Acute tubulointerstitial nephritis with or without uveitis: a novel form of post-acute COVID-19 syndrome in children

Marina Avramescu[1,33], Pierre Isnard[2,3,33], Sarah Temmam[4,34], Agnès Chevalier[5,34], Paul Bastard[6,7,8], Mikael Attia[9], Romain Berthaud[1], Marc Fila[5], Claire Dossier[10], Julien Hogan[10], Tim Ulinski[11], Damia Leguevaques[12], Férielle Louillet[13], Edouard Martinez Casado[13], Jean-Michel Halimi[14], Sylvie Cloarec[15], Ariane Zaloszyc[16], Camille Faudeux[17], Caroline Rousset-Rouvière[18], Stéphanie Clavé[18], Jérôme Harambat[19], Edouard Rollot[19], Thomas Simon[20], Megan Nallet-Amate[21], Bruno Ranchin[22], Justine Bacchetta[22], Florence Porcheret[23], Josselin Bernard[23], Amélie Ryckewaert[24], Anne Jamet[25], Jacques Fourgeaud[25], Nicolas Da Rocha[4], Philippe Pérot[4], Nicolas Kuperwasser[26], Naïm Bouazza[27,28,29], Marion Rabant[2], Jean-Paul Duong Van Huyen[2], Matthieu P. Robert[30], Julien Zuber[31], Jean-Laurent Casanova[6,7], Marc Eloit[4], Isabelle Sermet-Gaudelus[32] and Olivia Boyer[1]

[1]Pediatric Nephrology, Maladies REnales Héréditaires de l'Enfant et de l'Adulte (MARHEA) Reference Center, Institut National de la Santé Et de la Recherche Médicale (INSERM) U1163, Imagine Institute, Paris Cité University, Necker-Enfants Malades Hospital, Assistance Publique - Hôpitaux de Paris (APHP), Paris, France; [2]Department of Pathology, Necker-Enfants Malades Hospital, Assistance Publique - Hôpitaux de Paris (APHP), Paris, France; [3]Université de Paris, Institut National de la Santé Et de la Recherche Médicale (INSERM) U1151, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 8253, Institut Necker Enfants Malades, Département « Croissance et Signalisation », Paris, France; [4]Pathogen Discovery Laboratory, Department of Virology, Institut Pasteur, Paris, France; [5]Department of Pediatric Nephrology, Arnaud-de-Villeneuve Hospital, University Hospital of Montpellier, Montpellier, France; [6]Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut National de la Santé Et de la Recherche Médicale (INSERM) U1163, Imagine Institute, Necker-Enfants Malades Hospital, Paris, France; Paris Cité University, Paris, France; [7]St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, New York, USA; [8]Department of Pediatrics, Necker-Enfants Malades Hospital, Assistance Publique - Hôpitaux de Paris (AP-HP), Paris, France; [9]Molecular Genetics of RNA Viruses, Department of Virology, Institut Pasteur, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 3569, Paris, France; [10]Department of Pediatric Nephrology, Robert Debré Hospital, Paris Cité University, Paris, France; [11]Department of Pediatric Nephrology, Armand Trousseau Hospital, Paris, France; [12]Department of Pediatric Nephrology, Centre Hospitalier Régional Universitaire (CHRU) de Lille, Lille, France; [13]Department of Pediatrics, Rouen University Hospital, Rouen, France; [14]Department of Adult Nephrology, Centre Hospitalier Régional Universitaire (CHRU) de Tours, Tours, France; [15]Department of Pediatric Nephrology, Clocheville Hospital, Centre Hospitalier Régional Universitaire (CHRU) de Tours, Tours, France; [16]Department of Pediatric Nephrology, centre Hospitalier Universitaire (CHU) de Strasbourg, Strasbourg, France; [17]Department of Pediatrics, Pediatric Nephrology Unit, L'Archet Hospital, University Hospital of Nice, Nice, France; [18]Department of Multidisciplinary Pediatrics, Pediatric Nephrology Unit, Assistance Publique - Hôpitaux de Marseille (APHM), Marseille, France; [19]Department of Pediatrics, Pediatric Nephrology Unit, SoRare Reference Center, Bordeaux University Hospital, Bordeaux, France; [20]Department of Pediatric Nephrology, SoRare Reference Center, Toulouse University Hospital, Toulouse, France; [21]Pediatric Department, Dijon University Hospital, Dijon, France; [22]Department of Pediatric Nephrology, centre Hospitalier Universitaire (CHU) de Lyon, Lyon, France; [23]Pediatric Department, Nantes University Hospital, Nantes, France; [24]Department of Pediatric Nephrology, centre Hospitalier Universitaire (CHU) de Rennes, Rennes, France; [25]Department of Clinical Microbiology, Necker Enfants-Malades Hospital, Assistance Publique - Hôpitaux de Paris (AP-HP), Paris Cité University, Paris, France; [26]Paris Cité University, Institut National de la Santé Et de la Recherche Médicale (INSERM) U1151, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 8253, Necker Enfants Malades Institute, Department « Croissance et Signalisation », Paris, France; [27]Equipe Associée (EA) 7323, Université Paris, Pharmacologie et évaluations thérapeutiques chez l'enfant et la femme enceinte, Paris, France; [28]Unité de Recherche Clinique, Hôpital Tarnier, Paris, France; [29]Centre d'Investigation Clinique (CIC)-1419 Institut National de la Santé Et de la Recherche Médicale (Inserm), Cochin-Necker, Paris, France; [30]Department of Ophthalmology, Necker-Enfants Malades Hospital, Assistance Publique - Hôpitaux de Paris (APHP), Paris Cité University, Paris, France; [31]Department of Nephrology and Kidney Transplantation, Necker-Enfants Malades Hospital, Assistance Publique - Hôpitaux de Paris (APHP), Paris Cité University, Paris, France; and [32]Department of Pediatric Pulmonology and Allergology, Necker-Enfants Malades Hospital, Assistance Publique - Hôpitaux de Paris (APHP), Paris, France

Correspondence: *Olivia Boyer, Pediatric Nephrology, MARHEA Reference Center, INSERM U1163, Imagine Institute, Paris Cité University, Necker-Enfants Malades Hospital, APHP, 149, rue de Sèvres, 75015 Paris, France. E-mail: olivia.boyer@aphp.fr*

[33]MAv and PI contributed equally to the work.

[34]ST and AC contributed equally to the work.

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Multiorgan sequelae of coronavirus disease 2019 (COVID-19) beyond the acute phase of infection are increasingly described as clinical experience expands. In children, acute COVID-19 appears to be generally asymptomatic or mild. Yet, the multisystem inflammatory syndrome in children (MIS-C) may be a severe postinfectious complication following exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] During the first pandemic year, we observed a striking increase in the incidence of acute tubulointerstitial nephritis (aTIN) without or with uveitis (TINUs) among children. Causes of aTIN include drugs, infections, and systemic diseases, but often remain undetermined. The rare TINUs syndrome associating aTIN and uveitis is considered to result from a still ill-characterized immune-mediated process. The observed increased incidence of idiopathic aTIN/TINUs prompted us to examine whether SARS-CoV-2 might be the initial trigger.

RESULTS

Increased incidence of aTIN and TINUs during the pandemic

Between April 1, 2020, and March 31, 2021, 48 children with a median age of 14.7 years (range, 9.4–17.6 years) were diagnosed with aTIN (n = 25) or TINUs (n = 23) of undetermined cause in France (Figure 1a and b) compared with the 8 to 10 cases/year of idiopathic aTIN recorded in 2018 to 2019, and 46 TINUs cases over an 18-year period between 2000 and 2018.[2] This translates into 3- and 12-fold increases in the incidence of aTIN and TINUs, respectively (from 1 to 3

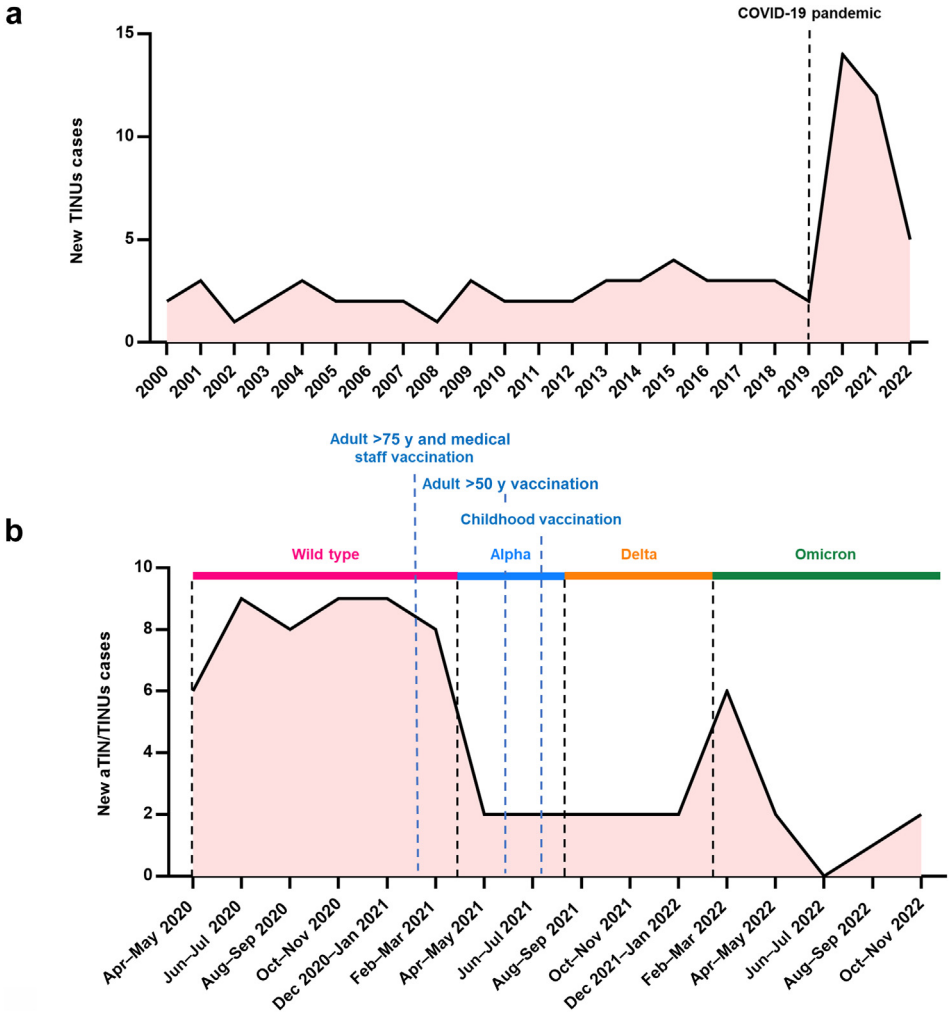

Figure 1 | Incidence of nationwide pediatric acute tubulointerstitial nephritis (aTIN)/aTIN with uveitis (TINUs) cases in France and anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody levels and profiles in children with aTIN/TINUs. (**a**) Annual incidence of nationwide pediatric TINUs cases in France from 2000 to 2022. (**b**) Bimonthly incidence of nationwide pediatric aTIN/TINUs cases in France from April 2020 to November 2022. The approximate dates of circulation of the different strains of SARS-CoV-2 and the dates of launch of the vaccination campaigns according to age are shown in the figure. All analyzed cases of aTIN/TINUs were recorded during the circulation of the original virus and the Alpha variant. Interestingly, only 21 cases of aTIN/TINUs were later recorded between April 2021 and November 2022, when the Delta and the Omicron variants were the predominant viral strains. Of note, none of the patients had received any vaccination against SARS-CoV-2 before the diagnosis of aTIN/TINUs as the campaign for children aged >12 years was launched on June 15, 2021, in France. (Continued)

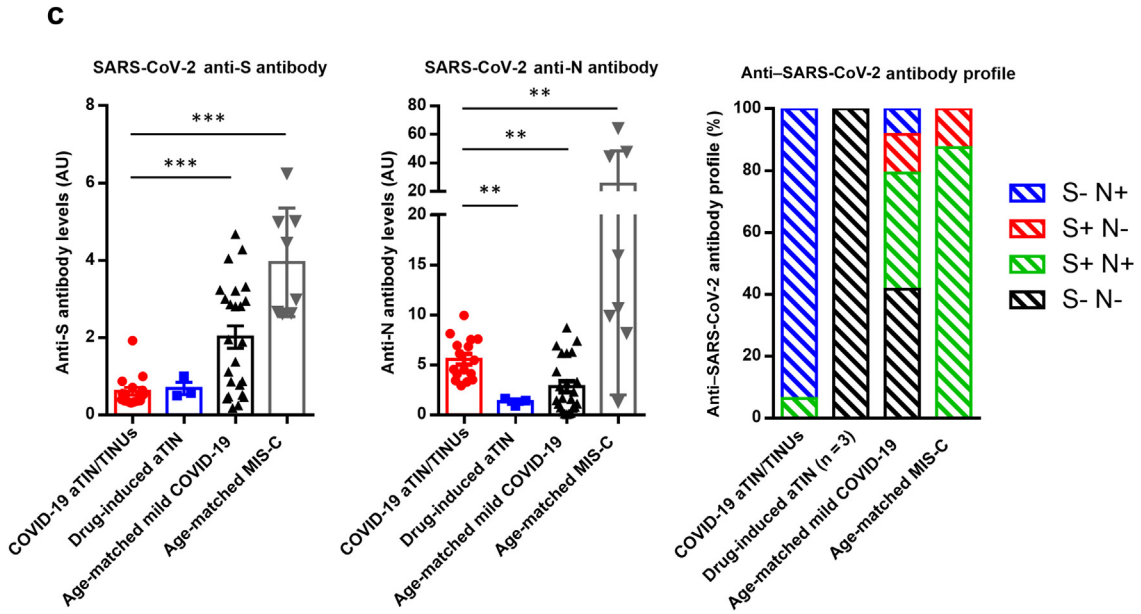

Figure 1 | (Continued) (**c**) Anti–SARS-CoV-2 anti-spike (S) and anti-nucleocapsid (N) antibody levels and profiles in children with aTIN/TINUs compared with contemporaneous age-matched children with ibuprofen-induced aTIN (n = 3; aged 12, 14, and 15 years) or hospitalized for mild coronavirus disease 2019 (COVID-19; n = 24; median age, 14.4 years [range, 12–18 years]) or multisystem inflammatory syndrome in children (MIS-C; n = 8; median age, 12.4 years [range, 10–14 years]), all sampled during the first pandemic year.[4] Wilcoxon-Mann-Whitney *U* test was applied to test the significance of the difference. $^{**}P < 0.01$, $^{***}P < 0.001$. AU, arbitrary unit.

per million children, and from 0.12 to 1.4 per million children, respectively).

Virological findings

The SARS-CoV-2 nasopharyngeal swab test result was negative in all children tested at diagnosis of aTIN/TINUs (37/48), and only 4 of 48 had presented symptoms of COVID-19 in the preceding weeks. Routine serologic SARS-CoV-2 testing targeting the nucleocapsid (N) protein was negative in all 29 tested children, and anti-spike (S) serology was positive only in patient 9 at onset of the kidney disease and before initiation of therapy, at the local centers (Supplementary Table S1).

To investigate the potential association between aTIN/TINUs and COVID-19, we retrospectively performed SARS-CoV-2 serologic tests using a luciferase immunoprecipitation assay (LIPS) presenting a specificity of 100% and sensitivities ranging from 83.1% (LIPS-N) to 99.4% (LIPS-full S)[3,4] (Supplementary Methods and Supplementary Figure S1). Serum at the time of the aTIN/TINUs diagnosis was available for 16 children. Strikingly, all serum samples tested positive for high-level anti-N IgGs, whereas only one (patient 9) had positive anti-S IgGs (Figure 1c and Supplementary Table S1). As controls, 3 children diagnosed with ibuprofen-induced aTIN during the study period, which was not suspected to be related to a preceding COVID-19 infection, had negative LIPS results. Moreover, COVID-19 ELISpot was positive in the 5 children for whom peripheral blood cells were available. Together, these data were consistent with a SARS-CoV-2–specific immune response resulting from an infection because the patients had not yet been vaccinated against COVID-19, and the available vaccines exclusively elicit an anti-S antibody response. We also performed a pseudoneutralization assay to detect the presence of virus-neutralizing antibodies in 16 samples. Neutralizing activity against SARS-CoV-2 was only detected in 2 cases, in line with the absence of anti-S antibodies.

We next compared the antibody titers and serologic profiles of patients in this study with those of age-matched controls (aged 9–17 years) hospitalized with mild COVID-19 or MIS-C during the same period (Figure 1c).[4] Children with MIS-C displayed predominantly the N+/S+ profile, with significantly higher antibody levels than the other 2 groups. Children with mild COVID-19 had higher levels of anti-S antibodies but lower levels of anti-N antibodies than children with aTIN/TINUs.

Clinicobiological features

We investigated whether the phenotype of the TINUs observed during the pandemic differed from those of the historical French pediatric cohort of TINUs from 2000–2018 (Supplementary Table S2).[2] No statistically significant difference was observed. The typical presentation was sudden weight loss, polyuria, fever, and biological markers of inflammation. All patients had reduced estimated glomerular filtration rate at diagnosis (median, 31.9 ml/min per 1.73 m^2 [range, 8.4–87.8 ml/min per 1.73 m^2]), leading to kidney biopsy. Estimated glomerular filtration rate increased to 86.0 ml/min per 1.73 m^2 (range, 66.8–134.5 ml/min per 1.73 m^2) at 12-months follow-up, whereas 32% of patients had chronic kidney disease. Of note, none of the 6 patients with aTIN who

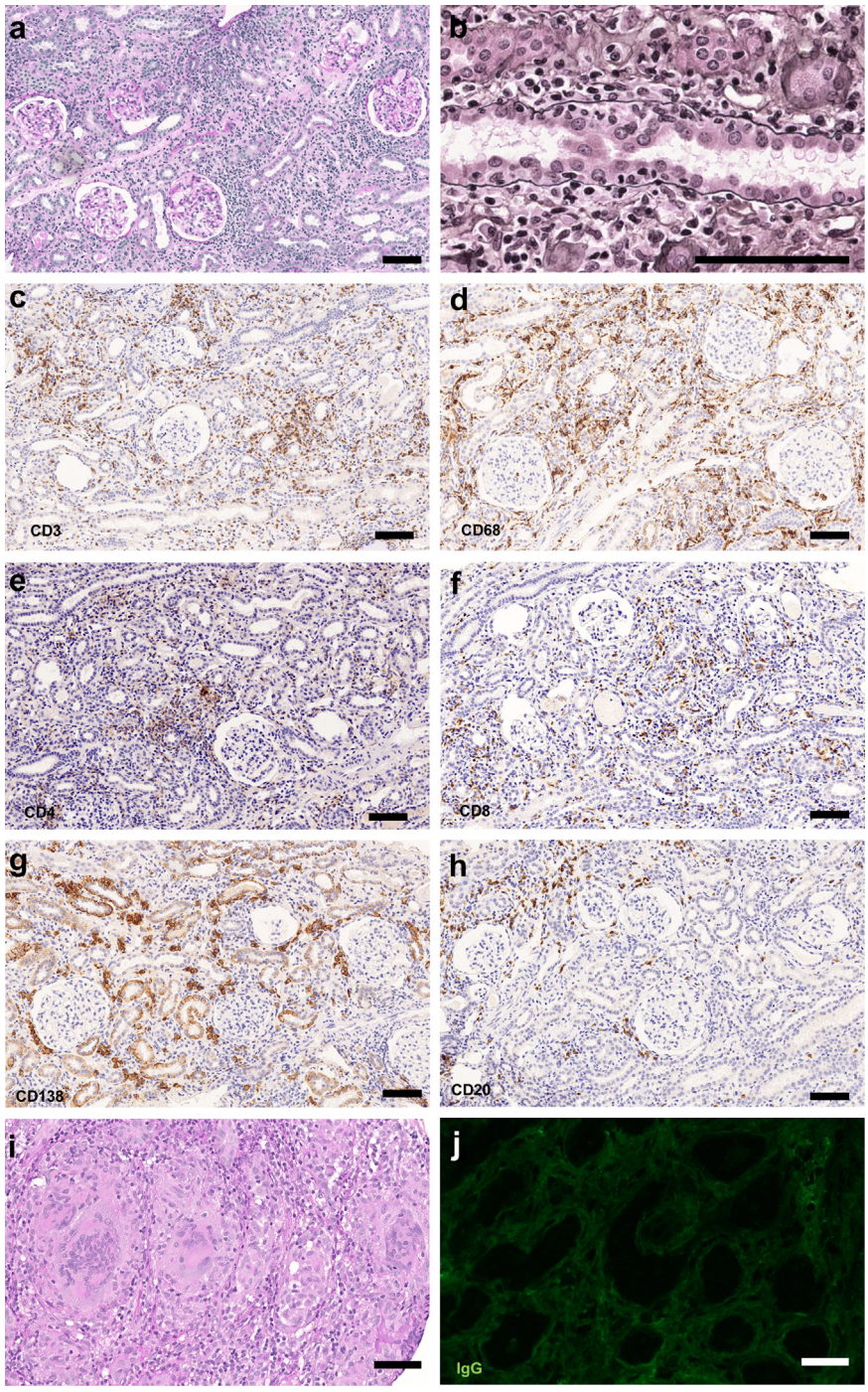

Figure 2| Light microscopy and immunohistochemistry analysis of kidney biopsies. Representative images of optical microscopy. (**a**) Light microscopy (original magnification ×100) using periodic acid–Schiff staining, showing diffuse interstitial infiltrate mainly composed of mononuclear cells. (**b**) Light microscopy (original magnification ×400) using silver staining, showing numerous tubulitis lesions. (**c**) Immunohistochemistry analysis (original magnification ×100) targeting CD3, showing an important T-cell infiltrate. (**d**) Immunohistochemistry analysis (original magnification ×100) targeting CD68, showing an important macrophage infiltrate. (**e,f**) Immunohistochemistry analysis (original magnification ×100) targeting CD4 and CD8, showing a relatively balanced distribution of CD8 and CD4 T cells. (**g**) Immunohistochemistry analysis (original magnification ×100) targeting CD138, showing numerous plasma cells. (**h**) Immunohistochemistry analysis (original magnification ×100) targeting CD20, showing a small B-cell infiltrate. (**i**) Light microscopy (original magnification ×100) using periodic acid–Schiff staining, showing granulomas with multinucleated giant cells in a minority of patients. (**j**) Immunofluorescence analysis (original magnification ×100) targeting IgG, showing no tubular basal membrane deposits. Bar = 100 μm. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

were tested had autoantibodies to neutralizing type I interferons (Supplementary Figure S2).

Pathology findings

Thirty-nine (81%) patients underwent a kidney biopsy. Biopsies from the 16 patients with extensive serologic workup were blindly reassessed by 3 kidney pathologists (PI, MR, and J-PDVH; Supplementary Table S1). Light microscopy showed acute interstitial nephritis, characterized by an infiltrate of predominantly mononuclear cells associated with numerous tubulitis lesions and no Ig deposits, as classically observed in TINUs (Figure 2). We did not notice significant morphologic differences between our cohort and a series of 14 prepandemic TINUs, except a tendency toward more fibrosis and fewer eosinophils in the present series (Supplementary Table S3 and Supplementary Figures S3 and S4). To determine whether this kidney inflammation could be directly related to a specific pathogen, we performed metagenomic next-generation sequencing from 11 kidney biopsy samples, and no definite pathogens were identified (Supplementary Table S1). We next performed SARS-CoV-2 reverse transcriptase polymerase chain reaction and detected low viral loads of SARS-CoV-2 mRNA in 2 of 11 kidney samples. Of note, SARS-CoV-2 immunostaining of kidney biopsies was negative (Supplementary Figure S5).

DISCUSSION

To our knowledge, this is the first study showing an increased incidence of pediatric aTIN and TINUs superimposed to the first wave of the COVID-19 pandemic. To date, the association of aTIN and COVID-19 has been suggested in a few cases.[5,6] We inferred from our study that aTIN/TINUs could be considered as a novel form of post–COVID-19 disease in children. As a matter of fact, epidemiologic data, positive SARS-CoV-2 serologic test results, and ELISpot in all tested patients (16/16 and 5/5, respectively), and *in situ* detection of SARS-CoV-2 in 2 kidney biopsies, strongly support a causal link. COVID-19 was asymptomatic in 44 of 48 (90%) children at the acute phase, as reported in most post–COVID-19 MIS-C series.[1]

Notably, patients had a unique N+/S– serologic profile, which differs from those observed in adults with COVID-19 and children with MIS-C who develop both anti-S and anti-N IgGs. Similarly, children with mild COVID-19 predominantly generate anti-S IgGs.[4] The presence of anti–SARS-CoV-2 IgGs in all tested children in the present series further exemplifies the postinfectious nature of COVID-19–related aTIN/TINUs. Moreover, the lack of detection of SARS-CoV-2 mRNA in most kidney samples further supports a resolved infection.

Of interest, the clinical and histologic features in the present series were similar to those of prepandemic TINUs, and affected mostly adolescents.[2] This suggests that SARS-CoV-2–induced aTIN/TINUs cases share common pathogenic pathways with other forms.

We therefore propose that SARS-CoV-2 could prime autoinflammation through molecular mimicry,[7] as reported in other post–COVID-19 long-term symptoms. SARS-CoV-2 uses the receptors angiotensin-converting enzyme 2 and transmembrane protease serine 2 for cell entry and protein S priming, respectively. More important, these 2 proteins are expressed in kidney tubules and in eye components.[8,9] Therefore, we hypothesize that both tissues were infected during the acute phase, triggering an overly potent and long-lasting detrimental immune response, despite subsequent clearance of the virus. Of note, the presence of a predominant lymphohistiocytic infiltrate and the absence of Ig deposits are in favor of a cell-mediated mechanism.

Overall, our relatively large cohort of this rare disease provides additional insights into the pathophysiology of TINUs and suggests that SARS-CoV-2 should be considered among the infectious agents responsible for pediatric aTIN/TINUs.

The limitations of this study are mainly the recruitment through biopsy-based registry, potentially subject to selection bias; the retrospective design, precluding fresh sampling for functional immunologic testing, including T-cell response to interferon; and the absence of available serologic samples from prepandemic patients with aTIN/TINUs. However, the 3 contemporaneous children with ibuprofen-related aTIN/TINUs had a negative SARS-CoV-2 LIPS serology.

Interestingly, the aTIN/TINUs incidence declined after April 2021. The vaccination campaign, herd immunity, and emergence of new variants might have contributed to this trend. Nevertheless, our data should raise awareness that post–COVID-19 aTIN/TINUs may be responsible for chronic kidney damage in adolescents that may compromise kidney function in adulthood.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

MAv and PI contributed equally to the work. MAv designed the study, handled regulatory ethics submissions, collected and analyzed clinical and serologic data, collected available samples, and wrote the first draft of the manuscript. MAv contacted PI to collaborate on this study. PI analyzed the histology data, designed and interpreted microbiological molecular data, and wrote the manuscript. ST and AC contributed equally to the work. ST performed and analyzed the serologic assays and edited the manuscript. AC collected and analyzed clinical data and edited the manuscript. PB performed the interferon experiments. MAt performed the pseudoneutralization assay. AJ and JF performed the next-generation sequencing on kidney biopsies. NDR and PP assisted with serologic testing. NK proof edited the manuscript in English and critically reviewed its scientific content. MR and J-PDVH analyzed the kidney histology. MPR reviewed the ophthalmologic data and examined the patients where possible. JZ and J-LC critically reviewed the manuscript with their expertise in immunology. ME led the virological experiments. IS-G coordinated the COVID-19 cohort in

children locally, handled regulatory ethics submissions, analyzed the data, and edited the manuscript. OB designed the study, coordinated all experiments, and wrote the manuscript. All the authors observed the patients, collected clinical data, facilitated the study, and declare they have seen and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary Methods.

Supplementary Results.

Supplementary Table S1. Histologic, immunohistochemical, and virological findings in the 16 coronavirus disease 2019 (COVID-19)–associated children with acute tubulointerstitial nephritis (aTIN)/aTIN with uveitis (TINUs) with available serum samples (sera) stored at onset of kidney disease.

Supplementary Table S2. Clinical and biological characteristics of the 48 children of the present cohort compared with the historical nationwide French cohort of children diagnosed with acute tubulointerstitial nephritis with uveitis (TINUs) from 2000–2018.

Supplementary Table S3. Comparison of histologic lesions in the 16 children with coronavirus disease 2019 (COVID-19)–associated acute tubulointerstitial nephritis (aTIN)/aTIN with uveitis (TINUs) and available serum samples (sera), with a series of 14 children with prepandemic TINUs.

Supplementary Figure S1. Seasonal coronavirus seroprevalences between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid (N)+ and N– patients.

Supplementary Figure S2. Neutralizing auto-antibodies (Abs) against interferon (IFN)-α2, IFN-ω, or IFN-β in children with coronavirus disease 2019 (COVID-19)–related acute tubulointerstitial nephritis (aTIN)/aTIN with uveitis (TINUs).

Supplementary Figure S3. Acute polymorphonuclear leukocyte infiltrate on patient 1 kidney biopsy.

Supplementary Figure S4. Light microscopy showing interstitial fibrosis and tubular atrophy.

Supplementary Figure S5. Immunohistochemical detection of nucleocapsid (N) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

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