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# Role of Xenosialylation in Post-Infectious and Post-Vaccination Complications, Including Covid-19 and Anti-SARS-CoV-2 Vaccination

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**Abstract:** The host glycosylation mechanism, with sialic acids as a key component, is essential for synthesizing carbohydrate components in viral glycoproteins. We hypothesize a correlation between the presence of the Neu5Gc on the host tissue and the development of infectious complications, adverse vaccine reactions, and autoimmune diseases. In certain mammals, including humans, the loss of the Cytidine Monophospho-N-Acetylneuraminic Acid Hydroxylase gene (negative-CMAH) prevents the synthesis of Neu5Gc, which acts as a Mammalian-associated Carbohydrate Antigen (MCA), (XeSiAs-Neu5Gc). When negative-CMAH species consume products from positive-CMAH mammals or are exposed to non-human cell-derived medicines, Neu5Gc can be integrated into their glycocalyx through a process called xenosialylation, eliciting an inflammatory response (xenosialitis) and prompting the production of circulating anti-Neu5Gc antibodies aimed at eliminating Neu5Gc. We hypothesize that in the case of xenosialylation, neutralizing antiviral antibodies from infections or vaccinations—including those for SARS-CoV-2—may cross-react with the XeSiAs-Neu5Gc glycans, as these resemble viral envelope antigens produced by the host's glycosylation. Additionally, circulating anti-Neu5Gc antibodies may also react with other circulating antibodies, including newly formed antiviral ones with a XeSiAs-Neu5Gc-contaminated Fc region. This can lead to the serum removal of the anti-inflammatory antibodies, leaving only hyperinflammatory IgG agalactosylated antibodies. Such conditions are also seen in various inflammatory and autoimmune diseases. We hypothesize that the combination of antibody cross-reaction and the removal of the XeSiAs-Neu5Gc-contaminated Fc region anti-inflammatory antibodies may intensify severe inflammatory responses like cytokine storms and coagulopathies in COVID-19 patients and those vaccinated. Assessing serum levels of total XeSiAs-Neu5Gc antibodies could be a valuable method for identifying patients at risk of severe complications from viral infections and vaccinations, including SARS-CoV-2. This strategy may also deepen our understanding of the pathogenesis of autoimmune diseases linked to post-infectious and post-vaccination complications, particularly for viruses utilizing the host glycosylation machinery, such as SARS-CoV-2, IAV, EBV, and others.

**Keywords:** sialic acid, Neu5Gc, xenosialitis, COVID-19, SARS-CoV-2 vaccination

## Aims and Objectives of This Work

This is a "hypothesis article" which means that the article presents a novel idea or theory without direct experimental evidence. Therefore, this hypothesis article does not present any new experimental data or a meta-analysis. The hypothesis was formulated based on a thorough review of the scientific literature concerning hyper-immune responses, autoimmune diseases, and exaggerated responses to viral infections and vaccinations, with particular reference to the SARS-CoV-2 pandemic."

This presentation aims to explore the potential connection between the non-self-antigen Neu5Gc and the severity of various health conditions, including SARS-CoV-2 infections, adverse vaccine reactions, and autoimmune diseases.

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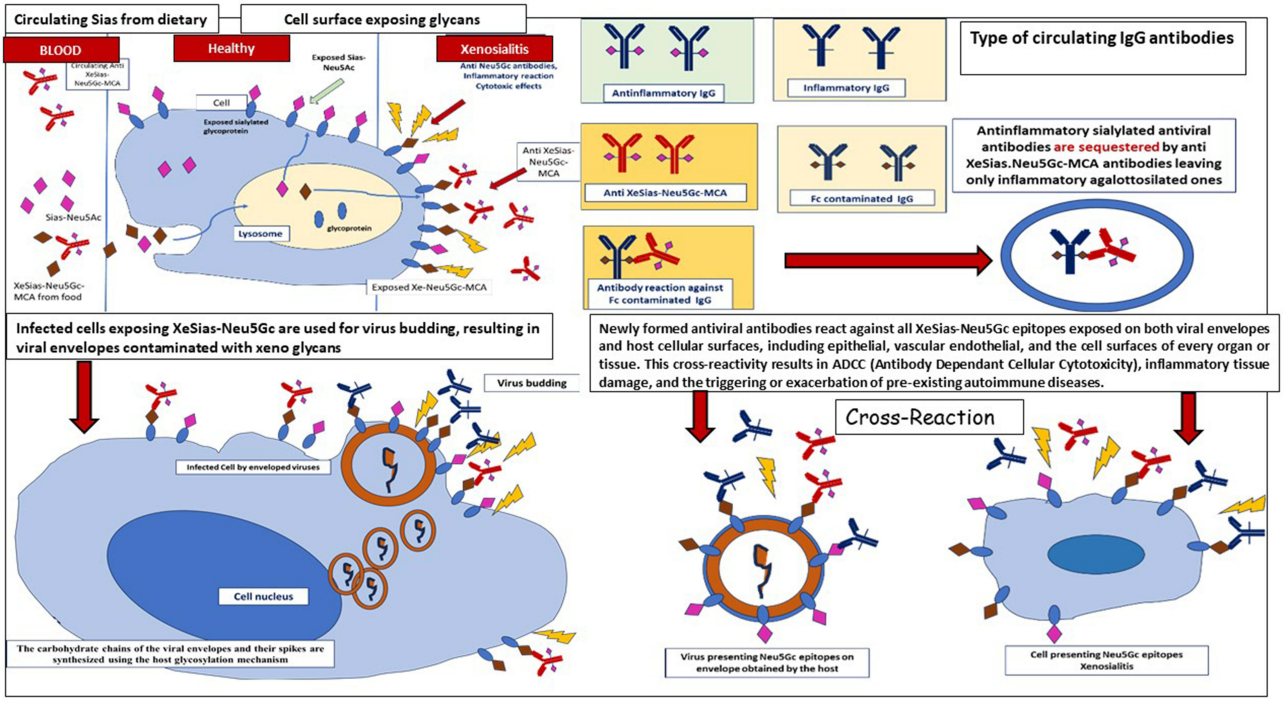
**Graphical Abstract**




1. Objective 1: Correlation Analysis – The primary objective was to demonstrate the correlations between seemingly unrelated events or those not fully explained by the scientific literature regarding infectious complications, post-vaccination side effects, and autoimmune diseases.
2. Objective 2: Verification Tests – Additionally, this presentation aimed to propose verification tests for clinical pathologies using biological samples (see Chapter "Tips for Hypothesis Testing"), including retrospective study methods, to validate the correlations explained by the hypotheses presented here.

By elucidating the association between Neu5Gc and various health or pathogenic conditions, this presentation aims to contribute to a more comprehensive understanding of their underlying pathophysiological mechanisms and potential therapeutic interventions.

## Introduction/Background

Most mammals express two common sialic acids (SiAs): N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc).[1,2] 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.[3,4]

Natural antibodies against Neu5Gc, synthesized by negative-CMAH mammalian species including humans, represent an adaptation strategy against viral zoonoses for blocking viral infections affecting positive-CMAH species.[5,6] By producing anti-Neu5Gc 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.[5] The production of anti-XeSias-Neu5Gc antibodies may result in adverse effects like autoimmune diseases and hypersensitivity reactions to viral infections and vaccines which we propose is due to a xenosialylation condition (see below).

In humans, Neu5Gc (originally called non-human-SiA),[7] here named XeSiAs-Neu5Gc, can be assimilated by dietary products,[8–10] 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.[11,12] 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,[5,9] causing xenosialylation.[8–13] It has been shown that XeSiAs-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 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.[2,9,10,13] 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 XeSiAs-Neu5Gc, lymphocytic infiltration, and tissue damage (inflammatory condition or *xenosialitis*) and, secondly, by lymphocytes B stimulation, which starts to produce circulating anti- XeSiAs-Neu5Gc antibodies, which permanently react against all incorporated XeSiAs-Neu5Gc[5,13–16] including circulating antibodies contaminated at the Fc region by XeSiAs-Neu5Gc.[15–17]

As recently described in patients with suspected and confirmed primary Sjögren's Disease (SjD), glycans attached to the fragment crystallizable region (Fc) of IgG antibodies, influence their pro- or anti-inflammatory effector function.[18] SjD patients exhibited significantly lower sialylation and galactosylation levels versus asymptomatic patients, demonstrating that lower sialylation and galactosylation levels are significantly associated with an increase in B-cell activation markers and distinct autoantibody profiles, particularly with multiple autoantibody reactivities.[18]

The incorporated XeSiAs are Mammalian-associated Carbohydrate Antigens (MCAs),[5] which trigger an autoimmune reaction against the xeno-contaminated epitopes[5,18] like after exposure to mammalian serum [serum sickness][5,19] or xenograft.[20,21]

The appearance of circulating anti- XeSiAs-Neu5Gc-MCAs antibodies, which act as autoimmune antibodies[9,15–19] has already 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.[22,23] Introducing the XeSiAs-Neu5Gc into the host gut has an important impact on the intestinal microbiome composition.[24,25] It is currently known that a XeSias-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) productions against XeSiAs-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 (ie, clostridia and bacteroides). Bacterial sialidases can cleave all types of sialic acid residues from complex glycan structures. Free XeSiAs-Neu5Gc, not used as a carbon source by intestinal bacteria, are released and can be:

1. assimilated by intestinal cells and exposed on the surface of enterocytes;[2,9,10,13]
2. appear in circulation at a steady-state level for several hours, followed by the metabolic incorporation into multiple glycoproteins and glycolipids of the entire body tissues;[10,11,13]
3. incorporated on the surface of intestinal bacteria, transforming the intestinal microbiome into a "Xeno-enhanced Microbiome" and determining gut hypersensitivity to bacterial metabolites and toxins, recurrent intestinal inflammation, and infant and adult autoimmune diseases.[24,26–28]

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,[27,29] recurrent intestinal inflammation,[19] predisposing to inflammation-induced gut permeability (known as *leaky-gut* condition), a condition almost always associated with autoimmune diseases development in infant and adult,[24,26–28] thus facilitating also the onset of diet-related colon carcinomas.[19,30]

It has been documented that the circulating anti-Neu5Gc antibodies are also involved in "Latent Autoimmune Diabetes in Adults" (LADA).[31–33] Furthermore, Multiple sclerosis (MS), Guillain-Barré syndrome (GBS), Rheumatoid arthritis (RA),

Systemic lupus erythematosus (SLE), Autoimmune thyroid disease, Kawasaki Syndrome (KS), post-COVID-19 Multisystem Inflammatory Syndrome in Children (MIS-C), and Adults (MIS-A), are some of the autoimmune diseases in which autoantibodies are always detectable in the host serum.[15,34] Moreover, interestingly, one Autoimmune Disease (AD) may coexist with others (ie, poly-autoimmunity), which may exhibit different autoantibodies with different specificities supporting the correlation with polyclonal anti Neu5Gc antibodies directed to several organs/tissues.[35]

The inflammatory function of antibodies is inversely related to the level of galactose incorporation into glycan residues: glycans that have no galactose residues (G0), only one (G1) or two (G2) galactose residues.[36] In inflammatory diseases, a notable enrichment of agalactosylated glycan Fcs (G0) is observed; conversely, high levels of galactosylation/sialylation are associated with reduced inflammatory activity of antibodies.[15,16,36] Recently it has been found that SARS-CoV-2 infected individuals display, at diagnosis, variations in the glycans composition of circulating IgGs.[37] The authors demonstrated that a deficiency on galactose and sialic acid structures on IgG Fc in COVID-19 patients appears to induce NK cells activation associated with increased release of IFN-γ and TNF-α, which indicates the presence of pro-inflammatory immunoglobulins and higher immune activation, associated with a poor disease course.[37]

More recently, other authors have investigated the role of antibody glycans in Covid-19 severity.[38] They reported an increase in di- and tri-sialylated glycans and altered mannose glycans in total serum IgM in severe COVID-19 compared to moderate COVID-19 patients. IgM *N-glycosylation* modifies T cell proliferation and alters complement activation rates; the degree of IgM mannosylation and sialylation of these patients was correlated with the levels of IL-16 and IL-18 cytokines, and was also significantly correlated with markers of disease severity: D-dimer, blood urea nitrogen (BUN), creatinine, potassium, and early anti-SARS-CoV-2 IgG, IgA, and IgM commenting that this result seems to be in direct contrast with the decrease of sialic acid found on the serum IgG from the same cohorts.[38] However, this observation is consistent with studies in which circulating desialylating IgG is closely related to the severity of Covid-19[37,39] as well as many autoimmune diseases.[15,16,36,40]

## Hypothesis

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 specific mammalian host.[5] In individuals with a pre-existing, severe state of xenosialitis characterized by high levels of circulating anti-XeSiAs-Neu5Gc antibodies,[19] we hypothesize that:

1. During viral infections, neutralizing antiviral antibodies produced to combat the infection may indiscriminately cross-react with all xenosialylated XeSiAs-Neu5Gc epitopes. This is due to their antigenic similarity with the viral envelope antigens of viruses that utilize the host glycosylation machinery to synthesize their envelope glycoproteins,[5] such as SARS-CoV-2 or other enveloped viruses like influenza A virus (IAV).
2. This cross-reactivity could exacerbate the pre-existing condition of xenosialitis and related autoimmune diseases.
3. The massive production of neutralizing viral antibodies, to fight the ongoing viral infection or after immune stimulation induced by vaccination, causes massive XeSiA contamination of the Fc glycans of newly formed antibodies, mainly IgG, but also IgM which are always highly sialylated.[38]
4. These newly formed IgGs, with their xeno-XeSiA-Neu5Gc-contaminated Fc regions, will be targeted and sequestered by the inflammatory response of T lymphocytes, thus altering the balance between circulating inflammatory and anti-inflammatory antibodies in favor of hyper-reactive ones (agalactosylated antibodies)[15,35] typically observed in many inflammatory/autoimmune diseases[15,16,18,36,40] and also in severe Covid-19.[37–39]
5. The massive removal of newly formed xeno-contaminated neutralizing antibodies, in favor of those hyperreactive agalactosylated antibodies that do not contain Sias in the Fc region, contributes to the amplification and exacerbation of all post-infectious and post vaccination inflammatory/autoimmune reactions thus enhancing the Antibody Dependent Cellular Cytotoxicity (ADCC).[36]
6. In relation to the grade of contamination by XeSiAs-Neu5Gc-MCAs of the Sias epitopes (severe state of xenosialitis) and circulating neutralizing antibodies serum level, the host's immune reaction becomes exasperated, massive, and widespread.

The effect of the removal of newly formed xeno-contaminated neutralizing antibodies, in favor of those hyperreactive agalactosylated antibodies that do not contain Sias in the Fc region is illustrated in the Figure 1

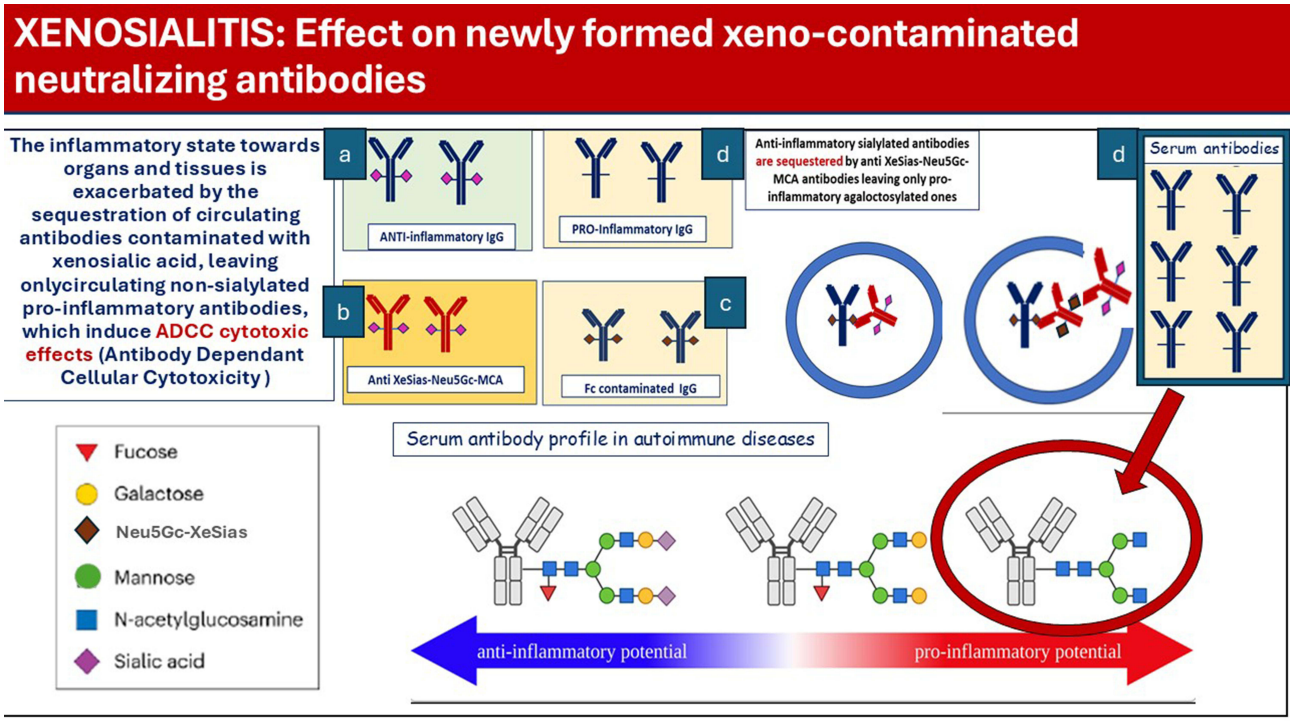



**Figure 1** Schematically represents **(a)** IgG antibodies containing terminal sialic acid (Blu IgG with violet diamonds) in the Fc region. In patients with high levels of xenosialylation, antibodies produced to neutralize an ongoing infection or following vaccination are assembled at the Fc level using the circulating Neu5Gc-XeSias, resulting in xenocontaminated IgGs **(b)** (IgG containing Neu5Gc-XeSias) (Blu IgG with brown diamonds). This triggers the production of anti-antibodies that sequester all xenocontaminated antibodies **(c)**, leaving only non-sialylated antibodies in circulation **(d)**. Non-sialylated antibodies are highly inflammatory and induce cytotoxic effects (ADCC - Antibody Dependent Cellular Cytotoxicity). This explains the severe inflammatory reactions in cases of viral infections with enveloped viruses (such as coronavirus or influenza virus), the associated adverse vaccine reactions, and the exacerbation or onset of autoimmune diseases.

# Discussion of the Hypothesis

## Current Knowledge About Xenosialysation

The individual state of heavy xenosialisation could justify and explain all the various forms of cytokine storm around 15 days after Covid-19 diseases and an excessive immune cell recruitment and activation, dysregulated inflammation, coagulopathy, and neuropathies.

1. All immune hyper-reactive forms, currently observed in the latest SARS-CoV-2 pandemic, both in adults and children[41,42] can be considered adverse immunological reactions linked to the state of xenosialylation of the host (immune reaction against every XeSiAs-Neu5Gc-MCAs-contaminated cell surface exacerbated by the sequestering of xeno-Fc-region-contaminated antibodies in favor of inflammatory ones), such as the severe inflammatory clinical entity of Covid 19 and Long Covid syndrome,[37–39] also observed after other severe pandemics (mainly IAV pandemics).[43]
2. The same mechanism of immune hyper-reactive form comes into play when the vaccine alarm is activated in a highly xenosialylated host. The immune system activated by the inoculated antigen indiscriminately reacts against every XeSiAs-Neu5Gc contaminated cell surface and newly formed xeno-Fc-region-contaminated antibodies, altering the balance of circulating antibodies in favor of hyper-reactive agalactosylated antibodies, thus triggering all the different degrees of immunological post-vaccinal side effects reported in the literature such as onset or exacerbation of different types of autoimmune disorders reported after the anti-SARS-CoV-2 vaccination.[44,45]
3. The individual state of heavy xenosialylation is, also, able to explain the observation reported in the literature that the same massive production of autoantibodies is observed both in the Long Covid complications and post-vaccination adverse reactions.[42,44,45]

4. The individual high level of xenosialylation, also, explains why the circulating antibodies both after viral infection, vaccination and autoimmunity diseases are inflammatory agalactosylated not sialylated ones as reported by the literature.[18,36,40]
5. Also, the clinical entities of dysregulated immune reactions, which are differentiated between the genders,[46] 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.[47,48]
6. The xenosialitis inflammatory level, 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, 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.[44,47,48]
7. Moreover, the high frequency of all types of coagulopathies observed both during the Covid-19 pandemic and after anti SARS-CoV-2 vaccination can be explained by the xeno-contamination of the endothelial vascular system, in which the contact with the Anti-MCAs of the xenosialylated vascular endothelial surface triggers the platelet activation and NETs formation, inducing the thrombotic phenomenon in patients which never have been exposed to heparin.[45,49,50]
8. Based on this hypothesis, it is highly plausible that also heterogeneous autoimmune clinical entities such as Type I diabetes and LADA, RA, MS, GBS, SLE, and many others, including thrombotic disorders, could be caused by the immunological reaction to the individual incorporation and distribution of XeSiAs-Neu5Gc self-antigens in the specific districts/organs.[32,33,45]
9. In the same manner, the neurological syndrome observed both in the Long Covid complications and after the anti-SARS-CoV-2 vaccination can be explained by the high concentration of XeSiAs-Neu5Gc at the level of the structural gangliosides of the Nervous System (75% of sialic acid is concentrated in the Nervous System)[51,52] for which the xeno-contamination of this district can trigger serious inflammatory/autoimmune consequences.[53] The heavy xeno-contamination of the nervous system and related inflammatory/autoimmune reactions can be the cause, and therefore explain, all polyneuropathies (including GBS) reported both after spontaneous viral infection and vaccinations.[16,45] It is now known that COVID-19 patients exhibited unique anti-glycan antibody profiles compared to healthy controls.[53] A striking difference in COVID-19 patients compared to controls was the presence of autoantibodies to numerous self-carbohydrates, including gangliosides, N-linked glycans, and Lewis's antigens. The antibody signals observed in COVID-19 patients were >20 times higher than the largest signal in the control group. Data reported by Butler et al (2022)[54] suggested that these antibodies could arise from the recognition of gangliosides/glycolipids incorporated into the SARS-CoV-2 envelope, as we hypothesized.

## Typs for Hypothesis Testing

The hypothesis presented here can be verified by utilizing biological sample banks (primarily blood or plasma) stored during the SARS-CoV-2 pandemic. We suggest comparing the levels of polyclonal anti-XeSias-Neu5Gc antibodies in biological samples from several cohorts: patients with severe adverse reactions to SARS-CoV-2 vaccines, patients with mild or no reactions, and patients with pre-existing or newly diagnosed autoimmune diseases, with or without COVID-19.

1. It is proposed to measure polyclonal anti-XeSias-Neu5Gc antibody titers on these samples (using an ELISA test that encompasses them all) and correlate these with the severity and course of COVID-19, severe adverse reactions to SARS-CoV-2 vaccines and patients with pre-existing or newly diagnosed autoimmune diseases.
2. Validation of the hypothesis predicts that the level of polyclonal anti-XeSias-Neu5Gc antibodies will be significantly elevated and directly correlated with the severity of symptoms or adverse reaction and disease course, while the inverse is expected in patients with mild or moderate courses or no adverse reactions.
3. To validate this hypothesis a study must include the analysis of the glycosylation status of circulating antibodies to identify the levels of anti-inflammatory versus pro-inflammatory antibodies and correlate them with the severity of

symptoms and disease course. The hypothesis predicts a direct correlation between the level of pro-inflammatory antibodies and disease severity.

4. In the case of patients with coagulation disorders accompanied by related clinical pictures in the context of SARS-CoV -2 infection or by adverse vaccination reactions to the relevant vaccines, the hypothesis would explain the positive correlation between high levels of coagulation markers[55] (High levels of interleukin-6, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and fibrinogen, D-dimer, interleukins 1, 2, 4, 6, 7, 10, 12, and 17, TNF-α, Macrophage colony-stimulating factor (MCSF), and interferon-gamma (IFN-γ) and the serum anti XeSias-Neu5Gc titer.

## Conclusions and Future Directions

The severe complications observed in some Covid-19 patients, both elderly and very young, and after anti-SARS-CoV-2 vaccinations are like those observed in all other viral infections/vaccinations (typically after infection and/or vaccination against IAV, EBV). This means that the mechanism of such exasperated inflammatory/autoimmune reaction is common to all viral infections and related vaccinations. The collected pandemic data demonstrated/confirmed that most of these patients are affected by other comorbidities associated with an autoimmune cause predisposing to a higher incidence of those severe complications.

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] including xeno contaminated newly formed antibodies, as a common cause of all the severe inflammatory/autoimmune reactions observed both in severe cases of Covid-19 and/or anti SARS-CoV2-vaccinations.

The hypothesis suggests framing and investigating, even retrospectively, serious and persistent adverse post-infectious and/or post-vaccination phenomena in the light of xenosialylation as a possible innovative tool to identify, understand and prevent serious post-infectious complications, such as Long-Covid syndrome, both in case of exposure to natural SARS-CoV-2 infection and in case of related vaccinations.

Particularly, the retrospective analysis for the anti Neu5Gc levels of sera collected from patients who presented serious complications from Covid-19 or post-vaccination side effects could quickly confirm or deny the hypothesis.

Furthermore, the hypothesis will be confirmed or denied by the positive correlation of the serum titer of anti Neu5Gc levels with the sialylation/desialylation level of the circulating antiviral antibodies titer of patients developing severe viral infection complications or side effect vaccinations, including SARS-CoV-2 and related vaccinations.

The confirmation of this hypothesis would be of great help for understanding the causes of autoimmune diseases and their already observed correlation with post-infectious and post-vaccination complications for all viral infections, at least for those viruses that use the host glycosylation machinery for newly synthesized their envelope glycoproteins (SARS-CoV-2, IAV, EBV, etc).

## Abbreviations

ACE2, angiotensin-converting enzyme 2; AD, Autoimmune Disease; ADCC, Antibody Dependent Cellular Cytotoxicity; BUN, blood urea nitrogen; CMAH, Cytidine Monophospho-N-Acetylneuraminic Acid Hydroxylase; COVID-19, coronavirus disease; EBV, Epstein-Barr virus; Fc, Fragment crystallizable; GBS, Guillain-Barré syndrome; IAV, Influenza A virus; KS, Kawasaki Syndrome; LADA, Latent Autoimmune Diabetes in Adults; MCAs, Mammalian-associated Carbohydrate Antigens; MIS-A, Multisystem Inflammatory Syndrome in Adult; MIS-C, Multisystem Inflammatory Syndrome in Children; MS, Multiple sclerosis; NETs, Neutrophil Extracellular Traps. Neu5Ac, N-acetylneuraminic acid; PAMPs, Pathogen Associated Molecular Patterns; RA, Rheumatoid arthritis; SAMPs, Self-Associated Molecular Patterns; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SiAs, Sialic Acids; SLE, Systemic lupus erythematosus; XeSiAs-Neu5Gc, Neu5Gc, N-glycolylneuraminic acid.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no competing interests.

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