lungs) and gastrointestinal system. At necropsy, lungs showed mild-to-moderate interstitial pneumonia and swollen lymph nodes. In humans, viral replication is most prominently detected in the upper respiratory epithelia and may be found in other parts of the body [101]; in addition to severe respiratory failure, human autopsy data show systemic involvement of multiple organ systems, including cardiovascular, renal, and neurologic, but apart from the respiratory tract, it is challenging to describe morphological alterations attributable to SARS-CoV-2 infection [102]. Innate and adaptive immune responses to SARS-CoV-2 infection paralleled those observed in humans, including adaptive humoral (e.g., anti-SARS-CoV-2 nucleocapsid (N) and spike (S) IgG antibodies) and cellular (e.g., antigen-specific T cell) responses. After infection, innate and adaptive cytokine/chemokine production in NHPs also mirrors human responses, though the dysregulated cytokine storm described in humans with severe disease has not been observed in NHPs.

In short, current NHP models of COVID-19 (rhesus monkeys, crab-eating (cynomolgus) macaques, grivets/vervets, and common marmosets) recapitulate mild-to-moderate disease in humans in both clinical and radiological presentation; severe and fatal disease has not yet been effectively modeled [8,18–20,31,39,58,103–107].
routine screening and diagnosis or in patients with mild symptoms or without symptoms [1,10,11]. However, chest imaging may complement the initial evaluation to inform management in patients with lower respiratory symptoms and signs and/or in patients with worsening respiratory status as the clinical syndrome evolves and/or in patients with potential alternative diagnoses. When indicated, CXR and CT are predominantly used. CXR is a fast, relatively inexpensive, portable, and widely available imaging technique frequently used in clinical settings [9,12–35]. Particularly early during the course of infection, and especially in patients with mild disease, the sensitivity of CXR compared with RT-PCR is relatively low, at only 69% [although the overall positive predictive value (PPV) remains high at 95.7%] [1,3,36–41]. By contrast, multiple studies and meta-analyses have shown chest CT to be more sensitive (94% versus 89% for RT-PCR [42]), although at a recognized cost of specificity, which is dependent on local prevalence and pretest probability [42]. Notably, chest CT has been able to identify patients with COVID-19 even when RT-PCR testing was negative early during disease progression [43]. The emerging evidence base regarding the use of CXR and CT imaging in COVID-19 has led to consensus guidelines that detail relative strengths and weaknesses and provide recommendations [1,10,11]. These sensitivity limitations also apply to imaging of SARS-CoV-2-exposed NHPs, and CXR has been widely used in research studies. Although most human CXR examinations are inconclusive during the early stages of COVID-19 [37], 28 studies of SARS-CoV-2-exposed NHPs detected CXR abnormalities early after exposure [9,13–17,20–23,26,27,29,32,35,44–46]. However, CXR imaging was inconclusive in other studies [12,13,23–25,28] and was generally found to be uncorrelated with the degree of lung abnormalities at necropsy (Figure 1).

Although consensus guidelines [1,10,11] have not clearly defined the relative merits of CXR versus CT imaging in assessing COVID-19, chest CT is recommended for the evaluation of patients with COVID-19, typically to inform clinical management in patients who are severely ill or deteriorating, including the exclusion of other diagnoses, such as pulmonary embolism. CT imaging has been applied in 16 published studies of SARS-CoV-2-exposed NHPs (Table 1) [47]. As a tomographic imaging modality that acquires data in 3D and at higher resolution compared with CXR, chest CT is more sensitive and affords more detailed qualitative and quantitative characterization of lung abnormalities [38]. The risks and benefits associated with chest CT versus CXR are only partly applicable to NHPs in a research setting: higher radiation exposure has less consequence because most studies are terminal; staff in contact with research animals are always in biosecure personal protective equipment; and potential contamination of imaging suites is mitigated by scheduled rigorous decontamination [1,48].

In NHP studies, CT imaging generally mirrored imaging of human lung abnormalities both in character and distribution. The reported prevalence of common CT findings in SARS-CoV-2-exposed NHPs is shown in Table 1 and Figure 2. Compared with human disease, CT abnormalities (most commonly GGOs) in NHPs were detected at a much earlier disease stage and resolved fairly rapidly, albeit with considerable intersubject heterogeneity; most abnormalities were first observed starting at 2 days post-exposure and resolved within 2 weeks [33].

CXR and chest CT are mainly anatomical imaging modalities that provide structural characterization (i.e., the location, volume, morphology, and texture of lung abnormalities). By contrast, PET-CT combines the high-resolution anatomical information from CT with quantitative functional assessments by PET. When performed with the metabolic radiotracer [fluorine-18]fluoro-2-deoxyglucose ([18F]-FDG), a glucose analog that is internalized but not metabolized by metabolically active inflammatory cells, PET-CT enabled the quantification of heightened metabolic activity as a proxy of inflammation in lung parenchyma, regional lymph nodes, heart, kidneys, bone marrow, brain, and other organs in the context of COVID-19 [8,9,49–51]. However, its limited availability in many
countries and emergency settings has restricted its application as a frontline COVID-19 diagnostic imaging modality in the clinic. In preclinical research settings, PET-CT is being increasingly used in NHP studies to evaluate human respiratory diseases, most notably tuberculosis [52]. However, even in preclinical settings, this imaging modality is not widely available in biocontainment laboratories; PET-CT imaging has been used in only six studies of SARS-CoV-2-exposed NHPs (Table 1 and Figure 1)[8,9,34,49–51]. Other imaging modalities can be applied in both humans and NHPs for diagnosis of COVID-19 (Box 2).

Qualitative and quantitative analysis of COVID-19-related lung abnormalities detected by CT and PET-CT

Reliable quantification of lung abnormalities identified by in vivo imaging within (and among) experimental NHP groups is of great interest to allow meaningful correlations with histopathological data and for the evaluation of both therapeutic and prophylactic treatment efficacy [8,33,35,49,53,54]. Although data continue to accumulate, COVID-19-related lung abnormalities detected by CT have already been successfully matched with gross pathology of NHP samples (Figure 3). Several semiquantitative scoring systems based on lesion volume and type proportion of positive results in statistics and diagnostic tests that are true positives.

Translocator protein (TSPO): transmembrane protein located on the outer mitochondria membrane and mainly expressed in glial cells in the brain and on activated macrophages.

Somatostatin receptor 2 (SSTR2): protein-coding gene acting as a general inhibitor of the release of hormones and secretory proteins in the cerebrum and kidneys.

Standard uptake value (SUV): a way of determining activity in PET. Computer software converts the visual data into numerical values for the measured activity, normalized for body weight/surface area, and injected dose.

Figure 1. Comparison of chest X-ray (CXR), computed tomography (CT), and positron emission tomography–computed tomography (PET-CT) imaging. To compare results in non-human primate (NHP) lungs after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) exposure, images on the same row originate from the same animal and were obtained on the same day (day 4 post-exposure). For the animal in the upper row, the lesion located in the middle part of the left lung (marked by the crosshairs and arrows) is clearly visible using all three imaging modalities. By contrast, alterations in lung density for the animal shown in the bottom row could only be clearly distinguished from healthy lung using CT (marked by the crosshairs and arrows) but are hardly visible on CXR and PET-CT. Adapted from [9].
Table 1. Summary of SARS-CoV-2 studies in NHPs meeting inclusion criteria

| Animal | Exposure | Imaging method | Imaging results | Refs |
|--------|----------|----------------|-----------------|------|
| Type   | Number and sex | Age (y) | Weight (kg) | SARS-CoV-2 isolate | Dose and volume | Incoculation route | Modality | Frequency | Analysis method | Lesion type | Time |
| 'African green monkey' | 6 (4 f + 2 m) Adult NA | P3 INMI11/2020/Italy | 4.6 × 10^5 PFU (6 ml) | Intratracheal + intranasal | CXR | D1, D2, D3, D4, D5 | Radiologists interpret + veterinarian/ veterinary pathologist | Did not reflect degree of lesions and hemorrhage of lungs seen at necropsy | Inconclusive [12] |
| | 8 (4 m + 4 f) NA 3.5-6 | P4 USA_WA1/2020 | 1.82 × 10^6 PFU (6.5 ml) | Intranasal (1 ml) + intratracheal (4 ml) + oral (1 ml) + ocular (0.5 ml) | CXR | Pre-infection, D1, D3, D5, D7, D10 | NA | NA | NA [13] |
| AGM: 14 (m) CEM: 4 (2 m + 2 f) 4.2–8 AGM: 5.6–7.55 CEM: 2.3–3.9 USA_WA1/2020 | Intranasal CXR | D3, D7 | Pulmonary infiltrates | D1–D7 [14] |
| AGM: 14 (m) CEM: 4 (2 m + 2 f) 4.2–8 | Intratracheal CXR | D3, D7 | Pulmonary infiltrates | D6–D7 [15] |
| AGM: 14 (m) CEM: 4 (2 m + 2 f) 4.2–8 | Intratracheal CXR | D3, D7 | Pulmonary infiltrates | D1–D12 [16] |
| AGM: 14 (m) CEM: 4 (2 m + 2 f) 4.2–8 | Intratracheal CXR | D3, D7 | Pulmonary infiltrates | D1–D6 [17] |
| 4 (±2 re-exposure control, +1 mock) | WH-09/human/2020/CHN isolated in their laboratory | 0.7 × 10^6 PFU (1 ml) for both exposures | Intratracheal CXR | Re-infection, D7, D25, D33 (< 5 dpr) | Radiological interpretation | Bilateral GGOs, pneumonia | D7 [18] |
| | 5 (n = 3), 15 (n = 2) | 3–5 | 0.7 × 10^6 PFU (1 ml) | Intratracheal CXR | Pre-infection, D5, D6, D9, D10, D11, D13, D15 | Interstitial infiltration | D5 old, D7 young [19] | (continued on next page) |
| Animal | Exposure | Imaging method | Imaging results | Refs |
|--------|----------|----------------|-----------------|------|
| Type   | Number and sex | Age (y) | Weight (kg) | SARS-CoV-2 isolate | Dose and volume | Inoculation route | Modality | Frequency | Analysis method | Lesion type | Time |
| 12 (m + f) | 3.6–5.7 | P3 nCoV-WA1-2020 | 1.1 × 10⁷ PFU (6 ml) | Intranasal, oral, ocular, intratracheal | CXR | Pre-infection, D1, D3, D5, D7 | Clinical veterinarian interpretation | Lobe involvement, pulmonary infiltration | D1–D7 | [20] |
| 5 (m) | 3–5 | NA | WH-09/human/2020/Orin | 0.7 × 10⁶ PFU | Conjunctival (n = 2) or intratracheal (n = 1) or intraoral (n = 2) | CXR | Pre-infection, D1, D3, D5, D7 | NA | Interstitial infiltration, costophrenic angles, patchy lesions | D3–D7 | [21] |
| 8 (m + f) | 11–17 | NA | 2019-nCoV/USA-WA1/2020 | 1.1 × 10⁶ PFU (2 ml) | Intranasal + intratracheal | CXR | D5, D2, D4, D7, D10 | Radiological interpretation, radiograph score | Pulmonary infiltration, GGOs for 2/4 untreated group, 0/4 treated group, 1/4 untreated group – severe pneumonia | D2–D10 | [22] |
| 16 (f) | 3.5–10 | 4.5–10 | P3 nCoV/USA-WA1-2020 | 2.8 × 10⁷ PFU (6.5 ml) | Intratracheal (4 ml), intranasal (1 ml), oral (0.5 ml) | CXR | Pre-infection, D1, D3, D5, D7 | Lung radiography score | NA | D1–D7 | [23] |
| 12 (m + f) | 3–11 | 4–10 | Unspecified | 0.7 × 10⁶ PFU (5 ml) | Intrabronchial (4 ml) + intranasal (1 ml) | CXR | D2B, D27, D25, D-21, D14, D7, D0, D1, D3, D5, D7 | NA | Mild, no evidence of frank consolidative pneumonia | NA | [24] |
| 5 | 6–7 | NA | BetaCoV/Wuhan/WW04/2019 | 0.7 × 10⁶ PFU | Intratracheal | CXR | Pre-infection, D3, D6 | NA | NA | NA | [25] |
| 10 + 1 (mock) (m) | NA | NA | From CDC Guangdong Province, China | 1 × 10⁶ PFU (1 ml) | Intragastric by gavage and intranasal | CXR | Pre-infection, D1, D4, D7, D14 | NA | Nodular lesions caused by pulmonary infiltrates | D4–D7 | [26] |
| 12 (6 m + 6 f) | 3.5–6.5 | 5.82–12.81 | USA_WA1/2020 | 2.5 × 10⁶ PFU (2.5 ml) | Intratracheal (2 ml) + intranasal (0.5 ml) | CXR | Pre-infection, D1, D3, D5, D7 | Veterinary radiologists | Pulmonary infiltrates | D3–D7 | [27] |
| 44 (m) | 3.5–8 | NA | USA_WA1/2020 | 1.0 × 10⁶ PFU (1.01 ml) | Intratracheal (0.5 ml) + intranasal (0.5 ml) + ocular (0.01 ml) | CXR | D6, D13, D20 | CXR score | Subtle radiographic changes | D6–D20 | [28] |
| Animal Model | Dose (PFU) | Route | Pre-infection, Post-infection, Control | Radiologist interpretation |
|--------------|------------|-------|---------------------------------------|---------------------------|
| Crab-eating macaques | 16 (m + f) | 3-5 (m), 16-23 (f) | P4 USA_WA1/2020 | Intrad tracheal, intranasal (1 ml) + oral (1 ml) + ocular (0.5 ml) | CXR score by clinical veterinarian | Pulmonary inlattes D1-D2 |
| Pigtail macaques | 2+4 | 7-10 | USA_WA1/2020 | Intratracheal (4 ml) + intranasal (1 ml) + oral cavity, intratra ch canal | NA | Mock, pneumonia; vaccinated, no lesion D1-D7 |
| Crab-eating macaques/ rhesus monkeys/ common marmosets | OMEM: 6; RM: 14 (m + f); CM: 6 (m + f) | NA | From CDC Guangdong Province, China | Intratracheal, intranasal | NA | Interstitial pneumonia |
| African green monkeys/ crab-eating macaques/ rhesus monkeys | AGOM: 3; CEM: 4; RM: 4 (m + f) | Adult | nCoV-WA1-2020 (R4717) | Aerosol (1-3 μm MMAD) | CXR | D1, D3, D5, D7, D9, D11, D15, D18 | Increased lung opacity and presence of infiltrates D3-D11 |
| Rhesus monkeys/ crab-eating macaques | RM: 7 (m + f); CEM: 1 (f) | NA | USA_WA1/2020 | Mucosal atomization (1 ml) + intratra chnal (4 ml) | CXR | Control: pre-infection, D2, D3, D4, D5, D7, D9, D10, D14, D21; vaccinated: pre-infection, D2, D4, D6, D8 | Control: infiltrates, GGGs, consolidation, crazy-paving pattern, linear opacities; vaccinated: lacked abnormalities D2-D5 |
| Rhesus monkeys | 12 (m + f) | 2-4 | P3 Victoria/01/2020 | Intranasal (1 ml) and intratra chnal (2 ml) | CT | Pre-infection, D5 | Medical radiologist; COVID pattern score and zone score | Predominantly bilateral disease, GGGs, consolidation D5, n = 3/6 vaccinated and n = 5/6 unvaccinated [57] |

(continued on next page)
| Animal | Type | Number and sex | Age (y) | Weight (kg) | SARS-CoV-2 isolate | Dose and volume | Inoculation route | Imaging method | Imaging results | Lesion type | Time |
|--------|------|----------------|---------|------------|-------------------|----------------|----------------|----------------|----------------|-------------|------|
| Crab-eating macaques | 18 (m + f) | 3-6 | >4.5 | R3 Victoria/01/2020 | 5 × 10^6 PFU (3 ml) | Intranasal (1 ml) and intratracheal (just above carina, 2 ml) | CT | Pre-infection, D5 | CT score | Relatively mild and only affected less than 25% of lung | D5, n = 3/6 vaccinated high dose, n = 6/6 vaccinated low dose, n = 5/6 unvaccinated | [54] |
| | 18 (9 m + 9 f) | 2.5–3.5 | >4 | R3 Victoria/01/2020 | 5.0 × 10^6 PFU (3 ml) | Intratracheal (2 ml) + intranasal (1 ml) | CT | Pre-infection, D5 | CT score by medical radiologist | COVID pattern score + zone score | D5 | [73] |
| Crab-eating macaques | 39 (m + f) | 3 | 2.85-4.82 | P5 hCoV-19/ France/IDF0372/2020 | 1.0 × 10^6 PFU (4.5 ml) | Intranasal + intratracheal (just above carina) | CT | Pre-infection, D2, D5, D11/13 | CT score | | | D2-D11/13 | [56] |
| | 10 (f) | 3-6 | NA | BetaCoV/France/ IDF/0372/2020 | 1 × 10^6 PFU (4.5 ml) | Intranasal + intratracheal | CT | D3 | CT score, including lesion type and volume by two people | Small lung lesions, GGOs | D3 is N9/10 positive | [53] |
| | 8 | NA | NA | hCoV-19/France/ IDF0372/2020 | 1.0 × 10^6 PFU (5 ml) | Intratracheal (4.5 ml) + intranasal (0.5 ml) | CT | D3, D7, D10, D14 | CT score including lesion type and volume | Nonextended GGOs | D3-D14 | [74] |
| Crab-eating macaques/ rhesus monkeys | 10 (3 m+7 f) | 2-4 | 2.3-6.0 | Unspecified | 0.7 × 10^6 PFU + RE: 0.7 × 10^6 PFU (n = 2) | Intratracheal (0.9/1.0 ml) + intranasal (0.1 ml), RE: intratracheal | CT (slice of third, sixth, ninth thoracic vertebrae used) | CT, pre-infection, (D3), D5, D7, D10, D12, D14, RE: D0, D2, D7 (n = 3) | Visual interpretation | Pneumonia | D3-10/12 | [60] |
| | 12 (m + f) | 2.80-4.85 | 5.0 × 10^6 PFU (3 ml) | P3 Victoria/01/2020 | Intranasal + intratracheal just above carina | CT (n = 4) | Radiologist interpretation | GGOs, pulmonary abnormalities and peripheral consolidations that involved <25% of lung | | | D18 | [65] |
| Rhesus monkeys | 28 (+5 mock) (m) | 3-9 | 4.6–11.7 | USA WA1/2020 | 3.2 × 10^6 PFU | Intranasal + intratracheal | PET-CT (n = 6) | Pre-infection and D4/5 | Whole-lung uptake, ROI based on SUV > 1.5 SUVCMR | No vaccine-associated enhanced respiratory disease found | NA | [122] |
| Species/strain                  | Gender | Total   | Age  | Route(s)                         | Virus dose | Virus delivery | CT/PET-CT timepoints | CT/PET-CT findings                                      |
|--------------------------------|--------|---------|------|----------------------------------|------------|----------------|----------------------|---------------------------------------------------------|
| Hamadryas baboons/ rhesus monkeys/ common marmosets | HB: 12 RM; 16 CM;   RM: 6; Study 1: 4 RM; Study 2: 6 RM (m + f) | 2-20 | 5.6-27.5 | 8.82 × 10⁵ PFU (0.42 ml CM) | 1.05 × 10⁶ PFU (0.5 ml HB + RM); 8.82 × 10⁵ PFU (0.42 ml CM) | Study 1: CT: post-infection, D1, D2, D3; Study 2: CT: D6, D12 | Veterinary radiological interpretation (2); CT score generally minimal or mild, not consistently associated with viral challenge | All time points |
| Rhesus monkeys                 | 21 (m) +6 (m) mock | 2-4 | NA | P6 USA-WA1/2020                 | 1.05 × 10⁵ PFU (0.5 ml) | CT: pre-infection, D2, D4, D6, D8, D10, D12, D19, D30; CT score including lesion type and volume; PET: SUVmean, SUVpeak | Various schedules with D4/5 CXRs; pre-infection, D1, D3, D6, D10/EOP; CT: pre-infection, D3/4, EOP | Generally minimal or mild, not consistently associated with viral challenge |
| Crab-eating macaques           | 3 (+3 mock) (m + f) | 4-4.5 | 3.17-4.62 | 2019-nCoV/ USA-WA1- A12/2020 | 3.65 × 10⁶ PFU (4 ml) | Direct bilateral primary post-carinal intrabronchial instillation | CT: pre-infection, D2, D4, D6, D8, D10, D12, D19, D30; RET-CT: pre-study, D2, D6, D12 | PET-max SUV, GGOs, consolidations, interlobular septal thickening | D2-D12 |
| Crab-eating macaques/ rhesus monkeys | 12 (5 m + 7 f) | 3.6-3.8 | hCoV-19/ France/ IDR372/2020 | 1.0 × 10⁵ PFU (5 ml) | Intrapulmonary (4.5 ml) and intranasal (0.5 ml) | CT: pre-infection, D5/6; RET-CT: pre-infection, D5/6; PET: SUVmean, SUVpeak | CT score including lesion type and volume; PET: SUVmean, SUVpeak | GGOs, consolidations, interlobular septal thickening |
| Crab-eating macaques/ rhesus monkeys | 8 (m) | 4-6 + 16 (CEM) | 3.3-9.7 | P5 BetaGoV/ BaxPat1/2020 | 0.7 × 10⁶ PFU (5 ml) | Intrapulmonary (upper part, 4.5 ml) and intranasal (0.25 ml per nostril) | CT: pre-infection, D2, D4, D6, D8, D10, D12, D14, D16, D22; RET-CT: D2, D16, D22, D29, D35 | CT: CT score, PET-CT: LN volume, LN HU density, LN SUVmean, SUVpeak; PET: SUVmean, SUVpeak | D5-14 |
| African green monkeys/ rhesus monkeys | AGM: 4/2 (m + 2 f); RM: 4 (3 m + 1 f) | 4-6 | AGM: 3.5-7.4; RM: 7.5-16.0 | USA-WA1/2020 | 1.4 × 10⁷ PFU (aerosol, n = 4); 3.61 × 10⁶ PFU (N = 4) | Aerosol (+2 μm MMAD) (AGM + 2RM); multiple routes (oral, 1 ml; nasal, 1 ml; intratracheal, 1 ml; conjunctival, 50 μL per eye) | CT: pre-infection, D5/6, D14; PET-CT: pre-infection, D5/6; CT: CT score, PET-CT: LN volume, LN HU density, LN SUVmean, SUVpeak; PET: SUVmean, SUVpeak | GGOs, consolidations, crazy-paving pattern; SUVs on lymph nodes; one showed left ocular region | D2-D35 |
| African green monkeys/ rhesus monkeys | AGM: 4/2 (m + 2 f); RM: 4 (3 m + 1 f) | 4-6 | AGM: 3.5-7.4; RM: 7.5-16.0 | USA-WA1/2020 | 1.4 × 10⁷ PFU (aerosol, n = 4); 3.61 × 10⁶ PFU (N = 4) | Aerosol (+2 μm MMAD) (AGM + 2RM); multiple routes (oral, 1 ml; nasal, 1 ml; intratracheal, 1 ml; conjunctival, 50 μL per eye) | CT: pre-infection, D5/6, D14; PET-CT: pre-infection, D5/6; CT: CT score, PET-CT: LN volume, LN HU density, LN SUVmean, SUVpeak; PET: SUVmean, SUVpeak | Radiological interpretation | D7-D8, D21-D22 | [34] |

(continued on next page)
Table 1. (continued)

| Animal                      | Exposure                                                                 | Imaging method          | Imaging results                      | Refs  |
|-----------------------------|--------------------------------------------------------------------------|-------------------------|--------------------------------------|-------|
| Rhesus monkeys              | 28 (f)                                                                  | 4-8                     | 5.4–12.1                             | 0.7 × 10⁵ PFU (5 ml) | CT (gated) (CBCT), LUS | Pre-infection, D2, D7, D14 | CT score, LUS score | CT: GGOs, consolidations, crazy paving patterns LUS: B-lines with or without pleural abnormalities | D2-14 | [67] |
| 'African green monkeys'     | 4 (+2 mock) (m)                                                         | 3.5                     | 4.2–6.3                              | P3 München-1.1/2020/29 (Munich) virus | 1.0 × 10⁷–⁴.2 (aerosol); 2.5 × 10⁵ PFU (5 ml) | CXR; CT (CBCT); PET-CT | CXR: every sediment = pre-infection, D2, D4, D7, D11, D14, D2 CT: D0, D4, D11 (±18) PET-CT: D0, D4, D11 (±18) | CXR: radiologist interpretation PET: SUV̄ total lung, SUV̄ max LNs | CXR: mild unspecific infiltrates; CT: GGOs, thickened vessel structures, foot; PET: SUV̄ max in total lung and thoracic nodes | D2-D4; D4-D11; D4-D11 | [69] |

*Abbreviations: AGM, 'African green monkeys'; CBCT, conebeam CT, used in the MultiScan LFER 150 (Mediso Medical Imaging Systems); CEM, crab-eating (aka 'cynomolgus') macaques; CM, common marmosets; D, day (as in 'D2'); dpi, days post-infection; dpr, days post-re-exposure; EOP, end of experiment; f, female; HB, hamadryas baboons; LN, lymph node; LUS, lung ultrasound; m, male; MMAD, median mass aerodynamic diameter; p, passage; PFU, plaque-forming unit; RE, re-exposure; RM, rhesus monkeys; ROI, region of interest; SUV̄ CMR, standard uptake value; cylinder-muscle ratio.*
(e.g., GGOs, consolidation, crazy-paving pattern, and pleural thickening) have been developed to allow monitoring of disease progression by lung CT of NHPs (Table 1). These scores reflect the extent and longitudinal course of qualitative findings. However, poor scoring standardization across studies makes it challenging to compare experimental data from different research groups in detail outside the determination of a general pattern and peak. Recognizing that semiquantitative scoring relies on qualitative radiological assessments potentially subject to bias, several research groups have invested in the development of semiautomated, user-independent, quantitative readouts of radiographic abnormalities. For instance, normalized changes from pre-exposure baselines can be measured longitudinally as the percent change in lung hyperdensity (PCLH) [55] using CT data (Figure 4) [8,33]. Further development of such quantitative readouts should be encouraged.

A limited number of NHP studies have used PET-CT to measure $^{18}$F-FDG uptake as a proxy for metabolic activity in the lungs and regional lymph nodes via anatomical co-registration with CT. Average, maximum, or peak (as robust alternative to maximum values, which could be affected by noise) standard uptake values (SUVs) in lung lesions, lymph nodes, or total lungs were used as measures of $^{18}$F-FDG uptake and as surrogates of inflammatory activity. $^{18}$F-FDG uptake was generally found to co-register with CT-identified structural abnormalities (e.g., GGOs and

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**Table 1. Semiquantitative Scores for Monitoring Disease Progression**

| Score | Description | Formulation |
|-------|-------------|-------------|
| GGOs  | Ground glass opacities | - |
| Consolidation | Accumulation of gas in alveoli or acini | - |
| Crazy-paving pattern | Interlobular septal thickening | - |
| Pleural thickening | Thickening of the pleura | - |

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**Figure 2. Common lung abnormalities detected by computed tomography (CT) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-exposed macaques.** Distribution of CT scan abnormalities in a 3D reconstruction image of a SARS-CoV-2-exposed macaque at peak disease, day 4 (left panel: blue, airways; gray, normal lung; red, vessels; yellow, imaging abnormalities). Selected characteristic abnormalities include: (i) peri-bronchial consolidation in the left accessory lobe (top-middle panel day 2: top inset, red arrow); (ii) posterior ground glass opacities (GGOs) with reticulation in the posterior right lung (top-middle panel day 2: bottom inset, red arrow); (iii) bilateral posterior GGOs with reticulation (top-right panel day 2: top and bottom insets, red arrows); (iv) GGOs with superimposed crazy-paving pattern (interlobular septal thickening) in right posterior lung (bottom-middle panel day 4: top inset, red arrow) and mixed GGOs with pleural-based consolidation in left posterior lung (bottom-middle panel day 4: bottom inset, red arrow); and (v) pleural-based mixed GGOs and consolidation (bottom-right panel day 6: inset, blue arrow). Adapted from [8]. Abbreviation: COVID-19, coronavirus disease 2019.
Consolidation) during the course of the studies. Higher \(^{18}\)F-FDG uptake was detected 2 days \([8]\) or 4 days post-exposure \([9]\) and resolved by day 11 or day 12 but increased again at later time points (up to day 35) \([49]\). Imaging from NHPs vaccinated against SARS-CoV-2 showed lower or no \(^{18}\)F-FDG uptake in abnormalities within the lungs compared with unvaccinated control animals \([50]\).

Challenges in applying medical imaging for the evaluation of SARS-CoV-2 infection in NHPs

Medical imaging of SARS-CoV-2-exposed NHPs is providing deeper insight into the pathophysiological processes related to lung abnormalities in patients with COVID-19. Indeed, the majority of clinical disease would be inapparent by clinical assessment (observation, physical examination, and laboratory blood markers), which justifies higher-resolution imaging evaluation. However, many challenges need to be overcome before noninvasive imaging readouts of the lungs can be reliably used as COVID-19 markers and to fully exploit their potential complement to standard immunological and virological assays. Some of these challenges are related to the NHP model. The clinical disease elicited by SARS-CoV-2 exposure in NHPs is generally mild to moderate (even radiographically), although some abnormalities associated with more severe disease (e.g., alveolar consolidation) have been reported occasionally \([8,22,45,49,51,53,56–58]\). Mid-to-moderate disease progression in NHPs mirrors that observed in humans (e.g., in the evolution of initial GGOs to organizing pneumonia and consolidation). Unsurprisingly, in (typically) young healthy animals without significant comorbidity, consistent radiographic abnormalities associated with acute respiratory distress syndrome (ARDS) in humans are absent, and the model has failed to assist in the evaluation of more serious lung pathology. Since COVID-19 severity in humans (and, therefore, also likelihood of hospitalization) has been associated with several risk factors, including advanced age and obesity \([59]\), a handful of experiments used comorbid or aged NHPs to increase the likelihood of severe COVID-19 development. The few studies performed with aged NHPs \([29,33,34,60–63]\) suggested
that local inflammatory innate immune responses in the lungs are induced earlier than in younger NHPs and are responsible for protection against severe disease, although final outcomes were not significantly affected [33,60]. Similar to body mass index (BMI) in humans, obesity in NHPs is measured with a weight-for-height index (WHI). Values above 62 kg/m².7 and 67 kg/m² are indicative of obesity in crab-eating macaques and rhesus monkeys, respectively [64]. On average, these values correspond to body weights over 10 kg. Most NHPs used in SARS-CoV-2-related studies (Table 1) weighed less than 10 kg; therefore, the effect of obesity on NHP disease progression has yet to be addressed.

The SARS-CoV-2 exposure route may also be a factor influencing lung abnormality pattern and severity in NHPs. NHPs have been exposed to SARS-CoV-2 in various ways, most commonly via combined intranasal and intratracheal inoculation [12,22,27,35,49,50,53,54,56,57,65–67]. Multiple routes [9,13,16,20,23,26,28,29,31,33,34,46] lead to SARS-CoV-2 shedding. However, although infection is not necessarily dependent on respiratory tract exposure, it is plausible that the route of exposure impacts the progression of disease. Exposure deeper in the trachea was found to induce lung lesion development earlier compared with exposure in the upper trachea (Table 1) [9,49,54,56,65]. Small-particle aerosol exposure is an alternative to direct inoculation [9,32,34,44]. Overall, viral load, clinical disease progression and severity, plasma cytokine concentrations, and pathology are similar in aerosol-exposed NHPs compared with those exposed via other routes [34]. Since SARS-CoV-2 particles are ~100–120 nm in diameter, aerosols with droplet sizes of 1–3 μm have the potential to contain up to 120–460 virions per droplet [68]. The half-maximum infection dose (ID₅₀) of SARS-CoV-2 is unknown for both humans and NHPs but is likely to be less than 400 virions, which is the ID₅₀ of SARS-CoV [69].

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**Figure 3.** Lung abnormalities detected by computed tomography (CT) matched with gross pathology. Obtained just before euthanasia (day 7 post-exposure), these images were matched to gross pathology photographs. Lesions found reflecting the same location are marked with similarly colored circles. Adapted from [61]. Abbreviation: NHP, non-human primate.
suggest that one droplet will suffice to initiate infection. Studies to determine the impact of aerosol particle size on the pulmonary disease phenotype (quality and quantity) are ongoing.

Other challenges are posed by the potential incompatibility of some experimental procedures with medical imaging experiments. For example, bronchoalveolar lavage (BAL) is often used to detect viruses and local immune responses in the lower respiratory tract [70,71]. In general, BAL and imaging should be concurrently used with caution, as BAL may produce CXR or CT imaging artifacts when performed on consecutive days [72], interfere with pulmonary pathogenesis (within-lung spread), and potentially confound histopathology. Of the 41 NHP SARS-CoV-2 studies examined, 24 used BAL during the infection phase and included imaging [12–16,20,22–24,27–30,33,35,50,51,53,54,56,65,73,74]. Eight included single BAL immediately before or at necropsy [12,20,22,33,54,57,65,73]. In one study, chest opacities and abnormalities might have been caused by the BAL procedure [12]. In two studies, BAL was deliberately not performed due to concerns of interference with imaging [8,49]. To guard against confounding findings due to BAL, medical imaging and necropsies should be performed on days not consecutive with BAL [72]. Additionally, incorporating quantitative, longitudinal medical imaging in high animal biosafety level (ABSL-3) or maximum containment settings (ABSL-4), as required for research with SARS-CoV-2 [75], is not without challenges, including increased experimental costs; medical imaging equipment.

Figure 4. Data analysis of lung abnormalities detected by computed tomography (CT) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-exposed macaques via percent change in lung hyperdensity (PCLH). Top: representative axial CT images in three SARS-CoV-2-exposed macaques for each indicated study day (D). The gray scale represents radiodensity in Hounsfield units (HU). Bottom: percentage change in volume of lung hyperdensity (PCLH) measured over time in the same SARS-CoV-2 inoculated macaques shown in the upper panel. Adapted from [8].
is not routinely available in most ABSL-3 and -4 settings; the maximum frequency of applied anesthesia required for imaging and the maximum number of NHPs that can practically be imaged at a single time point may create bottlenecks that limit optimal experimental design; and imaging conditions and technology vary among studies and sites, which makes it challenging to directly compare data obtained at separate sites (as highlighted herein). We foresee that advanced medical imaging of SARS-CoV-2-exposed NHPs may be enhanced by the development of best-practice principles and strategies to enable collaborative harmonization and standardization of data across studies, research groups, and organizations. Specific recommendations include choice of imaging modality (oriented to the appropriate scientific question), timing of evaluations, standardized data collection, centralized data repositories, and agreed-upon analysis approaches. Key principles, specific recommendations, and complex questions, based on the current knowledge of imaging NHPs focused on, although not solely applicable for, SARS-CoV-2 infection, are enumerated in Table 2 and the Outstanding questions section (see Outstanding questions).

Value of medical imaging, lessons from the clinic, and future directions
Although the absence of severe COVID-19 in the NHP model limits the translatability of imaging findings to some degree, medical imaging is established as a much-needed complementary readout to clinical, virological, or immunological assessments in the preclinical arena. Effectively modeling COVID-19 in NHPs, especially for non-fatal disease, requires meaningful measurements of infection and disease that include, but should not be limited to, viral quantification. In the absence of apparent clinical disease and viremia, RT-qPCR assessment of nasal and/or tracheal swab samples to measure viral load is often used as a proxy for disease severity. However, truly quantifiable measurements are challenging due to varying swab materials and quantities, sample site locations, RT-qPCR processes, and exposure routes, all of which may influence results [39]. Perhaps, the most important drawback is that SARS-CoV-2 infection involves the lower respiratory tract, which can only partly be sampled by invasive BALs. Furthermore, virological assessment alone cannot provide a complete picture of disease; indeed, histopathological and virological assessment of tissue (after necropsy) have both been required to

Table 2. Key principles and recommendations for imaging of SARS-CoV-2-exposed NHPs

| Topic                | Key principles and recommendations                                                                 |
|----------------------|------------------------------------------------------------------------------------------------------|
| Modality             |                                                                                                      |
| CXR                  | Low sensitivity and reader dependence limits utility of CXR in a mild-to-moderate infection model     |
| LUS                  | High sensitivity, visibility of centrally located lesions is limited due to aeration, which limits its  |
|                      | use in a mild-to-moderate infection model                                                             |
| CT                   | Recommended modality for detection of pulmonary abnormalities in SARS-CoV-2-exposed NHPs             |
| PET-CT               | Provides limited additional value for detection of lung abnormalities compared with CT (with 18F-FDG)  |
|                      | High value for functional characterization and quantification of LNs                                   |
| Frequency and time   |                                                                                                      |
| Obtain baseline image | Baseline image before infection                                                                        |
|                      | Minimal imaging frequency after infection, one image in first 4 days post-exposure, one on day 5–10,   |
|                      | and one on day 11–15.                                                                                |
|                      | Imaging frequency can be reduced but not stopped at 14 days post-exposure                             |
| Analysis method      | Qualitative evaluation of extent, distribution, type, and evolution of abnormality by expert readers is |
|                      | sufficient for general conclusion                                                                    |
|                      | Quantitative analysis is preferred, preferably in automated and user-independent manner. In general,    |
|                      | quantification of percentage of lung involvement, independent of type of abnormality, has been useful   |
| Lung abnormality     | Appearance of lung lesions should be correlated with gross pathology score but not necessarily         |
|                      | RT-PCR values obtained from upper or lower respiratory tract sampling                                  |
|                      | Other experimental procedures in lungs (e.g., BAL) can influence appearance of lung lesions during      |
|                      | both imaging and necropsy                                                                           |

Abbreviations: LN, lymph node; LUS, lung ultrasound.
describe the consequences of disease after infection, either at the organ/tissue or systemic level. Therefore, particularly in the lower respiratory tract, medical imaging of pulmonary disease contributes uniquely to the ability to understand and measure the consequences of infection [76]. Advanced medical imaging of the lungs of SARS-CoV-2-exposed NHPs shows promise in detecting and longitudinally evaluating disease. Without higher-resolution CT or PET-CT imaging, lung abnormalities reminiscent of COVID-19 (in character, distribution, location, and evolution; Figure 1) would not have been detected and could not have been assessed longitudinally in this model or matched with gross pathology (Figure 3). To fully exploit the potential of medical imaging in this setting, additional efforts should be devoted to correlate imaging results (on a per-lung or per-lobe basis) with histopathological abnormalities measured using quantitative scoring systems [16,60].

A limited number of human observational studies or clinical trials [77] have used medical imaging as part of the evaluation of both therapeutic and prophylactic treatment efficacy, including evaluations of tocilizumab (CT/CXR, qualitative) [78,79], favipiravir (CT, semiquantitative score), convalescent plasma (CT, qualitative) [80], and hydroxychloroquine versus febuxostat (CT, percent involvement) [81]. Generally, these have relied, at best, on semiquantitative CT scoring systems as secondary outcomes and often report only qualitative pre-/post-treatment changes. With regard to evaluation of MCMs in NHPs, a limited number of studies have been performed [82]. A recent study evaluating the efficacy of dalbavancin in rhesus monkeys resulted in fewer pulmonary infiltrates detectable by CXR in treated versus non-treated animals [83]. Other studies have instead relied on the characterization of CXR abnormalities using standard scoring systems. In the evaluation of remdesivir [20] and baricitinib [22] in rhesus monkeys, this approach was able to capture significant differences in lung involvement between treatment and control groups; however, in another study, no difference between groups could be observed by in vivo imaging despite detection of a treatment effect by lung pathology scores. An efficacy evaluation of hydroxychloroquine (either alone or in combination with azithromycin) in SARS-CoV-2-exposed crab-eating macaques used a semiquantitative CT score, compounding information on lesion type and volume, and found no evidence of antiviral activity or clinical benefit (as confirmed by recent meta-analyses) [56,84].

Recognizing that expert-generated qualitative or semiquantitative scoring systems (Figure 2) require dedicated personnel, need true blinding, and are intrinsically susceptible to bias, it is reasonable to foresee that current efforts in standardizing fully quantitative measures of lung disease, either by semiautomatic quantification of CT imaging abnormalities (e.g., longitudinally tracking the PCLH; Figure 4) or PET-CT abnormalities (e.g., tracking the SUV in specific regions of interest or globally), are likely to add value in forging host–virus–disease correlations and evaluating MCMs. In this evolving landscape, the ability to serially evaluate quantitative noninvasive (i.e., without serial euthanasia) measures of lung disease in a validated well-controlled experimental setting would significantly advance the evaluation of MCMs in these models. Furthermore, the large data sets derived from advanced imaging analyses have promise for the application of artificial intelligence (AI)-enabled machine-learning approaches as ‘agnostic’ evaluation of the fundamental relationship between infection and disease [85]. These are being explored in human clinical settings [86–91] but may be of higher yield in the controlled experimental settings afforded by animal modeling, including in uncovering findings otherwise unattainable with current readouts, such as in detecting subclinical organ involvement at unexpected sites for which pathological significance would be determined subsequently. Harmonization of scoring systems, or bidirectional/multidirectional exchange of CT images among research groups, and collaborations toward this end would likely move the field forward.

Clinician’s corner

At present, NHP models of COVID-19 recapitulate only mild-to-moderate human disease.

While the development of an NHP model of severe COVID-19 is being pursued, current models highlight the added benefit of longitudinal medical imaging to characterize and quantify otherwise apparent or mild clinical disease.

Thorough studies in NHP models and standardization of quantitative imaging readouts may shed light on COVID-19 lung pathophysiology beyond what can be learned in the clinical setting.

The application of novel imaging technologies in the preclinical arena may further enhance the value of noninvasive imaging in the assessment of COVID-19.
Although requiring the adaptation of dedicated settings or hardware to preclinical ABSL-3/4 imaging settings, these and other techniques that are gaining interest in the clinic (Box 2) may provide additional insight into the pathophysiology of lung involvement in COVID-19 and may be applied in NHPs.

In NHPs, PET-CT deploying radiotracers other than 18F-FDG may provide additional insight into specific pathophysiological processes related to COVID-19 to fulfill more hallmarks of the ideal PET radiotracer for imaging inflammation, such as specificity and diagnostic value [92]. Some of these PET radiotracers have already been examined in small animal models of COVID-19, and studies in NHPs are ongoing. For example, 124I-iodo-N,N-diethyl-2,5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-acetamide (DPA-713), a radiotracer with translocator protein (TSPO) as a target selectively trapped by activated macrophages, was found to accumulate in pulmonary lesions in SARS-CoV-2-exposed golden hamsters [93]. The inflammation-specific peptide DOTATATE (paired with gallium-68 (68Ga) or copper-64 (64Cu)), a radiotracer with specificity for somatostatin receptor 2 (SSTR2), was developed to improve the diagnosis of neuroendocrine tumors [94]. However, because SSTR2 is expressed by several inflammatory cells, DOTATE is being investigated as a potential tracer for evaluation of several conditions, including cardiovascular [95] and infectious diseases [96], and appears to be particularly advantageous for imaging cardiac inflammation [92]. In one case report, DOTATE was detected in an axillary lymph node after COVID-19 vaccination [97,98]. PET radiotracers targeted to the ACE2 receptor are also being developed [99]. Further in-depth studies investigating the expression levels of imaging target molecules in key cellular populations (e.g., macrophages) may prove invaluable in shaping the future landscape of PET-CT imaging in the context of COVID-19 [100].

Concluding remarks
Here, we have provided a detailed review of medical imaging in NHP models of COVID-19. Our search was limited to studies of medical imaging in SARS-CoV-2-exposed NHPs published online and found within online repositories. Several studies were limited in terms of sample size, data availability, and methodological quality, and the reported findings should be interpreted within that context. Nevertheless, these studies reflect current approaches. We argue that advanced imaging tools add unique insight into (so far) poorly understood relationships of SARS-CoV-2 exposure, infection, the host response, and disease presentation in the lungs. Challenges to advanced imaging characterization in NHP models require careful consideration and upstream investment that is likely well worth the return, not only in the current moment (for SARS-CoV-2/COVID-19), but also in the longer term for numerous respiratory viruses and diseases, known or as yet unencountered. Careful characterization of NHP models may enable exploration of complex determinants and progression of disease (see Outstanding questions). Advanced medical imaging of adequate resolution is vital to truly characterize (quality) and reliably measure (quantity) disease without bias to understand and provide meaningful longitudinal readouts of disease (in animal studies) and bridge to humans; thus, medical imaging will be an increasingly important component of the NHP modelers’ toolkit.

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Declaration of interests
The authors declare no competing interests.

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