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Perspective

Kawasaki like disease in SARS-CoV-2 infected children – a key role for neutrophil and macrophage extracellular traps

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Abstract: Less than 2% of children are reported to test positive for SARS-CoV-2. However, increasing reports have described COVID-positive children demonstrating symptoms like Kawasaki disease (KD), an acute vasculitis in medium sized vessels. Characteristic clinical features of KD include fever, conjunctivitis, mucosal alterations, rashes, and cervical lymphadenopathy. We searched PubMed with six keywords including neutrophil and macrophage extracellular traps (NETs/METs), Kawasaki disease, vasculitis, COVID-19 and SARS-CoV-2. We discussed here how SARS-CoV-2 infection is accompanied by activation of proinflammatory cytokines, specifically IL-1β and production of neutrophil and macrophage extracellular traps (NETs/METs), structures formed through specialized types of cell death. In this review, we propose that the KD-like pathogenesis observed in COVID-19-infected children could arise from infection of resident macrophages resulting in activation of NLRP3 inflammasomes and release of IL-1β in a genetically predisposed subset of infected children, mediated via NET/MET dysregulation and overproduction. We also propose potential avenues of diagnosis and treatment that could be utilized to aid such patients.

Keywords: COVID-19; Kawasaki syndrome; macrophage extracellular traps; neutrophil extracellular traps; SARS-CoV-2; thrombosis; vasculitis

1. Introduction

COVID-19 has caused significant mortality and morbidity in adults globally, but seems to have largely left children under 18 years of age unaffected [1], with less than 2% testing positive for SARS-CoV-2 [1,2]. Perhaps this could be due to inadequate diagnostic testing of this particular subset of asymptomatic population. In adults, a proportion of COVID-19-positive patients developed severe symptoms, characterized by acute respiratory distress syndrome (ARDS), coagulopathy, vasculopathy and multiorgan failure [3]. However, it seems rare for occurrence of severe disease in COVID-19-positive children, with children traditionally representing vehicles of infection for more vulnerable populations (the elderly and adults with co-morbidities) [1,2].

However, the World Health Organization (WHO) has raised attention towards increasing reports of COVID-19 positive children demonstrating similar symptoms to a rare disorder known as Kawasaki disease (KD) [4]. First described by Tomisaku Kawasaki in Japan [5], KD represents the most common cause of acquired heart disease in children, and is reported to be 10–30 times higher in Japan than the United States of America or Europe [6]. Usually a self-limiting pathogenesis of acute vasculitis in medium sized vessels, the characteristic clinical features of KD include fever, conjunctivitis, mucosal alterations, rashes, and cervical lymphadenopathy [7]. Many children in the acute phase of the disease are hemodynamically unstable, and present as Kawasaki shock syndrome (KSS) [8], while some may also present symptoms of macrophage activation syndrome (MAS) with a secondary cytokine storm due to loss of cytolytic function of $CD8^+$ and Natural Killer (NK) cells [9–12].

Recently, Verdoni et al. [13] reported a cluster of 10 cases with symptoms resembling those of KD and KSS in the epicenter of the Italian COVID-19 outbreak, with symptoms observed including fever, polymorphic rash, induration of hands & feet, non-purulent conjunctivitis, and bilateral cervical lymphadenopathy. Of the 10 cases examined, 6 exhibited echocardiographic abnormalities, with 2 presenting with coronary vessel aneurisms [13]. Subsequently, a number of other studies have also identified similar clusters and associations of Kawasaki like disease (KLD) and KSS at the center of COVID-19 outbreaks in children (Table 1) [13–19], potentially suggesting a link between KLD and COVID-19 in children with severe disease symptoms. We hypothesize, here that KLD in COVID-19 infected children is due to dysregulation of innate immune cells including neutrophils and macrophages and excessive production of neutrophil and macrophage extracellular traps. In this paper we analyzed the association between dysregulation of innate immune cells like neutrophils and development of Kawasaki like disease in children with COVID-19- infection to propose a pathophysiological mechanism.

Table 1. Summary of clinical features from case studies and case series of the children recently affected by Kawasaki like disease associated with COVID-19.

Clinical Features	Verdoni et al. [13] (n = 10)	Riphagen et al. [15] (n = 8)	Rivera et al. [16] (n = 1)	Jones et al. [14] (n = 1)
COVID-19 status	All 10 positive	2 were positive 2 were exposed	COVID-19 positive	COVID-19 positive
KD-like clinical features	Conjunctivitis Rash Mucosal changes Swelled extremities. Shock and hypotension	All were acutely ill. High fever > 4 days 3 conjunctivitis and rash; 2 conjunctivitis but no rash; 1 rash with no conjunctivitis	High fever > 8 days Tachypnea Hypotension Rash Swelling (palms and soles) Conjunctivitis	High fever > 5 days Tachycardia and tachypnea Subcostal retractions Rash Conjunctivitis Swelling of extremities.
Other features	5 with diarrhea and 4 with neurological complications	6 had diarrhea abdominal pain and vomiting. 3 had odynophagia	Decreased appetite, diarrhea, dysuria, and abdominal pain	-
Cardiac abnormalities	6 with cardiac abnormalities and 2 with coronary aneurisms	All 8 patients had cardiac abnormalities	Enlarged heart with pericardial effusion	Normal with no cardiac abnormalities
Investigations	5 demonstrated MAS and 4 showed KSS on investigation	Inflammation with increased CRP, Procalcitonin, Ferritin, TAG's and D-dimer. Echo-bright coronary vessels were a common finding on echocardiography	Leukocytosis Anemia Thrombocytopenia Elevated ESR, CRP, procalcitonin, ferritin, liver enzymes, and troponins Hyponatremia Hypoalbuminemia	Left shifted WBC with bandemia. Elevated CRP, ESR. Hyponatremia and hypoalbuminemia. CXR with faint opacity in left midlung zone. Normal echocardiography
Treatment	All were administered intravenous immunoglobulins 2 given Aspirin 8 given methylprednisolone	Intravenous immunoglobulins Antibiotics: (Ceftriaxone and Clindamycin) 6/8 children were given Aspirin.	High flow oxygen, Fluid boluses for the hypotension, and intravenous immunoglobulin which was discontinued due to worsening hypotension and restarted with pretreatment of Diphenhydramine and methylprednisolone. Medium-dose Aspirin.	The patient was treated per guidelines with intravenous immunoglobulin high-dose Aspirin

2. Associations between Kawasaki disease and extracellular traps

Also referred to as Multisystem Inflammatory Syndrome in Children (MIS-C), the pathophysiology of KLD conditions are not yet well understood, with suggestions indicating the causative factor is due an abnormal immune response to the SARS-CoV-2 virus. Although MIS-C may mimic Kawasaki disease (KD), MIS-C presents with a different immunophenotype [21]. Numerous criteria are now applied when diagnosing MIS-C. At a primary level, investigations include a complete blood count (CBC), comprehensive metabolic panel (CMP), erythrocyte sedimentation rate (ESR), c-reactive protein (CRP), and SARS-CoV-2 testing (PCR and/or serology). Further diagnostics and testing can include (if required) an electrocardiogram (EKG) and/or echocardiogram (ECHO), as well as laboratory investigations of troponin T and B-type natriuretic peptide (BNP)/N-terminal proBNP, D-dimer, ferritin, procalcitonin and LDH. Blood cultures have also been recommended during initial evaluations due to the potential for patients to present with or mimic septic shock or toxic shock syndrome [20,21].

The mechanisms underlying KD seem complex and multifaceted, with causative factors including genetics, disease seasonality, infectious agents, and host inflammatory responses [22]. While specific causative mechanisms remain unknown, theories suggest a number of potential etiologies including: (1) the super antigen theory, (2) RNA virus theory, (3) infectious vasculitis theory and (4) autoantigen theory [22]. Of these, most opinion regards the RNA virus-based theory as most encompassing of observed symptoms and etiologies, particularly in the context of COVID-19-infected children [7], which suggests that asymptomatic infection of RNA viruses in children could cause KLD in a genetically predisposed subset [7]. In such assertions, viral proteins are thought to persist in respiratory epithelial cells and macrophages as inclusion bodies, culminating in an adaptive immune response that damages coronary vessels [7,23–25], such as the mechanism proposed for SARS-CoV-2.

3. Neutrophil extracellular traps (NETs)

Excessive release of a phenomenon termed neutrophil extracellular traps (NETs) has been observed at significant levels in sera of KD patients [26], while similarly high levels of NETs have also been observed in sera of adult COVID-19 patients compared to healthy controls [27,28]. Perhaps this co-association highlights a potential dysregulated innate immune response to viral pathogens in KD patients. Considering the commonalities between both etiologies, we propose that the KD-like pathogenesis observed in COVID-19 pathogenesis could arise from infection of resident macrophages resulting in activation of NLRP3 inflammasomes and release of IL-1β in a genetically predisposed subset of infected children. The continuous activation of neutrophils by IL-1β culminates in excessive release of NETs causing acute disease in these patients. Additionally, persistence of viral particles as cytoplasmic inclusion bodies in respiratory epithelium and macrophages with intermittent shedding will cause an adaptive immune response which damages coronary vessels.

Neutrophils represent one of the first tiers of the immune response to invade the inflamed area. Activated neutrophils can release a mesh-structure of DNA-rich material combined with proteinases,

called neutrophil extracellular traps (NETs), which can entrap and eliminate microbes [29]. Such structures are formed through a specialized type of cell death seen in neutrophils called "NETosis", induced during inflammatory responses through cytokines such as IL-1β, culminating in release of proteinase-containing granules, and chromatin de-condensation and discharge from the nucleus [29]. While representing an elegant immune mechanism, excessive NETosis is detrimental, whereby increasing NET levels activate neighboring macrophages to induce further cytokine production, eliciting a IL-1β-NET feed-forward-loop [27].

4. Macrophage extracellular traps (METs)

Similar structures to NETs can also be produced by macrophages in response to various stimuli, resulting in macrophage extracellular traps (METs) produced by pro-inflammatory (M2) macrophages in response to neutrophils undergoing NETosis [30]. Thus, the IL-1β-NETs loop could also potentially result in excessive production of METs by macrophages. Indeed, the acute phase of COVID-19-mediated KD-like disease could be driven by macrophages, whereby infection activates the NLRP3 inflammasome complex [31,32]. This activation would result in a further release of IL-1β and recruitment of increasing levels of neutrophils and NETosis [27], which would in turn activate macrophages to produce METs. The spilling over of NETs and METs into circulation can result in vasculitis, thrombosis and multiorgan failure seen in these patients (Figure 1).

5. The role of extracellular traps in thrombosis, vasculitis, and Kawasaki Disease

Excessive NETs release has been associated with alveolar damage and accumulation of edema, endothelial injury and coagulopathy, elevated platelet activation, and thrombogenesis [27]. NETs may induce thrombin formation by developing scaffolds that trap platelets and pro-thrombogenic factors, forming large aggregates (both with and without fibrin), capable of blocking microvasculature without activation of coagulation pathways and thrombus formation [33]. Furthermore, individual NETs components such as DNA, histones, and proteases may also induce thrombosis [33,34]. Excessive NETs production promotes anti-neutrophil cytoplasmic antibody (ANCA) production, also linked to vasculitis occurrence [35], leading to ANCA-associated vasculitis (AAV), which affects small vessels and it is accompanied by elevated ANCA levels in COVID-19 patient serum [35]. Collectively, such mechanisms may represent a further indirect pathway towards increased small/medium vessel thrombogenesis.

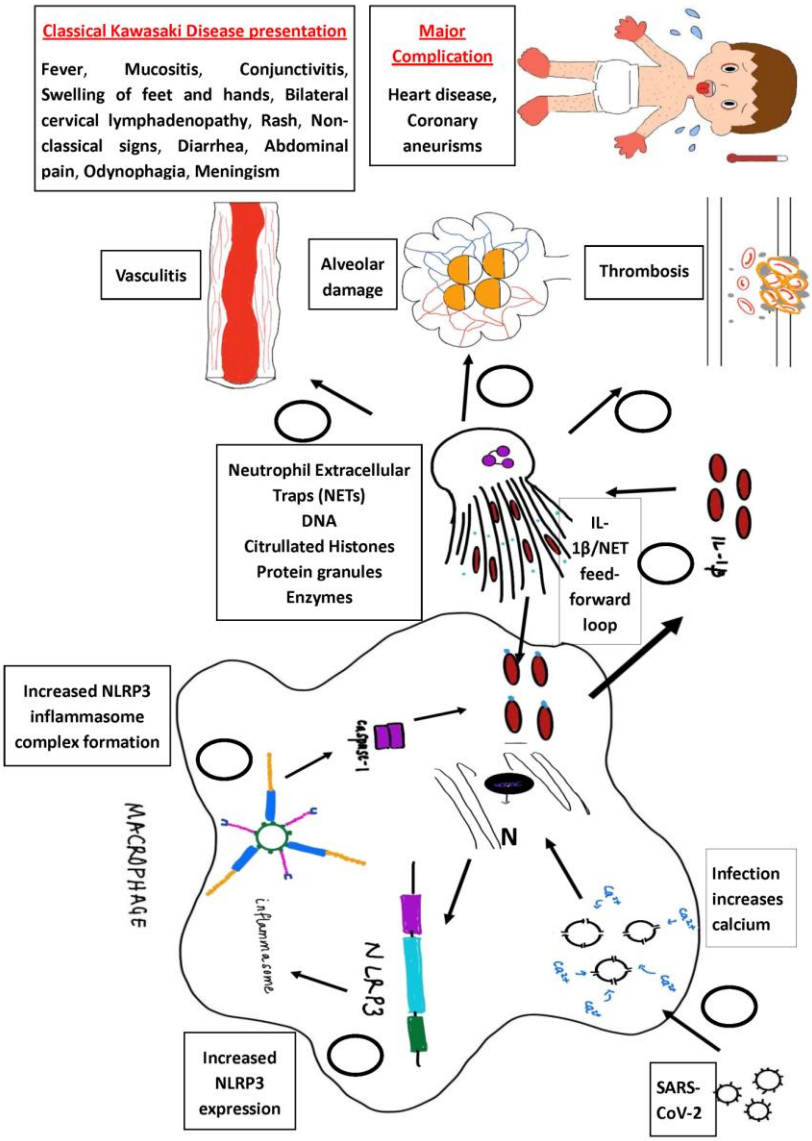

Figure 1. Pathophysiology of Kawasaki like disease. Schematic visualization of the proposed mechanism underlying increased Neutrophil Extracellular Trap (NET) production, mediated via NLRP3 inflammasomes and elevated production of IL-1β, presented chronologically in response to SARS-CoV-2 infection of macrophages, potentially leading to occurrence of Kawasaki Disease-like symptoms in infected children (steps 1–7). Viral infection increases calcium ionic (Ca^{2+}) influx, enhancing NLRP3 expression and increased formation of NLRP3 inflammasome complexes within the macrophage. Elevated inflammasome complex levels in turn elevates cleavage of pro-IL-1β to IL-1β via increased caspase production. Elevated IL-1 β will enter a 'feed-forward loop' with the NLRP3 inflammasome, and further enhance levels of NET production. Such highly increased levels NET levels will result in increased clot formation, endothelial damage, and alveolar damage associated with COVID-19, also contributing towards thrombosis and vasculitis contributing towards the typical presentations observed in children diagnosed with KD.

Macrophages also recognize pathogen-associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) via pattern recognition receptors (PRRs), releasing a number of cytokines including IL-1β, IL-6, and TNF-α in response to infection [36]. These cytokines act as endogenous pyrogens, increasing the thermoregulatory set-point in the hypothalamus resulting in fever [37]. Induction of IL-6 by IL-1β can also induce acute-phase proteins from the liver [38], while TNF-α causes local vasodilation and vascular leakage resulting in rubor and edema [39]. Viral antigens can also persist in the respiratory epithelium and macrophages in KD patients, which are intermittently shed into circulation and target coronary vessels [7], stimulating IgA secreting antigen-specific plasma cells and $CD8^+$ cells culminating in coronary vessel damage [7,40,41]. Perhaps it is the combination of both mechanisms that could result in occurrence of KD-like pathogenesis in COVID-19 children, resulting in damage to coronary vessels in the post-infectious phase.

6. Discussion

A number of studies have identified genes and associated specific single nucleotide polymorphisms (SNPs) associated with the occurrence of KD, most of which have an immune-regulatory function [42]. However, one of the more recent and more relevant KD-associated pathways to be identified involves the inositol-triphosphate 3-kinase (ITPKC) gene, expression of which mediates intracellular Ca^{2+} release [43,44] thought to act as a key second messenger in T cell receptor signaling, potentially influencing a greater and more prolonged expansion of inflammation, increasing risk/severity of KD [45]. Critically in the case of NET/MET-mediated thrombosis and vasculitis in COVID-19 patients, Alphonse et al. suggested that ITPKC action is macrophage-dependent, influencing NLRP3 activation through intracellular Ca^{2+} levels leading to an increased IL-1β and IL-18 production [44].

Considering the above discussion, we suggest that cytokine profiling of such patients (including IL-1β, IL-6 and TNF-α) should be investigated. Additionally, biomarkers for circulating NETs including cell free DNA (cfDNA), DNA-enzyme complexes, citrullinated histone 3 (H3-Cit) and NET-associated enzymes (such as MPO) should also be investigated. Additionally, these patients should be evaluated for ANCA positivity. Standard treatment in such patients involve the use of intravenous immunoglobin, aspirin, and corticosteroids [13]. Considering that inflammasome complex activation and subsequent excessive release of NETs/METs in the acute phase of this disease, we suggest drugs that block/disrupt the IL-1β-NETs feedback loop, such as Ankinara which can which block IL-1β production [27]. Recombinant DNase-1 (Dornase alfa) can also be used to neutralize circulating NETs [46], while Silvelestat which is a NET-enzyme inhibitor could also be considered. Silvelestat is also currently approved for treatment of acute respiratory distress syndrome can be added to evaluate its efficacy in limiting alveolar and endothelial damage in these patients [47].

Conflict of interest

Authors declare no conflict of interest.

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