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SHORT COMMUNICATION

SARS-CoV-2-specific IgG and NCP in vulnerable patients without symptoms

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SUMMARY

Patients with severe COVID-19 both seroconvert earlier and develop higher concentrations of SARS-CoV-2-specific IgG than patients with mild symptoms. In this retrospective study we considered different categories of patients defined as "vulnerable" because affected by other pathologies, such as patients with genetic and cardiovascular diseases; patients with autoimmune dermatological disease; kidney and lung transplant patients, and pregnant women because the prevalence of Covid-19 infection during pregnancy is not known. This study was performed at IRCCS San Matteo Hospital in Pavia, North Italy, a zone considered at high risk during the COVID-19 pandemic from June to December 2020. None of the positive screened patients had symptoms of COVID-19 infection at the time of inclusion in this study.

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INTRODUCTION

The most-studied antigens and almost all developing techniques concentrate on detecting the IgM and IgG antibodies produced against coronavirus structural proteins (Assadiasl *et al*., 2020). Patients with severe Covid-19 both seroconvert earlier and develop higher concentrations of SARS-CoV-2-specific IgG than patients with mild symptoms. SARS-CoV-2 is a single-stranded RNA virus; the glycoprotein of the spike has antigenic properties and facilitates antigenic interaction with the host's cell surface receptors (Walls *et al*., 2020). SARS-CoV-2 infects cells by attaching to the principal viral entry receptor, angiotensin-converting enzyme2 (ACE2) via S^B (binding of domain B) to enter target cells (Xing-Yi *et al*., 2013; Rajpoot *et al*., 2021). The expression of this receptor has been reported in single-cell messenger-RNA-sequencing data on epithelial cells in the oral mucosa, liver, kidney, intestine, heart, and at the protein level in alveolar epithelial cells (Minotti *et al*., 2020). However, the presence of IgG antibodies is indicative of current or previous infection (Zavaglio *et al*., 2021; Cassaniti *et al*., 2021).

In this retrospective study we considered different categories of patients defined as "vulnerable" because affected by other pathologies, such as patients with genetic and cardiovascular diseases; patients with autoimmune dermatological disease; kidney and lung transplant patients, and pregnant women to detect the prevalence of COVID-19 infection during pregnancy (Tsatsaris *et al*., 2021). Patients with impaired immune response are more prone to higher morbidity and mortality (Esposito *et al*., 2021; Fekadu *et al*., 2021).

People living with different pathologies have a higher risk of developing severe COVID-19 symptoms than individuals without the condition, although the authors found no statistically significant increased risk of severe COVID-19 in immunosuppressed patients (Gao *et al*., 2020).

Key words:
IgG anti SARS-CoV-2 (S); nucleic capsid protein (NCP); tumor nerosis factor alpha (TNFα); Interleukin-17 (IL-17)

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This study was performed at IRCCS San Matteo Hospital in Pavia, North Italy, a zone considered at high risk during the COVID-19 pandemic (Cavagna *et al.*, 2020; Piccinini *et al.*, 2020) from June to December 2020; serum samples were collected in this period during ambulatorial visits and stored at -80°C. The study was approved by the local Ethics Committee (P-20200046007). All the positive screened patients had no symptoms of COVID-19 infection at the time of inclusion in this study; median age of positive patients was 50 years (range 29-76). The major risk factors for a worse outcome remain advanced age (Brodin, 2021); obesity, diabetes and cardiovascular problems (Minotti *et al.*, 2020). Genetic defects or immunosenescence may account for a reduced type-I IFN response and severity of COVID-19 (Jamilloux *et al.*, 2020).

Patients with underlying co-morbidity, and immunosuppressive drugs such as dyalitic or transplant patients (Rampino *et al.*, 2021), appeared to be more susceptible severe illness and at higher risk of death due to complications of COVID; this condition makes patients more vulnerable to infections and requires continuous follow-up to manage their disease (Fekadu *et al.*, 2021).

The aim of this study was to determine if the risk of infection of COVID-19 among patients who are more vulnerable to complications was higher because of comorbidity conditions, although these patients were without symptoms.

Screened patients (*Table 1*):

a) 121 patients with genetic and cardiovascular diseases were screened for IgG anti SARS-CoV-2 and 8 positive patients (6.5%) were identified, 3 of them (2.5%) were also positive for anti-SARS-CoV-2 nucleic capsid protein (NCP), and 113 (93%) patients were negative;
b) 132 patients with autoimmune dermatological disease (Psoriasis and Pemphigus), 13 of them (10%) were positive for IgG antibodies and 7 of them (5.5%) were also positive for NCP, 119 (90%) patients were negative;
c) 328 kidney and lung transplant patients, 13 (4%) were positive for IgG antibodies and 8 (2%) were also positive for NCP, 315 (96%) patients were negative.).

None of the patients had symptoms of COVID-19 infection at the time of inclusion.

d) We also screened 320 pregnant women in their third month of pregnancy undergoing routine monitoring at the obstetric ward, to detect the prevalence of COVID-19 infection during pregnancy (Tsatsaris *et al.*, 2021), knowing that the symptoms of SARS-CoV-2 such as dyspnea, pneumonia and acute respiratory distress syndrome appear to be the same as those of other patients affected by COVID disease in the general population. We found that 10 of them (3.1%) were positive for IgG antibodies and 3 (0.9%) were also positive for NCP, 310 (97%) women were negative. The positive women had no symptoms, like the other patients considered in this study. The low seroprevalence is possibly explained by the fact that pregnant women were more accurate in accepting and applying national recommendations to limit the spread of the virus (Tsatsaris *et al.*, 2021), *Table 1*.

Statistical analysis was performed, comparing the IgG anti-Spike and IgG/NCP values of different groups. The IgG/NCP values between kidney and lung transplant patients vs. pregnant women ($p=0.037$) were the only statistically significant group. Numerical variables were expressed as median value with range and compared with the Mann-Whitney U test (for two-group comparison). Analysis was performed with GraphPad Prism version 6 and a p-value<0.05 was considered statistically significant (*Figure 1*).

In this work we did not perform a study of gender to detect immune-system differences between men and women related to the infection. Brodin reported that men are over-represented among patients with severe disease, while long COVID seems to be more likely in women than in men.

Serum samples were tested for detection of IgG antibodies to SARS-CoV-2 spike (S) and NCP protein by ELISA assay (Euroimmun, Lübeck, Germany), according to the manufacturer's instructions. The semi-quantitative (IgG) results were expressed as a ratio with respect to an internal calibrator: a ratio of <0.8 was considered negative, ≥ 1.1 was considered positive and intermediate results were considered borderline. More than one IgG anti SARS-CoV-2 and NCP protein by ELISA assay was performed for each patient to rule out the possibility of a false negative or false positive antibody test, until resolution of symptoms, if present, and the negativization of nasopharyngeal swab.

The most common symptoms reported by patients affected by SARS-CoV-2 were fever, cough, asthenia, diarrhea, headache and dyspnea. Symptomatic patients developed pneumonia or acute respiratory distress syndrome (ARDS); some of them were hospitalized and the others were monitored at home (Gandhi *et al.*, 2020; Bruce *et al.*, 2022). A growing number of studies are pointing to asymptomatic infection in a significant fraction of individuals and indicate that transmission events occur from presymptomatic and asymptomatic individuals (Brodin, 2021). In our cohort in particular, time from transplant and ongoing intensive immunosuppression were not found to be associated with infection (Visco-Comandini *et al.*, 2022).

Although an unresolved issue about COVID-19 is the influence of immunosuppressive therapy minimization on disease course, in a previous study (Asti *et al.*, 2021) we showed that SARS-CoV-2 infection is not a

Table 1

Patients	*Sex*	*Age*	*IgG anti SARS-CoV-2 (s)*	*IgG anti SARS-CoV-2 (NCP)*	*Neg anti-IgG/NCP*	*Therapy*
Dermatological disease (psoriasis/ pemfigo) Tot. 132			N. pos. 13 (10%)	N. pos. 7 (5,3%)	119 (90%)	
1	M	52	7	2,2		anti-TNFα
2	M	65	5,3	3,2		anti-IL-17
3	F	58	8,7	3,6		anti-IL-17
4	M	64	3	1,6		anti-TNFα
5	M	56	2,1	<0,8		anti-TNFα
6	M	68	5,5	4,5		anti-TNFα
7	F	68	1,4	<0,8		anti-TNFα
8	M	44	5,5	<0,8		anti-TNFα
9	M	53	1,3	<0,8		anti-TNFα
10	M	61	1,3	<0,8		anti-TNFα
11	M	73	6,6	1,4		anti-TNFα
12	M	47	2,3	<0,8		anti-TNFα
13	M	55	6,7	4,5		anti-TNFα
Genetic and Cardiovascular disease Tot. 121			N. pos.8 (6,5%)	N. pos. 3 (2,5%)	113 (93%)	
1	F	69	1,7	<0,8		/
2	F	69	1,6	<0,8		/
3	F	50	1,2	<0,8		/
4	M	10	3,3	1,1		/
5	M	54	4,6	<0,8		/
6	F	46	2	<0,8		/
7	F	59	6,2	1,9		/
8	M	49	6,5	3,2		/
Pregnant women Tot.320			N. pos. 10 (3,1%)	N. pos. 3 (1%)	310 (97%)	
1	F	31	1,3	<0,8		/
2	F	33	2,2	1,3		/
3	F	25	1,9	<0,8		/
4	F	33	2,8	<0,8		/
5	F	41	1,1	<0,8		/
6	F	29	1,6	1,9		/
7	F	27	1,9	1,2		/
8	F	34	5,5	<0,8		/
9	F	41	1,6	<0,8		/
10	F	31	3,3	<0,8		/
Kidney and Lung Transplant Tot. 328			N. pos. 13 (4%)	N. pos. 8 (6%)	315 (96%)	
Kidney 1	M	60	2,8	<0,8		Tac/mmf/predn
Lung 2	M	40	7,1	1,5		Tac/mmf/predn
Lung 3	F	49	1,3	<0,8		Tac/ever/predn
Kidney 4	M	66	1,3	<0,8		Tac/predn/mmf
Kidney 5	M	62	4,7	1,3		Sirol/predn/mmf
Kidney 6	M	58	1,6	2,2		Predn/ever/mmf
Kidney 7	F	40	2,4	<0,8		Tac/ever/predn
Lung 8	F	63	6	3,1		Tac/ever/predn
Kidney 9	M	49	9,4	4,2		Tac/mmf/predn
Lung 10	M	76	5	1,9		CyA/mmf/predn
Kidney 11	M	42	8,6	1,4		Tac/mmf/predn
Kidney 12	M	66	10,2	3,6		Tac/ever/predn
Kidney 13	F	40	3	<0,8		Tac/mmf/predn

Legend: CyA: Cyclosporine A; mmf: Mycopenolate mofetil; Tac: Tacrolimus; ever: everolimus; ster:steroids; predn: metilprednisolone; sirol: sirolimus; anti-IL-17: biological therapy; anti-TNFα: biological therapy.

major trigger of allo-reaction in kidney transplant recipients.

Lung transplantation (LT) recipients are particularly scrutinized because the main injury of severe acute respiratory syndrome (SARS-CoV-2) infection is respiratory. Patients sometimes have impaired respiratory function; clinical presentation may be altered by local factors but may not be a risk factor for a more severe disease course than in the general population, and immunocompromised adults appear not to have a greater risk of more severe pulmonary involvement (D'Antiga, 2020). Studies have shown that especially for transplant recipients, malignancy was sometimes associated with severe disease, but not necessarily followed by a worse outcome (Minotti *et al*., 2020).

Whether organ transplant recipients are more susceptible to COVID-19 and whether immunosuppressive treatments have harmful (or protective) effects on disease progression needs to be assessed in large-scale studies (Visco-Comandinia *et al*., 2022).

Pre-existing cardiovascular disease seems to be linked with worse outcomes and increased risk of death in patients with COVID-19, whereas COVID-19 itself can also induce myocardial injury, arrhythmia, acute coronary syndrome and venous thromboembolism (Nishiga *et al*., 2020). The high burden of systemic inflammation associated with COVID-19 has been proposed to accelerate the development of subclinical disorders or cause de novo cardiovascular damage (Nishiga *et al*., 2020; Madjid *et al*., 2020). These symptoms were not present in patients we enrolled because they were all asymptomatic, but even for fragile patients, risk factors for a worse outcome were the same as in the general population (D'Antiga, 2020). The effect of COVID-19 on the cardiovascular system in immunocompromised patients, such as those with cancer or those who have undergone organ transplantation, is largely unknown (Madjid *et al*., 2020).

Patients with autoinflammatory skin disease such as Psoriasis, a papulo-squamous and desquamative disorder, and Pemphigus, an autoimmune pathogenesis that affects the skin and mucous membranes (Ávalos-Díaz *et al*., 2013), were the highest number of positive patients in this study compared with the other categories of patients considered. The epidermis provides protection for the human body through specialized cells involved in immunity that are distributed throughout the organ. Autoimmune and autoinflammatory skin diseases as relevant conditions are polygenic diseases exhibiting a prominent autoimmune component (McGonagle *et al*., 2006).

The dermatological patients considered in this study were subjected to biologic therapy (*Table 1*); it is currently unknown how biologic therapy for psoriasis might impact patients with psoriasis and COVID-19 (Brownstone *et al*., 2020). Biologic medications (*Table 1*) modulate the immune system by inhibiting tumor necrosis factor alpha (TNFα), interleukin-17 (IL-17). Patients do not show substantial increases in infection risk compared to placebo (Gooderham *et al*., 2018).

In a systematic review, Gao *et al.* stated that immunosuppression and immunodeficiency were associated with increased risk of severe COVID-19 disease, although the statistical differences were not significant and immunodeficiency was not listed among

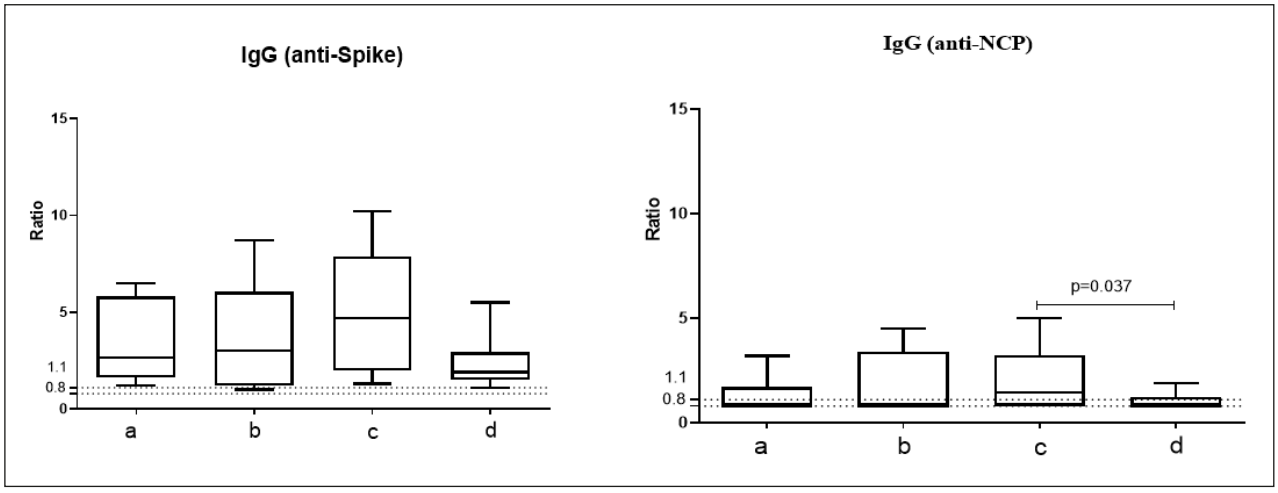

Figure 1 - *Statistical analysis has been performed comparing IgG anti-Spike and IgG/NCP values of different groups. The IgG/NCP values between kidney and lung transplant patients vs pregnant women (p=0,037) results to be the only group statistically significant. Numerical variables were expressed ad median value with range and compared with the Mann- Whitney U test (for two-group comparison). Analysis was performed with GraphPad Prism version 6 and a p-value < 0.05 was considered statistically significant.*

Legend: a=patients with generic and cardiovascular diseases; b=patients with autoimmune dermatological disease; c=kidney and lung transplant patients; d=pregnant women.

the main comorbidities found in patients with a worse outcome.

An early type-I IFN response is required as the first line of defense to suppress viral replication and spread; data derived from SARS-CoV-2 have revealed that coronaviruses suppress type-I IFN response by interfering with type-I IFN receptor-signaling pathways and induce T cell apoptosis (Jamilloux Y. *et al*., 2020). Other studies have revealed early type-1 INF response in peripheral blood from patients with mild/moderate to severe forms during the first week of COVID-19 (Trouillet-Assant *et al*., 2020). Our results show that among all patients screened for SARS-CoV-2-specific IgG antibodies and who developed detectable IgG using two validated commercial clinical methods, the highest positive patient number (10%) is among dermatological patients with psoriasis and pemphigoid. To avoid a rise in non-COVID-19-related morbidity and mortality, it is important that patients with chronic diseases continue to receive care in spite of the pandemic (Tsatsaris *et al*., 2020). Given that individuals with SARS-CoV-2 infection might be asymptomatic, routine screening of donor tissues is necessary during the pandemic because clinical presentation may be altered by local factors.

In conclusion, immunosuppression may not be a predisposing factor for COVID-19. This might be explained by a hypothetical protective role of a weaker immune response, determining a milder disease presentation and instead being protective, avoiding damage to tissues, otherwise caused by a dysregulation in innate immune response (Minotti *et al*., 2020). This study demonstrated that the different groups of vulnerable asymptomatic patients considered in this work did not present more serious complications of SARS-CoV-2 infection than did healthcare workers in the same hospital in the period considered (Rovida *et al*., 2021).

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