A pilot study on Covid and Autism: Transmission, Clinical presentation and Vaccine side effects

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Abstract

Background: several neurobiological mechanisms has been proposed to support the hypothesis of higher Covid-19 risk in individuals with autism spectrum disorders (ASD). However, no real data are available on this population.

Methods: we evaluated period prevalence (March-May 2020) of Covid-19 infection and symptom presentation in a sample of individuals with severe ASD and comorbid intellectual disability (n=36) using anti-SARS-Cov-2 antibody positivity as a proxy of infection. Additionally, we evaluated vaccine side effect in the same group.

Results: Considering the entire sample, there was no significant difference between prevalence of Covid 19 positivity between autistic participants and staff personnel (Chi-square=0.71, p=0.39). Levels of antibodies against the spike protein and the RBD were not significantly different between autistic and staff participants. Level of antibodies against the NTD domain were higher in autistic individuals. There was a significant difference between prevalence of symptomatic Covid-19 in autistic participants compared to staff personnel (9.1% vs 92.3%, Chi-square=19.3, p<0.001). The most frequent side effect among autistic participants was light fever.

Conclusion: The present study provides preliminary data on Covid 19 transmission and presentation in ASD. Our data did not support the hypothesis of a higher susceptibility and severity of Covid-19 in ASD.

Key words: Autism Spectrum Disorders, Covid-19, susceptibility, antibody response, vaccine
Introduction
The Covid-19 pandemic has disrupted the lives of billions of people, adding uncertainty, and causing psychological distress to the world population. Individuals with Autism Spectrum Disorder (ASD) represent a population potentially more vulnerable in time of pandemics. Autistic individuals of all ages, as well as their caregivers, have been considerably impacted by stay-at-home orders, routine disruptions, and new social distancing standards (Brondino, Damiani, & Politi, 2020). Additionally, ASD has been proposed as a potential risk factor for the development of Covid-19 infection. In fact, autistic individuals, especially if cognitive impairment is present, may experience difficulties in maintaining social distancing and in using personal protective equipment (PPE) correctly due to sensory difficulties. Moreover, literature data point out that autistic people are more prone to both bacterial and viral infections (Sabourin et al., 2019). Specific ASD pathophysiological mechanisms have been advocated as potential risk factor for Covid-19 (Lima, Barros, & Aragão, 2020). First, the presence of an altered immune response, characterized by increase in pro-inflammatory cytokines and impairment in cellular-mediated response, has been frequently reported in ASD (Masi, Glozier, Dale, & Guastella, 2017; Meltzer & Van de Water, 2017; Pangrazzi, Balasco, & Bozzi, 2020). Second, atypical antipsychotics, such as risperidone, frequently prescribed to treat irritability in ASD (Fusar-Poli et al., 2019), have been shown to exert anti-inflammatory effects and disrupt both innate and adaptive immune responses (May, Slitzky, Rostama, Barlow, & Houseknecht, 2020) and thus represent an adjunctive risk factor for Covid-19 in the autistic population. Third, six genes previously associated with ASD have been demonstrated to be differentially regulated in individuals with severe Covid-19 (Quincozes-Santos et al., 2021). Finally, several ASD individuals showed melatonin deficiency or alteration in the melatonin genes: as the melatonin system acts both as an immune regulator and an oxidant scavenger, impairment in this system has been hypothesized to impact Covid-19 susceptibility in autistic people (Brown, Karthikeyan, Pandi-Perumal, & Cardinali, 2021). Despite these premises, real data on Covid-19 transmission and prevalence among autistic individuals are lacking. To our knowledge, only two papers have been published on the topic: the first one is a case report describing a high viral load in an autistic child suffering from Covid-19 in a residential facility (Grossi & Terruzzi, 2021); the second case series considered the characteristics of infection among autistic residents of a neuropsychiatric facility which was transformed in a Covid-19 ward, thus not providing real data about transmission (Nollace et al., 2020).
The aims of the present study are: a) to assess susceptibility of autistic patients to Covid-19, comparing the prevalence rates of Covid-19 in autistic patients and staff of two Italian facilities specifically dedicated to autistic adolescents and adults; b) to evaluate the clinical presentation of Covid-19 in autistic patients and staff; c) to record side effects of Covid-19 vaccination in the same population.

Material and methods

Susceptibility and clinical presentation

The study of natural infection by SARS-CoV-2 was conducted between March 2020 and May 2020 in two centers: the daycare center “Il Tiglio” and the farm community “Cascina Rossago” which are a day center and a farm community specifically designed for individuals with severe ASD and comorbid cognitive impairment, located in the Lombardy Region, Italy, near two regional nature parks.

The daycare center “Il Tiglio” accommodates 18 individuals, and the daily staff is composed of 7 therapists, 1 psychologist, 1 kinesiologist, 2 care assistants. Constant contact between ASD individuals and staff was present for two weeks before general lockdown of the country (March 9, 2020), during which several accommodations were made, but PPE was not provided for both staff and ASD subjects. Immediately after closure, almost all the staff developed symptoms consistent with Covid-19 infection and, after two weeks, the same happened in some families of autistic patients. Therefore, all autistic individuals in this setting could be considered exposed to SARS-CoV-2. The daycare center reopened at the end of May 2020 and, before reopening, each subject and staff member underwent a blood sample in order to determine the presence of SARS-CoV-2 antibodies according to the standard Italian National Health System (NHS) determination.

The farm community “Cascina Rossago” accommodates 22 adults (17 males and 5 females) and the daily staff is composed of 17 therapists, three nurses and 4 care assistants. The farm community was closed to external visits between March 2020 and May 2020. Similar to the daycare center, nurses and some staff members developed symptoms consistent with Covid-19 in that period. Autistic individuals due to sensory issues were not able to attain to the use of PPE (while all staff started to wear PPE as soon as they were provided) and they lived all together as in a household. Therefore, all autistic subjects could be considered exposed to SARS-CoV-2. At the end of May 2020 each subject and staff member underwent a blood sample
in order to determine the presence of anti-SARS-CoV-2 antibodies according to the standard Italian NHS determination.

Procedure

All autistic subjects were diagnosed according to DSM 5 criteria by a senior psychiatrist with wide expertise in ASD. Staff was used as a natural control group, as it could be considered exposed to the same conditions and environment as the autistic participant.

At the time of blood sampling required for reopening or for control purposes (May 2020), written informed consent was provided by patients or their legal representative before entering the study. The protocol was approved by the Ethic Committee of IRCCS Policlinico San Matteo, Pavia, Italy.

Blood samples were drawn from the antecubital vein in the morning between 9.00 and 11.00 AM. Serum was immediately extracted, aliquoted and stored at -80°C until the analysis. The assays for detection of SARS-CoV-2 antibodies were conducted independently by our NHS laboratory and into two different university laboratories.

To detect specific antibodies, recombinant SARS-CoV-2 Spike RBD was produced using polyethyleneimine-based transient transfection of Freestyle HEK293 Cells (Life Technologies) cultivated in suspension according to Faravelli and colleagues (Faravelli et al., 2021). The pCAGGS plasmids for production of the C-terminal His-tagged SARS-CoV-2 Spike RBD (#NR_52310), were obtained from BEIRESOURCES (NY, USA). Serum was tested the presence IgA and IgG against the receptor-binding domain of the SARS-CoV-2 Spike protein by means of the ELISA-based assay as described in (Krammer and Simon, 2020) and in (Bruni et al., 2020). The method has excellent specificity and sensitivity for IgGs (sensitivity 97% specificity 86%) and proved suitable for detection of anti-SARS-CoV-2 IgAs with excellent sensitivity and specificity as well. Briefly, the SARS-CoV-2 Spike RBD was diluted in Phosphate Buffered Saline (PBS tablets E404-200TABS, VWR) at a final concentration of 2 µg/ml, and 50 µl of solution were used to coat a 96-well plates (Nunc MaxiSorp™ flat-bottom, ThermoFisher) overnight at 4°Celsius. The day after, the coating solution was removed, and the plates were washed thrice with 200 µl of 0.1% of Tween-20 (Sigma, P1379) in PBS (hereafter, called PBST). A total of 200 µl of 3% Bovine Serum Albumin (BSA, Sigma A7030) diluted in PBS (blocking solution) were added to each well for at least 1 hour
at room temperature. In the meantime, samples were centrifuged at 4000 rpm for 10 minutes at room temperature and then diluted 1:50 and 1:200 with 1% BSA in PBST (hereafter, reagent solution). After 1 hour of incubation, the blocking solution was removed and the plates washed as described above, then 50 µl of the diluted samples were plated and incubated for 1½ - 2 hours at room temperature. After the incubation step, the plates were again washed, and 50 µl of a 1:3000 dilution of mouse anti-human IgG-horseradish peroxidase (HRP) conjugated secondary antibody (BD, clone G18-145) or a 1:12000 dilution of HRP-conjugated donkey anti-human IgA (Biolegend, clone Poly24110), both prepared in reagent solution, were added to the plate and incubated for 1h at room temperature. Next, the plates were washed and 50 µl of tetramethylbenzidine (TMB) Substrate Reagent (Sigma, T0440, ready to use) were added. The reaction was stopped after 10 minutes with 50 µl H₂SO₄ 1N and the absorbance was measured at 450 nm in a Glomax plate reader. According to Bruni et al., (2020), the threshold line for IgG and IgA were set as 0.277 and 0.295, respectively.

Additionally, for validation of positive hits emerging from the initial serological investigations, IgG against different subdomains belonging to the ectodomain of the Spike protein of SARS-CoV-2 was evaluated using ELISA-based immunodetection. Antibody titers against the following Spike subdomains (S1-S2 with D614G mutation -res. 14-1208-, NTD -res. 14-303-, RBD-SD1 -res. 319-591- and S2 -res. 686-1208-) were measured by means of ELISA-based immunosampling starting from an initial serum dilution of 1:314. Measurements were conducted in 384-well plates as described in (Bertoglio et al., 2021).

An ad-hoc questionnaire evaluating Covid-19 symptoms was completed for each participant and review of medical charts of each autistic participant of the previous three month before blood sampling was conducted.

**Vaccine side effects**

The study of vaccine side effects was conducted in March 2021, when patients and staff were all fully vaccinated with Comirnaty vaccine. The UKU side effect rating scale (Lingjaerde, Ahlfors, Bech, Dencker, & Elgen, 1987) for adverse events was completed for each participant.

**Statistical Analysis**
Descriptive statistics (mean and standard deviation, percentage and counts) was provided for each variable. Difference in period prevalence rates and symptoms (fever, cough, diarrhea, pneumonia/dyspnea, hospitalization) between patients and staff was evaluated by means of Chi square test. Potential differences between autistic individuals taking antipsychotics compared to subjects not on antipsychotic medications was determined by means of Chi square test. EC$_{50}$ determination of IgG titration curves were determined with the ELISA plate reader (Epoch, BioTek) software Gene Five 3.03. Difference in antibodies titers between patients and staff was evaluated by means of Mann-Whitney test. A two-tailed p-value <0.05 was regarded as statistically significant. IBM SPSS 23.0 was used for all calculation.

Results

General characteristics of the study participants for each center are reported in Table 1. Fourteen autistic individuals in the daycare center and 22 in the farm community agreed to participate. Eighteen staff subjects agreed to participate in the determination of IgG and IgA antibodies (n=10 for the daycare center and n=8 for the farm community) while all agreed (n=11 for the day care center and n=24 for the farm community) to report about Covid-19 positivity (NHS measurement), symptoms and vaccine side effects.

**Susceptibility to SARS-CoV-2 infection**

At the daycare center, NHS antibody positivity for Covid-19 was present in 81.8% of the staff personnel and in 42.8% of the autistic subjects. The difference between the two groups was statistically significant (Chi-square=3.75, p=0.05). At the farm community, NHS antibody positivity for Covid-19 was detected in 20% of the staff personnel and in 13.6% of the autistic subjects. The difference between the two groups was not statistically significant (Chi-square=0.33, p=0.57). At the daycare center, SARS-CoV-2 IgG positivity was present in 81.8% of the staff personnel and in 42.8% of the autistic subjects. At the farm community, SARS-CoV-2 IgG positivity was detected in 12.5% of the staff personnel and in 18.1% of the autistic subjects. At the daycare center, SARS-CoV-2 IgA positivity was present in 18.1% of the staff personnel and in 7.1% of the autistic subjects. At the farm community, SARS-CoV-2 IgA positivity was detected in 12.5% of the staff personnel and in 0.9% of the autistic subjects.

Considering the entire sample, there was no significant difference between prevalence of NHS Covid 19 positivity between autistic participants and staff personnel (Chi-square=0.71, p=0.39). The same effect was
applied also to SARS-CoV-2 IgG positivity (Chi-square=2.60, p=0.10) and IgA positivity (Chi-square=0.60, p=0.44).

Levels of antibodies against the spike protein and the RBD were not significantly different between autistic and staff participants (U=25, p=0.49; U=27, p=0.63; respectively). Level of antibodies against the NTD domain were significantly different between autistic participants and staff personnel (U=16, p=0.05), being higher in autistic individuals.

No significant difference in NHS Covid-19 positivity was detected between participants taking antipsychotics or not (Chi-square=0.01, p=0.9).

Clinical presentation

There was a significant difference between prevalence of symptomatic Covid-19 in autistic participants compared to staff personnel (9.1% vs 92.3%, Chi-square=19.3, p<0.001). Additionally, while two cases of severe Covid-19 pneumonia requiring hospitalization were present in staff personnel (both females, one age 41 years and the other 40 years), no cases were present among autistic participants. Of note, among the ten autistic participants showing positivity to Covid-19, only one presented fever (max 38°C) for two days and cough for one week not requiring medications.

Vaccine side effects

The most frequent side effect among autistic participants was light fever (37.5°C) (n=7), followed by fatigue (n=2). One autistic participant showed a worsening in problem behaviors for a week following vaccination. The most frequent side effect among staff participants was fatigue and light fever (<37.5°C) (n=14), which determined 18 lost workdays. All staff participants experience pain at the site of injection.

Discussion

Despite the growing numbers of hypotheses on the potential higher risk for covid infection among autistic individuals (Brown et al., 2021; Lima et al., 2020), we did not observe a higher rate of infection among ASD subjects compared to staff personnel. Although the difference in total prevalence rate was not statistically significant, in the daycare center almost all the staff acquired the infection at some point during the pandemics, as detected as antibody positivity, while less than half of the autistic subjects had antibody
positivity to SARS-CoV-2. This may be explained by the inability of those subjects to produce specific IgG antibodies or, alternatively, may indicate natural resistance to the acquisition of SARS-CoV-2 infection. Additionally, in the farm community the number of infected autistic individuals was very low considering that patients were unable to wear PPE and prone to be in close proximity to each other, as it happens in every household. Moreover, the younger age of autistic patients should have conferred a higher susceptibility (Goldstein, Lipsitch, & Cevik, 2021). One possible limitation of this finding is that autistic individuals were confined in the farm community or may have had limited social activities outside the daycare center compared to the staff: however, in that period the Italian government issued a stay-at-home order which lasted until May, impacting both the staff personnel and the.

ASD subjects displayed a better clinical presentation compared to staff personnel. This could be partly due to a younger age in the autistic population compared to staff (Levin et al., 2020). However, the low rate of infection together with a silent clinical presentation could at least raise the hypothesis that ASD may provide a sort of protection against the acquisition of SARS-CoV-2 infection, instead of an additional risk. This is in contrast with the higher rate of infection usually observed in this patient population. It could be hypothesized that early and frequent infection (Sabourin et al., 2019), such as influenza and common cold, could have primed the immune system to a more adequate response to a novel viral threat. Additionally, it has been hypothesized that presence of higher level of IFN-gamma, displayed by ASD subjects (Croonenberghs, Bosmans, Deboutte, Kenis, & Maes, 2002), could be protective against Covid-19 (Hu et al., 2020). Furthermore, the presence of a pro-inflammatory status (Brondino et al., 2019; Emanuele et al., 2010) in ASD could be protective against the cytokine storm which could determine severe Covid symptoms (Bhardwaj et al., 2021). This is in line with a recent pre-print meta-analysis (https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3832645) which did not observe and increase mortality or risk for severe Covid-19 in patients with developmental disabilities.

Covid vaccine administration was not associated to significant side effect in autistic participants, which, in general, showed a better response compared to staff. This topic is of particular importance, given the fact the several parents of autistic individuals, despite the evidence of a non-association between ASD and vaccines (Gerber & Offit, 2009), still are extremely reluctant to provide consent to vaccination. In our sample, three subjects did not receive Covid vaccine due to consent denial from the parents. Additionally, prioritizing
vaccination in people with severe mental illness is both a health and political issue (De Picker, Dias, et al., 2021; De Picker, Yolken, et al., 2021), and in Italy vaccination for people with mental disorders has been scattered and unequal between different regions.

The present study provides preliminary data on Covid 19 transmission and presentation in ASD and advocates for a paradigm shift in the conception of ASD, which should be considered not only as a vulnerable population in need of protection but also as a group with unsuspected strengths needing further investigation.

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References


Table 1. General characteristics of the sample.

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>ASD participants (n=36)</th>
<th>Staff participants (n=35)</th>
<th>ASD Daycare Center (n=14)</th>
<th>ASD Farm Community (n=22)</th>
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<tbody>
<tr>
<td></td>
<td>Mean or n</td>
<td>SD or %</td>
<td>Mean or n</td>
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Note. Percentage of Covid 19 symptoms were calculated on the sample displaying NHS positivity (n=10 for the ASD sample and n=15 for the staff sample)

<sup>a</sup>Covid 19 positivity was tested in all the autistic sample and in 18 staff subjects.