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Lung perfusion assessment in children with long-COVID: A pilot study

Daniele Antonio Pizzuto PhD[1] | Danilo Buonsenso PhD[2,3] | Rosa Morello MD[2] | Cristina De Rose MD[2] | Piero Valentini MD[2] | A. Fragano MD[1] | Fabiana Baldi MD[4] | Daniela Di Giuda MD[1,5]

[1]Department of Radiology, Radiotherapy and Hematology, Nuclear Medicine Unit, Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy

[2]Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A Gemelli IRCCS, Rome, Italy

[3]GlobalHealth Research Institute, Istituto di Igiene, Università Cattolica del Sacro Cuore, Rome, Italy

[4]Division of Respiratory Medicine, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy

[5]University Department of Radiological Sciences and Hematology, Section of Nuclear Medicine, Uniiversità Cattolica del Sacro Cuore, Rome, Italy

Correspondence
Danilo Buonsenso, Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A Gemelli IRCCS, Largo A. Gemelli 8, 00168, Rome, Italy.
Email: danilobuonsenso@gmail.com

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Abstract

Background: There is increasing evidence that chronic endotheliopathy can play a role in patients with Post-Covid Condition (PCC, or Long Covid) by affecting peripheral vascularization. This pilot study aimed at assessing lung perfusion in children with Long-COVID with ^{99m}Tc-MAA SPECT/CT.

Materials and Methods: lung ^{99m}Tc-MAA SPECT/CT was performed in children with Long-COVID and a pathological cardiopulmonary exercise testing (CPET). Intravenous injections were performed on patients in the supine position immediately before the planar scan according to the EANM guidelines for lung scintigraphy in children, followed by lung SPECT/CT acquisition. Reconstructed studies were visually analyzed.

Results: Clinical and biochemical data were collected during acute infection and follow-up in 14 children (6 females, mean age: 12.6 years) fulfilling Long-COVID diagnostic criteria and complaining of chronic fatigue and postexertional malaise after mild efforts, documented by CPET. Imaging results were compared with clinical scenarios during acute infection and follow-up. Six out of 14 (42.8%) children showed perfusion defects on ^{99m}Tc-MAA SPECT/CT scan, without morphological alterations on coregistered CT.

Conclusions: This pilot investigation confirmed previous data suggesting that a small subgroup of children can develop lung perfusion defects after severe acute respiratory syndrome coronavirus 2 infection. Larger cohort studies are needed to confirm these preliminary results, providing also a better understanding of which children may deserve this test and how to manage those with lung perfusion defects.

KEYWORDS
children, CPET, Long Covid, SPECT/CT

Daniele Antonio Pizzuto and Danilo Buonsenso are both first authors.

1 | INTRODUCTION

Three years after the first description of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there is increasing understanding that the impact of this virus on humans is not limited to acute infection, as a large number of previously infected people develop a significant burden of post-acute sequelae.[1] In particular, different independent studies from multiple countries have documented that 5%–40% of infected patients develop a cohort of chronic and unexplained symptoms like severe fatigue, postexertional malaise (PEM), thromboembolism, headache and neurocognitive problems, rashes, gastrointestinal and muscle-skeletal issues, and many others.[2] This condition is known as Long Covid (a term initially coined by patients) or PostCovid Condition (PCC) and is fully recognized by the World Health Organization (WHO). PCC has been initially described in adults,[3] but a growing number of studies have later documented that also children can develop it, in a range of 1% −10% according to the different methodologies and classifications used by different studies.[4]

Although it is still unclear why some patients develop PCC, researchers worldwide are putting several efforts into studying biomarkers of PCC, specifically in adults. So far, preliminary studies have demonstrated that a number of pathological events can lead to PCC, including immune dysregulation, reactivation of latent viruses, viral persistence, gut dysbiosis, and chronic endothelial damage leading to microclots and peripheral perfusion abnormalities.[5] ^{99m}Tc-labeled macroaggregated albumin (^{99m}Tc-MAA) scintigraphy is a very feasible tool for assessing lung perfusion abnormalities in patients with newly suspected or long-term evaluation of thromboembolism.[6–8] For this reason, lung MAA planar scintigraphy was previously inserted in the international procedural guidelines for the detection and the assessment of the extent of vascular involvement in patients with previous pulmonary thromboembolism at follow-up.[9] Subsequently, the implementation of a three-dimensional and hybrid imaging approach (i.e., Single Photon Emission Computed Tomography [SPECT/CT]) resulted promising, as it was reported to be able to reduce the rate of non-diagnostic scans for this specific topic to as low as 0%–5%.[10–13] During the pandemic era, ventilation/perfusion (V/Q) SPECT/CT imaging has been proposed as a useful diagnostic tool to assess the possible presence of vascular impairment at the arteriolar level and to compare it with the possible involvement of pulmonary airways at the same time, thus configuring a match or a mismatch panel. Alternatively, the ventilation scintigraphy could be at least partially replaced by the evaluation of abnormal densitometry areas of lung parenchyma on CT scans, such as atelectasis zones, which could indirectly be an expression of airway impairment. Hence, lung ^{99m}Tc-MAA (SPECT/CT) could represent a well-established tool to detect lung perfusion defects, even at the peripheral small-vessels level.[6] More specifically, a peripheral, mottled and patchy perfusion defect, in absence of ventilation abnormalities in the same pulmonary zone, was described as a possible expression of vascular endothelial damage at a small vessel level (microangiopathy).[6] Previous studies in adults and a pediatric case report[7,8] showed abnormal lung perfusion in patients with PCC, leading to the implementation of new larger studies investigating lung perfusion in Covid-19 survivors. However, the available evidence is still scarce, particularly in children. Further evidence on this topic may serve to better inform the development of PCC services.

With this pilot study, we aimed at investigating lung perfusion in a well-characterized prospective cohort of children with PCC using ^{99m}Tc-MAA SPECT/CT.

2 | MATERIALS AND METHODS

This study is part of a prospective cohort study of children younger than 18 years of age with a previous microbiologically confirmed diagnosis (based on SARS-CoV-2 detected on nasopharyngeal swab by Real Time Polymerase Chain Reaction—RT-PCR) of SARS-CoV-2 infection, that were assessed in our pediatric post-Covid outpatient clinic in Rome, Italy. In our outpatient clinic, we evaluate children that had fully recovered from acute infection and those that presented with persisting symptoms. Children can be sent to the post-Covid unit either after discharge from our Institution (after admission or emergency department evaluation), or directly sent from the family pediatricians (and therefore not seen at baseline during acute infection). The pediatric post-Covid unit is running since mid-2020. Therefore, the following categories of children are daily evaluated in our outpatient unit:

2.1 | Fully recovered children

This group included those that reported no persisting symptoms after acute SARS-CoV-2 infection at the time of follow-up post-onset of acute COVID-19 symptoms (at least 8 weeks).

2.2 | PCC cases

According to a recently released Delphi definition and a previous WHO definition, we defined PCC as children having persisting symptoms for at least 12 weeks, with these symptoms having a negative impact on daily life and not otherwise explained.[14]

We developed a protocol to assess children with PCC, which has been described previously.[4] Specifically for this study, children recognized as possible PCC cases (symptoms persisting for at least 12 weeks), during the time window of 3-6 months after initial infection, undergo routine blood tests, autoimmunity screening including celiac disease, thyroid function, lung ultrasound, ECG and cardiac ultrasound to exclude other possible diagnoses. Those with persisting symptoms and negative diagnostics performed cardiopulmonary exercise testing (CPET). In case CPET documented any abnormal finding (reduction of functional capacity expressed as a reduced consumption of Oxygen “VO2 peak” due to signs of lower cardiogenic efficiency and/or muscle decondition, with associated

indirect signs of pulmonary vasculopathy), children were proposed to perform ^{99m}Tc-MAA SPECT/CT to study lung perfusion.

2.3 | Inclusion and exclusion criteria for ^{99m}Tc-MAA SPECT/CT sub-study

We included children younger than 18 years of age with a previous laboratory confirmed (RT-PCR) diagnosis of acute SARS-CoV-2 infection that were assessed at least 24 weeks (6 months) after the first positive test, and had a CPET documenting signs of fatigue or other cardiopulmonary abnormalities, in the absence of alternative diagnoses.

Exclusion criteria included: suspected PCC children but eventually diagnosed with celiac diseases, anemia, autoimmune diseases, hypothyroidism, diabetes, hepatitis, blood malignancies, as per our protocol[4]; confirmed or suspected primary or acquired immune compromising conditions, recent or current administration of immune suppressive therapies, or other diseases affecting the immune system, or known coagulation disorder or any ongoing treatment with anticoagulants/antiaggregants; children fulfilling WHO's criteria for MIS-C (ongoing or previous).

2.4 | ^{99m}Tc-MAA SPECT/CT imaging

A dual-head gamma camera system (Symbia Intevo 2; Siemens) was used for both planar and SPECT imaging. Low-dose free-breathing coregistered CT was employed for attenuation correction of photons and more precise anatomic localization of abnormal functional findings. Care dose mA was applied on CT acquisition, which consists of dose reductions with automated dose modulation for individual patient size and shape while producing optimal image quality. More specific technical features are provided by a table on Supporting Information: 1.

^{99m}Tc-MAA was intravenously administered to all patients in the supine position immediately before the scan; dose activities were properly chosen according to the EANM guidelines for lung scintigraphy in children.[15] The planar scan consisted of anterior, posterior, right and left lateral, anterior right oblique and posterior left oblique, anterior left oblique and posterior right oblique projections (matrix: 128 × 128 pixels; zoom factor: 1.0). Not any post-processing was performed after the planar acquisition.

SPECT/CT protocol acquisition and raw data reconstruction algorithm were listed in Supporting Information: 1. The choice of acquisition and reconstruction parameters was derived from clinical practice, as no specific reproducible evidence exploring the role of MAA SPECT/CT during the follow-up of children after SARS-CoV 2 infection is available in the literature as a reference.

Planar and SPECT images were visually analyzed and classified according to the presence/absence and the shape of abnormal findings, as follows:

1. normal: no evidence of areas with reduced uptake of ^{99m}Tc-MAA in both lungs;
2. abnormal: evidence of irregular (i.e., patchy, mottled, and/or peripheral) ^{99m}Tc-MAA distribution possibly associated with typically triangle-shaped segmental/subsegmental defects.

First, functional images provided by SPECT and morphologic data resulting from CT scans were evaluated separately; hence, SPECT and CT were simultaneously assessed in fused images. The presence of abnormal ^{99m}Tc-MAA uptake was compared with the possible presence of densitometric alterations in the same region. Patterns were independently classified by two experienced nuclear physicians (D. D. G. and D. A. P), as follows:

1. Match: irregular/reduced MAA uptake areas corresponding to morphological alterations on CT.
2. Mismatch: irregular/reduced MAA uptake findings in absence of corresponding morphological alterations on CT or the presence of morphological CT alterations without corresponding areas of reduced MAA uptake.

2.5 | Variables and outcome

The following data were collected for each child: disease severity during acute infection classified as asymptomatic, mild, moderate, and severe, according to the adapted classification by Buonsenso et al.[16]; time of follow-up since the first infection and probably SARS-CoV-2 variant infection (defined according to the most prevalent variant (>75% of isolates) circulating in Italy during the time of infection, available at 1. https://www.epicentro.iss.it/coronavirus/pdf/sars-cov-2-monitoraggio-varianti-rapporti-periodici-27-maggio-2022.pdf); persisting symptoms; CPET results^{99m}; Tc-MAA SPECT/CT findings.

The primary objective was to describe lung perfusion findings in children with PCC examined with ^{99m}Tc-MAA SPECT/CT. Secondary outcomes consisted of an understanding of CPET findings associated with lung perfusion abnormalities, an understanding of demographic and clinical data during acute Covid-19 in relation to lung perfusion abnormalities; an understanding of persisting symptom clusters associated with lung perfusion abnormalities.

2.6 | Statistical analysis

Given the lack of evidence in the recent literature, this investigation was as designed as a pilot study, using convenience sampling. As such, no formal sample size calculation was needed and inclusion was based on standards for pilot studies, recommending a minimum sample size of 10 subjects.[17] A descriptive study of ^{99m}Tc-MAA SPECT/CT imaging and clinical characteristics of the enrolled children was thus performed.

TABLE 1 Main clinical characteristics of the study population.

Patient	Gender	Age (y)	Severity of Acute infection (as/mild/mod/sev)	Probably infecting SARS-CoV-2 variant	Time from initial infection (months)	Persisting symptoms
1	F	13	Mild	Wild	5	1. Fatigue 2. Smell and taste disturbances 3. Cough 4. Headache 5. Exertional dyspnea
2	M	12	Mild	Wild	6	1. Fatigue 2. Headache 3. Muscle pain 4. Exertional dyspnea
3	M	16	Asymptomatic	Wild	13	1. Fatigue-headache 2. Muscle and joint pain 3. Exertional dyspnea
4	M	12	Mild	Wild	15	1. Fatigue-headache 2. Brain fog syndrome 3. Muscle and joint pain 4. Fatigue 5. Chest pain
5	F	15	Mild	Alfa	3	1. Headache 2. Exertional dyspnea 3. Tachycardia 4. Fatigue 5. Sleep disturbances
6	M	11	Mild	Alfa	7	1. Fatigue 2. Muscle pain 3. Exertional dyspnea
7	F	17	Mild	Alfa	5	1. Fatigue-exertional dyspnea 2. Chest pain 3. Headache 4. Muscle and joint pain 5. Concentration problems
8	M	16	Moderate	Delta	15	1. Fatigue 2. Headache 3. Muscle and joint pain
9	F	11	Mild	Omicron	7	1. Fatigue 2. Exertional dyspnea
10	M	9	Mild	Delta	3	1. Fatigue 2. Nausea and vomiting 3. Gastrointestinal pain
11	F	13	Moderate	Alfa	7	1. Exertional dyspnea 2. Chest pain 3. Tachycardia 4. Fatigue 5. Brain fog syndrome
12	M	17	Moderate	Alfa	4	1. Exertional dyspnea 2. Chest pain 3. Tachycardia
13	F	15	Mild	Alfa	18	1. Tachycardia 2. Exertional dyspnea
14	M	12	Mild	Omicron	3	1. Tachycardia 2. Exertional dyspnea 3. Muscle and joint pain

TABLE 2 SPECT/CT and cardiopulmonary exercise testing (CPET) findings in the study population.

Patient	MAA SPET/CT Results	Details of abnormal distribution pattern	CPET: muscolar deconditioning and fatigue	CPET: Likehood of pulmonary vasculopathy (VE/VCO2 slope)	CPET: cardiogenic efficiency (pulse of O2)	functional capacity (VO2 peak)
1	Abnormal	Nonhomogeneous distribution with localized perfusion defect	Mild	Likely	Reduced	Moderate reduction
2	Normal	/	Moderate	Unlikely	Reduced	Normal?
3	Abnormal	Nonhomogeneous distribution with localized perfusion defect	Severe	Unlikely	Reduced	Moderate reduction
4	Normal	/	Moderate with desaturation	Unlikely	Normal	Normal
5	Abnormal	Nonhomogeneous distribution	Moderate	Unlikely	Reduced	Mild reduction
6	Normal	/	Moderate	Unlikely	Reduced	Mild reduction
7	Abnormal	Nonhomogeneous distribution	Moderate	Unlikely	Normal	Normal
8	normal	/	moderate	Not evaluable for early fatigue and pains	Not evaluable for early fatigue and pains	Not evaluable for early fatigue and pains
9	Normal	/	Mild	Suspected	Normal	Normal
10	Abnormal	Nonhomogeneous distribution	Severe	Suspected	Normal	Normal
11	Normal	/	Moderate	Unlikely	Reduced	Mild reduction
12	Normal	/	Severe	Unlikely	Reduced	Severe reduction
13	Normal	/	Moderate	Suspected	Normal	Normal
14	Mild hypoperfusion	Mild hypoperfusion right upper lobe in the posterior area	Severe	Likely	Significantly reduced	Severe reduction

Note: The CPET is done on a cycle ergometer with RAMP Incremental Exercise Test protocol variable between 7–13 W/min for a time of minimum 8 min and maximum 12 min. The Hansen–Wasserman equation is used to calculate the predicted normal values. The anaerobic threshold (AT) was determined by the V-slope. The test provides information about oxygen consumption (VO_2), production of CO_2 (VCO_2), pulmonary ventilation (VE) and cardiac parameters (heart rate -HR) obtained during a controlled exercise testing. Matching this values we can obtain important other parameters such as VO_2/HR (pulse of oxygen) that explain cardiogenic efficiency or VE/VCO_2 slope (determined using the linear regression analysis of VE and VCO_2 obtained throughout the exercise period) that in absence of respiratory abnormalities can express if over 30 as value an indirect sign of pulmonary vasculopathy. Moreover matching VE/VCO_2 @AT and (end tidal CO2) $PETCO_2$@AT we can calculate a likehood of pulmonary vasculopathy.

3 | RESULTS

Fourteen children fulfilling PCC diagnostic criteria, and complaining of chronic fatigue and PEM after mild efforts, underwent ^{99m}Tc-MAA SPECT/CT scans and were included in this study. All children were aged 9 years or more (range: 9–17 years) and showed persisting symptoms for 3 months or more. None had severe acute SARS-CoV-2 infection. Fatigue and PEM were the most frequently reported chronic symptoms. Further demographic and clinical details of the study population, including the infecting variant, are reported in Table 1. All children had pathological CPET findings (Table 2).

Six out of 14 (2.8%) children showed perfusion defects on ^{99m}Tc-MAA SPECT/CT scan, without morphological alterations on co-registered CT (Table 2 and Figure 1). In particular, 5/6 (83.3%) children with lung perfusion abnormalities were previously affected by a mild acute infection, whereas a single child (16.7%) was asymptomatic during SARS-CoV-2 infection. However, during the follow-up, persisting symptoms (e.g., headache and dyspnea during exercise) were detected in 6/6 (100%) patients. Among children with pathological 99mTc-MAA SPECT/CT, 2/5 were probably infected with wild virus, 2/6 with alpha, 1/6 with Delta and 1/6 with Omicron. Details about ^{99m}Tc-MAA SPECT/CT findings and images are reported in Table 2 and Figure 1, respectively.

4 | DISCUSSION

This is the largest study showing details of lung perfusion assessed by ^{99m}Tc-MAA SPECT/CT in a cohort of previously healthy children that developed PCC after SARS-CoV-2 infection. Overall, we provided evidence of abnormal lung perfusion in five children, confirming our previous case report published earlier during the pandemic.[7]

Although the pathological events leading to PCC are still unclear, several hypotheses, based on preliminary studies, have been proposed, including immunological dysfunction, SARS-CoV-2 persistence, intestinal dysbiosis and chronic endotheliitis.[5] It is thought that all these events may play a role in PCC, with some abnormalities prevailing over others in different patients and contributing to different phenotypes of PCC.[4] However, chronic endotheliitis is the most investigated theory. The concept of chronic endotheliitis has been implemented after early observations that acute Covid-19 is characterized by vascular involvement with higher risks of thromboembolic events in the acute and post-acute phase,[18] as also hypothesized in the Multisystem Inflammatory Syndrome.[19] Later during the pandemic, Pretorius and her team investigated the peripheral blood of patients with PCC and found evidence of circulating microclots, even in the absence of clearly pathological coagulation tests and thrombotic events. Their findings have been reported in different studies and controlled cohorts,[20–23] including

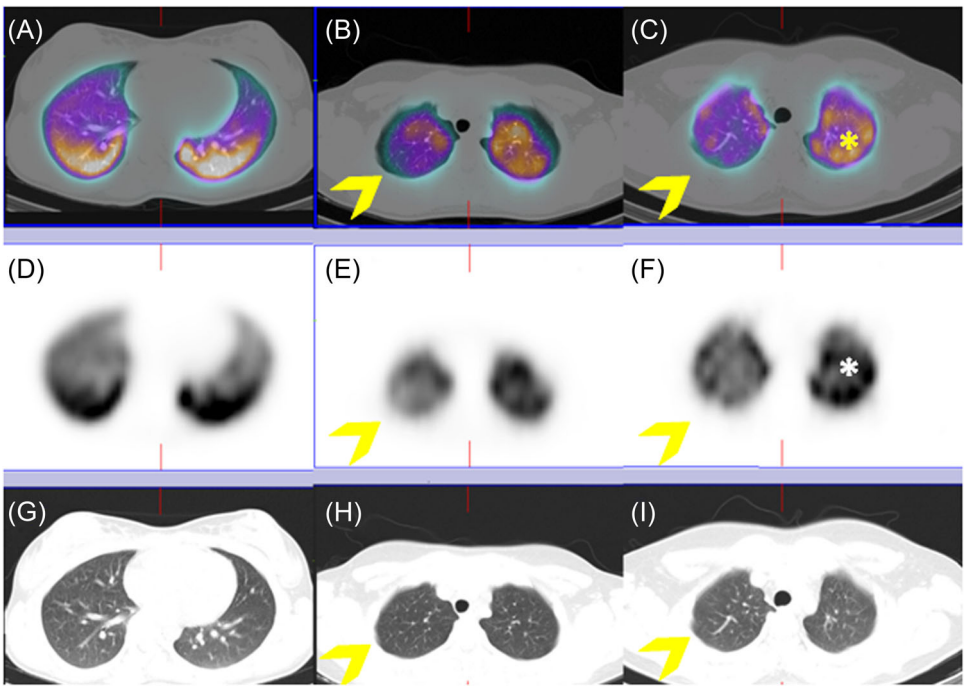

FIGURE 1 Lung SPECT/CT scan with 99mTc-MAA in three Long-COVID children. No significant lung perfusion alterations were detected in a patient with Long-COVID symptoms (A, D, F) on both axial hybrid images and functional slices (A, D). Conversely, MAA SPECT/CT showed an area of mild hypoperfusion in the apical segment of the right upper lobe, clearly evident on both axial fused and functional slices in another patients with Long-COVID syndrome (B, E; arrow). Lastly, an area of moderate hypoperfusion was detected on a third long COVID patient, clearly evident on hybrid imaging, as well as on the functional slices(C, F; arrow), which is associated to a diffuse and heterogenous distribution of radiopharmaceutical in both lung parenchyma (*). All these abnormal findings do not correspond to parenchymal alterations on co-registered CT images (G, H, I). MAA, macroaggregate albumin; SPECT/CT, single photon emission computed tomography with coregistered CT.

patients with Chronic Fatigue Syndrome,[24] a clinical condition that shares several similarities with PCC. The observations of circulating microclots and hyper activated platelets suggest that some, still unknown, inflammatory events give continuous stimuli to the endothelial systems. A recent study from the same team, using proteomics, confirmed the presence of proinflammatory molecules that may also contribute to a failed fibrinolysis phenomenon. In addition, they also found that many proteins remain entrapped in circulating fibrin amyloid microclots and, therefore, they might otherwise be missed on routine blood tests.[25] Altogether, these findings suggest that chronic endotheliitis may possibly explain why individuals with PCC suffer from chronic fatigue, dyspnea, or cognitive impairment.

Given current evidence, researchers have begun investigating coagulation[26] and organ perfusion also in children, as this population can also develop PCC, although less frequently than adults.[27,28] In a previous case report, we highlighted that a child with unexplained chronic symptoms and CPET abnormalities had evidence of lung perfusion defects.[7] Similar findings were later confirmed by Heiss et al, using MRI-derived functional proton ventilation and perfusion parameters: they found persistent pulmonary dysfunction in both children and adolescents recovered from COVID-19 and with PCC (performed within research studies and not as routine diagnostics).[29] These results seem to be in line with our larger cohort, showing lung perfusion defects in 6/14 (42.8%) children investigated. All together, these findings need attention and long term follow-up in children with PCC, as early reports from adult practice suggest a potential link between chronic endothelitis and future development of chronic pulmonary hypertension, if these subclinical conditions are not promptly recognized.[30–34]

Even though our pilot study reinforces the presence of unknown microvascular problems in some children with PCC, it is important to interpret cautiously our findings for a few reasons. Although the administrated 99mTc-MAA dose activity was as low as reasonably achievable (ALARA), namely tailored to the children according to the late EANM procedure guidelines,^{99m}Tc-MAA SPECT/CT involves gamma ray exposure; therefore, its use should be limited to those patients at highest risk of having a pathological test. Also in our cohort, about 60% of patients had a normal test despite persisting unexplained symptoms and a pathological CPET showing muscular fatigue in all patients but only indirect signs of abnormal vascularization in some patients. For these reasons, children with PCC may initially undergo echocardiographic screening for pulmonary hypertension (eg. hypertophy of the right ventricle, tricuspid insufficiency or abnormal shape of the pulmonary doppler curve) and, in case of abnormal findings, ruling out ongoing thrombotic processes with laboratory measurement of D-Dimers.^{99m}Tc-MAA SPECT/CT may be considered as a third-step test in children with abnormal findings at noninvasive tests (eg echocardiography and/or CPET) or those with severe persisting cardiorespiratory symptoms (this is currently also our approach.[35] Given the small number of patients in our cohort, we were not able to perform statistical analyses to understand the risk factors for having an abnormal ^{99m}Tc-MAA SPECT/CT.

To the best of our knowledge, to date, no evidence has been described in the literature exploring the potential clinical role of ^{99m}Tc-MAA SPECT/CT for screening and monitoring children with previous Sars-COV 2 infection. Abnormal perfusion findings on ^{99m}Tc-MAA SPECT/CT related to a chronic endothelial dysfunction at a small vessel level typically assume a patchy, mottled peripheral pattern, which is associated with no densitometric alterations on co-registered CT, or with no evidence of abnormal findings on simultaneous ventilation SPECT/CT scan (mismatch).[6] A similar pattern was described in patients with pulmonary arterial hypertension-associated microangiopathy,[36,37] further reinforcing that, at least theoretically, such chronic subtle events, if not recognized nor treated, may lead to chronic pulmonary hypertension.[29–34] In our cohort, the pathological perfusion pattern presented with a similar non-homogenous distribution of ^{99m}Tc-MAA within both lung parenchyma. However, the peripheral feature was not clearly detected in our patients. An explanation might rely on the coexistence of motion artifacts resulting from free breathing during the scan, which could make the detection of this feature more difficult. This limitation might have partially affected the identification of further subtle vascular impairment and the correct interpretation of the perfusion abnormal findings. Hence, the meaning of an abnormal ^{99m}Tc-MAA SPECT/CT is not fully clear and, therefore, it is also uncertain which therapeutic approach to implementing. In this context, it is important that such studies are performed within research projects and not as routine diagnostics. Nevertheless, a positive MAA SPECT/CT seems to be more likely associated with the lung infection phenomena, which were driven by the more aggressive variants of the virus from the pandemic era, so suggesting a high pre-test probability of positive MAA SPECT/CT in the case of severe involvement of lung respiratory system.

Our study has limitations to address. First, the small sample size did not allow us to understand risk factors for pathologic MAA SPECT/CT. Second, for ethical issues (radiation exposure in children) we did not include a control group of healthy children, although the observation that the majority of examined lungs were normally perfused suggests that those areas abnormally perfused are real pathologic findings. SARS-CoV-2 variants were defined according to the most circulating variant in Italy at time of infection, but not by genotyping of each isolated virus.

In conclusion, our study provides further evidence that a subgroup of children with PCC has abnormal lung perfusion which may reflect the chronic endothelial abnormalities described in adults with the same condition. Although these data are preliminary and require further investigations, they add another piece to the difficult-to-understand puzzle of PCC.

AUTHOR CONTRIBUTIONS

Daniele Antonio Pizzuto: Investigation; writing—original draft. **Danilo Buonsenso**: Conceptualization; investigation; funding acquisition; writing—original draft; methodology; writing—review and editing; supervision; data curation; project administration. **Rosa Morello**: Investigation; data curation. **Cristina De Rose**: Investigation; writing—

original draft. **Piero Valentini**: Supervision; resources; project administration. **A. Fragano**: Investigation. **Fabiana Baldi**: Investigation. **Daniela Di Giuda**: Supervision; Resources.

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CONFLICT OF INTERESTS STATEMENT

DR Danilo Buonsenso has received grants to study Long Covid from Pfizer and Roche.

DATA AVAILABILITY STATEMENT

The data available upon request to the corresponding author

ETHICS STATEMENT

The study was approved by the Ethics Committee of our Institution (ID 3078). Written informed consent was obtained from all participants or legal guardians. All procedures performed were in accordance with the ethical standards defined by the 1964 Helsinki Declaration and its later amendments.

ORCID

Daniele Antonio Pizzuto http://orcid.org/0000-0001-8567-2639
Cristina De Rose http://orcid.org/0000-0002-5394-8335

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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