Changes and evolution among SARS-CoV-2 hospitalised patients in terms of severity, mortality and virus genome in a Spanish cohort

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Abstract

A comparison between pandemic waves could help to understand the evolution of this disease. The objective of this work was to study the evolution of COVID-19 hospitalized patients on different pandemic waves in terms of severity and mortality. We performed an observational retrospective cohort study of hospitalized patients (5,220) with SARS-CoV-2 infection from February to September in Aragon, Spain. In a comparative way, we analyzed ICU admission and 30-day mortality, clinical characteristics and risk factors, of first and second waves. SARS-CoV-2 virus genome were analyzed in 236 samples. Patients in the first wave (n=2,547) were older (74 y, IQR: 60-86 vs. 70 y, IQR: 53-85; $p<0.001$) and showed worse clinical and analytical parameters related to severe COVID-19 than in the second wave (n=2,673). The probability of ICU admission at 30 days was 16% and 10% in the first and second wave, respectively ($p<0.001$). The cumulative 30-day mortality rates were 38% in the first wave and 32% in the second one ($p=0.007$). Survival differences were observed among patients aged 60 to 80 years. There was variability among death risk factors and virus genome between waves. Therefore, the two COVID-19 pandemic waves analyzed were different, in terms of disease severity and mortality.

Introduction

Covid-19, caused by SARS-CoV-2, is the first major pandemic humankind has faced in over 100 years. Pursuing naturally-acquired herd immunity is not a feasible strategy [1]. Therefore, while awaiting an widespread vaccination to minimize the impact of SARS-CoV-2, we must rely on early diagnosis, isolation, tracing contacts of infected individuals, and population-based non-pharmacological interventions, including physical distancing, the use of masks, and enhanced hand hygiene, among others [2, 3, 4, 5, 6].

As opposed to SARS-CoV-1, the transmission of SARS-CoV-2 is expected to resemble that of pandemic influenza, with several pandemic waves, followed by seasonal circulation as it might have previously happened with other known coronaviruses [7, 8].

Aragón is an autonomous Spanish community that experienced the first pandemic wave between February and May, 2020. After a period of full lockdown, community transmission of SARS-CoV-2 decreased markedly [9]. After several local outbreaks in June and July, SARS-CoV-2 transmission was widespread, and Aragón had the highest midsummer incidence in the European region [10, 11]. Although there are indications that the second wave was less severe than the first [12], there are no studies that support this hypothesis in a localized cohort like ours. Likewise, although studies have been reported on the identification of risk factors for the severity of the disease in Spain [13], there is a lack of studies comparing the changes in the evolution of hospitalized patients in terms of severity and mortality with patient's demographic, clinical and laboratory information.

The present study aimed to study the evolution of those hospitalised with SARS-COV-2, over a period of time characterised by two distinct pandemic waves, in terms of severity and mortality.

Methods

Design and setting

This observational retrospective cohort study was conducted in Aragon, a region in Northeastern Spain, with 1,328,753 inhabitants (January 1, 2020). The publicly-funded healthcare system (SALUD) covered the entire population. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cohort studies [14].

The testing strategy in Aragón was adapted to the capabilities of the healthcare system. In March and early April 2020, only patients that had symptoms consistent with moderate or severe disease were tested. After late April, all patients with suspected COVID-19 could be tested, regardless of symptom severity.

The beginning and end of each wave was defined, based on the variation in SARS-CoV-2 infection incidences observed from February 27 to September 23. We observed two well-defined waves: the first from February 27 to May 27, 2020, and the second from June 17 to September 23, 2020 (Supplementary Figure S1). However, dated September 23rd, the second wave has not fully declined.

Patients, Definitions, And Data Source

We included all patients with SARS-CoV-2 infections, confirmed with RT-PCR, that were hospitalized in the SALUD hospital network. Hospitalization was considered chronologically related to a SARS-CoV-2 infection, when it occurred within the first 20 days after, and no more than 10 days before, the first positive SARS-CoV-2 PCR test.

Our primary data source was the Aragón Healthcare Records Database, which we accessed through the *BIGAN Gestion Clinica* platform of the Aragón Department of Health. This database contained demographic and clinical information on all individuals covered by SALUD.

We collected data on demographics, comorbidity, and drugs prescribed in the 6 months prior to hospitalization. Vital signs were recorded upon arrival at the emergency room. Laboratory variables measured in the first 24 h were available only for the largest two hospitals of the SALUD network, which represented 60.6% of all patients admitted with COVID-19 in our region. The observation period lasted up to 30 days after the last patient was included in the analysis. The main outcomes were: admission into the intensive care unit (ICU) and 30-day all-cause mortality after hospital admission.

Virus Genome Analysis

Whole genome sequences (> 29,000 bp) of SARS-CoV-2 from Aragon (n = 295) were retrieved from the database established by the global initiative on sharing all influenza data (GISAID). We evaluated how these sequences were distributed among different phylogenetic clades. Sequences were aligned with an iterative refinement method implemented in MAFFT version 7 software [15], and sequences were manually edited with Bioedit v7.2.5 [16]. The phylogeny of

the alignment was inferred with IQ-Tree software v2.1.1 [17], and node support was assessed with an ultrafast bootstrap approximation. The TIM2 substitution model, with unequal base frequencies and a proportion of invariant sites (TIM2 + F + I), was selected as the best-fit model, according to the Bayesian information criterion. In Aragón, the prevalence of the D614G mutation was tracked throughout the pandemic by aligning this region in all 295 genomes.

Statistical analysis

We performed a comparative descriptive analysis of the first- and second-wave cohorts. Continuous variables are presented as the median and interquartile range (IQR), and categorical variables are presented as absolute and relative frequencies. Comparisons were performed with the Mann-Whitney test for continuous variables and the Chi-square test for categorical variables or proportions.

In addition, we performed two longitudinal analyses in the group of hospitalized patients to evaluate the management of ICU admission and the occurrence of death. The follow-up time started from the hospitalization date and ended at either the ICU admission date or the discharge/death date. We used cumulative incidence curves to analyze longitudinal data, and the Gray test to compare variables between waves, for groups stratified by sex or age.

Finally, we analyzed univariate and multivariate logistic regression models to evaluate associations between the study variables and death. The discriminatory capacities of significant risk factors were evaluated by measuring the area under the receiver operating characteristic (ROC) curve (AUC). Results are expressed as odds ratios (OR) and p-values.

The threshold p-value was set at 0.05. Analyses were performed with R version 3.6.2 language programming (R Foundation for Statistical Computing, Vienna, Austria) and Python version 3.7 provided by Jupyter (jupyter.org).

Ethics Declaration

The research protocol was approved by the Clinical Research Ethics Committee of Aragón (PI 20/183). Due to the retrospective observational nature of this study, data could be fully anonymized, and informed consent was waived.

Results

Differences in hospitalized patients between the first and second waves

Between February 27 and September 23, 2020, 33,498 patients were diagnosed with SARS-CoV-2 infections in Aragón (Fig. 1). The number of individuals with confirmed SARS-CoV-2 infections increased considerably (five-fold) between the first and second waves, due to the greater availability of diagnostic tests, but the absolute number of hospitalized patients with SARS-CoV-2 infections was similar in both waves. Of these, during the first and second waves, respectively, 332 (13%) and 198 (7.4%) were admitted to the ICU ($p < 0.001$), and 779 (30.6%) and 501 (18.7%) died ($p < 0.001$). Comparison of cumulative cases between waves is represented in Fig. 2.

Age and sex distributions between waves for patients that were hospitalized, admitted to the ICU, or deceased are shown in Supplementary Figure S2. Among all patients hospitalized with COVID-19, the mean age was significantly different in the two waves (Table 1). Patients in the first wave were older (74 y, IQR: 60–86 vs, 70 y, IQR: 53–85; $p < 0.001$) and had more comorbidities, including cerebrovascular disease and dementia and also previous pneumonia, than patients in the second wave. However, the second wave had a higher frequency of diabetes. In addition, previous drug treatments in hospitalized patients differed between the two pandemic waves.

Table 1
Comparisons of clinical and laboratory variables between the two COVID-19 pandemic waves, Aragon, Spain, February-September 2020.

Variable	Hospitalized patients					ICU patients					Deaths				
	First wave		Second wave		p-value	First wave		Second wave		p-value	First wave		Second wave		p-value
	n	%	n	%		n	%	n	%		n	%	n	%	
Sex, %															
Male	1,365	53.6	1,372	51.3	0.097	219	66.0	131	66.2	1.000	427	54.8	256	51.1	0.20
Female	1,182	46.4	1,301	48.7		113	34.0	67	33.8		352	45.2	245	48.9	
Age (years), median (IQR)	74 (60–86)	NA	70 (53–85)	NA	< .001	70 (60–76)	NA	62 (54–72)	NA	< .001	85 (77–90)	NA	87 (81–91)	NA	0.01
Emergency room															
Systolic pressure (mm Hg), median (IQR)	126 (112–141)	NA	128 (114–142)	NA	0.126	127 (114–143)	NA	130 (115–144)	NA	0.319	122 (108–141)	NA	129 (113–145)	NA	0.00
Diastolic pressure (mm Hg), median (IQR)	71 (62–80)	NA	72 (64–80)	NA	0.111	73 (63–81)	NA	73.5 (66–81)	NA	0.210	68 (59–78)	NA	69 (61–78)	NA	0.29
Heart rate (bpm), median (IQR)	86 (75–98)	NA	83 (73–97)	NA	0.001	90 (78–101)	NA	88 (77–100)	NA	0.265	87 (74–100)	NA	85 (73–98)	NA	0.18
Respiratory rate (bpm), median (IQR)	25 (20–32)	NA	26 (22–32)	NA	0.487	26 (20.5–32)	NA	27.5 (23.7–32)	NA	0.329	30 (24–34)	NA	31 (24–36)	NA	0.19
Temperature (°C), median (IQR)	36.8 (36.3–37.5)	NA	36.6 (36.2–37)	NA	< .001	37 (3.5–37.8)	NA	36.7 (36.3–37.3)	NA	0.001	36.9 (36.3–37.5)	NA	36.6 (36.3–37.2)	NA	0.00
Oxygen saturation (%), median (IQR)	95 (92–97)	NA	95 (93–97)	NA	< .001	94 (90–97)	NA	94 (92–96)	NA	0.374	94 (90–96)	NA	94 (92–96)	NA	0.00
Oxygen treatment, %	278	12.7	239	9.6	0.001	41	13.7	18	10	0.262	75	11.9	50	10.5	0.49
Capillary blood glucose (mg/dl), median (IQR)	147 (118–194)	NA	150 (121–215)	NA	0.329	147 (117.7–195.5)	NA	151 (126–210)	NA	0.590	162 (124.5–218)	NA	180 (140–255)	NA	0.09
Laboratory, median (IQR)															
Glucose (mg/dl)	113 (97–139)	NA	116 (97–144)	NA	0.434	156.5 (117–211)	NA	164 (123–203)	NA	0.481	123 (101–164)	NA	132 (104–172)	NA	0.10
Creatinine (mg/dl)	0.94 (0.74–1.29)	NA	0.89 (0.69–1.19)	NA	< .001	0.93 (0.71–1.31)	NA	0.73 (0.58–0.99)	NA	< .001	1.22 (0.91–1.86)	NA	1.18 (0.84–1.64)	NA	0.09
Urea (g/l)	0.421 (0.3–0.69)	NA	0.4 (0.29–0.62)	NA	0.003	0.51 (0.36–0.74)	NA	0.47 (0.34–0.62)	NA	0.123	0.71 (0.5–1.06)	NA	0.63 (0.43–0.94)	NA	0.01
Chloride (mmol/l)	101 (98–105)	NA	102 (99–105)	NA	0.032	103 (99–106)	NA	104 (102–107)	NA	< .001	103 (99–107)	NA	102 (99–106)	NA	0.41
Potassium (mmol/l)	4.17 (3.84–4.53)	NA	4.19 (3.86–4.52)	NA	0.904	4.14 (3.77–4.59)	NA	4.11 (3.85–4.41)	NA	0.561	4.31 (3.84–4.68)	NA	4.25 (3.83–4.59)	NA	0.24
Ionic Calcium (mmol/l)	1.17 (1.13–1.22)	NA	1.15 (1.11–1.19)	NA	< .001	1.13 (1.08–1.17)	NA	1.13 (1.09–1.17)	NA	0.812	1.18 (1.13–1.22)	NA	1.16 (1.11–1.2)	NA	0.00
Alanine aminotransferase (ALT) (U/l)	23 (15–40)	NA	24 (15–40)	NA	0.953	36 (23–58)	NA	35 (21–49)	NA	0.227	20 (13–32)	NA	19 (13–29.5)	NA	0.28

ICU: intensive care unit; IQR: interquartile range; NA: not applicable

Patients were grouped as hospitalized patients (total number of patients), admitted to the ICU, and deceased. Laboratory and clinical variables were baseline values. Units are shown in parentheses. Values of comorbidities and previous treatment are displayed as n and the percentage of total patients (%).

	Hospitalized patients					ICU patients					Deaths				
Aspartate aminotransferase (AST) (U/l)	33 (24-49.75)	NA	32 (23-47)	NA	0.235	47 (31-74.5)	NA	38 (27-54)	NA	0.004	35 (23-53)	NA	34 (24-49)	NA	0.61
Lactate dehydrogenase (LDH) (U/l)	292 (230-398)	NA	286 (224-372)	NA	0.018	465 (345-600)	NA	438 (332-546)	NA	0.247	319 (244-439)	NA	310 (238-458)	NA	0.60
Prothrombin activity (APT) (%)	85 (74-96)	NA	90 (78-102)	NA	< .001	81 (68.5-94)	NA	83 (73-97.75)	NA	0.077	81 (66-91)	NA	84 (71-99.5)	NA	0.00
International normalized ratio-prothrombin time (INR-PT)	1.12 (1.06-1.23)	NA	1.08 (1.02-1.18)	NA	< .001	1.16 (1.07-1.29)	NA	1.13 (1.05-1.24)	NA	0.027	1.17 (1.08-1.36)	NA	1.13 (1.03-1.26)	NA	0.00
Active partial thromboplastin time (RATIO-APTT) (seconds)	1 (0.91-1.1)	NA	0.97 (0.89-1.06)	NA	< .001	0.97 (0.9-1.08)	NA	0.91 (0.83-0.99)	NA	< .001	1.01 (0.92-1.11)	NA	0.97 (0.89-1.08)	NA	0.00
D-Dimer (microgr/l)	958 (519-1770)	NA	758 (428-1431)	NA	< .001	1334 (808-2418)	NA	1129 (612-2567)	NA	0.310	1495 (972-3603)	NA	1327 (772-3893)	NA	0.13
Fibrinogen (mg/dl)	700 (599-709)	NA	657 (550-700)	NA	< .000	700 (657-847)	NA	700 (577-764)	NA	0.004	680 (571-715)	NA	650 (531-700)	NA	0.00
Leukocytes (mil/mm3)	6.8 (5.1-9.3)	NA	6.64 (4.9-9)	NA	0.055	9 (6.77-12.1)	NA	9.6 (7.05-12.5)	NA	0.276	8 (5.8-10.95)	NA	7.55 (5.3-10.3)	NA	0.09
Lymphocytes (mil/mm3)	0.95 (0.67-1.38)	NA	1.02 (0.70-1.48)	NA	0.001	0.64 (0.44-0.94)	NA	0.68 (0.46-0.96)	NA	0.279	0.81 (0.53-1.26)	NA	0.77 (0.56-1.10)	NA	0.37
Lymphocytes (%)	14.6 (8.7-22.6)	NA	16.4 (9.9-24.5)	NA	< .001	7.1 (4.4-12.0)	NA	6.9 (4.5-11.9)	NA	0.787	10.1 (6-17.4)	NA	10.7 (6.3-18.0)	NA	0.68
Monocytes (mil/mm3)	0.50 (0.35-0.68)	NA	0.49 (0.34-0.70)	NA	0.530	0.41 (0.27-0.64)	NA	0.51 (0.34-0.72)	NA	0.010	0.51 (0.34-0.73)	NA	0.48 (0.31-0.69)	NA	0.09
Monocytes (%)	7.6 (5.3-9.99)	NA	7.6 (5.3-10.1)	NA	0.636	4.8 (3.2-7.015)	NA	5.3 (3.8-7.45)	NA	0.158	6.4 (4.2-9.4)	NA	6.365 (4.1-9.2)	NA	0.39
Neutrophils (mil/mm3)	5.00 (3.46-7.39)	NA	4.82 (3.24-6.99)	NA	0.011	7.91 (5.48-10.7)	NA	8.16 (5.77-10.9)	NA	0.548	6.19 (4.25-9.33)	NA	6.07 (3.83-8.67)	NA	0.33
Neutrophils (%)	76 (66.6-84.4)	NA	74.1 (64.8-82.9)	NA	< .001	88.2 (80.2-91.2)	NA	86.6 (80.3-91.1)	NA	0.348	81.7 (72.8-88)	NA	81.6 (72.3-89)	NA	0.79
Basophils (mil/mm3)	0.02 (0.01-0.03)	NA	0.02 (0.01-0.03)	NA	0.423	0.02 (0.01-0.03)	NA	0.01 (0.00-0.03)	NA	0.001	0.02 (0.01-0.04)	NA	0.02 (0.01-0.03)	NA	0.16
Basophils (%)	0.3 (0.2-0.5)	NA	0.3 (0.2-0.5)	NA	0.776	0.2 (0.1-0.3)	NA	0.1 (0.1-0.2)	NA	< .001	0.265 (0.1-0.4)	NA	0.27 (0.1-0.4)	NA	0.46
Eosinophils (mil/mm3)	0.007 (0-0.036)	NA	0.008 (0-0.039)	NA	0.471	0 (0-0.016)	NA	0 (0-0)	NA	0.004	0.002 (0-0.021)	NA	0.001 (0-0.016)	NA	0.47
Eosinophils (%)	0.1 (0-0.5)	NA	0.1 (0-0.6)	NA	0.408	0 (0-0.1)	NA	0 (0-0)	NA	0.002	0.04 (0-0.3)	NA	0.01 (0-0.2)	NA	0.50
Red blood cells (mil/mm3)	4.52 (4.08-4.87)	NA	4.52 (4.09-4.91)	NA	0.281	4.17 (3.79-4.52)	NA	4.24 (3.80-4.59)	NA	0.280	4.31 (3.84-4.70)	NA	4.34 (3.84-4.72)	NA	0.58

ICU: intensive care unit; IQR: interquartile range; NA: not applicable

Patients were grouped as hospitalized patients (total number of patients), admitted to the ICU, and deceased. Laboratory and clinical variables were baseline values. Units are shown in parentheses. Values of comorbidities and previous treatment are displayed as n and the percentage of total patients (%).

	Hospitalized patients					ICU patients					Deaths				
Erythroblasts (mil/mm3)	0.001 (0-0.009)	NA	0.002 (0-0.01)	NA	0.088	0.001 (0-0.01)	NA	0 (0-0.01)	NA	0.695	0.001 (0-0.01)	NA	0.002 (0-0.01)	NA	0.45
Erythroblasts (%)	0.04 (0-0.1)	NA	0.06 (0-0.1)	NA	0.007	0.01 (0-0.1)	NA	0 (0-0.1)	NA	0.627	0.04 (0-0.1)	NA	0.04 (0-0.1)	NA	0.64
Mean corpuscular hemoglobin concentration (MCHC) (g/dl)	33.4 (32.8-34)	NA	33.5 (32.9-34.1)	NA	0.022	33.4 (33.0-34.1)	NA	33.5 (33.0-34.1)	NA	0.327	33.1 (32.6-33.7)	NA	33.1 (32.5-33.6)	NA	0.78
Hemoglobin (g/dl)	13.5 (12.3-14.6)	NA	13.6 (12.3-14.7)	NA	0.554	12.6 (11.3-13.7)	NA	12.8 (11.5-1.8)	NA	0.367	13 (11.7-14.2)	NA	1.1 (11.8-14.3)	NA	0.37
Hematocrit (%)	40.4 (36.9-43.5)	NA	40.4 (36.9-43.7)	NA	0.893	37.6 (34.1-40.4)	NA	37.7 (34.6-40.9)	NA	0.392	39.2 (35.4-42.8)	NA	39.4 (35.8-43.1)	NA	0.42
Mean corpuscular volume (MCV) (fl)	90.3 (86.6-93.6)	NA	90 (86.15-93.6)	NA	0.158	90.2 (87.6-93.3)	NA	90.4 (86-93.8)	NA	0.701	91.8 (87.7-95.3)	NA	92.2 (88.5-95.6)	NA	0.41
Platelet count (mil/mm3)	188 (143-246)	NA	190 (148-246)	NA	0.610	227 (169-288)	NA	242 (173-312)	NA	0.334	177 (136-230)	NA	172 (131-215)	NA	0.24
Mean platelet volume (MPV) (fl)	9.1 (8.4-9.9)	NA	9.2 (8.5-9.9)	NA	0.197	8.9 (8.275-9.7)	NA	8.9 (8.3-9.6)	NA	0.881	9.2 (8.7-10.2)	NA	9.4 (8.6-10.1)	NA	0.68
Interleukin-6 (pg/ml)	41.51 (17.18-50)	NA	32.5 (12.6-58.6)	NA	0.161	50 (21.5-82.2)	NA	50 (16.9-94.4)	NA	0.825	50 (36.3-84.2)	NA	50 (25.9-102.2)	NA	0.83
C-reactive protein (mg/l)	8.18 (2.75-15.07)	NA	5.52 (1.64-11.23)	NA	< .001	14.56 (5.9-24.61)	NA	8.31 (2.44-15.5)	NA	0.001	11.15 (5.52-1.8)	NA	9.69 (2.48-17.52)	NA	0.06
Procalcitonin (mg/l)	0.13 (0.07-0.32)	NA	0.11 (0.07-0.24)	NA	0.084	0.36 (0.16-0.94)	NA	0.17 (0.09-0.57)	NA	< .001	0.24 (0.13-0.73)	NA	0.24 (0.12-0.59)	NA	0.66
Ferritin (ng/ml)	452 (220-1003)	NA	614 (306-1190)	NA	0.007	1277 (583-2727)	NA	1145 (659-2026)	NA	0.971	460 (216-997)	NA	704 (360-1268)	NA	0.05
Comorbidities/ Previous diagnosis, %															
Ischemic Cardiopathy	207	8.1	180	6.7	0.054	27	8.1	17	8.6	0.880	95	12.2	62	12.4	0.93
Hypertension	835	38.2	877	35.3	0.050	115	38.5	56	31.1	0.108	319	50.6	234	49.1	0.63
Intermittent claudication	109	4.3	97	3.6	0.261	19	5.7	10	5.1	0.854	66	8.5	24	4.8	0.01
Cerebrovascular disease	292	11.5	248	9.3	0.013	21	6.3	11	5.6	0.841	156	20.0	106	11.2	0.66
Dementia	354	13.9	279	10.4	< .001	21	6.3	2	1.0	0.002	200	25.7	125	24.9	0.80
Diabetes	493	19.4	588	22.0	0.018	72	21.7	56	28.3	0.096	198	25.4	147	29.3	0.15
Obesity	366	4.4	414	15.5	0.251	69	20.8	55	27.8	0.090	96	12.3	82	16.4	0.04
Chronic obstructive pulmonary disease (COPD)	367	14.4	375	14.0	0.717	52	15.7	31	15.7	1.000	137	17.6	96	19.2	0.50
Previous pneumonia	381	15.0	230	8.6	< .001	76	22.9	20	10.1	< .001	117	15.0	47	9.4	0.00
Malignancy	132	5.2	124	4.6	0.362		4.8	8	4.0	0.824	57	7.3	32	6.4	0.58

ICU: intensive care unit; IQR: interquartile range; NA: not applicable

Patients were grouped as hospitalized patients (total number of patients), admitted to the ICU, and deceased. Laboratory and clinical variables were baseline values. Units are shown in parentheses. Values of comorbidities and previous treatment are displayed as n and the percentage of total patients (%).

	Hospitalized patients					ICU patients					Deaths				
Previous Treatments, %															
Gastric secretion inhibitors	1045	41.0	970	36.3	< 0.001	124	37.3	72	36.4	0.856	454	58.3	273	54.5	0.18
Antidiabetics	408	16.0	471	17.6	0.128	61	18.4	56	28.3	0.008	164	21.1	114	22.7	0.47
Antithrombotics	739	29.0	688	25.7	0.007	78	23.5	43	21.7	0.669	359	46.1	219	43.7	0.43
Beta-blockers	400	15.7	364	13.6	0.034	55	16.6	27	15.6	0.367	177	22.7	105	21.0	0.46
Potassium-sparing diuretics	115	4.5	94	3.5	0.07	9	2.7	5	2.5	1.000	61	7.8	20 (	4.0	0.00
Anxiolytics	276	10.8	237	8.9	0.019	46	13.9	23	11.6	0.511	184	23.6	102	20.4	0.20
Antidementia drugs	41	1.6	37	1.4	0.559	11	3.3	1	0.5	0.057	88	11.3	68	13.6	0.26
Nutritional supplements	194	7.6	188	7.0	0.198	9	2.7	1	0.5	0.088	78	10.0	31	6.2	0.02
ICU: intensive care unit; IQR: interquartile range; NA: not applicable															
Patients were grouped as hospitalized patients (total number of patients), admitted to the ICU, and deceased. Laboratory and clinical variables were baseline values. Units are shown in parentheses. Values of comorbidities and previous treatment are displayed as n and the percentage of total patients (%).															

Compared to the second wave, patients hospitalized in the first wave showed signs of greater disease severity, including a higher heart rate, higher temperature, lower oxygen saturation, and higher levels of creatinine, C-reactive protein, LDH, and fibrinogen (Table 1). Other disease severity parameters, such as neutrophilia and lymphopenia, also indicated worse disease in patients infected in the first wave compared to those infected in the second wave.

Among patients admitted to the ICU, those in the first wave were significantly older (70 y, IQR: 60–76 vs. 62 y, IQR: 54–72) and had a higher rate of dementia, compared to those admitted to the ICU in the second wave. Patients in the first wave also took antidiabetic drugs less frequently and exhibited higher levels of both clinical and analytical markers of serious illness compared to patients in the second wave (Table 1).

Unlike patients that were hospitalized or admitted to the ICU, patients that died in the first wave were younger than those that died in the second wave (Table 1) (85 y, IQR: 77–90 vs. 87 y, IQR 81–91). Moreover, they were also less obese than in the second wave. Nevertheless, patients that died in the first wave showed higher disease severity parameters than those that died in the second wave.

Risk factors of death based on univariate and multivariate analyses

The univariate analysis results (Supplementary Table S1) showed that mortality was best predicted in the first wave (based on the ROC AUC) by urea (AUC = 0.81), age (AUC = 0.79), D-dimer (AUC = 0.73), procalcitonin (AUC = 0.73), and creatinine (AUC = 0.72). The best predictors of mortality in the second wave were age (AUC = 0.82), urea (AUC = 0.77), procalcitonin (AUC = 0.74), D-dimer (AUC = 0.71), and lymphocytes (AUC = 0.70).

The multivariate analysis results (Fig. 3) showed that, in both pandemic waves, age, elevated LDH levels, impaired kidney function, and taking diuretics and nutritional supplements before hospitalization were independent risk factors associated with mortality. Conversely, normal platelet and red blood cell counts were protective factors. However, the waves also showed some significant differences. For example, female sex was a protective factor (OR = 0.643) in the first, but not the second wave. In contrast, obesity (OR = 1.815) and immunosuppressant treatment (OR = 5.988) were risk factors for mortality in the second, but not the first wave. Other significant factors identified in the first, but not the second wave, included previous treatment with antidepressants (OR = 1.708), anti-dementia drugs (OR = 2.049), or cardiac vasodilators (OR = 3.076). Our multivariate models for the first and second waves had AUC values of 0.88 (0.86–0.91) and 0.88 (0.86–0.90), respectively ($p = 0.750$), which showed that the models had similar predictive value.

Longitudinal Data Analysis

The cumulative incidences of ICU admission (Fig. 4) were significantly different between waves ($p < 0.001$). The probability of an ICU admission ranged from 13% at 10 days to 16% at 30 days, during the first wave, and from 8% at 10 days to 10% at 30 days during the second wave. After stratifying by sex, the ICU admission probability among men ranged from 16–20% (10–30 days) during the first wave, and from 10–13% (10–30 days) during the second wave ($p < 0.001$). The probability of ICU admission among women was lower; it ranged from 10–12% (10–30 days) during the first wave, and from 5–7% (10–30 days) during the second wave ($p < 0.001$).

When stratified by age, we found that the need for an ICU admission was significantly different between waves for the 0–60year group ($p = 0.05$), the 60–80year group ($p < 0.001$), and the > 80year group ($p < 0.001$). For these three age groups, the rates of ICU admission were 13%–19%, 18%–25%, and 5%–7%, respectively, in the first wave, and 10%–15%, 14%–18%, and 1%–2%, respectively, in the second wave.

Overall survival was significantly different between waves ($p = 0.007$) (Fig. 5). The probability of death ranged from 18% at 10 days to 37% at 30 days in the first wave, and from 11% at 10 days to 32% at 30 days in the second wave. Mortality was greater in the first wave for patients with either short or long hospitalizations. When stratified by sex, for men, mortality ranged from 16–38% in the first wave, and from 10–32% in the second wave ($p = 0.02$). For women,

mortality ranged from 19–36% in the first wave, and from 11–32% in the second wave ($p = 0.2$). In both waves, mortality was greater among men than among women.

Mortality increased with age in both waves. However, overall survival was significantly different between waves in the 60-80-year age group ($p = 0.02$), but not in the 0-60-year or > 80 -year age groups ($p = 0.20$). In the first wave, the probabilities of death at 10 and 30 days were 2%-7%, 13%-32%, and 30%-54% for hospitalized patients aged 0-60-years, 60-80-years, and > 80 -years, respectively. Of note, the probability of dying was lower in the youngest group than in the two older groups. Remarkably, the probability of death was higher for longer hospitalization periods, particularly among the oldest patients. In the second wave, the probabilities of death were 1%-5%, 5%-24%, and 21%-48% for the three age groups, respectively. These values were lower, but the trends were similar, compared to those observed in the first wave.

Differences In The SARS-Cov-2 Virus Genome

We analyzed 236 virus samples from the first wave and 56 from the second. The distribution of the D614G spike protein mutation was different between waves. It was present in 66% of viruses studied in the first wave and 100% of viruses studied in the second. According to the GISAID classification, 32% of the viruses analyzed during the first wave belonged to clades S (characterized by L84S mutation in the NS8 protein) and V (with G251V mutation in the NS3 protein); but these virus strains disappeared in the second wave. In contrast, 98.2% of viruses studied in the second wave belonged to clade G (characterized by D614G spike protein mutation) and 1.8% belonged to clade GR (with D614G spike protein mutation and the G204R mutation in the nucleocapsid protein).

Discussion

Aragon was one of the first regions in Europe to experience the emergence of this second pandemic wave of SARS-CoV-2 infection. This experience allowed early analyses and comparisons of the two waves, which could shed light on the future evolution of the pandemic.

Although the number of hospitalized patients was similar in both waves, we found significant differences in the increase of cases over time. The intensity of the first surge was very difficult for the healthcare system to manage, which led to its collapse or near-collapse. However, that degree of intensity did not occur during the second wave.

Another difference between the two waves was that the patients hospitalized were younger in the second wave than in the first wave. This difference was probably due to the greater exposure of younger people in the community and the greater protection of older people, who were more concerned about the perils of the disease.

Compared to the first wave, the second wave had less marked clinical (high fever) and analytical (lymphopenia, elevated D-dimer levels) predictors of worse outcomes [18, 19]. Moreover, age-related comorbidities, such as cerebrovascular disease and dementia, were less prevalent in the second wave than in the first wave. Interestingly, diabetes was more prevalent in the second wave.

Patients transferred to the ICU had somewhat different characteristics between waves. Compared to the first wave, patients in the second wave were younger and more frequently took antidiabetic drugs. Also, the frequency of cognitive impairments was lower in the second wave. It is likely that patients with these features were also infected in the first wave, but the number was obscured by the larger number of older and seriously ill patients. In the second wave, older and seriously ill patients were less numerous than in the first wave.

However, of note, although patients that required hospitalization and ICU care were younger in the second wave than in the first wave, the median age of death was higher in the second wave.

The most relevant finding of our study was that the overall 30-day mortality of hospitalized patients declined during the second wave. This significant decline in mortality affected essentially all patients aged 60 to 80 years old. A potential cause for this finding was that hospitalized patients had, overall, less severe parameters in the second wave than in the first wave. These parameters included vital signs and the clinical inflammation markers that serve as prognostic factors of severity [20–22]. Another explanation for the difference in mortality between waves could be improvements in the clinical management of patients. Although no antiviral drugs have clearly increased survival rates in patients with COVID-19, other advances in the management of patients with more severe infections, have been associated with improved outcomes [23–25]. Additionally, unlike the rapid rise observed in the first wave, during the second wave, the number of cases increased gradually. Therefore, although the health system has been overloaded, health resource use has increased gradually, which has prevented a system collapse. Indeed, this situation might be associated with better outcomes.

Finally, we could not rule out the possibility that mutations in the virus might have reduced the virulence of SARS-Cov-2. We analyzed genomes from the first and second waves and found that the spike 614G mutation, which was abundant only at the end of the first wave, was present in all genomes isolated in the second wave. These findings have been described throughout the country [26]. Initially, this mutation was associated with greater disease severity [27]. However, a recent study indicated that, although the G614 variant was related to greater infectivity and higher viral loads, there is no evidence that it was associated with disease severity [28].

This study has some limitations. In the first two months of the pandemic, there was a significant shortage of diagnostic tests. This situation prevented an overall comparison between pandemic waves. Due to these limitations, we focused the comparison on patients that were admitted to hospitals. Both the hospital admission criteria and the number of patients admitted with COVID-19 were similar in the two waves. Other limitations of this study were primarily due to its retrospective nature and the data source (electronic medical records). Additionally, this study included data from the entire region; therefore, different hospitals might have employed different management criteria and different resource allocations. However, some of these limitations were compensated by the high number of patients included.

Declarations

Competing interests

The authors declare no competing interests.

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Author contribution

RA, JRP, RH and TS were responsible for the conception and design of the study. RH

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Data availability

The clinical and demographic data analysed were retrieved through the *BIGAN Gestion Clinica* platform of the Aragón Department of Health, which contains the information from the Aragón Healthcare Records Database.

Whole genome sequences of SARS-CoV-2 were retrieved from the database established by the global initiative on sharing all influenza data (GISAID).

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Figures

Figure 1

Selection and analysis of study participants, Aragon, Spain, February-September, 2020 (n=33,498). Note that substantial underdiagnosis occurred in the first wave, due to the scarcity of available tests.

Figure 2
Comparison of cumulative cases between waves, Aragon, Spain, February-September, 2020. Prevalence of SARS-COV-2 cases, confirmed with RT-PCR positive tests, during the first (purple) and second waves (green) are shown for hospitalized patients (left panel), ICU admissions (middle panel), and deaths (right panel). ICU: intensive care unit.

Figure 3
Forest plot of significant predictors of death. Multivariate analysis results show odds ratios for potential predictors during (left panel) the first wave and (right panel) the second wave, Aragon, Spain, February-September, 2020. Colors indicate: red: demographics; blue: laboratory measurements; green: medical history. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Figure 4

Cumulative incidences of ICU admissions for (top row) all patients; (middle row) patients stratified by sex and (bottom row) patients stratified by age, Aragon, Spain, February-September, 2020. Colors indicate: by sex: purple: men, green: women; by age: green: 0-60 years, red: 60-80 years, blue: >80 years. Solid lines: first wave; dashed lines: second wave.

Figure 5

Cumulative incidences of deaths for (top row) all patients; (middle row) patients stratified by sex and (bottom row) patients stratified by age, Aragon, Spain, February-September, 2020. Colors indicate: by sex: purple: men, green: women; by age: green: 0-60 years, red: 60-80 years, blue: >80 years. Solid lines: first wave; dashed lines: second wave.

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