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Serology-based therapeutic strategy in SARS-CoV-2-infected patients

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ABSTRACT

SARS-CoV-2 infection can be a life-threatening disease. The optimal treatment of patients is not yet standardized. We use a serology-based therapeutic strategy based on the presence of antibodies against the SARS-CoV-2 virus, in which patients with positive serology receive aggressive anti-inflammatory treatment with high-dose dexamethasone and/or tocilizumab and patients with negative serology receive early convalescent plasma therapy. We also analyze the immunological impact of this therapy in the recovery of T cells, B cells and NK cells during hospitalization in a COVID-19 infectious ward. Our results suggest that aggressive therapy with early administration of convalescent plasma and high-dose dexamethasone may be of benefit in patients with SARS-CoV-2 infection and might avoid progression of lung damage or need of admission in intensive care. This strategy did not impair immune responses against SARS-CoV-2, as 93% of the patients generated antibodies against the virus. Independently of previous immunological status of the patients, serology-guided therapy might benefit even patients with a high CIRS-G score, immunosuppressed or medically debilitated individuals and elderly patients. T cell disturbances were most frequent in patients who required high-dose dexamethasone, and B cell depletion was most frequent in patients who received tocilizumab. Early passive immunotherapy with convalescent plasma does not affect lymphoid recovery.

1. Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has spread worldwide. SARS-CoV-2 has adapted to inhibit immune responses at the initial stage of infection and evade the immune system. Contradictory publications on therapy for COVID-19 patients and the use of dexamethasone [1], convalescent plasma (CP) [2] and tocilizumab [3] during the three waves of high hospital pressure in Spain have contributed to several changes in evidence-based therapeutic strategies [4]. To avoid this bias, a small cohort of patients was treated using a serology-based therapeutic strategy, in which the presence of antibodies against the virus guides treatment and distinguishes patients with a delayed immune response and negative serology from those at increased risk of inflammatory complications with a fully established immune response. The diagnostic could be used to identify patients with infection and susceptibility to severe disease who would benefit from passive immunotherapy [5]. Passive immunotherapy with CP with antibodies against SARS-CoV-2 should be used before the onset of symptoms in order to be an early intervention to avoid viremia [5]. Overall, specific antibodies and cell mediated immunity against virus start at 4 to 10 days after infection [6], for this reason a negative serology against SARS-CoV-2 suggests early infection or delayed immune response. Interestingly, the most effective therapy for hypogammaglobulinemia is immunoglobulin replacement. Patients with antibody deficiencies like common variable immune deficiency, chronic lymphocytic leukemia and multiple myeloma with immunoparesis, benefit from immunoglobulin therapy despite most viral infections are cleared normally by CD8 T cells.

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However, lack of humoral immunity enhances susceptibility to viral reactivation [6]. However, if serology is positive indicates inflammatory induced complications; for this reason antibody-based antiviral therapies should be only prescribed to antibody-negative patients.

2. Materials and methods

We categorized patients into two groups according to the detection of IgG or IgM against virus SARS-CoV-2:

(A) Patients with delayed immune response to viral infection (negative serology against SARS-CoV-2), group (A).
(B) Patients with increased risk of inflammatory complications (positive serology against SARS-CoV-2), group (B).

Patients in group (A) received infusions of convalescent plasma (300 ml) every week until IgG or IgM antibodies against SARS-CoV-2 were detected or until clinical improvement (especially in immunosuppressed patients). We adopted this strategy because the use of convalescent plasma is associated with minimal side effects and potentially great benefit in patients with SARS-CoV-2 infection. Protective cardiovascular effects of dexamethasone have been described previously; for this reason, we administered an initial simple dose of 10–20 mg of dexamethasone (high or very high dose of glucocorticoid equivalent to ≥100 mg prednisone per day) to patients who received convalescent plasma, which was then generally tapered off as soon as the inflammatory conditions were under control. We considered a medium dose of glucocorticoid equivalent to 30 mg prednisone per day the optimal dose during the viral phase in group (A) patients. Some patients in group (A) worsened in their clinical and inflammatory parameters; in that case, the patient was treated like group B. The use of corticosteroids used wisely can provide relief the deleterious effects of excessive immune system activation such as cytokine storm, without causing profound immunosuppression.

Patients in group (B) did not receive infusions of convalescent plasma, because an immune response was fully established, in concordance with the presence of anti-SARS-CoV-2 IgM or IgG antibodies. Patients in group (B) received treatment with medium doses of glucocorticoids equivalent to 30 mg of prednisone. However, if clinical and/or inflammatory parameters worsened, treatment with very high doses of glucocorticoids consisting of 20–40 mg of dexamethasone was initiated. If levels of IL-6 (>40 pg/mL) or D-dimer (>1500 ug/L) increased despite this therapy, the patient received treatment with tocilizumab, a monoclonal antibody against IL-6 (600 mg in patients > 75 kg or 400 mg in patients < 75 kg) in combination with high-dose dexamethasone (20–40 mg/day). The key points for this therapy is to use high doses of dexamethasone as premedication before plasma or when the patient has an established immune response as indicated by the appearance of anti-SARS-CoV-2 antibodies (Fig. 1).

All patients received venous thromboprophylaxis at doses of 1 mg/kg/day of low-molecular-weight heparin (LMWH).

2.1. Subjects

Sixty-two patients were admitted to the COVID-19 infectious ward. Sixteen patients were excluded because they could not receive the immunological therapeutic strategy, did not give their consent to participate in the study, had insufficient clinical data, were patients in palliative care or asymptomatic patients with SARS-CoV-2 infection with an alternative medical reason transferred from another medical department. Seven patients required admission to intensive care. Forty-six anonymized patients were primarily treated with this strategy and

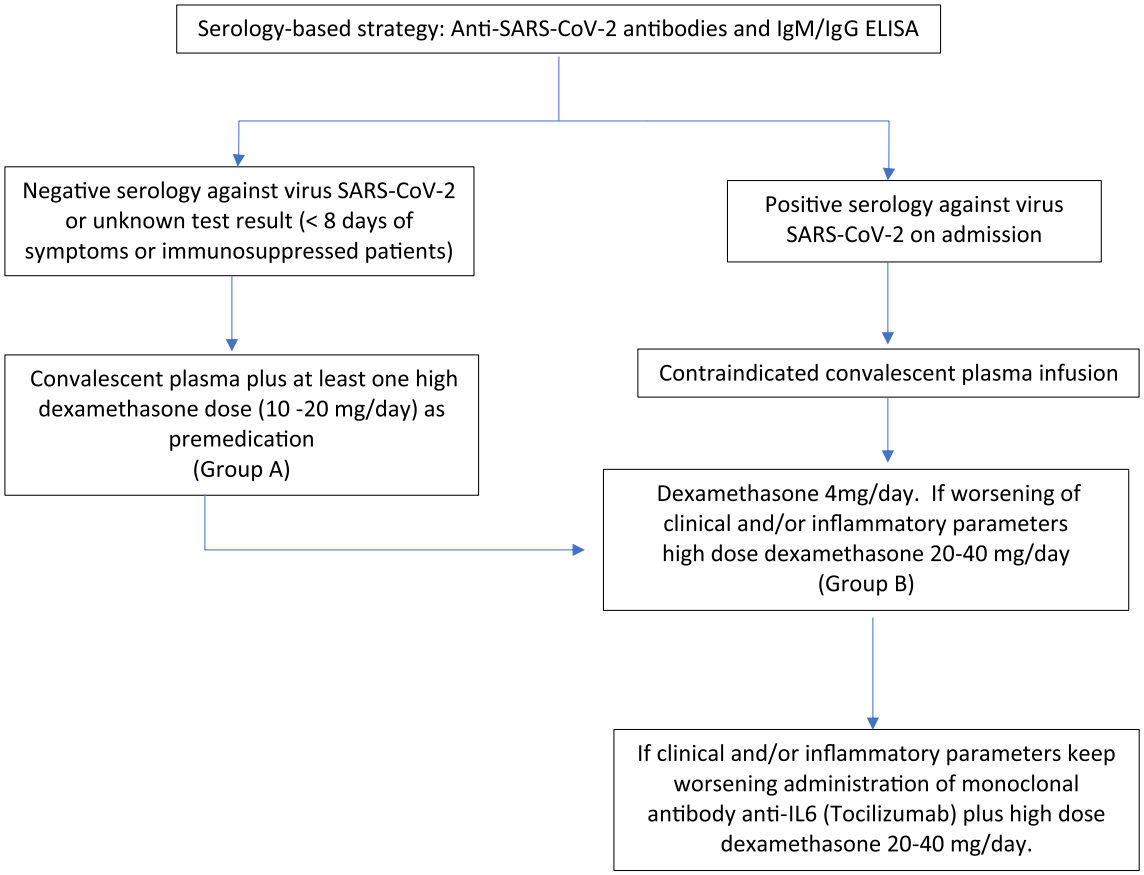

Fig. 1.

retrospectively analyzed. Pharyngeal swabs were collected and used for detection of SARS-CoV-2. All patients involved in this study were hospitalized in the COVID-19 infectious ward of San Pedro Hospital in Logroño, Spain.

2.2. Data collection

Patients characteristics included medical history, comorbidities, X-ray results, laboratory findings, flow cytometry analysis and treatment and were obtained from the electronic medical record system. ***The ethics committee granted access due to pandemic emergency.*** The date of hospital admission, diagnosis and the date of discharge from the COVID-19 infectious ward were also recorded. Severity, risk and comorbidities of each patient were evaluated through three different scales (CURB64 [7], FINE SCALE [8] and CIRS-G [9]). High comorbidity was defined such as six or more points on the CIRS-G scale.

2.3. Laboratory testing

Convalescent plasma was obtained from convalescent donors with positive antibodies against nucleocapsid (N) antigen using Elecsys® Anti-SARS-CoV-2 in an immunoassay for the in vitro qualitative detection of antibodies (including IgG) to SARS-CoV-2. Enzyme Immuno Assay (ELISA) for the determination of IgG and IgM antibodies to COVID19-specific Nucleocapsid ("Core") and Spike antigen were also performed for testing human antibodies in plasma from all donors in accordance with the manufactureŕs instructions. (REF COV19M.CE.96, 192 Test and COVI19G.CE.96,192 Tests. DIA. PRO. Diagnostic Bioprobes Srl. Via G. Carducci n° 27). Both types of analysis were performed to all patients to guide the selection of treatment, plasmatherapy in patients with negative antibodies anti-SARS-CoV-2 or anti-inflammatory treatment in patients with positive antibodies anti-SARS-CoV-2.

Laboratory results including interleukin-6 (IL6), C-reactive protein (CRP), D-dimer, ferritin; lymphocytes, T cells (CD3), helper T cells (CD3+, CD4+), cytotoxic T cells (CD3+, CD8+), B cells (CD19+) and natural killer cells (CD56+) were collected on admission and at discharge from the COVID-19 infectious ward or in the last determination after in transfer following resolution of SARS-CoV-2 infection, transfer to intensive care or death. Serial determinations of antibodies against SARS-CoV-2 were also collected (data not shown) to confirm the immune response and serological conversion in patients of group (A) during the hospital stay. Laboratory data of some patients were missing due to the absence of tests or delayed results.

2.4. Statistical analysis

The Wilcoxon signed-rank test, Sign test and Mann-Whitney *U* test were used to estimate the statistical significance of the differences observed. Statistical analysis was performed using Social Science Statistics Software (www.socscistatistics.com). The tests with P value of <0.05 were considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics

A total of 46 patients initially diagnosed with COVID-19 were retrospectively analyzed in this study. Forty-five patients (98%) tested positive for SARS-CoV-2 in their pharyngeal swabs. One patient with clinical picture compatible with COVID-19 infection was treated according to the therapeutic protocol; however, the final diagnosis was rhinovirus infection in an immunosuppressed cancer patient with recent chemotherapy-induced interstitial pneumonitis. The median age of patients was 71 years, ranging from 41 to 97 years.

3.2. Clinical outcomes

The patients remained hospitalized a median of eight days in the COVID-19 infection ward (X_{me} = 8; 1–29 days). 85% of patients with negative SARS-CoV-2 antibodies at admission presented anti-SARS-CoV-2 positive at discharge (23/27). (Table 1) 96% of patients presented clinical improvement (44/46) and 59% of patients presented radiological improvement (27/46) (Table 2) 30 day overall survival was 96% (44/46).

Around 57% were male and 43% female. Twenty-one patients (46%) had moderate to high mortality risk calculated by the CURB65 Scale. Sixteen patients (35%) had a moderate to high risk calculated by the FINE SCALE. Twenty-eight patients (60%) had increased comorbidity calculated by the CIRS-G Scale. (Table 3)

3.3. Treatment

Group (A): Patients with delayed immune response to viral infection (negative serology against SARS-CoV-2): **Twenty-six patients received**

Table 1

Patient	Serology to SARS-CoV-2 on admission	Seroconversion during hospitalization Anti SARS-CoV-2: 85%/Total patients 93%		
		Totales (1 YES, 2 NO)	IgG	IgM
1	Negative	YES	YES	YES
2	Negative	YES	YES	YES
3	Positive	YES	YES	YES
4	Negative	YES	YES	YES
5	Negative	YES	YES	YES
6	Negative	NO	NO	NO
7	Positive	YES	YES	YES
8	Positive	YES	YES	YES
9	Positive	YES	YES	YES
10	Negative	YES	YES	YES
11	Positive	YES	YES	YES
12	Negative	NO	NO	NO
13	Positive	YES	YES	YES
14	Negative	NO	NO	NO
15	Negative	YES	YES	YES
16	Negative	YES	YES	NO
17	Positive	YES	YES	YES
18	Positive	YES	YES	NO
19	Negative	YES	YES	YES
20	Negative	YES	NO	YES
21	Negative	YES	YES	YES
22	Negative	YES	YES	YES
23	Negative	YES	YES	NO
24	Positive	YES	YES	YES
25	Negative (Rhinovirus)	NO	NO	NO
26	Positive	YES	YES	YES
27	Positive	YES	YES	NO
28	Positive	YES	YES	NO
29	Positive	YES	YES	NO
30	Positive	YES	YES	YES
31	Negative	YES	YES	YES
32	Negative	YES	YES	YES
33	Negative	YES	YES	YES
34	Negative	YES	YES	YES
35	Negative	YES	YES	NO
36	Negative	YES	NO	YES
37	Negative	YES	YES	YES
38	Positive	YES	YES	NO
39	Positive	YES	YES	YES
40	Delayed results (IgM + IgG -)	YES	YES	YES
41	Negative	YES	YES	YES
42	Unknown at admission Positive CLL	YES only 24 h after CP	YES	NO
43	Negative	YES	YES	YES
44	Negative	YES	YES	YES
45	(unknown at admission) Positive	YES	YES	YES
46	Negative	YES	YES	YES

Table 2

Patient	AGE	Radiology Admission	Dischargue	1 month	Intensive care unit	DEATH
1	91	IB	IB-	N	No	No
2	87	IB	IB-	N	No	No
3	62	IB	IB	N	No	No
4	90	IB	D	ND	No	No
5	96	IB	D	D	No	No
6	59	IB	IB-	N	No	No
7	54	IB	IB	N	No	No
8	70	IB	D	N	No	No
9	77	IB	IB	N	No	No
10	65	IB	N	N	No	No
11	69	IB	IB	N	No	No
12	84	IB	IB	ND	No	No
13	61	IB	IB	N	No	No
14	60	D	N	ND	No	No
15	72	IB	N	ND	No	No
16	73	D	N	ND	No	No
17	73	N	N	ND	No	No
18	83	D	D-	N	No	No
19	89	IB	IB-	N	No	No
20	76	IB	D	N	No	No
21	46	IB	N	ND	No	No
22	42	IB	N	ND	No	No
23	67	IB	I	ND	No	No
24	77	N	N	ND	No	No
25	69	IB	IB	ND	No	No
26	86	IB	IB	IB-	No	No
27	97	IB	D	D	No	No
28	50	IB	N	N	No	No
29	61	IB	IB	N	No	No
30	60	IB	N	N	No	No
31	52	N	N	N	No	No
32	67	IB	N	N	No	No
33	72	IB	IB-	IB-	Yes	No
34	77	IB	IB-	IB-	No	No
35	43	D	D	N	No	No
36	85	N	N	ND	No	No
37	63	D	D-	N	No	No
38	66	IB	IB	IB-	No	No
39	49	N	N	ND	No	No
40	86	IB	IB-	N	No	Yes
41	83	IB	IB-	N	No	No
42	76	IB	IB-	N	No	No
43	61	IB	IB	N	No	No
44	84	IB	IB-	N	No	No
45	41	IB	N	N	Yes	No
46	86	IB	D	ND	No	No

IB: Bilateral interstitial pneumonia/ID: Right lung infiltrate/I: Left lung infiltrate/ND Not done -: improvement N: normal N

convalescent plasma (negative and 3 unknown serology on admission) (Table 3**)** Only three patients with unknown serology on admission were treated with convalescent plasma: one patient with 7 days of symptoms, who required admission to intensive care; one patient with IgM anti-SARS-CoV-2 positive but IgG negative, who presented an intense inflammatory reaction that required tocilizumab with progressive worsening and death; and one immunosuppressed patient with chronic lymphocytic leukemia and multiple sclerosis, previously treated with rituximab. This patient presented positive serology after plasmatherapy but 24 h after infusion of convalescent plasma became negative in two separated serology determinations, so we assume that the antibodies detected were from the infused plasma. This patient received weekly plasma infusions during 4 consecutive weeks. This patient is a proof of principle that the infused plasma has anti-SARS-CoV-2 antibodies. The median number of days from diagnosis to convalescent plasma infusion was four days (range 1–9 days) and 26/46 patients (56%) received a convalescent plasma infusion at least once. (Fig. 2 **and** Table 3**).**

Group (B): Patients with increased risk of inflammatory complications (positive serology against SARS-CoV-2). **Nineteen patients presented antibodies against SARS-CoV-2 on admission (contraindicated convalescent plasma infusion), of whom 19/46 (41%) received dexamethasone. (**Fig. 2 **and** Table 3**).**

Glucocorticoid: 13/46 patients (28%) received medium doses of dexamethasone (<10 mg/day) and 32/46 (70%) received at least one high dose of dexamethasone (>or = 10 mg/day). **(**Fig. 2 **and** Table 3**).**

Tocilizumab: Only 8/46 patients (17%) received treatment with the monoclonal antibody tocilizumab. The median number of days from diagnosis to tocilizumab treatment was eight (range 6–16 days). **(**Fig. 2 **and** Table 3**).**

3.4. Humoral immunity

IgM and/or IgG antibodies anti-SARS-CoV-2 were detected in 43/46 patients (93%) during their admission in the COVID-19 unit (**several patients presented seroconversion from antibodies anti-SARS-COV2 negative at admission to positive at discharge**) **(**Table 1**).** The median time from diagnosis to antibody detection was eight days (range 1–20 days). Only 2/46 patients (4%) recruited in the study required admission to the intensive care unit, and only 1/46 (2%) died due to COVID-19 or as a consequence of post-COVID-19 pulmonary fibrosis.

3.5. Other infections

Besides SARS-CoV-2, other infections were detected in some of the study patients: *Streptococcus pneumoniae, Escherichia coli, Enterococcus faecium, Enterococcus faecalis, Staphylococcus hominis, Staphylococcus epidermidis, Candida albicans, rhinovirus and cytomegalovirus.*

3.6. Immunological findings

The performed 45 blood tests on patient admission revealed lymphopenia in 21/45 patients (46%), defined as < 900 lymphocytes/μl (X_{me} = 900 cls/μl [200–4600 cls/μl]), normal range 900–5200 cls/μl. However, in only 15/45 patients (33%) lymphopenia persisted upon discharge from the COVID-19 unit (X_{me} = 1300 cls/μl [200–4100 cls/μl]) (*P < 0.05)*. Lymphocyte immunophenotyping of 35 patients revealed a severe T cell (CD3 +) lymphocytopenia on admission (X_{me} = 295 cls/μl [40–2660 cls/μl]), normal range 714–2266 CD3 + T cells/μl, with only partial recovery upon discharge (X_{me} = 590 cls/μl [144–20870 cls/μl]), which resulted statistically significant (*P < 0.05)* **(**Table 4**).**

3.7. Helper T cells (CD3+ CD4+)

A severe depletion in helper T cells (CD4 +) was detected on admission, with a median of <200 T CD4 + cells (X_{me} = 198 cls/μl [29–1730 cls/μl]), normal range 359–1565 CD4 + T cells/μl, showing a partial recuperation upon discharge (X_{me} = 380 cls/μl [75–1340 cls/μl]) (*P < 0.05)*.

3.8. Cytotoxic T cells (CD3+ CD8+)

Cytotoxic T cell (CD8 +) lymphopenia was also detected (X_{me} = 102 cls/μl [11–986 cls/μl]), normal range 178–853 CD8 + T cells/μl, with again partial recuperation upon discharge (X_{me} = 171 cls/μl [20–642 cls/μl]) *(P < 0.05)*.

3.9. B cells (CD19+)

B cells remained within normal levels on admission (X_{me} = 64 cls/μl [1–1310 cls/μl]), normal range 61–321 CD19 + B cells/μl, with a significant increase at discharge (X_{me} = 173 cls/μl [1–2420 cls/μl]) *(P < 0.05)*.

Table 3

Patient	AGE	CURB-65	FINE	CIRS-G > 6	TOCILIZUMAB	PLASMA	DEXAMETHASONE > 10 mg/day
1	91	2	4	YES	NO	YES	YES
2	87	1	3	YES	NO	YES	YES
3	62	0	1	NO	NO	NO	YES
4	90	3	5	YES	NO	YES	YES
5	96	2	5	YES	NO	YES	YES
6	59	0	3	YES	NO	YES	YES
7	54	0	2	NO	NO	NO	YES
8	70	1	2	NO	YES	NO	YES
9	77	1	3	YES	NO	NO	NO
10	65	1	3	NO	NO	YES	YES
11	69	1	3	NO	NO	NO	NO
12	84	2	4	YES	NO	NO	NO
13	61	0	1	YES	NO	NO	YES
14	60	0	2	YES	NO	NO	NO
15	72	2	3	YES	NO	YES	YES
16	73	2	3	YES	NO	YES	YES
17	73	2	4	NO	NO	NO	NO
18	83	1	3	NO	YES	NO	YES
19	89	1	3	YES	YES	NO	YES
20	76	2	5	YES	YES	NO	YES
21	46	0	1	NO	NO	YES	NO
22	42	1	3	YES	NO	YES	YES
23	67	2	3	YES	NO	YES	YES
24	77	3	4	YES	NO	NO	NO
25	69	2	4	YES	YES	YES	YES
26	86	2	4	YES	NO	NO	YES
27	97	2	4	YES	NO	NO	YES
28	50	1	2	YES	NO	NO	YES
29	61	0	2	NO	NO	NO	NO
30	60	0	2	NO	NO	NO	NO
31	52	0	3	NO	NO	YES	NO
32	67	1	1	NO	NO	YES	NO
33	72	2	1	NO	NO	YES	YES
34	77	3	4	YES	YES	YES	YES
35	43	0	3	NO	NO	YES	YES
36	85	2	4	YES	NO	YES	YES
37	63	0	3	NO	NO	YES	YES
38	66	2	4	YES	NO	NO	YES
39	49	1	2	NO	NO	NO	NO
40	86	2	4	YES	YES	YES	YES
41	83	2	4	YES	NO	YES	YES
42	76	1	3	YES	NO	YES	YES
43	61	0	2	NO	NO	YES	NO
44	84	2	3	YES	NO	YES	YES
45	41	0	2	NO	YES	YES	YES
46	86	4	5	YES	NO	YES	YES

3.10. NK cells (CD56+)

NK cell (CD56 +) depletion was detected on admission (X_{me} = 105 cls/μl [1–359 cls/μl]), normal range 126–729 CD56 + NK cells/μl, without significant recovery upon discharge (X_{me} = 104 cls/μl (1–319 cls/μl]) *(P = NS)*.

3.11. Comorbidity

Patients with high comorbidity determined by CIRS-G index (CIRS-G ≥ 6) presented marked immune disturbances in lymphocyte populations in contrast to patients with low comorbidity. The initial blood count in patients with CIRS-G ≥ 6 showed lymphopenia (X_{me} = 700 cls/μl [200–2200 cls/μl]), severe decrease of CD4 + T cells (<200 cls/μl) (X_{me} = 170 cls/μl [29–818 cls/μl]), low CD8 + T cells (X_{me} = 86 cls/μl [11–208 cls/μl]), low NK cells (X_{me} = 91 cls/μl [12–359 cls/μl]) and low B cell counts (X_{me} = 32 cls/μl [4–1310 cls/μl]). Interestingly, patients with low comorbidity (CIRS-G < 6) showed normal lymphocyte count (X_{me} = 1150 cls/μl [300–4600 cls/μl]), CD4 + T cells (X_{me} = 385 cls/μl [97–1730 cls/μl]), NK cells (X_{me} = 140 cls/μl [1–314 cls/μl]) and B cells (X_{me} = 90 cls/μl [1–110 cls/μl]). However, patients with low comorbidity determined by CIRS-G index also showed low CD8 + T cell counts (X_{me} = 153 cls/μl [19–986 cls/μl]) similar to patients with high comorbidity (CIRS-G ≥ 6).

Improvement of lymphocyte counts after applying serologic-based therapeutic strategy was similarly recognized in patients with CIRS-G ≥ 6 (1200 cls/μl) and patients with CIRS-G < 6 (1400 cls/μl), *P = NS*.

We identified 30 patients with determination of lymphoid subpopulations on hospital admission and before discharge from the COVID-19 infection ward (20 with CIRS-G ≥ 6 and 10 with low comorbidity). In this population, we analyzed the recovery of the immune system after applying a serologic-based therapeutic strategy.

Patients with CIRS-G ≥ 6 improved CD4 + T cells count from severe lymphopenia (<200 cls/μl) to normal levels of CD4 + T cells. Comparable recovery was also detected in the B cell compartment with a significant improvement in CD19 B cells. Intriguingly, the major antiviral cells such as CD8 + T cytotoxic cells and NK cells remained below normal reference values in patients with CIRS-G ≥ 6, despite significant recovery of CD8 + T cells. Low levels of NK cells remained stable in patients with CIRS-G ≥ 6 during SARS-CoV-2 infection.

Lymphocyte recovery in patients with CIRS-G ≥ 6 (n = 20) (Table 4)

Patients without comorbidities maintained similar levels of CD4 + T cells, CD8 + T cells and NK cells on admission and at discharge from the COVID-19 infection ward. However, we detected significant increases in the numbers of B cells. Interestingly, a similar compartment of NK cells was detected in patients without comorbidities and patients with CIRS-G ≥ 6.

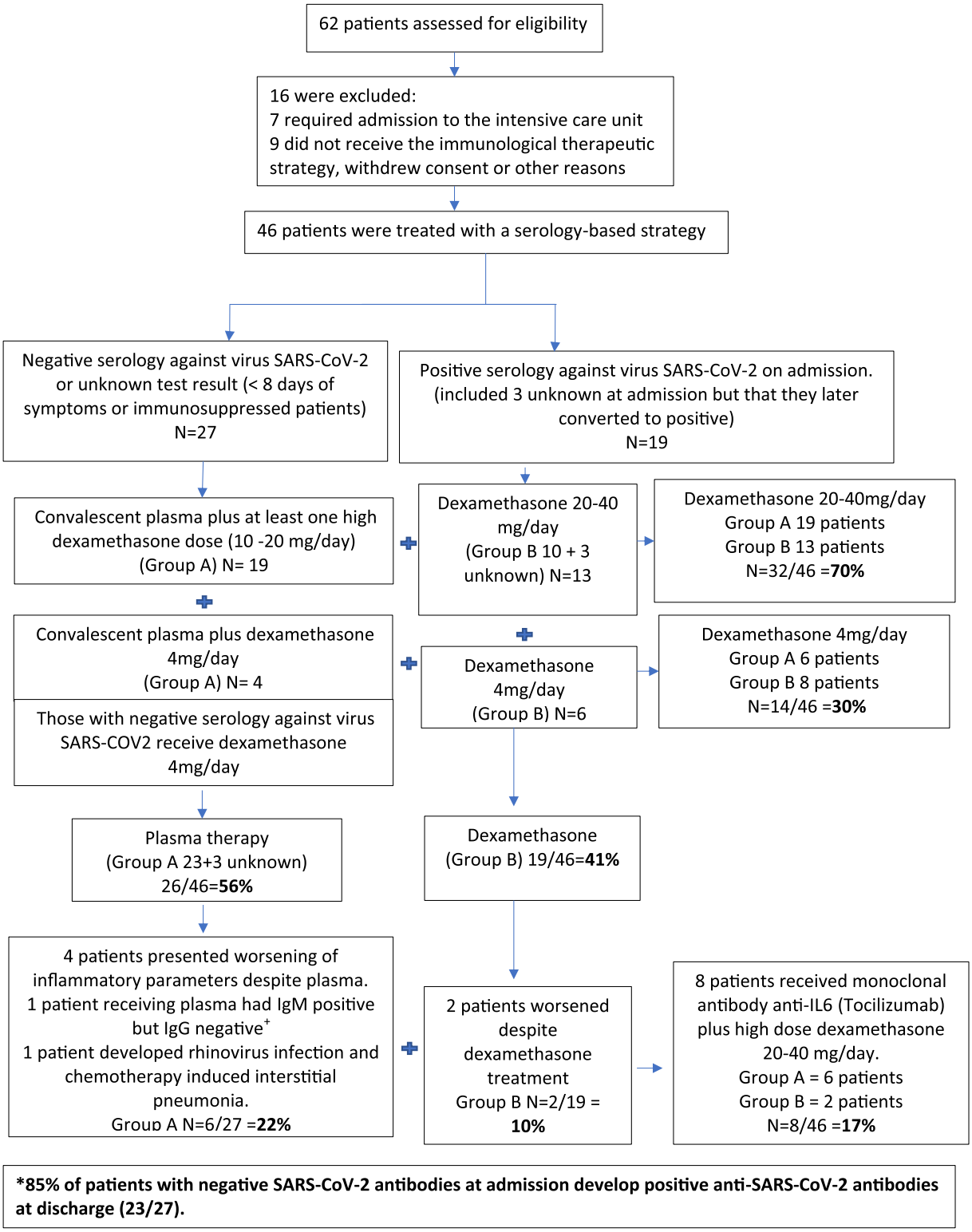

Fig. 2.

Lymphocyte recovery in patients with low comorbidity (n = 10) (Table 4)

3.12. Dexamethasone

Thirty-two patients received high-dose dexamethasone (≥10 mg/daily) and fourteen patients received low doses of dexamethasone (6 or 8 mg/daily). No statistically significant differences were detected in total lymphocyte counts between the high-dose (750 cells/μl) and low-dose dexamethasone groups (990 cells/μl), $P = NS$. However, we were able to study thirty-three patients with immunophenotype, twenty-five of whom received high-dose dexamethasone and only eight a low dose. We observed significantly lower levels of both CD4 + T cells and CD8 + T cells on admission in patients who received high-dose

Table 4

Table 4. **Immunological Findings**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
HELPER T CELLS (CD4 +) cells/μl	198 (29–1730)	380 (75–1340)	359–1565	p < 0.05
CYTOTOXIC T CELLS (CD8 +) cells/μl	102 (11–986)	171 (20–642)	178–853	p < 0.05
B CELLS (CD19 +) cells/μl	64 (1–1310)	173 (20–642)	61–321	p < 0.05
NK CELLS (CD56 +) cells/μl	105 (1–359)	104 (81–319)	126–729	p = NS

Table 4. **Lymphocyte recovery in patients with CIRS-G>6 (n=20)**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
HELPER T CELLS (CD4+) cells/μl	170 (29–818)	390	359–1565	p<0.05
CITOTOXIC T CELLS (CD8+) cells/μl	86 (11–208)	75–1340	178–853	p<0.05
B CELLS (CD19+) cells/μl	32 (4–1310)	131	61–321	p<0.05
NK CELLS (CD56+) cells/μl	91 (12–359)	22–642	126–729	p=NS

Table 4. **Lymphocyte recovery in patients with low comorbidity (n=10)**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
HELPER T CELLS (CD4+) cells/μl	385 (97–534)	326 (124–778)	359–1565	p=NS
CITOTOXIC T CELLS (CD8+) cells/μl	153 (19–345)	181 (20–329)	178–853	p=NS
B CELLS (CD19+) cells/μl	90 (1–419)	215 (1–553)	61–321	p<0.05
NK CELLS (CD56+) cells/μl	140 (1–229)	100 (1–200)	126–729	p=NS

Table 4. **High dose dexamethasone lymphocyte patient recovery (n=25)**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
HELPER T CELLS (CD4+) cells/μl	180 (29–818)	370 (75–1340)	359–1565	p<0.05
CITOTOXIC T CELLS (CD8+) cells/μl	95 (11–256)	158 (20–642)	178–853	p<0.05
B CELLS (CD19+) cells/μl	42 (4–1310)	144 (5–2420)	61–321	p<0.05
NK CELLS (CD56+) cells/μl	91 (12–359)	121 (5–319)	126–729	p=NS

Table 4. **Low dose dexamethasone Lymphocyte patient recovery (n=8)**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
HELPER T CELLS (CD4+) cells/μl	479 (159–1730)	403 (215–778)	359–1565	p=NS
CITOTOXIC T CELLS (CD8+) cells/μl	146 (85–986)	194 (108–329)	178–853	p=NS
B CELLS (CD19+) cells/μl	95 (1–110)	231 (1–553)	61–321	p=NS
NK CELLS (CD56+) cells/μl	91 (1–314)	121 (1–270)	126–729	p=NS

Table 4. **Convalescent plasma patient lymphocyte patient recovery**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
LYMPHOCYTES (n=26)	800 (300–2200)	1300 (300–4100)	900–5200	p<0.05
HELPER T CELLS (CD4) cells/μl (n=23)	188 (29–818)	355 (75–340)	359–1565	p<0.05
CITOTOXIC T CELLS (CD8+) cells/μl (n=23)	102 (11–345)	161 (20–642)	178–853	p<0.05
B CELLS (CD19+) cells/μl (n=23)	51 (1–1310)	151 (5–2420)	61–321	p<0.05
NK CELLS (CD56+) cells/μl (n=23)	105 (1–359)	100 (1–319)	126–729	p=NS

Table 4. **Positive anti- SARS-COV2 antibody at admission lymphocyte patient recovery (n=8)**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
HELPER T CELLS (CD4+) cells/μl	179 (88–507)	403 (215–778)	359–1565	p<0.05
CITOTOXIC T CELLS (CD8+) cells/μl	84 (24–228)	117 (45–329)	178–853	p<0.05
B CELLS (CD19+) cells/μl	62 (6–81)	120 (6–442)	61–321	p<0.05
NK CELLS (CD56+) cells/μl	104 (13–187)	91 (18–200)	126–729	p=NS

Table 4. **Tocilizumab lymphocyte patient recovery**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
LYMPHOCYTES (n=8)	400 (200–2200)	950 (200–2500)	900–5200	p<0.05
HELPER T CELLS (CD4+) cells/μl (n=6)	142 (29–353)	353 (75–676)	359–1565	p=NS
CITOTOXIC T CELLS (CD8+) cells/μl (n=6)	76 (11–118)	109 (22–210)	178–853	p=NS
B CELLS (CD19+) cells/μl (n=6)	11 (4–42)	53 (6–204)	61–321	p<0.05
NK CELLS (CD56+) cells/μl (n=6)	76 (26–151)	122 (5–200)	126–729	p=NS

Table 4. **Lymphocyte recovery in absence of tocilizumab treatment**

	ADMISSION	DISCHARGE	REFERENCE VALUE	p VALUE
LYMPHOCYTES (n=37)	1000 (300–2200)	1400 (300–4100)	900–5200	p<0.05
HELPER T CELLS (CD4+) cells/μl (n=25)	268 (97–818)	374 (124–1340)	359–1565	p<0.05
CITOTOXIC T CELLS (CD8+) cells/μl (n=25)	106 (41–345)	161 (34–448)	178–853	p<0.05
B CELLS (CD19+) cells/μl (n=25)	69 (1–1310)	164 (1–2420)	61–321	p<0.05
NK CELLS (CD56+) cells/μl (n=25)	114 (1–339)	94 (1–319)	126–729	p=NS

dexamethasone (CD4 + T cells 180 cells/μl and CD8 + T cells 95 cells/μl) in comparison with the low-dose group (CD4 + T cells 479 cells/μl/ CD8 + T cells 146 cells/μl), P < 0.05**.** No differences in B cell and NK cell counts were detected between different doses of dexamethasone on admission (high-dose dexamethasone CD19 + B cells 42 cells/μl/ NK cells 114 cells/μl vs low-dose dexamethasone CD19 + B cells 95 cells/μl/ NK cells 91 cells/μl), *P = NS*.

Patients who received high-dose dexamethasone (n = 32) showed a significant improvement in the counts of total lymphocytes from admission (750 cells/μl) to the time of discharge (1250 cells/μl), P < 0.05**.** Recovery was at the expense of CD4 + T cells, CD8 + T cells and CD19 + B cell counts at discharge. Low levels of NK cells remained stable during SARS-CoV-2 infection in patients on high doses of dexamethasone.

High dose dexamethasone lymphocyte patient recovery (Table 4**)**

Patients who received low-dose dexamethasone maintained similar levels of lymphocyte counts on admission (990 cells/μl) and at discharge (1400 cells/μl), $P = NS$. No significant increase in the numbers of CD4 + T cells, CD8 + T cells, B cells and NK on admission and at discharge from the COVID-19 infection ward were identified, $P = NS$.

Low dose dexamethasone lymphocyte patient recovery (Table 4)

3.13. Convalescent plasma

Twenty-six patients received convalescent plasma, the other nineteen patients presented antibodies against SARS-CoV-2 on admission. No statistically significant differences were detected in total lymphocyte counts between patients who received convalescent plasma (800 cells/μl) and patients with positive antibodies on admission (1100 cells/μl), $P = NS$. However, we were able to study twenty-four patients with immunophenotype that received convalescent plasma and nine patients with positive antibodies on admission. No statistically significant differences were detected in CD4 + T cells, CD8 + T cells, B cells and NK cells on admission or at discharge in patients who received convalescent plasma and patients with positive antibodies on admission. Interestingly, we observed a significant recovery of levels of CD4 + T cells, CD8 + T cells and B cells at discharge in patients who received convalescent plasma or those with anti-SARS-CoV-2 antibodies in comparison with admission levels. Patients with positive serology on admission maintained similar lymphocyte counts on admission (1100 cells/μl) and at discharge (1400 cells/μl), $P = NS$.

Convalescent plasma patient lymphocyte patient recovery (Table 4**).**

Positive anti-SARS-COV-2 antibody at admission lymphocyte patient recovery (Table 4**).**

3.14. Tocilizumab

Eight patients who received treatment with tocilizumab showed a significant reduction of lymphocytes (400 cells/μl) on admission in comparison with thirty-eight patients (1050 cells/μl) who did not received tocilizumab. Six patients who received tocilizumab had peripheral blood lymphocyte immunophenotype study performed. CD4 + T cells, CD8 + T cells and NK cells remained on a similar level when compared with the other patients on admission and at discharge. However, patients who received tocilizumab had significantly reduced B cells (11 cells/μl) on admission in comparison with the non-tocilizumab group (69 cells/μl), $P < 0.05$. This significant reduction in the B cell compartment in patients who received tocilizumab was maintained at discharge (53 cells/μl in tocilizumab patients vs 185 cells/μl in the other patients), $P < 0.05$.

Significant recovery of the total lymphocyte numbers was detected in patients who received tocilizumab from admission to discharge, $P < 0.05$

Patients who received tocilizumab showed a recovery of the levels of CD4 + T cells, CD8 + T cells and NK cells from admission to discharge from the COVID19 infection ward, although without reaching statistical significance. However, significant increases in the numbers of B cells were detected in these patients.

In patients without tocilizumab treatment, the levels of CD4 + T cells, CD8 + T cells and B cells on admission increased statistically significantly at discharge from the COVID-19 ward, $P < 0.05$. Increases in NK cells were also detected, although not significant, $P = NS$.

Tocilizumab lymphocyte patient recovery (Table 4**)**

Lymphocyte recovery in absence of tocilizumab treatment (Table 4**)**

4. Discussion

Patients with a mild clinical presentation may not initially require hospitalization, but clinical signs and symptoms may worsen, with progression to severe lung disease in the second week of illness, coinciding with an exaggerated immune response and the generation of antibodies. We used this point to decide on an initial aggressive therapeutic approach using passive immunotherapy with convalescent plasma in patients with viral lung disease, or treatment high dose dexamethasone +/- tocilizumab in patients with established immunological response with the presence of anti-SARS-CoV-2 antibodies. The risk factors for progression to severe illness include older age and underlying chronic medical conditions [10]**;** however, in our immunologically based treatment these factors were irrelevant for the therapeutic decision. We used the CIRS-G scale to reflect chronic medical conditions. 60% of the patients treated with this protocol presented high CIRS-G scores. This population may be at risk of progressing to severe lung disease because their immune system is debilitated by previous conditions [10]**,** and this is the reason why the use of early convalescent plasma or aggressive anti-inflammatory treatment could be beneficial.

Our results suggest that aggressive therapy with early administration of convalescent plasma and high-dose dexamethasone may be of benefit in patients with SARS-CoV-2 infection and might avoid progression of lung damage or need of admission in intensive care. Interestingly, this strategy does not impair immune responses against SARS-CoV-2 and 93% of the patients generate anti-SARS-CoV-2 antibodies. Independently of the previous immunological status of the patients, serologically guided therapy might be of benefit even in patients with a high CIRS-G score, immunosuppressed or medically debilitated individuals and elderly patients.

The most important antiviral cells such as CD8 + T cytotoxic cells [11,12] and NK cells remained below normal reference values in patients with CIRS-G ≥ 6. Early passive immunotherapy with convalescent plasma in combination with dexamethasone such treatment against SARS-CoV-2 infection may be of benefit and not affect the immune recovery or individual immune responses against the virus. High-dose dexamethasone does not affect the generation of humoral immune responses in patients infected with SARS-CoV-2 and does not have a severe impact on lymphocyte recovery. Patients who require aggressive anti-inflammatory therapy with dexamethasone and tocilizumab showed lower levels of lymphocytes on admission. Patients who required high doses of dexamethasone showed increased T cells disturbances in the CD4 + and CD8 + T cell compartment [11,12], in sharp difference to patients who received tocilizumab, and showed low levels of B cells with a potential impaired humoral immune response. Depletion of NK cells in patients with SARS-CoV-2 infection is the most recognized immune disorder on admission and at discharge, reflecting an innate immune dysfunction. Because antibody dependent cellular cytotoxicity (ADCC) is mainly mediated by NK lymphocytes [13] and the 20 mg dose of dexamethasone is used as premedication in antibodies specifically designed to increase ADCC without any decrease in its efficacy [14,15], we considered that its use as premedication when plasma is administered is safe and not very immunosuppressive [16]**.** We think that the decrease in ADCC in the case of patients infected with SARS-CoV-2 is more related to the decrease or depletion on NK cells.[17]**.** Although the number of patients in this cohort is limited, high-dose dexamethasone therapy based on the presence of antibodies to the SARS-CoV-2 virus is novel ant should be explored in future clinical trials.

Administration of convalescent plasma in patients with positive anti-SARS-CoV2 serology on admission may be unsuccessful [18]; however, in patients with negative anti-SARS-CoV2 serology has great utility to rescue patients. The median number of days from diagnosis to convalescent plasma infusion was four (range 1–9 days) and patients remained hospitalized in the COVID-19 infection ward a median of eight days (range 1–29 days). A recent review indicates that mortality of patients with immunosuppression who were treated with convalescent plasma was 16%, with 60% of patients demonstrating rapid clinical improvement within 5 days after convalescent plasma therapy [19]. Other studies reporting on the success of plasma when transfused within 3 days

support our strategy and findings [20,21].

5. Conclusion

Serological status on admission may be of interest to guiding an aggressive therapy with convalescent plasma or anti-inflammatory treatment in patients with acute infection of SARS-CoV-2, independent of age and comorbidities. This approach might diminish the progression of lung injury and the necessity of admission to intensive care.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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