Post COVID-19 hyperinflammatory acute kidney injury and background ovarian insufficiency: Renal functional restoration after combined angiotensin-converting enzyme inhibitor with estrogen replacement

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Research Article

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Abstract

Adolescents with COVID-19 and subsequent multisystem inflammatory injury share key features with Kawasaki disease and toxic shock syndrome. As referenced in a United Nations WHO brief from May 2020, findings from North America and Europe drew notice to an acute illness accompanied by severe, diffuse hyperinflammation leading to multiorgan failure. While females diagnosed with COVID-19 generally have more favorable outcomes than males, this protective effect is negated by any low/absent estrogen state. Our research focuses on secondary amenorrhea from pre-adulthood ovarian insufficiency where *RUNX2, SALL1* and *SAMD9* variants were identified by exon sequencing in a healthy, phenotypically normal 46,XX adolescent. Against this background, COVID-19 infection occurred necessitating hospital admission where multisystem inflammatory syndrome in children (MIS-C) was diagnosed. Although renal function was compromised secondary to COVID-19 infection, this damage was transient and apparently reversed by angiotensin-converting enzyme inhibitor plus transdermal estrogen replacement therapy. This research is the first to study the intersection of multigene variants, ovarian insufficiency, COVID-19, and acute kidney injury as a component of MIS-C. The background transition from adolescence to adulthood is also considered, where successful recovery of renal function was attained using combined enalapril and supplemental estrogen.

Introduction

Secondary amenorrhea from ovarian insufficiency before age 40yrs is uncommon and is exceptionally rare in adolescence [1]. If present, renal disease complicates management of hypoestrogenism as impaired kidney function will impact elimination half-life of hormone replacement [2, 3]. When the Coronavirus disease 2019 (COVID-19) damage sequence extends to multisystem inflammatory syndrome, direct renal cytopathic effect, tubular injury from cytokine storm, or immune-mediated glomerulonephritis are acutely relevant [4]. In this condition, enalapril and other ACE-inhibitors conserve kidney tissue by lowering intraglomerular pressure [5] and estrogen can also play a beneficial role. In practice, renal status is usually monitored via estimated glomerular filtration rate (eGFR) derived from serum creatinine clearance, but this is unreliable in underweight conditions where serum cystatin C is preferred [6, 7]. Here an unusual alignment of *RUNX2, SALL1*, and *SAMD9* variants is described where pediatric COVID-19 infection and renal injury emerged in the context of ovarian insufficiency.

Clinical Presentation

Accompanied by her mother, an 18 year old nonsmoking Caucasian female attended for second opinion to discuss secondary amenorrhea, low serum estrogen, elevated serum FSH and creatinine and prior hospitalization for COVID-19. There was no history of proteinuria or electrolyte imbalance. Menarche was at age 11 and normal ovarian, uterine, and cervical structures were confirmed on abdominal ultrasound and pelvic computed tomography. However, menses had completely ceased by age 13 and serum FSH was consistently above 100mIU/ml. Serum anti-Mullerian hormone was undetectable thereafter. She was evaluated by a reproductive endocrinologist where clomiphene challenge test showed bilateral follicular response. However, by age 15 no specific explanation for 'ovarian failure' was offered.

In late 2020, the patient—then age 17½—was evaluated for fever, headache, fatigue, nausea and emesis. Pregnancy test and COVID-19 screen were negative; oral vancomycin was initiated for *C. difficile* which was diagnosed incidentally. When temperature increased to 39.7°C two days later, a second COVID-19 test was positive. Now underweight, the patient was admitted to hospital where supportive care was provided and vancomycin was adjusted to i.v. administration. Three days later, her body mass index was 16.8kg/m^2 as fever, diarrhea, and vomiting continued. Proteinuria was accompanied by elevated serum creatinine (3.5 mg/dL). The diagnosis was revised to COVID-19-associated multisystem inflammatory syndrome in children (MIS-C) and she was transferred to intensive care unit (ICU). Next, vancomycin was disconnected while remdesivir, cefepime, dexamethasone, and intravenous immunoglobulin therapy were started. Erythrocyte sedimentation rate, C-reactive protein, and D-dimer level - all of which had been markedly abnormal - gradually normalized in ICU. The patient was discharged home after 15 days with local outpatient renal clinic consult and comprehensive genetics panel set for follow-up.

Kidney function was assessed in local outpatient nephrology unit by eGFR formulae based on serum creatinine (Cr) alone [8]

$$\text{Cr eGFR} = 142 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.200} \times 0.9938^{age} \times 1.012;$$

serum cystatin C alone [9],

$$\text{Cystatin C eGFR} = 133 \times \min(S_{cys}/0.8, 1)^{-0.499} \times \max(S_{cys}/0.8, 1)^{-1.328} \times 0.996^{age} \times 0.932;$$

and both [10],

$$\text{Cr + cystatin C eGFR} = 135 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.200} \times \min(S_{cys}/0.8, 1)^{-0.323}$$

$$\times \max(S_{cys}/0.8, 1)^{-0.778} \times 0.9961^{age} \times 0.963$$

where:

eGFR = estimated glomerular filtration rate; S_{cr} = serum creatinine (mg/dL); S_{cys} = serum cystatin C (mg/L); $\kappa = 0.7$ (for females); $\alpha = -0.241$ (for females); $\min(S_{cr}/\kappa, 1)$ = min S_{cr}/κ or 1.0; $\max(S_{cr}/\kappa, 1)$ is the maximum of S_{cr}/κ or 1.0; age (yrs).

Precautionary enalapril 2.5mg/d was prescribed while renal biopsy was scheduled; the steep rise in cystatin C was sufficiently alarming (see Fig. 1) that the patient and her family were advised about renal transplant options. Meanwhile, exome sequencing identified variants Q253H in *SALL1* and R824Q in *SAMD9* plus a previously unreported multiexon 3' terminal duplication of *RUNX2* [11] none of which were considered related to ovarian insufficiency [1]. On renal

biopsy, six of 19 fields showed extensive/total fibrosis and two were partially scarred with foot process effacement and mild interstitial chronic inflammation, all consistent with focal segmental glomerulosclerosis (see Fig. 2). Transdermal estradiol (phase-in at 25mcg, then increased to 100mcg) was initiated within one month of angiotensin-converting enzyme inhibitor therapy, as steady declines in serum creatinine & cystatin C showed substantial improvement in kidney function. Management is coordinated across nephrology, endocrine, and genetics clinics with regular checkups and monthly complete blood counts to guide the need for repeat renal biopsy, or other interventions.

Discussion

Concurrence of genetic variants, secondary amenorrhea from ovarian insufficiency, COVID-19, and MIS-C with renal injury presents an unusual opportunity to report how these factors can merge clinically. As the understanding of MIS-C and COVID-19 continues to grow, data on *RUNX2, SALL1* and *SAMD9* mutations in this setting remain sparse. *SAMD9* has been placed among 'hub genes' from protein-protein network analysis during COVID-19 [12] but thus far no research has linked either *RUNX2* or *SALL1* with Coronavirus or MIS-C. Of note, Yale experts found the same R824Q variant in *SAMD9* in a boy age 15 months with adrenal insufficiency, ambiguous genitalia, dysmorphic features and global developmental delay [13]. Myelodysplasia and monosomy 7 were seen on bone marrow biopsy, but his *SAMD9* variant occurred in the absence of any *RUNX2* or *SALL1* mutation.

Why might this *SAMD9* variant fail to produce the expected gain-of-function effect via heightened action of the gene's growth repressor product? The answer may be that in isolation, an altered SAMD9 protein can be resolved by monosomy 7 or secondary somatic nonsense/frameshift loss-of-function change. This adaptive reversion rectifies a solitary SAMD9 disruption [14, 15], but the co-presence of *RUNX2* and *SALL1* variants here cannot be discounted. Indeed, the convergence of all three variants together appear to have enabled a multiplex effect to permit a milder phenotype. While dysfunction at *RUNX2, SALL1*, or *SAMD9* could be unrelated to ovarian insufficiency, simultaneous changes across these 'master regulators' support an autosomal influence on overall gonadal function [16, 17].

Females with COVID-19 generally have better prognoses than males—a protective effect attributed to estrogen—but this advantage disappears after menopause [18, 19]. Recent research has found an androgen-sensitive transmembrane serine protease facilitates SARS-CoV-2 access to cells, so women with relative androgen surplus might be at increased risk of COVID-19 infection by this pathway [20]. Because our patient was effectively post-menopausal for at least one year before her Coronavirus infection, the usual benefits of functional ovaries could not be realized.

The hyperinflammatory renal presentation of MIS-C is congruent with features of Kawasaki disease and toxic shock syndrome [21–23], particularly high fever and severe multisystem involvement (*e.g.*, cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic, or neurologic) [24]. CDC national surveillance data on COVID-19 patients age ≤ 24yrs find only 2.5% of this group require hospitalization, and fewer still (0.8%) need ICU support [25]. Plouffe *et al* (2021) published on multifocal renal infarcts in a 6-year-old boy suffering from MIS-C nine weeks after a febrile illness characteristic of COVID-19. While their patient also had Factor V Leiden mutation, this was considered insufficient to account for the full clinical picture [26]. Boudhabay *et al* (2021) described post-COVID-19 MIS-A (adult) in a 46-year-old man with biopsy-proven renal thrombotic microangiopathy, where complement inhibition with eculizumab dramatically improved renal function [27].

This work also underscores different ways to measure renal function, so we incorporated eGFR calculations using all three age-adjusted formulaic standards. Cystatin C is present in essentially all human tissues as an amino acid product of nucleated cells, and is a highly sensitive marker for preclinical loss of renal function [28–30]. The age-related rise in serum cystatin C is also a good predictor for health outcomes such as cardiovascular death and diminishing cognitive function [31]. Interestingly, osteoblast response to cystatin C has been examined in a murine model with special focus on bone matrix mineralization and bone growth. Activity of RUNX2 was boosted in cystatin C-treated cells, suggesting that cystatin C drives osteoblast differentiation, mineralization, and bone formation [32]. Moreover, renal failure itself has been shown to cause enhanced RUNX2 expression via dampened microRNA-93 levels [33]. MicroRNA-93 inhibits the Wnt/β-catenin pathway, targeting Transcription factor 4 (TCF4) with improved renal function by reducing vascular calcification [33]. While nephrons—like oocytes—are not believed to retain a replenishment capacity in adult mammals, *in situ* regrowth of nephrons has been observed in an animal model and specific expression of SALL1 was observed among markers promoting this regeneration [34]. The role of ACE-inhibitors in managing renal disease is well established [5] and estradiol can also provide protection in acute injury [35]. Our case validates application of both agents in tandem, and ovarian and renal findings described here should extend the characterization of how these specific variants impact quality of life.

Declarations

Authors' Contributions

ESS developed the research plan and organized initial drafts; ESS, SHW, and APHW reviewed the literature; SHW and APHW supervised the project and editorial aspects. All authors read and approved the final manuscript.

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Written permission was provided by the patient and family for publication of this manuscript.

Disclosures

None.

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Figures

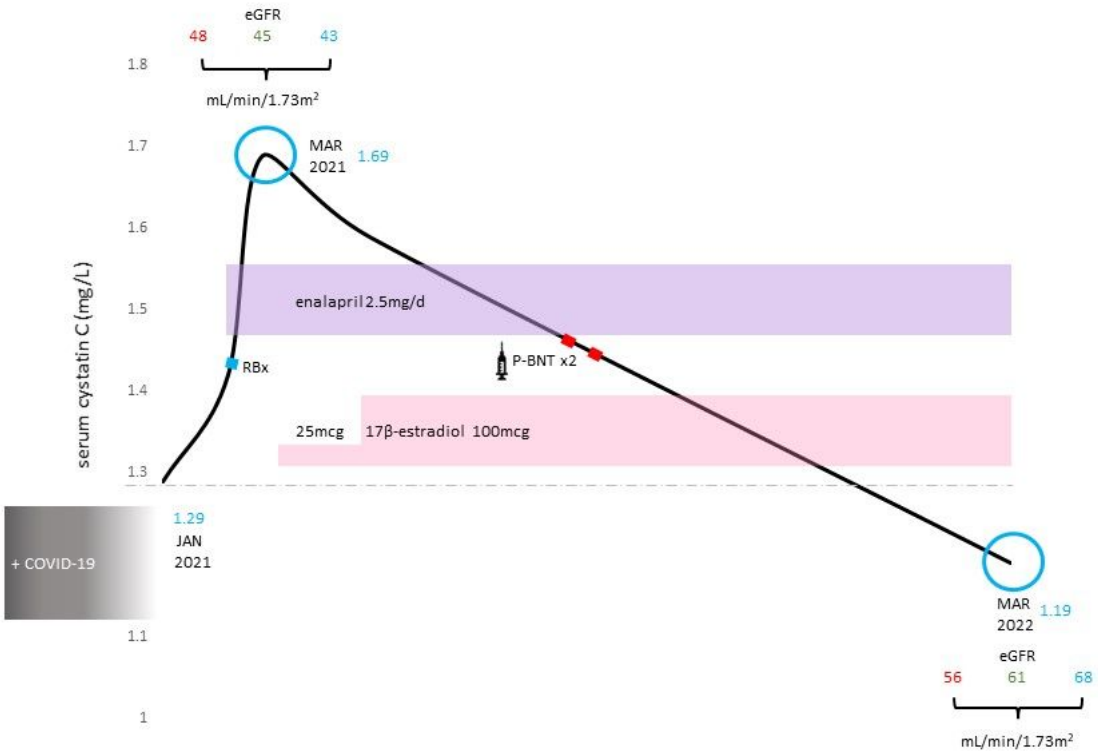

FIG. 1

Figure 1

Post COVID-19 serum cystatin C levels, demonstrating onset of acute kidney injury within 6-8 weeks of infection. Maximum cystatin C (upper circle) corresponds to apex of renal impairment which responded favorably to daily enalapril (purple bar) and tapered transdermal estrogen replacement (pink bar). Estimated glomerular filtration rate (eGFR) calculations derived from serum creatinine (red), and serum cystatin C (blue) are shown. Calculation using both eGFR markers (green) is included. Renal biopsy = blue square; Pfizer/BioNTech coronavirus vaccination series = red square x2.

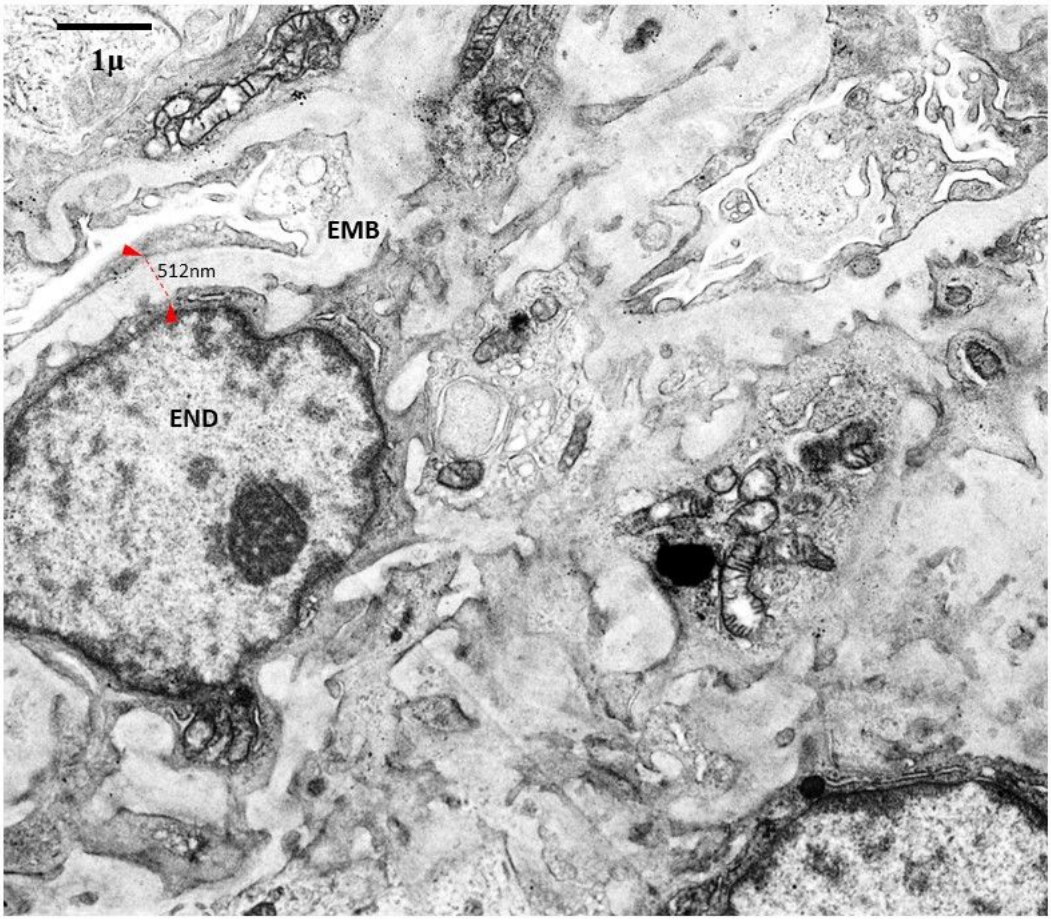

Figure 2

High-voltage (80kV) transmission electron microscopy view (x1500) of renal ultrastructure (female, age 17½ yrs) during post-COVID-19 multisystem inflammatory syndrome in children including acute kidney injury. END=endothelial cell, EBM=basement membrane thickness, 512nm (red).