Review

Xenosialylation: Role in SARS/CoV-2 Post-infectious and Post-vaccination Complications and Common Cause of Hyperimmune/Autoimmune Diseases

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Review

Xenosialylation: Role in SARS/CoV-2 Post-Infectious and Post-Vaccination Complications and Common Cause of Hyperimmune/Autoimmune Diseases

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Abstract: The host glycosylation mechanism synthesizes the carbohydrates of glycoproteins of viral envelopes. Due to the loss of the Cytidine Monophospho-N-Acetylneuraminic Acid Hydroxylase gene by some mammalian species, including humans (negative-CMAH), Neu5Gc is no longer synthesized. Uptake of Neu5Gc by negative-CMAH species, through the intake of food products derived from positive-CMAH mammals, leads to incorporating Neu5Gc-glycans in the glycocalyx (xenosialylation). Neu5Gc, being a Mammalian-associated Carbohydrate Antigen (MCA), acts as a non-self-antigen, inducing an inflammatory reaction (Xenosialitis), and triggering the production of circulating antiNeu5Gc antibodies to attack/remove all incorporated Neu5Gc. In the state of Xenosialitis, the virus-neutralizing antibodies produced by a heavily xenosialylated patient following exposure to viral infection (including SarsCoV2) or anti SarsCoV2 vaccination cross-react against all incorporated non-self Neu5Gc-MCA glycans due to their resemblance with viral envelope antigens synthesized by the host glycosylation mechanism. In addition, the circulating anti-XeSias antibodies determine the massive removal of the circulating neutralizing FC-xeno-contaminated antibodies by the serum, living only the hyper-inflammatory agalattosylated antiviral IgG antibodies. Therefore, we hypothesize that the combination of antibody cross-reaction against non-self Neu5Gc-MCA glycans and the massive removal of the xeno-contaminated newly formed neutralizing antibodies in favor of hyper reactive antibodies, could be the cause of the massive inflammatory reaction (cytokine storm, coagulopathies, neuropathies) observed in Covid 19 patients or after anti-SarsCoV2 vaccination. This analysis is an invitation to investigate the post-infectious and/or post-vaccination adverse phenomena in the light of xenosialization as a key to understanding the severe post viral and post vaccine complications, including SARS-CoV2 infection or related vaccination.

Keywords: sialic acid; sialylation; xeno-sialic acid; COVID-19; SARS-CoV-2

Introduction

Sialic acid (SiA) is a generic term to indicate a large family of nine-carbon acids that are present on the glyco-conjugated molecules (Glycans) associated with the membrane surfaces of most eukaryotic cells [1]. Sialylation is the addition of sialic acid units at the terminal ends of oligosaccharide chains of the glycans incorporated in the vertebrate cell surface and secreted glycoconjugates known as glycocalyx [2,3]. The presence of SiAs on glycans confers important physiological and immunological properties, such as a negative charge of water molecules to keep the cell surface hydrated, protection of glycoproteins from protease attack, intercellular adhesion, cell signaling, and immunological recognition [1–4]. Sialic acid (SiA) represents the main component of the mucous lining; it has the function of protecting the underlying cellular strata in organs such as the intestine, lung, uterus, oviduct, spermatic ducts, glandular tissue, and most of the body fluids, including saliva, from which the term "sialic" derives [3,5]. SiA always occupies the terminal part of all the polysaccharide structures of the cell membrane glycocalyx of vascular and ductal endothelial cells, including the more specialized structures of cell membranes such as the myelin sheath of the axons of the nerve cells of the Nervous System (NS), as well as the gangliosides lining the neuronal

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synaptic structures [6–9]. Sialic acid-containing glycans [sialoglycans] represent the specific self-associated molecular patterns (SAMPs) on the glycocalyx of healthy cells [10]. The sialoglycans are recognized as SAMPs by Sialic acid-binding immunoglobulins type lectins (Siglecs), the specific protein receptors exposed on the surface of several immune cells (such as monocytes, macrophages, dendritic cells, natural killer cells, B lymphocytes, neutrophils, and eosinophils), myeloid progenitors, trophoblasts and osteoclasts [11]. Siglecs, by interacting with SiA of the sialoglycan-SAMPs of the same cells, as well as adjacent ones, discriminate between the body's own (self) and foreign antigens (not-self). This means that Siglecs (e.g., CD33), as well as Factor H and CD24, have all been identified as potential SAMP-Recognizing Receptors (SAMP-RR), having the role of repressing immune responses [12,13]. There are at least 16 Siglecs expressed by different leukocyte populations [14]; some of these interpret "non-pathogen glycans" as "self" (SAMPs) and deliver inhibitory signals to immune cells to prevent them from becoming overstimulated [15]. Several studies suggested that any changes in glycan sialylation could modulate the inflammatory responses, regulate apoptosis, allow for viral immune escape, and promote cancer cell metastasis [3]. In particular, the sialylation of the Fc region of antibodies has shown to be a homeostasis that reduces the immune system's inflammatory reaction [13,16]. It has been proved that the sialylation of the IgG Fc-glycan of the circulating antibodies has a potent and stable anti-inflammatory activity [13], while the removal/reduction of the sialic acid induced an increase of the immune reactivity and inflammatory status [13,16]. Moreover, the rapid clearance of platelets from circulation occurs through the loss of terminal sialic acid, which represents the signal for their removal and destruction [17]. Removing terminal Sia of glycoconjugates by sialidases or oxidative damage initiates a series of cell-signaling events of innate immunity, especially in the nervous system [9].

It is not at all surprising that numerous pathogens and symbionts have evolved highly specific ways to recognize aspects of the dense and complex forest of cell surface glycans they encounter in host species. SiA viral recognition has been known to be a virulence factor for several pathogens [1]. Many bacteria and viruses, as well as Adenovirus, Reovirus, Orthomyxovirus [influenza virus], and Coronaviruses, have evolved to use SiA to evade the host's immune system. SiA participates, directly and indirectly, in the "coating" and "budding" of many enveloped viruses. Enveloped viruses use receptors containing SiA, for example, ACE2 receptors, to enter and subsequently exit the cells, thanks to their neuraminidases [enzymes capable of binding the terminal SiA of the specific receptor [18]. These interactions often involve Glycan-Binding Proteins [GBP] and can result in symbiosis, commensalism, or disease [3]. On the other hand, as a host defense mechanism, sialylated O-linked glycans covering mucins on mucosal cell surfaces provide a large layer of sialylated residues that act as a barrier, preventing pathogens from entering the cell by offering a decoy alternative binding site [3].

Xenosialylation

Most mammals express two common SiAs: N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) [19]. However, humans, ferrets (mustelids – weasel family), and few other mammals (bats, procyonids, pinnipeds) are deficient in Neu5Gc synthesis because of a specific mutation inactivating the Cytidine Monophospho-N-Acetylneuraminic Acid Hydroxylase (CMAH), gene responsible for converting CMP-Neu5Ac to CMP-Neu5Gc [20,21]. In humans, Neu5Gc (originally called non-human-SiA) [22], here named XeSiA-Neu5Gc, can be assimilated by dietary products, such as edible mammalian meat, milk, fish eggs (but not fish muscles) and edible echinoderms or can, also, be introduced by therapeutic administration of pharmaceutical preparations deriving from non-human cells cultured *in vitro*, as well as glycosylated biopharmaceutics such as recombinant glycoproteins [23,24]. Since human biochemical and cellular pathways cannot distinguish Neu5Gc from Neu5Ac, the assimilated XeSiAs-Neu5Gc are indifferently exposed on cell membrane glycans of the host cell surface, causing Xenosialylation [23–27]. It has been shown that XenoSiAs-Neu5Gc-glycoproteins ingested from food intake are incorporated into the enterocytes of the small intestinal tract and then appear in circulation at a steady-state level for several hours, followed by the metabolic incorporation into multiple

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glycoproteins and glycolipids of the entire body tissues as well as in vascular glycocalyx of the entire circulatory system or gangliosides of the peripheral and central nervous system in relation to the cellular SiAs turn-over [19,28]. The immune system recognizes the exposed XeSiAs-Neu5Gc, as a "not-self" (PAMPs) embedded in the context of the "self" (SAMPs), firstly by T lymphocytes TLRs (innate immune system), which trigger the release of local inflammatory cytokines against the XeSiA-Neu5Gc (inflammatory condition of Xenosialitis), and, secondly, by lymphocytes B stimulation, which starts to produce circulating anti-Neu5Gc antibodies (adaptative immune system), which permanently remove all incorporated XeSiAs-Neu5Gc (de-xenosialylation). The incorporated XeSiAs are Mammalian-associated Carbohydrate Antigens (MCAs) [30], which trigger an auto-immune reaction [30–32] like after exposure to mammalian serum [serum sickness] [22,32] or xenograft [29].

It has been demonstrated that a high levels of Xenosialylation of the cell surface, and relative inflammatory Xenosialitis condition, is associated with the long-term appearance of cancer, atherosclerosis, and several autoimmune diseases [31,32]. The appearance of circulating anti-Neu5Gc antibodies has been observed during the first year of life (early xenosialylation), and it is correlated with the presence of XeSiAs-Neu5Gc in the diet (mainly by mammalian xenosialylated milk and meats, particularly belonging to ruminants) of children not breastfed (bottle-feeding) or in early weaned ones [34,35]. Introducing the XeSiA-Neu5Gc into the host gut has an important impact on the intestinal microbiome composition [36,37]. It is currently known that a Neu5Gc-rich diet induces changes in the gut microbiota, which can assimilate the xeno-glycans derived from food and seems to be responsible for immunoglobulins (IgM, IgG, and IgA) variations against Neu5Gc. XeSiAs-Neu5GC bound to milk oligosaccharides and/or mucin/muscles-derived glycans of ruminants or other edible positive CMAH species are released by gut bacteria possessing sialidases (i.e., *clostridia* and *bacteroides*); free XeSiAs, not used as a carbon source by gut bacteria, are delivered and can be assimilated by intestinal cells or can be incorporated on the gut bacteria surface, determining gut hypersensitivity to bacterial metabolites and toxins, recurrent intestinal inflammation, and infant and adult autoimmune diseases [4,5,36,38,39]. Bacterial sialidases that cleave sialic acid residues from complex glycan structures are unaffected by innate immune mechanisms and can synergize with other virulence factors, such as toxins [40]. Many bacterial adhesins and major classes of toxins as bacterial surface structures, such as lipo-oligosaccharide (LOS), lipopolysaccharide (LPS), and polysaccharide capsules, contain SiA and glycans as receptors [40]. During biosynthesis, most human-adapted pathogens integrate on their bacterial surface structures, the SiA-Neu5Ac, as a prevalent form to mimic the host and subvert the human host immune system [40]. However, the bacterial sialidases can cleave all types of sialic acid residues from complex glycan structures, allowing the incorporation of the foodborn-free XeSiA-Neu5Gc by gut bacteria transforming the gut microbiome in “Xeno-enhanced Microbiome”. This mechanism exacerbates bacterial virulence factors, such as pathogen toxins [40], and enhances the host inflammatory immune reaction at the host gut level [32]. The consequences of exposure to a “Xeno-enhanced Microbiome” are chronic inflammatory response at the gut epithelial tract with gut hypersensitivity to bacterial metabolites and toxins, recurrent intestinal inflammation, predisposing to inflammatory gut permeability, thus facilitating the onset of infant and adult autoimmune diseases [4,5,36,38,39] and of diet-related colon carcinomas [32,38,41].

It has been reported that patients with cancer have circulating heterophile antibodies that agglutinate animal red cells via recognition of the mammalian cell surface XeSiA-Neu5Gc [33]. Also, it has been demonstrated that human anti-Neu5Gc antibodies interact with metabolically incorporated Neu5Gc (state of Xenosialylation) in the tissues or organs of the host to promote local chronic inflammation (inflammatory state of Xenosialitis), therefore contributing not only to diet-related carcinomas and tumor progression [41] but also to the onset of vascular inflammation (atherosclerosis, coagulopathies) and autoimmune diseases [32]. It has been documented that the circulating anti-Neu5Gc antibodies are also involved in atherosclerosis, type 2 diabetes, and “Latent Autoimmune Diabetes in Adults” (LADA) [31,42–44]. Furthermore, Multiple sclerosis (MS), Guillain-Barré syndrome (GBS), Rheumatoid arthritis (RA), Systemic lupus erythematosus (SLE), Autoimmune thyroid disease, Kawasaki disease (KD), post-COVID-19 Multisystem Inflammatory

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Syndrome in Children (MIS-C), and Adults (MIS-A), are some of the autoimmune diseases in which autoantibodies (Xeno-autoantibodies) are always detectable in the host serum [45–47]. Although clinically different, all autoimmune disorders share common immunopathogenic mechanisms. Moreover, interestingly, one Autoimmune Disease (AD) may coexist with others (i.e., poly-autoimmunity), which may exhibit different autoantibodies with different specificities supporting the correlation with polyclonal anti Neu5Gc antibodies directed to several organs/tissues [48].

Recurring Complications Related to Viral Infections: Autoimmune Disorders

This new pandemic has made it possible to collect a significant amount of data from all over the world about post-infective complications and post-vaccination side effects. The severe complications observed in some Covid-19 patients, both elderly and very young, and all the severe side effects after anti-SarsCoV2 vaccinations are very similar to the complications/side effects following many other viral infections/vaccinations. Based on epidemiological and experimental evidence, there is a growing awareness of the role of infectious diseases in the onset of autoimmune disorders [46]. Literature refers that most patients showing severe complications during or after Covid 19 diseases are affected by comorbidities, which recognize an autoimmune cause predisposing to a higher incidence of such severe complications. Generally, immunological diseases are classified into three major groups: autoinflammatory, autoimmune, and mixed pattern [49]. Many common diseases with strong inflammatory components could be classified as predominantly autoinflammatory, although most conditions also have evidence for autoimmunity in the clinical setting. This means that all immunological diseases can be conceptualized as being purely autoinflammatory or autoimmune or a combination of autoinflammatory-autoimmune mechanisms that variably interact in the phenotypic expression of disease [49]. Associations between viral infections and autoimmune diseases have been reported repeatedly in the literature [50]. Active human cytomegalovirus (HCMV) infection is often found in patients diagnosed with Immune Thrombocytopenic Purpura (ITP) [51] and in SLE [52]. Another virus with a reported association with SLE is the Epstein-Barr virus (EBV) [53,54]. High titers of anti-EBV antibodies are also present in patients affected by RA [55]. Other examples of viral infections linked to autoimmune diseases such as islet autoimmunity and type 1 diabetes include enteroviruses (e.g., coxsackievirus A4, coxsackievirus A2, and coxsackievirus A16) and measles, mumps and rubella [56,57]. Antibodies against several common human coronaviruses have been linked with autoimmunity in people with MS, SLE, Sjögren's syndrome, and mixed connective tissue disease [58–60]. Considering these associations between coronaviruses and autoimmunity and the sequence similarity of SARS-CoV-2 to these viruses, the same link between SARS-CoV-2 and autoimmunity is highly plausible [61]. To our knowledge, numerous studies demonstrated that COVID-19 is associated with a different degree of risk for various autoimmune diseases [62]. Many authors recognize a correlation between the complications of COVID-19 disease and the GBS, autoimmune hemolytic anemia, thyroiditis, Immune Thrombocytopenia (ITP), and KD [63–67]. Moreover, another series of studies has shown that COVID-19 leads to an aggravation of auto-immune pathologies already present in the patient, such as MS, SLE, or different forms of coagulopathy [68–73]. Most Covid-19 patients who develop severe complications are systematically affected by a large variability of autoimmune/chronic inflammatory immune-related comorbidities such as antiphospholipid syndrome (APS), systemic vasculitis, systemic autoimmune diseases, GBS, Kawasaki syndrome and MIS-C [66,74–79]. Studies report the concomitant or following COVID-19 disease of autoimmune-like and hyperinflammatory vascular/neurological manifestations such as the secondary hemophagocytic lymph-histiocytosis (HLH), ITP, Autoimmune Hemolytic Anemia (AIHA), thrombotic events associated with anti-Phospholipid antibodies (aPLs) and Lupus Anticoagulant (LAC) as in the Antiphospholipid Syndrome (APS), GBS and neurologic sensory-motor demyelinating syndrome resembling GBS, Miller Fisher Syndrome, Kawasaki-like disease, arthritis, skin manifestations [39,78,80]. A recent study reported an unusually high level of antibodies to GBS glycans in 15% of Covid-19 patients and to several other glycolipids not associated with GBS, such as GD3, fucosyl-GM1, GM2, and GM3 >35-fold higher than the largest signals in the control group; when considering all the glycolipids (GBS

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and non-GBS), 35% of the patients had high antibodies to at least one glycolipid [81]. Interestingly, antibody signals to gangliosides did not correlate with IgG titers to the spike protein, supporting the hypothesis that the severe complications resembling autoimmune diseases in Covid-19 are not due to viral circulation or direct viral cell damage [81]. After a SARS-CoV2 infection, often regardless of its severity, many patients continue to show clinical signs of debilitation, easy fatigue, concentration difficulties, and memory problems, even for several months, experiencing the so-called "Long COVID" syndrome [82]. This syndrome, more frequent in women, may result from poly-organ damage induced either by the excessive inflammatory response activated by the virus or by an autoimmune reaction unmasked by SARS-CoV-2 [82]. Women would be more exposed to this syndrome as they have stronger innate and acquired immunological responses than males, and both genes and hormones are involved in this sexual difference [83]. These different dynamics in immune response between women and men can explain why there is a higher incidence of autoimmune diseases in women, while in men, the susceptibility to neoplasms and infectious diseases is higher [83,84]. Morniroli et al., 2020 [85] postulated that the epidemiologic characteristics of COVID-19 [greater severity in male and older individuals] might be partially explained by the sex and age-related differences of sialome among humans and that COVID-19 clinical manifestations differ according to individual differences in the sialic acid expression on cell surfaces. Similarly, Torres-Acosta and Singer, 2020 [86] speculated that SARS-CoV-2's ability to affect T lymphocyte and myeloid cell physiology, coupled with age-related maladaptive biological phenomena (epigenetic alterations and mitochondrial dysfunction, telomere attrition, and cellular senescence, altered intercellular communication), explains the strong association between advanced age and increased risk of COVID-19-related morbidity and mortality. The authors explain the similarity of the ARDS, induced by both Sars-CoV-2 and Influenza A virus, as an "immunological reaction" to the presence of viral RNA in the host cell cytoplasm during the replication cycle. Indeed, they activate similar intracellular antiviral pathways and subsequent recruitment of similar immune cells to the respiratory epithelium [87–89]. This is the same mechanism observed in many autoimmune diseases, e.g., Kawasaki Syndrome [90], as well as in many other viral infections, including influenza, in which the Macrophage Activation Syndrome [MAS] - a life-threatening clinical entity - occurs [87]. However, these hypotheses do not explain why the same reactions, considered by all authors mentioned above to be related to age and aging, occur in very young patients with autoimmune syndromes such as Kawasaki Syndrome [90,91]. Furthermore, these hypotheses do not explain the constant correlation between the severe complications in some cases of Covid-19 and comorbidities such as diabetes, atherosclerosis, cardiovascular diseases, etc.

Adverse Reactions After SARS-CoV2 Vaccination

The commonly reported adverse events after SARS-CoV2 RNA vaccination are usually mild and self-limited, including pain, redness or swelling at the site of vaccine injection, headache, fever, fatigue, myalgia, muscle pain, nausea, vomiting, itching, chills, joint pain and rarely anaphylactic shock [92]. Generally, these reactions are not associated with serious illness. Most mild symptoms post-vaccination are very similar to the same non-serious symptoms of SarsCoV-2 infection but without the involvement of the respiratory tract [93]. Data monitoring conducted as part of the US vaccination program indicates that most non-serious adverse reactions occur within two days of vaccination and almost all within seven days [94,95]. Until now, only a case of reported COVID-19 vaccine-related liver injury, classified as autoimmune hepatitis, has been described [96] in a patient 26 days after the injection of the first dose of the vaccine (Oxford-AstraZeneca). The occurrence of adverse effects is reported to be lower in the Pfizer/BioNTech vaccine compared to the Moderna vaccine [93]. However, it is worth noting that more severe post-vaccination reactions to the anti SarsCoV2 vaccine are accompanied or followed by similar inflammatory/autoimmune syndromes induced by SarsCoV-2 infections in Covid-19 diseases [97,98], as well as by many other neurological and vascular disorders [99]. Sometimes, the phenomenon is accompanied by the reactivation of latent viruses, such as Epstein Barr Virus [100] or Herpes Viruses [99,101], particularly in patients with pre-existing autoimmune diseases.

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Similar complications, mainly at the neurological or vascular level, have also been reported in the case of IAV or Herpes Zooster vaccination [102–104]. Differences between sex and age have been observed in the vaccine biology field. It is well established that, compared to males, which showed a prevalence of myocarditis and pericarditis [105], females develop higher antibody responses related to autoimmune diseases and report more adverse reactions following vaccination [106,107]. In late February 2021, atypical thrombotic events following immunization with the adenoviral vector-based vaccine were first reported, and similar events have also been observed in recipients of other adenoviral vector-based vaccines and the mRNA-based vaccines [100,108–111]. Adverse reactions [thrombus formation] have been reported after the administration of Oxford–AstraZeneca chimpanzee adenovirus vectored vaccine ChAdOx1 nCoV-19 (AZD1222) [112], which has led several European countries to discontinue the administration of this vaccine [113].

These manifestations of coagulopathies, such as atypical thrombosis and thrombocytopenia following anti-SarsCoV2 vaccine immunization, are collectively referred as Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT) or Vaccine-Induced Prothrombotic Immune Thrombocytopenia (VIPIT) or Thrombosis with Thrombocytopenia Syndrome (TTS) [114–116]. The clinical features of the vaccine-induced phenomenon consisted of severe thrombocytopenia, aggressive thrombosis, and Disseminated Intravascular Coagulation (DIC), which resembles the coagulopathy seen in patients with Heparin-induced thrombosis (HIT) [117]. A study suggested that VITT is generated by a 2-step mechanism [109]. First, Platelet Factor 4 [PF4] interacts with vaccine components [including adenovirus hexon protein] to generate neo-antigens [109]. Second, anti-PF4 antibodies induce platelet and granulocyte activation 5–20 days after vaccination, driving the release of Neutrophil Extracellular Traps (NETs) that induce immune-thrombotic pathways, which may lead to clinical complications [109]. Although these events bear clinical resemblance to auto-immune heparin-induced thrombocytopenia (HIT), most cases had no known exposure to heparin before the onset of illness [109,118]. Alternatively, endogenous glycosaminoglycans present in the endothelial glycocalyx, resembling exogenous heparins, may play a role in the aberrant immune response in VITT cases. Damage to the glycocalyx may release fragmented forms of glycosaminoglycans that trigger an immune response upon electrostatic interactions with PF4 [119]. Early reported mortality rates amongst patients with Vaccine-Induced Thrombotic Thrombocytopenia (VITT), especially those with cerebral venous sinus thrombosis (CVST), were high, ranging between 30% and 60% [109]. However, it is still unclear why this immune-mediated thrombosis manifests mainly in the cerebral vessels and splanchnic circulation [118]. It should be noted that the risk of VITT remains far lower than the risk of thrombotic complications stemming from COVID-19 infection, especially for severe hospitalized cases, and thus the benefit of vaccination still outweighs the risks [100].

Discussion

Human natural antibodies against Neu5Gc-MCA, synthesized by negative-CMAH mammalian species, including humans, represent an adaptation strategy against viral zoonoses for blocking viral infections affecting positive-CMAH species [30]. By producing anti- Neu5Gc-MCA antibodies, negative-CMAH species, as humans, can block the potential zoonotic passage of viruses, normally confined to one or a few other positive-CMAH mammalian species [120]. The carbohydrate chains of the viral envelopes and their spikes are synthesized using the host glycosylation mechanism therefore, they are antigenically like the carbohydrate chains of a mammalian host [30]. However, a prolonged intake of Neu5Gc by humans, through administration of serum/pharmacologic derivatives [23;24] or, more frequently, through the intake of products derived from the tissues of positive CMAH mammals (meat, dairy) [28] - particularly in the presence of a microbiota poor in de-sialylating bacteria belonging to Clostridiales and Bacteroidales [36] - leads to progressive systemic contamination of the cell membranes by XeSia-Neu5Gc, also called Xenosialylation of the host, transforming all xenosialylated epitopes in a potential MCAs. The immunoreaction against the XeSiAs-Neu5GC-MCAs starts an inflammatory state characterized by lymphocytic infiltration, release of inflammatory cytokines, and tissue damage followed by production of specific anti-Neu5Gc antibodies, which attack/remove all the incorporated XeSiAs-Neu5GC-MCAs on host tissue

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(de-xenosialylation), including circulating antibodies contaminated at the FC region by Xeno-Neu5Gc. If xenosialic acid continues to be available from the diet or iatrogenic administrations, an uninterrupted vicious circle is established involving the persistence of related symptoms and the onset and/or exacerbation of autoimmune, neurological, or vascular disorders. It is well known that when an infectious agent infects the host (spontaneous viral infection) or is inoculated as a specific antigen for vaccination, the immune system triggers a systemic alarm to localize and identify the presence of the pathogen/antigen. We assume that, in a situation of pre-existing heavy xenosialylation and active state of Xenosialitis, with high levels of circulating anti-XeSiA-Neu5Gc-MCAs antibodies, neutralizing antiviral antibodies produced for fighting the SarsCoV2 infection, cross-react with all the xenosialylated XeSia-Neu5Gc-MCA epitopes due to their antigenic resemblance with the virus envelope antigens of those viruses that use the host glycosylation mechanism for forming their envelope, as SARS CoV-2 as well as other enveloped viruses (i.e., IAV). It is also plausible that the rapid and massive production of neutralizing viral antibodies to fight the ongoing viral infection causes the massive XeSiA contamination of the Fc-glycans of the newly formed antibodies (mainly IgG) which will be targeted by the T lymphocytes inflammatory reaction, thus altering the balance between circulating inflammatory and anti-inflammatory antibodies in favor of hyperactive ones (agalactosylated antibodies) [13], typically observed in inflammatory/autoimmune diseases [13,16,121]. Therefore, in relation to the grade of contamination of the MCA epitopes and circulating neutralizing antibodies serum level, the host's immune reaction becomes exasperated, massive, and widespread. This justifies and explains all the various forms of cytokine storm around 15 days after Covid-19 diseases, as well as the coagulopathies, neuropathies, and all the forms of adverse immunologic reactions currently observed in the last SarsCoV2 pandemic, both in adults and children, as reported in severe inflammatory clinic entity of Covid 19 and the Long Covid syndrome [90,122], but also observed after other severe pandemics (mainly IAV pandemics) [123].

The same mechanism comes into play when the vaccine alarm is activated in a highly xenosialylated host. In fact, the immune system activated by the inoculated antigen reacts against every xenocontaminated cell surface, also altering the balance of circulating antibodies in favor of hyperactive agalactosylated antibodies, thus triggering all the different degrees of immunological side effects reported in the literature. [43,44,124]. This phenomenon explains the onset or exacerbation of several types of the reported autoimmune disorders, after vaccine adverse reactions, including the anti SarsCoV2 vaccination. The same massive production of autoantibodies is reported both in the Long Covid complications and post-vaccination adverse reactions [122].

The clinical entities of dysregulated immune reactions, which are differentiated between the genders [125], are related to the different distribution of the xenosialylated epitopes. The xenosialylation involves all the ACE Receptors (which contain Sialic acid for virus entering), and sex hormones mediate their distribution in association with the protective effect of the estrogens during the fertile phase of a woman's life [83,84,106,107]. The state of Xenosialylation and, consequently, Xenosialitis inflammatory state can, therefore, explain why the coagulopathies and clot disorders are so frequent, especially in women than in men, and why men are more prone to severe Covid-19 diseases and myocarditis and cardiovascular disorders than women who, on the other hand, are more prone to develop autoimmune post-infective complications and post-vaccination side effects.

Moreover, the high frequency of all types of coagulopathies observed both during the Covid-19 pandemic and after antiSarsCov2 vaccination can be explained by the xeno-contamination of the endothelial vascular system, in which the contact with the Anti-MCAs of the xenosialylated endothelial surface triggers the platelet activation and NETs formation, inducing the thrombotic phenomenon also in patients which never have been exposed to heparin.

In our opinion, it is highly plausible that heterogeneous autoimmune clinical entities such as Type I, II, and LADA diabetes, Rheumatoid Arthritis, Multiple Sclerosis, GBS, SLE, and many others, as all the described Thrombotic disorders, could be caused by the immunological chronic reaction to the individual incorporation and distribution of XeSiAs-Neu5GC self-antigens in the specific districts/organs [43,44]. The neurological syndrome observed both in the Long Covid complications

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and after the anti-SarsCoV2 vaccination can be explained by the high concentration of sialic acid (75% of Sialic acid is concentrated in the Nervous System) at the level of the structural gangliosides of the Nervous System for which the xeno-sialization of this district can trigger serious inflammatory/autoimmune consequences [9]. The heavy xeno-contamination of the nervous system and related inflammatory/autoimmune reactions can cause the polyneuropathies reported both after spontaneous viral infection or vaccinations.

Conclusions and Future Directions

The severe complications observed in some Covid-19 patients, both elderly and very young, and after anti-SarsCoV2 vaccinations are like those observed in all other viral infections/vaccinations (typically after infection and/or vaccination against Influence Virus A - IAV, Epstein Barr Virus – EBV). This means that the mechanism of such exasperated inflammatory/autoimmune reaction is common to all viral infections and vaccinations. The collected pandemic data demonstrates/confirmed that most of these patients are affected by other comorbidities that recognize an autoimmune cause predisposing to a higher incidence of those severe complications. In this review, we illustrate a new theory to explain the relationship between the severe inflammatory reactions observed in Covid-19 patients or after the anti-SarsCoV2 vaccination and the inflammatory/hyperimmune/autoimmune diseases commonly observed.

We postulate the role of the not-self Neu5Gc (Xenosialic acid-XeSiA), incorporated on host Glycans as a self-antigen, acting as Mammalian-associated Carbohydrate Antigens [MCAs], as a common cause of all the severe inflammatory/autoimmune reactions observed both in severe cases of Covid-19 and/or anti SarsCoV2-vaccinations. The incorporation of Xenosialic acid on host organs and tissues triggers a simultaneous response of the innate immune system against all the previously incorporated xeno-antigen Neu5Gc, therefore initiating a "vicious circle" in which the presence of the xeno-antigen Neu5Gc triggers the inflammatory state of Xenosialitis with lymphocyte infiltration, cytokine activation against the exposed xeno-sialic antigens and produces anti xeno antigens antibodies which continuously attack/remove the xeno-Sias contaminated epitopes. The chronic low-grade inflammation towards the xeno-sialic acid, covering the cell surface of the organ/tissue, in which the turnover of xeno sialic acid is continuative, is the first step for determining the stochastic production of autoimmune antibodies and onset of relative organ autoimmune disease (type 1 diabetes, Hashimoto Thyroiditis, SLE, MS, RA, GBS, etc.). In case of a viral infection, such as that sustained by SARS CoV-2 or after anti-SarsCoV2-vaccination, in a heavy xenosialylated host presenting with a high grade of inflammatory state of Xenosialitis, it is highly probable and plausible that the virus-neutralizing antibodies, produced to combat the "antigenic alarm", indiscriminately cross-react against all MCA-contaminated XeSiA-Neu5Gc epitopes exacerbating the pre-existing condition of xenosialites and related forms of autoimmune pathology. Furthermore, the massive removal of newly formed xeno-contaminated neutralizing antibodies, in favor of those hyperreactive antibodies not containing Sias, also contributes to the amplification and exacerbation of all inflammatory/autoimmune reactions.

We are aware that the hypothesis presented here is merely speculative, based on the analysis and correlation of adverse phenomena reported by international literature and not on clinical studies. The intent of this analysis is, in fact, an invitation to clinical professionals dealing with patients with severe and persistent post-infectious and/or post-vaccination adverse phenomena to investigate these phenomena in the light of xenosialization as a possible innovative tool for identify and prevent the risk of serious complications, as well as Long-Covid syndrome, both in case of exposure to natural SARS-CoV2 infection and in case of exposure to related vaccinations.

Therefore, based on this hypothesis, further studies could focus on evaluating the total anti-MCA antibody titer in serum, in particular anti-Neu5GC antibodies as an easily verifiable parameter to explain the incidence and severity of viral infection and/or post vaccination adverse effects.

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References

1. Schauer R, Kamerling JP. Exploration of the Sialic Acid World. Adv Carbohydr Chem Biochem. 2018;75:1-213.
2. Schauer, R.. Chemistry, metabolism, and biological functions of sialic acids. Adv. Carbohydr. Chem. Biochem. 1982; 40:131–234. doi:10.1016/ S0065-2318[08]60109-2.
3. Varki, A. Biological roles of glycans. Glycobiology, 2017; 27, 3–49. doi: 10.1093/glycob/cww086
4. Severi E., Hood D.W., Thomas G.H. Sialic acid utilization by bacterial pathogens. Microbiology. 2007; 153:2817–2822. doi: 10.1099/mic.0.2007
5. Schauer, R. Sialic acids: Fascinating sugars in higher animals and man. Zoology 2004;107,49–64.
6. Brunngraber, E.G. Biochemistry, Function, and Neuropathology of the Glycoproteins in Brain Tissue. In: Davison, A.N., Mandel, P., Morgan, I.G. [eds] Functional and Structural Proteins of the Nervous System. Advances in Experimental Medicine and Biology, 1972; vol 32. Springer, Boston, MA.
7. Wang, B., Miller, B. J. The role and potential of sialic acid in human nutrition. Eur J Clin Nutr 2003; 57, 1351–1369.
8. Wang B. Sialic acid is an essential nutrient for brain development and cognition. Annu. Rev. Nutr. 2009; 29: 177-222.
9. Liao, H.; Klaus, C.; Neumann, H. Control of Innate Immunity by Sialic Acids in the Nervous Tissue. Int. J. Mol. Sci. 2020, 21, 5494. https://doi.org/10.3390/ijms21155494
10. Varki A. Since there are PAMPs and DAMPs, there must be SAMPs? Glycan "self-associated molecular patterns" dampen innate immunity, but pathogens can mimic them. Glycobiology. 2011 Sep;21[9]:1121-4. doi: 10.1093/glycob/cwr087. PMID: 21932452; PMCID: PMC3150115.
11. Wang X, Mitra N, Secundino I, Banda K, Cruz P, Padler-Karavani V, Verhagen A, Reid C, Lari M, Rizzi E, Balsamo C, Corti G, De Bellis G, Longo L; NISC Comparative Sequencing Program; Beggs W, Caramelli D, Tishkoff SA, Hayakawa T, Green ED, Mullikin JC, Nizet V, Bui J, Varki A. Specific inactivation of two immunomodulatory SIGLEC genes during human evolution. Proc Natl Acad Sci U S A. 2012 Jun 19;109[25]:9935-40. doi: 10.1073/pnas.1119459109. Epub 2012 Jun 4. PMID: 22665810; PMCID: PMC3382539.
12. Chen H.Y., Fermin A., Vardhana S., Weng I.C., Lo K.F., Chang E.Y.,] et al. Galectin-3 negatively regulates TCR-mediated CD4+ T-cell activation at the immunological synapse Proc Natl Acad Sci U S A, 2009; 106 [34, pp. 14496-14501.
13. Alter G., Ottenhoff T.H.M, Joosten S.A. Antibody glycosylation in inflammation, disease and vaccination. Semin. Immunol., 2018;39, pp. 102-110. ISSN 1044-5323.
14. Pillai S., Netravali I.A., Cariappa A., Mattoo H.. Siglecs and immune regulation Annu Rev Immunol, 2012; 30, pp. 357-392.
15. Maverakis, E.; Kim, K.; Shimoda, M.; Gershwin, M.E.; Patel, F.; Wilken, R.; Raychaudhuri, S.; Ruhaak, L.R.; Lebrilla, B.C. Glycans in the immune system and The Altered Glycan Theory of Autoimmunity: A critical review. J. Autoimmun. 2015, 57, 1–13.
16. Wong AHY, Fukami Y, Sudo M, et al, Sialylated IgG-Fc: a novel biomarker of chronic inflammatory demyelinating polyneuropathy. Journal of Neurology, Neurosurgery & Psychiatry 2016;87:275-279.
17. Sorensen AL, Rumjantseva V, Nayeb-Hashemi S, Clausen H, Hartwig JH, Wandall HH, Hoffmeister KM. Role of sialic acid for platelet life span: exposure of beta-galactose results in the rapid clearance of platelets from the circulation by asialoglycoprotein receptor-expressing liver macrophages and hepatocytes. Blood. 2009 Aug 20;114[8]:1645-54. doi: 10.1182/blood-2009-01-199414. Epub 2009 Jun 11. PMID: 19520807; PMCID: PMC2731641.
18. Byrd-Leotis, L.; Cummings, R.D.; Steinhauer, D.A. The Interplay between the Host Receptor and Influenza Virus Hemagglutinin and Neuraminidase. Int. J. Mol. Sci. 2017, 18, 1541. https://doi.org/10.3390/ijms18071541

19. Bergfeld A.K., Oliver M.T. Pearce, Sandra L. Diaz, Tho Pham, Ajit Varki. Metabolism of Vertebrate Amino Sugars with N-Glycolyl Groups: elucidating the intracellular fate of the non-human sialic acid N-glycolylneuraminic acid. Journal of Biological Chemistry. 2012; Volume 287, Issue 34.. Pages 28865-28881,ISSN 0021-9258 doi:10.1074/jbc.M112.363549.
20. Chou H.H., Takematsu H., Diaz S., Iber J., Nickerson E., Wright K.L., Muchmore E.A., Nelson D.L., Warren S.T., Varki A.. A mutation in human CMP-sialic acid hydroxylase occurred after the Homo-Pan divergence. Proc. Natl. Acad. Sci. USA. 1998; 95:11751–11756 10.1073/pnas.95.20.11751 - ù
21. Irie A., Koyama S., Kozutsumi Y., Kawasaki T., Suzuki A.The molecular basis for the absence of N-glycolylneuraminic acid in humans. J. Biol. Chem. 1998; 273:15866–15871 10.1074/jbc.273.25.15866 - DOI – PubMedDOI - PMC – PubMed.
22. Merrick, J.M.; Zadarlik, K.; Milgrom, F. Characterization of the Hanganutziu-Deicher [serum-sickness] antigen as gangliosides containing N-glycolylneuraminic acid. Int. Arch. Allergy Appl. Immunol. 1978, 57, 477–480.
23. Dicker, M.; Strasser, R. Using glyco-engineering to produce therapeutic proteins. Expert Opin. Biol. 2015, 15, 1501–1516.
24. Hombu R, Neelamegham S, Park S. Cellular and Molecular Engineering of Glycan Sialylation in Heterologous Systems. Molecules. 2021; 26[19]:5950.
25. Oetke, C., Hinderlich, S., Brossmer, R., Reutter, W., Pawlita, M., Keppler, O.T. Evidence for efficient uptake and incorporation of sialic acid by eukaryotic cells. European Journal of Biochemistry, 2001; 268 [16], pp. 4553-4561.
26. Bardor M, Nguyen DH, Diaz S, Varki A. Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells. J Biol Chem. 2005; 280:4228–37. doi: 10.1074/jbc.M412040200.
27. Collins, B.E., Fralich, T.J., Itonori, S., Ichikawa, Y., Schnaar, R.L. Conversion of cellular sialic acid expression from N-acetyl- to N-glycolylneuraminic acid using a synthetic precursor, N-glycolylmannosamine pentaacetate: Inhibition of myelin-associated glycoprotein binding to neural cells. Glycobiology, 2000; 10 [1], pp. 11-20.
28. Tangvoranuntakul P., Gagneux P., Diaz S., Bardor M., Varki N., Varki A., Muchmore E.. Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. Proc. Natl. Acad. Sci. USA. 2003; 100:12045–12050 10.1073/pnas.2131556100 - DOI - PMC – PubMed.
29. Paul A, Padler-Karavani V. Evolution of sialic acids: Implications in xenotransplant biology. Xenotransplantation. 2018 Nov;25[6]:e12424. doi: 10.1111/xen.12424. Epub 2018 Jun 22. PMID: 29932472; PMCID: PMC6756921.
30. Galili U. Human Natural Antibodies to Mammalian Carbohydrate Antigens as Unsung Heroes Protecting against Past, Present, and Future Viral Infections. Antibodies 2020; 9 [2], 25; https://doi.org/10.3390/antib9020025].
31. Padler-Karavani V., Yu H., Cao H., Chokhawala H., Karp F., Varki N., Chen X., Varki A. Diversity in specificity, abundance, and composition of anti-Neu5Gc antibodies in normal humans: potential implications for disease. Glycobiology. 2008; 18:818–830; 10.1093/glycob/cwn072.
32. Dhar C, Sasmal A and Varki A From “Serum Sickness” to “Xenosialitis”: Past, Present, and Future Significance of the Non-human Sialic Acid Neu5Gc. Front. Immunol. 2019; 10:807. doi: 10.3389/fimmu.2019.00807.
33. Zhu, A.; Hurst, R. Anti-N-glycolylneuraminic acid antibodies identified in healthy human serum. Xenotransplantation 2002; 9, 376–381.
34. Taylor RE, Gregg CJ, Padler-Karavani V, et al, Novel mechanism for the generation of human xeno-autoantibodies against the nonhuman sialic acid N-glycolylneuraminic acid. J Exp Med. 2010; 207[8]:1637-1646. doi:10.1084/jem.20100575.
35. Berger PK, Plows JF, Jones RB, Alderete TL, Yonemitsu C, Poulsen M, Ryoo JH, Peterson BS, Bode L, Goran MI. Human milk oligosaccharide 2'-fucosyllactose links feedings at 1 month to cognitive development at 24 months in infants of normal and overweight mothers. PLoS One. 2020; Feb 12;15[2]:e0228323. doi: 10.1371/journalpone.0228323. PMID: 32049968; PMCID: PMC7015316.
36. Zaramela LS, Martino C, Alisson-Silva F, Rees SD, Diaz SL, Chuzel L, Ganatra MB, Taron CH, Secrest P, Zuñiga C, Huang J, Siegel D, Chang G, Varki A, Zengler K. Gut bacteria responding to dietary change encode sialidases that exhibit preference for red meat-associated carbohydrates. Nat. Microbiol. 2019; 4:2082–2089.
37. Nishiyama K. and Mukai T. Sialic acid impact on the gut microbiome and function Glycoforum. 2022 Vol.25 [2], A3）DOI: https://doi.org/10.32285/glycoforum.25A3.
38. Byres E, Paton AW, Paton JC, et al, Incorporation of a non-human glycan mediates human susceptibility to a bacterial toxin. Nature. 2008; 456[7222]:648-652. doi:10.1038/nature07428.
39. Huang Y.L., Chassard C., Hausmann M., von Itzstein M., Hennet T. Sialic acid catabolism drives intestinal inflammation and microbial dysbiosis in mice. Nat. Commun. 2015; 6:8141. doi: 10.1038/ncomms9141.

40. Jennings M P., Day C J., Atack J M. How bacteria utilize sialic acid during interactions with the host: snip, snatch, dispatch, match and attach. Microbiology. 2022; Vol. 168, Issue 3, Pub: 22 March.
41. Hedlund M., Padler-Karavani V., Varki N.M., Varki A. Evidence for a human-specific mechanism for diet and antibody-mediated inflammation in carcinoma progression. Proc. Natl. Acad. Sci. USA. 2008; 105:18936–18941 10.1073/pnas.0803943105.
42. Stenström G., Gottsäter A., Bakhtadze E., Berger B., Sundkvist G. Latent Autoimmune Diabetes in Adults. Diabetes Dec 2005; 54 [suppl 2] S68-S 10.2337/diabetes.54.suppl_2.S68.
43. Bashir S, Leviatan Ben Arye S, Reuven EM, Yu H, Costa C, Galiñanes M, et al, Presentation mode of glycans affect recognition of human serum anti-Neu5Gc IgG antibodies. Bioconjug Chem. 2018 ; 30:161–68. doi: 10.1021/acs.bioconjchem.8b00817].
44. Löfvenborg, J.E., Ahlqvist, E., Alfredsson, L. et al, Consumption of red meat, genetic susceptibility, and risk of LADA and type 2 diabetes. Eur J Nutr 2020. https://doi.org/10.1007/s00394-020-02285-2.
45. Alisson-Silva F, Liu JZ, Diaz SL, Deng L, Gareau MG, Marchelletta R, et al, Human evolutionary loss of epithelial Neu5Gc expression and species-specific susceptibility to cholera. PLoS Pathog. 2018; 14:e1007133. doi: 10.1371/journalppat.1007133.
46. Rojas M, Restrepo-Jiménez P, Monsalve DM, et al, Molecular mimicry and autoimmunity. J Autoimmun. 2018; 95:100-123. doi:10.1016/j.jaut.2018.10.012.
47. Boligan KF, Oechtering J, Keller CW, Peschke B, Rieben R, Bovin N, Kappos L, Cummings RD, Kuhle J, von Gunten S, Lünemann JD. Xenogeneic Neu5Gc and self-glycan Neu5Ac epitopes are potential immune targets in MS. Neurol Neuroimmunol Neuroinflamm. 2020 Feb 3;7[2]:e676.
48. Anaya J.-M., The diagnosis and clinical significance of polyautoimmunity,Autoimmun. Rev. 13 2014; 423–426,https://doi.org/10.1016/j.autrev.2014.01.049.
49. McGonagle D, McDermott MF. A proposed classification of the immunological diseases. PLoS Med. 2006; 3[8]:e297.
50. Smatti MK, Cyprian FS, Nasrallah GK, Al Thani AA, Almishal RO, Yassine HM. Viruses and autoimmunity: a review on the potential interaction and molecular mechanisms. Viruses. 2019; 11[8]. 762-880.
51. DiMaggio D., Anderson A., Bussel J.B. Cytomegalovirus can make immune thrombocytopenic purpura refractory. Br. J. Haematol. 2009; 146:104–112.
52. Chen J, Zhang H, Chen P, et al, Correlation between systemic lupus erythematosus and cytomegalovirus infection detected by different methods. Clin Rheumatol. 2015; 34[4]: 691- 698.
53. Moon U.Y., Park S.J., Oh S.T., Kim W.U., Park S.H., Lee S.H., Cho C.S., Kim H.Y., Lee W.K., Lee S.K. Patients with systemic lupus erythematosus have abnormally elevated epstein-barr virus load in blood. Arthritis Res. 2004; 6:R295–R302.
54. Houen, G. and Trier, N.H., Epstein-Barr virus and systemic autoimmune diseases. Frontiers in immunology, 2021; p.3334.
55. Yokochi T., Yanagawa A., Kimura Y., Mizushima Y. High titer of antibody to the epstein-barr virus membrane antigen in sera from patients with rheumatoid arthritis and systemic lupus erythematosus. J. Rheumatol. 1989; 16:1029–1032.
56. Ramondetti F., Sacco S., Comelli M., Bruno G., Falorni A., Iannilli A., d'Annunzio G., Iafusco D., Songini M., Toni S., et al, Type 1 diabetes and measles, mumps and rubella childhood infections within the italian insulin-dependent diabetes registry. Diabet. Med. 2012; 29:761–766.
57. Honkanen H., Oikarinen S., Nurminen N., Laitinen O.H., Huhtala H., Lehtonen J., Ruokoranta T., Hankaniemi M.M., Lecouturier V., Almond J.W., et al, Detection of enteroviruses in stools precedes islet autoimmunity by several months: Possible evidence for slowly operating mechanisms in virus-induced autoimmunity. Diabetologia. 2017; 60:424–431.
58. Salmi A., Ziola B., Hovi T., Reunanen M. Antibodies to coronaviruses oc43 and 229e in multiple sclerosis patients. Neurology. 1982; 32:292–295.
59. Stewart J.N., Mounir S., Talbot P.J. Human coronavirus gene expression in the brains of multiple sclerosis patients. Virology. 1992; 191:502–505.
60. Magdi M., Rahil A. Severe immune thrombocytopenia complicated by intracerebral haemorrhage associated with coronavirus infection: A case report and literature review. Eur. J. Case Rep. Intern. Med. 2019; 6:001155.
61. Moody R, Wilson K, Flanagan KL, Jaworowski A, Plebanski M. Adaptive Immunity and the Risk of Autoreactivity in COVID-19. Int J Mol Sci. 2021; Aug 20;22[16]:8965.
62. Chang R., Yen-Ting Chen T., Wang S-I, HungY-M, Chen H-Y, Wei C-C-J,.Risk of autoimmune diseases in patients with COVID-19: A retrospective cohort study, eClinicalMedicine,Volume 2023; 56, 101783, ISSN 2589-5370,
63. https://doi.org/10.1016/j.eclinm.2022.101783.
64. Hosseini P, Fallahi MS, Erabi G, Pakdin M, Zarezadeh SM, Faridzadeh A, Entezari S, Ansari A, Poudineh M, Deravi N. Multisystem Inflammatory Syndrome and Autoimmune Diseases Following COVID-19:

Molecular Mechanisms and Therapeutic Opportunities. Front Mol Biosci. 2022; Apr 14;9:804109. doi: 10.3389/fmolb.2022.804109. PMID: 35495619; PMCID: PMC9046575.

65. Sheikh A. B., Chourasia P. K., Javed N., Chourasia M. K., Suriya S. S., Upadhyay S., et al, Association of Guillain-Barre Syndrome with COVID-19 Infection: An Updated Systematic Review. J. Neuroimmunology 2021; 355, 577577. 10.1016/j.jneuroim.2021.577577.
66. Saad M. A., Alfishawy M., Nassar M., Mohamed M., Esene I. N., Elbendary A. COVID-19 and Autoimmune Diseases: A Systematic Review of Reported Cases. Crr 2021; 17 [2], 193–204. 10.2174/1573397116666201029155856.
67. Toscano, G., Palmerini, F., Ravaglia, S., Ruiz, L., Invernizzi, P., Cuzzoni, M.G., Franciotta, D., Baldanti, F., Daturi, R., Postorino, P. and Cavallini, A., Guillain–Barré syndrome associated with SARS-CoV-2. New England Journal of Medicine, 2020; 382[26], pp.2574-2576.
68. Novelli, L., Motta, F., De Santis, M., Ansari, A.A., Gershwin, M.E. and Selmi, C., The JANUS of chronic inflammatory and autoimmune diseases onset during COVID-19–A systematic review of the literature. Journal of autoimmunity, 2021; 117, p.102592.
69. Garjani A., Middleton R. M., Hunter R., Tuite-Dalton K. A., Coles A., Dobson R., et al. COVID-19 Is Associated with New Symptoms of Multiple Sclerosis that Are Prevented by Disease Modifying Therapies. Mult. Scler. Relat. Disord. 2021; 52, 102939. 10.1016/j.msard.2021.102939.
70. Bonometti R., Sacchi M. C., Stobbione P., Lauritano E. C., Tamiazzo S., Marchegiani A., et al. The First Case of Systemic Lupus Erythematosus [SLE] Triggered by COVID-19 Infection. Eur. Rev. Med. Pharmacol. Sci. 2020; 24 [18], 9695–9697. 10.26355/eurrev_202009_23060.
71. Slimani Y., Abbassi R., El Fatoiki F. Z., Barrou L., Chiheb S. Systemic Lupus Erythematosus and Varicella-like Rash Following COVID-19 in a Previously Healthy Patient. J. Med. Virol. 2021; 93 [2], 1184–1187. 10.1002/jmv.26513.
72. Zamani B., Moeini Taba S.-M., Shayestehpour M. Systemic Lupus Erythematosus Manifestation Following COVID-19: a Case Report. J. Med. Case Rep. 2021; 15 [1], 29. 10.1186/s13256-020-02582-8.
73. Gracia-Ramos A. E., Saavedra-Salinas M. Á. Can the SARS-CoV-2 Infection Trigger Systemic Lupus Erythematosus? A Case-Based Review. Rheumatol. Int. 2021; 41 [4], 799–809. 10.1007/s00296-021-04794-7.
74. Alonso-Beato R., Morales-Ortega A., Fernández F. J. D. l. H., Morón A. I. P., Ríos-Fernández R., Rubio J. L. C., et al. Immune Thrombocytopenia and COVID-19: Case Report and Review of Literature. Lupus 2021; 30 [9], 1515–1521. 10.1177/09612033211021161.
75. Casciola-Rosen, L.; Thiemann, D. R.; Andrade, F.; Trejo Zambrano, M. I.; Hooper, J. E.; Leonard, E.; Spangler, J.; Cox, A. L.; Machamer, C.; Sauer, L.; Laeyendecker, O.; Garibaldi, B. T.; Ray, S. C.; Mecoli, C.; Christopher-Stine, L.; Gutierrez-Alamillo, L.; Yang, Q.; Hines, D.; Clarke, W.; Rothman, R. E.; Pekosz, A.; Fenstermacher, K.; Wang, Z.; Zeger, S. L.; Rosen, A., IgM autoantibodies recognizing ACE2 are associated with severe COVID-19. medRxiv 2020, 2020.10.13.20211664.
76. Civardi, C.; Collini, A.; Geda, D. J.; Geda, C., Antiganglioside antibodies in Guillain-Barré syndrome associated with SARS-CoV-2 infection. J Neurol Neurosurg Psychiatry 2020, jnnp-2020-324279.
77. Franke C.; Ferse, C.; Kreye, J.; Reincke, M.; Sanchez-Sendin, E.; Rocco, A.; Steinbrenner, M.; Angermair, S.; Treskatsch, S.; Zickler, D.; Eckardt, K.-U.; Dersch, R.; Hosp, J.; Audebert, H. J.; Endres, M.; Ploner, C. J.; Pruess, H., High frequency of cerebrospinal fluid autoantibodies in COVID-19 patients with neurological symptoms. medRxiv 2020, 2020.07.01.20143214.
78. Ramos-Casals M, Brito-Zerón P, Mariette X. Systemic and organ-specific immune-related manifestations of COVID-19. Nat Rev Rheumatol. 2021; Jun;17[6]:315-332. doi: 10.1038/s41584-021-00608-z. Epub 2021 Apr 26. PMID: 33903743; PMCID: PMC8072739.
79. Yazdanpanah N., Rezaei N. Autoimmune complications of COVID-19. J. Med. Virol., 2021; 94, pp. 54-62, 10.1002/jmv.27292.
80. Diaz P, Leveque M, Hautecloque G, Sellal F, Augereau O, Lita L, Biegle E, Belilita L, Bouterra C, Rerat P, Alvarez-Gonzalez A, Martinot M, Gerber V. The challenge of diagnosing Guillain-Barre syndrome in patients with COVID-19 in the intensive care unit. J Neuroimmunol. 2022; May 15;366:577842.
81. Qin C, Zhou L, Hu Z, et al, Dysregulation of immune response in patients with coronavirus 2019 [COVID-19] in Wuhan, China. Clin Infect Dis. 2020; 71[15]: 762- 768.
82. Butler DL, Gildersleeve JC. Abnormal antibodies to self-carbohydrates in SARS-CoV-2 infected patients. bioRxiv [Preprint]. 2020; Oct 16:2020.10.15.341479.
83. Ortona E, Malorni W. Long COVID: to investigate immunological mechanisms and sex/gender related aspects as fundamental steps for tailored therapy. Eur Respir J. 2022; Feb 3;59[2]:2102245. doi: 10.1183/13993003.02245-2021. PMID: 34531277; PMCID: PMC8462012.
84. Dupuis ML, Maselli A, Pagano MT, et al,. Immune response and autoimmune diseases: a matter of sex. Ital J Gender Specific Med 2019; 5: 11–20.
85. Klein S, Flanagan K. Sex differences in immune responses. Nat Rev Immunol 2016;16:626–638.doi:10.1038/nri.2016.90].

86. Morniroli D, Giannì ML, Consales A, Pietrasanta C and Mosca F. Human Sialome and Coronavirus Disease-2019 [COVID-19] Pandemic: An Understated Correlation? Front. Immunol. 2020; 11:1480. doi: 10.3389/fimmu.2020.01480.
87. Torres-Acosta MA, Singer BD. Pathogenesis of COVID-19-induced ARDS: implications for an ageing population. Eur Respir J. 2020; Sep 24;56[3]:2002049. doi: 10.1183/13993003.02049-2020. PMID: 32747391; PMCID: PMC7397945.
88. Schulert GS, Zhang M, Fall N, et al. Whole-exome sequencing reveals mutations in genes linked to hemophagocytic lymphohistiocytosis and macrophage activation syndrome in fatal cases of H1N1 influenza. J Infect Dis 2016; 213: 1180–1188. doi:10.1093/infdis/jiv550 .
89. Lerkvaleekul B, Vilaiyuk S. Macrophage activation syndrome: early diagnosis is key. Open Access Rheumatol 2018; 10: 117–128. doi:10.2147/OARRR.S151013.
90. Crayne CB, Albeituni S, Nichols KE, et al. The immunology of macrophage activation syndrome. Front Immunol 2019; 10: 119. Doi:10.3389/fimmu.2019.00119 .
91. Verdoni L. et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet. 2020; 395: 1771–78.
92. Rowley A.H. Understanding SARS-CoV-2-related multisystem inflammatory syndrome in children. Nat Rev Immunol 2020; 20, 453–454.
93. Hernàndez, A.F., Calina, D., Poulas, K., Docea, A.O., Tsatsakis, A.M. Safety of COVID-19 vaccines administered in the EU: should we be concerned? Toxicol. Rep. 2021; 8, 871–879.
94. Meo SA, Bukhari IA, Akram J, Meo AS, Klonoff DC. COVID-19 vaccines: comparison of biological, pharmacological characteristics and adverse effects of Pfizer/BioNTech and Moderna Vaccines. Eur Rev Med Pharmacol Sci. 2021; Feb;25[3]:1663-1669. doi: 10.26355/eurrev_202102_24877. PMID: 33629336.
95. Gee J, Marquez P, Su J, Calvert GM, Liu R, Myers T, et al, First Month of COVID-19 Vaccine Safety Monitoring- United States, December 14, 2020-January 13, 2021. MMWR Morbidity and Mortality Weekly Report. 2021; Feb 26;70[8]:283-8.
96. European Medicines Agency [EMA]. Pharmacovigilance Plan of the EU Regulatory Network for COVID-19 Vaccines. 2020.
97. Bril F., Al Diffalha S., Dean M., Fettig D. M. Autoimmune Hepatitis Developing after Coronavirus Disease 2019 [COVID-19] Vaccine: Causality or Casualty? J. Hepatol. 2021; 75 [1], 222–224. 10.1016/j.jhep.2021.04.003.
98. Dotan A, Shoenfeld Y. Perspectives on vaccine induced thrombotic thrombocytopenia. J Autoimmun. 2021; 121:102663. doi:10.1016/j.jaut.2021.102663.
99. Cimolai N. Untangling the Intricacies of Infection, Thrombosis, Vaccination, and Antiphospholipid Antibodies for COVID-19 [published online ahead of print, 2021; Jun 22]. SN Compr Clin Med. 2021;1-16. doi:10.1007/s42399-021-00992-3.
100. Segal, Y., Shoenfeld, Y. Vaccine-induced autoimmunity: the role of molecular mimicry and immune crossreaction. Cell Mol Immunol 2018; 15, 586–594.
101. Chen PW, Tsai ZY, Chao TH, Li YH, Hou CJ, Liu PY. Addressing Vaccine-Induced Immune Thrombotic Thrombocytopenia [VITT] Following COVID-19 Vaccination: A Mini-Review of Practical Strategies. Acta Cardiol Sin. 2021; Jul;37[4]:355-364. doi: 10.6515/ACS.202107_37[4].20210628A. PMID: 34257485; PMCID: PMC8261702
102. Bostan E., Yalici-Armagan B.J. Herpes zoster following inactivated COVID-19 vaccine: A coexistence or coincidence? Cosmet. Dermatol. 2021.
103. Isai A., Durand J., Le Meur S., Hidalgo-Simon A. and Kurz X. Autoimmune disorders after immunisation with Influenza A/H1N1 vaccines with and without adjuvant: EudraVigilance data and literature review. Vaccine. 2012; 30: 7123-7129.
104. Perricone C, Ceccarelli F, Nesher G, Borella E, Odeh Q, Conti F, Shoenfeld Y, Valesini G. Immune thrombocytopenic purpura [ITP] associated with vaccinations: a review of reported cases. Immunol Res. 2014; Dec;60[2-3]:226-35. doi: 10.1007/s12026-014-8597-x. PMID: 25427992.
105. Furer V., Zisman D., Kibari A., Rimar D., Paran Y., Elkayam O. Herpes zoster following BNT162b2 mRNA Covid-19 vaccination in patients with autoimmune inflammatory rheumatic diseases: A case series. Rheumatology.2021.
106. Machado BAS, Hodel KVS, Fonseca LMDS, Pires VC, Mascarenhas LAB, da Silva Andrade LPC, Moret MA, Badaró R. The Importance of Vaccination in the Context of the COVID-19 Pandemic: A Brief Update Regarding the Use of Vaccines. Vaccines [Basel]. 2022; Apr 12;10[4]:591. doi: 10.3390/vaccines10040591. PMID: 35455340; PMCID: PMC9027942.
107. Ciarambino T., Barbagelata E., Corbi G., Ambrosino I., Politi C., Lavalle F., Ruggieri A., Moretti A.M. Gender differences in vaccine therapy: where are we in COVID-19 pandemic? Monaldi Arch. Chest Dis. [2021].
108. Jensen A, Stromme M, Moyassari S, Chadha AS, Tartaglia MC, Szoeke C, Ferretti MT. COVID-19 vaccines: Considering sex differences in efficacy and safety. Contemp Clin Trials. 2022; Apr;115:106700.

109. Ciccone A. SARS-CoV-2 vaccine-induced cerebral venous thrombosis. Eur J Intern Med. 2021;89:19-21. doi:10.1016/j.ejim.2021; 05.026.
110. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. N Engl J Med. 2021 Jun.
111. Condorelli A, Markovic U, Sciortino R, Di Giorgio MA, Nicolosi D, Giuffrida G. Immune Thrombocytopenic Purpura Cases Following COVID-19 Vaccination. Mediterr J Hematol Infect Dis. 2021; 13[1]: e2021047. Published 2021 Mar 1. doi:10.4084/MJHID.2021.047.
112. Di Pietro M, Dono F, Consoli S, Evangelista G, Pozzilli V, Calisi D, Barbone F, Bonanni L, Onofrj M, De Angelis MV, Sensi SL. Cerebral venous thrombosis without thrombocytopenia after a single dose of COVID-19 [Ad26.COV2.S] vaccine injection: a case report. Neurol Sci. 2022; May;43[5]:2951-2956.
113. Casucci G, Acanfora D. DIC-Like Syndrome Following Administration of ChAdOx1 nCov-19 Vaccination. Viruses. 2021; 13[6]:1046. Published 2021 Jun 1. doi:10.3390/v13061046.
114. Alnima T.· Mulder M.M.G.· van Bussel B.C.T.· ten Cate H. COVID-19 Coagulopathy: From Pathogenesis to Treatment Acta Haematol 2022; 145:282–296.
115. Barnes GD, Cuker A, Piazza G, Siegal D. Vaccine-induced thrombotic thrombocytopenia [VITT] and COVID-19 vaccines: what cardiovascular clinicians need to know. Cardiology Magazine [online] 2021.
116. The International Society on Thrombosis and Haemostasis. ISTH Interim Guidance for the Diagnosis and Treatment on Vaccine-Induced Immune Thrombotic Thrombocytopenia. [Internet].
117. Furie KL, Cushman M, Elkind MSV, Lyden PD, Saposnik G; American Heart Association/American Stroke Association Stroke Council Leadership. Diagnosis and management of cerebral venous sinus thrombosis with vaccine-induced immune thrombotic thrombocytopenia. Stroke. 2021; 52[7]:2478-2482.]
118. Scully M, Singh D, Lown R, et al, Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. N Engl J Med. 2021; 384[23]:2202-2211.
119. Oldenburg J, Klamroth R, Langer F, Albisetti M, von Auer C, Ay C, Korte W, Scharf RE, Pötzsch B, Greinacher A. Diagnosis and Management of Vaccine-Related Thrombosis following AstraZeneca COVID-19 Vaccination: Guidance Statement from the GTH. Hamostaseologie, 2021.
120. Fraser DD, Patterson EK, Slessarev M, Gill SE, Martin C, Daley M, et al, Endothelial injury and glycocalyx degradation in critically Ill coronavirus disease 2019 patients: implications for microvascular platelet aggregation. Crit Care Explor. 2020; 2[9]:e0194.
121. Ochsenbein, A.F.; Fehr, T.; Lutz, C.; Suter, M.; Brombacher, F.; Hengartner, H.; Zinkernagel, R.M. Control of early viral and bacterial distribution and disease by natural antibodies. Science 1999; 286, 2156–2159.].
122. Goulabchand, Radjiv, et al. "Impact of autoantibody glycosylation in autoimmune diseases." *Autoimmunity reviews* 13.7 (2014): 742-750.
123. Khamsi R. Rogue antibodies could be driving severe COVID-19. Nature 2021; 590: 29–31. doi: 10.1038/d41586-021-00149-1.].
124. Latreille E, Lee WL. Interactions of Influenza and SARS-CoV-2 with the Lung Endothelium: Similarities, Differences, and Implications for Therapy. Viruses. 2021 Jan 22;13[2]:161. doi: 10.3390/v13020161. PMID: 33499234; PMCID: PMC7911974.
125. Ludwig DS, Hu FB, Tappy L, Brand-Miller J. Dietary carbohydrates: role of quality and quantity in chronic disease. BMJ. 2018; 361:k2340. doi: 10.1136/bmj.k2340.
126. Ghosh S, Klein RS. Sex drives dimorphic immune responses to viral in-fections. J Immunol 2017; 198:178–1790.