Characteristics, comorbidities and laboratory measures associated with disease severity and poor prognosis in young and elderly patients with COVID-19 admitted to medical wards in Emilia-Romagna region, Italy: a multicentre retrospective study

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ABSTRACT

Background and Objectives. A relatively small number of studies have investigated the characteristics, comorbidities and laboratory measures associated with prognosis in patients with COVID-19, admitted to Internal Medicine Units (IMU) in Italy. Therefore, we performed a retrospective multicentre study to identify baseline features, predisposing to severe disease and poor outcomes, in adult individuals with SARS-CoV-2 infection, hospitalized in 5 IMUs in the Emilia-Romagna region (Italy). *Materials and Methods.* We included 129 consecutive patients (male 75, median age 68 years) from 1st March 2020 to 31st October 2021. Patients' baseline characteristics, comorbidities, laboratory measures, and outcomes were collected. *Results.* At admission, the factors significantly associated with a higher risk of in-hospital mortality included: age (median 68 *vs*. 83 years in survived *vs*. dead patients, P=0.000), diabetes [Odds Ratio (OR) 4.00, P=0.016], chronic obstructive pulmonary disease (OR 4.60, P=0.022), cancer (OR 5.81, P=0.021), acute- (OR 9.88, P=0.000) and chronic-renal failure (OR 6.76, P=0.004). During the study period, 16 individuals died (12.4%), all over 70 years old. In deceased *vs*. non-deceased patients were detected: i) more elevated white blood cells and neutrophils-counts and lower lymphocytes count; ii) higher levels of total/direct bilirubin, creatinine, C-reactive-protein, lactate-dehydrogenase, ferritin, but only a slight Interleukin-6 increase; iii) a trend of lower vitamin D values. *Conclusions.* We proposed a new *I* index, a modified form of the Age-Adjusted Charlson Comorbidity Index, by considering pO_2/FiO_2 ratio, to better characterize the severity of COVID-19. Furthermore, we critically discuss our results with the current assumption which considers COVID-19 as a pathological condition associated with cytokine storm.

Introduction

In December 2019 the novel coronavirus SARS-CoV-2 has emerged in Wuhan (China). It has rapidly spread worldwide, becoming a very important public health problem.[1] Since the early months of 2020, several new genetic lineages of this virus, with potentially high pathogenicity in some variants (known as variants of concern or VOC) have periodically emerged worldwide. These different SARS-CoV-2 variants have caused major waves of infections across the globe with a high number of deaths in the general population, mainly in frail individuals.[2] Until now, this pathogen has infected about 763.7 million of people and its related disease, defined as Coronavirus disease-19 (COVID-19) by the World Health Organization (WHO), has caused more than 6.9 million deaths across the globe [World Health Organization, WHO Coronavirus (COVID-19) Dashboard accessed on 19 April 2023].[2] Several different vaccines and new pharmacological treatments have been approved and introduced into clinical practice with the aim of decreasing the burden of disease.[3-5] Nevertheless, considerable efforts are still needed to increase our knowledge concerning both the mechanisms involved in the pathogenesis of the syndrome caused by this pathogen and the factors modulating the viral spreading across the world. Several studies have shown that SARS-CoV-2 infects a wide range of cells in different host tissues, including not only lung and respiratory epithelium, but also vascular endothelium and cells of the intestine, liver, kidney, brain and immune cells.[5,6] Therefore, patients with COVID-19 present the involvement of multiple organs and systems, including nervous, respiratory, digestive, renal, and immune-systems as well as cardiovascular apparatus.[5,6] This property of SARS-CoV-2 causes a variety of clinical manifestations and complications detectable in infected subjects both in the acute and post-COVID phase of the disease.[6-8] The exuberant activation of the immune system, during the course of viral infection, is considered one of the main factors associated with the severity of the illness.[7] The immune response against SARS-CoV-2 infection involves cells, belonging both to the innate and to the adaptive arms of the immune system, such as neutrophils, Natural Killer, Killer-cells and macrophages B and T lymphocytes, and the release of mediators, such as Interleukin-1 (IL-1), IL-6, IL-8, Tumor necrosis factor (TNF)-α, Interferons (α and λ), reactive oxygen species and chemokines.[6,9-10] All these components promote an inflammatory status in host's tissues with different degrees of severity. The course of the disease and the outcome of patients with SARS-CoV-2 is strongly influenced by this process.[6,7] Early studies have suggested that individuals with severe forms of COVID-19 develop an inflammatory response with clinical and laboratory characteristics resembling those observed in the Cytokine Release Syndrome (CRS), sepsis, or acute pancreatitis (AP) and known as "cytokine storm". These patients have higher amounts of some pro-inflammatory cytokines (IL-1, TNF-α, IL-6, IL-8) in comparison with subjects without SARS-CoV-2 infection or patients with mild COVID-19.[11-12] In particular IL-6 was shown to be an important predictor of severe-COVID-19 in some studies and meta-analyses and some cut-off levels of this cytokine, potentially associated with a more serious disease, have been proposed by some authors.[13-14] However, other reports have suggested that IL-6 may not be the right target in critical forms of SARS-CoV-2 infection[15] and have questioned that a cytokine storm is involved in organ dysfunction in severe COVID-19.[16] A large series of biochemical parameters, including low levels of total white blood cells (WBC), lymphocytes and vitamin D, as well as high values of neutrophils, ferritin, D-dimer, C-reactive protein (CRP), alanine-transaminases (ALT), total and direct bilirubin, creatinine, lactate dehydrogenase (LDH) have been associated with a more severe clinical course and with a poor prognosis in SARS-CoV-2-related infection.[17-21] The coexistence of comorbidities was also correlated with a more severe course and a higher risk of death in patients with SARS-CoV-2 infection. Immune-compromised patients, such as individuals with cancer or with persistent pathological conditions, as well as elderly subjects with several diseases, including diabetes, hypertension, cardiovascular disease, obesity, autoimmune diseases, chronic kidney/lung/liver diseases, dementia, solid organ transplant[22,23] are at higher risk to develop severe forms of COVID-19 with a potentially poor outcome in comparison to younger and healthier individuals.[24,25] SARS-CoV-2 infection may cause an impairment in the function of kidney and gastrointestinal tract, mainly in elderly people,[26-27] inducing fluid and electrolyte imbalances (such as hyper/hyponatremia, hypokalemia, hypocalcemia) in those subjects who undergo a higher probability of complications and even a more elevated risk of death.[28-31] Furthermore, it has been suggested that low levels of vitamin D are associated with an increased risk of SARS-CoV-2 infection and a poorer prognosis in patients with deficiency of this micronutrient, but to date, no definitive results have been yet obtained.[32] A useful approach to predict the outcome in hospitalized subjects with COVID-19 may be represented by the use of comorbidity index scores. In particular, it is well-known that the Age-Adjusted Charlson Comorbidity Index (ACCI) functions as a predictor of severe clinical outcome in subjects who have been hospitalized, due to SARS-CoV-2 infection,[33] although additional comor-

bidity measures have been proposed with this purpose.[34] To date, a large series of studies have evaluated the general characteristics, management, and outcome of patients infected with SARS-CoV-2 in a wide range of hospital settings.[35-38] However, although Internal Medicine Wards (IMWs) have played a significant role in the management of patients with COVID-19 both worldwide and also in Italy, during the pandemic, by accepting and caring for the high number of infected individuals, relatively few data are available on baseline characteristics, clinical course and prognosis of subjects with SARS-CoV-2 infection, who have been admitted to these Wards. Most of the studies available on this topic are multicentric trials including a large number of patients, who have been enrolled in several hospitals across Italy.[39-43] Fewer studies have been carried out in a single region or Hospital Center,[44-45] or have examined only some peculiar aspects concerning the impact of viral infection in human pathology.[46-49] On the other hand, while well-designed multicenter studies allow for an improvement in the quality of the results obtained, the data collected may be heterogeneous and do not exactly reflect the characteristics, the clinical course and the prognosis of patients with COVID-19 belonging to narrower geographical areas. To date, few trials have investigated the trend of all these factors in patients with COVID-19, who have been admitted to the Internal Medicine/Cardiology Wards (IM/CW) in single Italian regions.[44-45] To our knowledge, so far in the Emilia-Romagna region only few studies have been published with this aim. These investigations have been performed in Piacenza and in Ferrara cities.[44-45]

The aim of our contribution was: i) to review the literature available on the association between the baseline characteristics, such as age, sex, comorbidities, routine biochemical findings and prognosis of symptomatic patients with SARS-CoV-2 infection who were hospitalized into Italian IM/CWs; ii) to retrospectively evaluate the above-mentioned features, parameters and outcome, such as length of hospital stay, admission to Sub-intensive-(SICU) or Intensive-Care Units (ICU), and death, in individuals with COVID-19, who were admitted to IMWs in some hospitals of Emilia-Romagna, an Italian region with more than 4 million inhabitants, in the period ranging from 1st March 2020 to 31st October 2021. Furthermore, we discuss the possible differences in risk factors associated with the course and outcome of patients with COVID-19 in some trials carried out in some Italian IMWs.

Materials and Methods

We performed this multicentre retrospective observational study recruiting adult patients who were consecutively admitted to participating IMWs in five Hospitals in the Emilia-Romagna region, in Italy, including UO Medicina Interna Budrio Hospital, UO Medicina Interna Bentivoglio Hospital and UO Cardiologia Bentivoglio Hospital (AUSL Bologna), UO Medicina d'Urgenza Faenza Hospital and UO Medicina Lugo Hospital (AUSL Romagna), due to symptomatic SARS-CoV-2 infection between 1st March 2020 to 31st October 2021.

Information about demographic characteristics (age and sex), medical history, laboratory blood and serum test parameters were collected.

Inclusion criteria

i) Age ranging from 18 to 100 years, hospitalization in ordinary hospital units, who were successively admitted to SICU or ICU, if needed;
ii) Confirmed diagnosis of SARS-CoV-2 infection with a positive polymerase-chain reaction nasopharyngeal swab test, according to WHO criteria;
iii) Clinical symptoms (fever, shortness of breath, sudden onset of anosmia/ageusia/dysgeusia) and radiological signs (computed tomography, ultrasonography or radiography) compatible with COVID-19.

Exclusion criteria

i) History of end-stage chronic renal failure (CRF), at stage 5 and/or end-stage liver disease (Child-Pugh C10);
ii) Life-threatening cardiac arrhythmias;
iii) Non-solvable upper airways obstructions;
iv) Bradypnea (<12 bpm) or gasping;
v) Need for airways protection, elevated risk of inhaling (*e.g.*, prolonged vomiting) and/or excessive secretion;
vi) History of recent trauma/surgery/facial deformation;
vii) Drowsiness (Kelly score >3).

Data collection

Several physicians (AC, MZ, FS, SC, GDM, FD, RF, PL) selectively collected all variables, using electronic medical records, gathered in an anonymized case report form on the unique episode number and independently reviewed them for their consistency. Patients were followed up until hospital discharge, death, or November 30th, 2021.

Ethics

The study was approved by the Health Research Ethics Committees of AVEC (Area Vasta Emilia Centro), Azienda Usl of Bologna and of Area Vasta Romagna, Azienda USL Romagna (protocol CO-VIT012022, SIRER ID 4285). The research project was conducted according to the ethical principles

of the Declaration of Helsinki. Data were last updated on 10 December 2022.

Statistics

After an initial descriptive analysis, univariate and multivariate methods for statistical analysis were used, when appropriate. In particular, data were statistically analyzed by comparing groups of patients with two main tests: robust multivariate analysis of variance (MANOVA) and Mann-Whitney test.[50,51] The first one aims to compare two or more groups of observations to decide if their multivariate distributions can be considered significantly different or not. The robustness of the test guarantees its applicability even if the normality of data is not fully respected. Mann-Whitney test, instead, has been used to evaluate if every single variable can be considered significant for the discrimination of the groups. Mann-Whitney test can be used also for non-Gaussian data. Furthermore, we have calculated an index based on a modified form of the Charlson Comorbidity Index (CCI).[52] In particular, we considered CCI corrected by age (ACCI), a score measuring the burden of complex comorbidities in a large subgroup of pathologies, such as cardiac, renal, liver-diseases and malignancies and from it we calculated a new index, in patients who survived hospitalization in IMWs. International scientific literature has suggested that ACCI represents the best predictor for severe clinical outcome in patients with COVID-19 infection admitted to Hospital.[33,53] We have indicated this parameter as *I*, expressing it as follows:

$$I = \frac{\text{ACCI}}{\text{PF}} * 100$$

where ACCI represents Charlson Index corrected by age and PF was defined as the worst pO_2/FiO_2 (P/F) measured during the in-hospital stay. Once calculated *I* for all patients, the quartiles of *I* were computed. All results were described in terms of P values. In all cases, P values can be considered significant (*i.e.*, indicating that the discrimination between groups is present) if their value is lower than the significance level, that in this case was chosen to be 0.05 (5%).

Results

The study cohort included 129 patients (75 males, 58.1%) with a median age of 68 years who were consecutively admitted to the above-mentioned Hospital Wards, between March 2020 to June 2021. Table 1 shows the clinical characteristics of the enrolled individuals, including sex, median age, associated diseases and the percentage of patients suffering from comorbidities.

Table 1. Clinical characteristics of the examined population (entire dataset: 129 patients); Odds ratios and relative P-values for the association between comorbidities and in-hospital mortality.

	Number of patients (% entire dataset)	Odds ratios (range)	P-value
Male sex	75 (58.1)		
Age (years)	68 (median)		
Hypertension	55 (42.6)	2.50 (0.76-8.99)	0.108
Diabetes	25 (19.4)	4.00 (1.11-13.9)	**0.016**
Ischemic heart disease	15 (11.6)	1.93 (0.309-8.60)	0.399
Acute myocardial infarction (AMI)	11 (8.5)	1.64 (0.517-9.20)	0.627
Chronic renal failure (CRF)	15 (11.6)	6.76 (1.63-27.1)	**0.004**
Acute renal failure (ARF)	15 (11.6)	9.88 (2.46-40.5)	**<0.001**
Chronic obstructive pulmonary disease (COPD)	15 (11.6)	4.60 (1.04-18.4)	**0.022**
Stroke/Transient ischemic stroke (TIA)	12 (9.3)	1.47 (0.142-8.01)	0.644
Polyvascular disease	9 (7.0)		
Peptic ulcer disease	4 (3.1)	0 (0-11.1)	1
Thyroid disease	13 (10.1)	0.56 (0.01-4.36)	1
Hepatic disease	3 (2.3)	0 (0-17.7)	1
Autoimmune disorders	6 (4.7)	1.44 (0.03-14.2)	0.556
Neoplastic disease	10 (7.8)	5.81 (1.05-28.9)	**0.021**
Thromboembolic disease	2 (1.6)	0 (0-38.4)	1

Bold values represent the significant variables.

During the hospitalization in IMWs, the outcome of 129 patients was as follows: 42 patients (32.5%) were transferred to SICUs, 7 (5.4%) to ICUs and 16 (12.4%) died; 64 patients (49.7%) were discharged alive from the IMWs.

In our analysis of data, we considered the following points:

Presence of comorbidities and higher risk of death

A significant association (P<0.05) between pre-existing comorbidities and in-hospital mortality was detected as OR (Table 1) for some conditions, including diabetes, CRF, ARF, COPD and cancer.

Age and mortality rate

16 patients (12.4%) died during their hospital stay. All of them were over 70 years old and the median age of deceased patients was higher than non-deceased individuals (median age was 83 years *vs*. 64 years respectively). The mortality rate progressively increased in the older age groups. In the group of patients over 70 years, the mortality rate increased from 24.1% in the decade 71-80 (29 patients in total) to 25.0% in the decade 81-90 (20 patients), to 42.8% in subjects over 90 years old (7 in total).

Length of in-hospital stay

The hospitalization length is resumed in Table 2 in order to compare similar reports from other studies, as considered in the discussion section.

Clinical characteristics in non-deceased and deceased patients

On the basis of the preliminary observation that all deceased patients were over 70 years old, we subdivided our study population (129 patients) into three groups (A, B and C respectively, Table 3). Group A included all non-deceased patients (113 patients), group B included the non-deceased patients older than 70 years (45 patients), group C included all deceased patients (16 patients, all of them are >70years). We compared the characteristics of the non-deceased patients with deceased patients (groups A and C) and those of all patients with non-deceased subjects older than 70 years (groups B and C). Then the non-deceased patients were subdivided into three groups: Q1 (54 patients) if *I* value was lower than the median value (1.5); Q2 (29 patients) if *I* value was comprised between the median and the third quartile (75%, at index value =2.6); Q3 (30 patients) if *I* value was higher than the third quartile. Then, differences between couples of the Q1, Q2, and Q3 groups were evaluated. We observed that the patients in the Q1 group were prevalently hospitalized in the general Medicine Units, whereas 8 of the 10 patients hospitalized in ICU were included in Q3, 1 in Q2, and 1 in Q1 respectively (the patient included in Q1 had a low ACCI due to the younger age, 46 years and the absence of co-morbidities).

Figure 1 shows the distribution of age in non-deceased individuals (Group A, in blue) and deceased subjects (Group C, in brown). Patients who died had a median age higher than 80 years and their ages

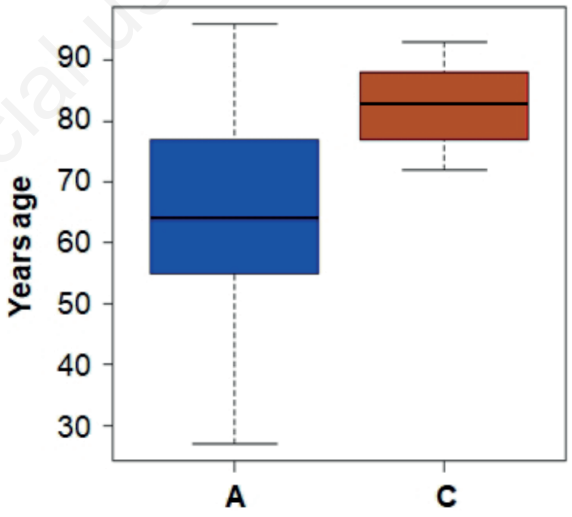

Figure 1. Distribution of age in non-deceased (A, blue) and deceased (C, brown) patients; the following values are marked: median (black bold), 25° and 75° percentiles, maximum and minimum.

Table 2. Length of patients' hospitalization is indicated in the different subclasses of subjects on the basis of their in-hospital stay outcome and is expressed in days (mean and median values).

Patients			Hospitalization length (days)		
	Number	%	Mean	Median	Range
All	129	100	10.5	9	3-34
Died in medicine wards	16	12.4	13.2	11	3-28
Discharged from medicine wards	64	49.6	8.5	8	4-30
Transferred to sub-intensive care units	42	32.6	11.3	10	4-34
Transferred to intensive care units	7	5.4	17.8	18	7-26

ranged between 70 and more than 90 years. Then, we considered the group of survived individuals over 70 years for further analysis.

Figure 2 shows the age distribution of the three groups of non-deceased patients. It can be seen that the median age increases from Q1 to Q3, but the age distributions are quite homogeneous.

Tables 3 and 4 show the P values calculated for all groups. Bold values represent the significant variables. In Table 4, the first line corresponds to the MANOVA test comparing the groups in a multivariate way. MANOVA test was carried out with the variables having less than 10 missing data. Table 4 describes the dead patients *vs*. non-deceased cases (C *vs*. A) and compares the three groups of non-deceased patients (Q1 *vs*. Q2, Q1 *vs*. Q3, Q2 *vs*. Q3). The symbol ● indicates variables that are not significant at the level 0.05, but these are slightly significant (P<0.1) and could have a partial effect on the group's discrimination.

The median values of the biochemical parameters considered in the different groups of patients and their range are shown in Table 4. Parameters differing significantly in the comparison between non-deceased and deceased patients, between patients >70 years who survived and subjects who died (see Table 3), and between the groups Q1, Q2, and Q3 (see Table 4) are indicated in bold.

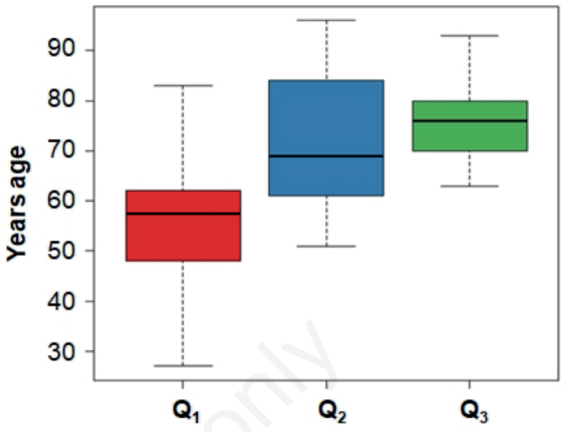

Figure 2. Age distribution of the three groups of non-deceased patients (Q1, Q2 and Q3).

Table 3. Prevalence of the underlying comorbidities; patients are reported as entire dataset and classified as all non-deceased patients (A), non-deceased classified according to *I* (Q1, Q2 and Q3 groups), non-deceased patients older than 70 years (B) and deceased patients (C, all of them are >70 years).

	Entire dataset	Non-deceased						Age >70			
	All (A)	Classified by *I* Q1		Q2		Q3		Non deceased (B)		Deceased (C)	
Number of patients	129	113		54		29		30		45		16	
Median age	68	64		58		69		76		79		83	
Age range	27-96	27-96		27-83		51-96		63-93		0-96		72-93	
Comorbidities	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Hypertension	45	39.8	15	27.8	12	41.4	18	60.0	26	57.8	10	62.5
Diabetes	18	15.9	4	7.4	5	17.2	9	30.0	7	15.6	7	43.8
Chronic renal failure (CRF)	9	8.0	1	1.9	3	10.3	5	16.7	7	15.6	6	37.5
Chronic obstructive pulmonary disease (COPD)	10	8.9	2	3.7	6	20.7	2	6.7	8	17.8	5	31.3
Stroke/Transient ischemic stroke (TIA)	10	8.9	1	1.9	3	10.3	6	20.0	7	15.6	2	12.5
Peptic ulcer disease	4	3.5	2	3.7	1	3.5	1	3.3	2	4.4	0	0
Thyroid disease	12	10.6	3	5.6	2	6.9	7	3.3	6	13.3	1	6.3
Hepatic disease	3	2.7	0	0	1	3.5	2	6.7	2	4.4	0	0
Autoimmune disorders	5	4.4	2	3.7	2	6.9	1	3.3	2	4.4	1	6.3
Neoplastic disease	6	5.3	0	0.0	2	6.9	4	3.3	4	8.9	4	25.0
Ischemic heart disease	12	10.6	3	5.6	2	6.9	7	3.3	9	20.0	3	18.8
Acute myocardial infarction (AMI)	9	8.0	2	3.7	3	10.3	4	3.3	6	13.3	2	12.5
Acute kidney injury (AKI)	8	7.1	0	0	2	6.9	6	20.0	7	15.6	7	43.8
Polyvascular arterial disease	6	5.3	1	1.9	1	3.5	4	3.3	4	8.9	3	18.8
Pulmonary thromboembolism	2	1.8	1	1.9	0	0	1	3.3	0	0	0	0

Patients included in the Q3 group had a worse P/F (lower values) measured during the hospital stay in comparison with the other Q groups, as was expected since the P/F is a validated index to assess the function of the lung.[54] Deceased patients had significantly higher values of maximum creatinine, total and direct bilirubin, ferritin, D-dimer, LDH, CRP, WBC and neutrophils and lower levels of lymphocytes in comparison with both non-deceased patients of a group (group A) and non-deceased subjects aged >70 years (group B). Among non-deceased patients, those with the more severe forms of the disease (Q3 worse than Q2 and worse than Q1) had significantly higher values of Urea, ALT and Aspartate transaminase (AST), D-dimer, IL-6, LDH, CRP, procalcitonin and neutrophils. Furthermore, we calculated the median level of vitamin D in the different groups of patients considered (Table 5). Its median value was 16.8 ng/ml (normal level > 20 ng/ml) for the subjects of the entire dataset, 17 ng/ml in non-deceased ones, 18 ng/ml in non-deceased ones >70 years and 14,05 ng/ml in deceased individuals, respectively.

This level did not significantly differ between distinct classes of patients' age, between Q1, Q2 and Q3 groups as well as between non-deceased and deceased patients. However, a trend of lower levels of this micronutrient was detectable in deceased subjects in comparison with individuals who did not (Table 5). A correlation between vitamin D values and death in our population was detected only for very low levels of vitamin D (≤7.5 ng/ml) (data not shown).

Starting from the assumption that CCI and then ACCI have been validated several years ago for estimating the risk of death in hospitalized patients, but that the predictive ability of ACCI in identifying individuals with a more compromised clinical status may

Table 4. P-values calculated in the comparison between deceased and non-deceased patients (groups C and A), between deceased and non-deceased patients aged >70 (groups B and C), and among Q1, Q2 and Q3 groups.

	Deceased (C) *vs.* non-deceased all data (A)	Deceased (C) *vs.* non-deceased >70 years (B)	Q1 *vs.* Q2	Q1 *vs.* Q3	Q2 *vs.* Q3
MANOVA	**2.90 10^{-11}**	**3.09 10^{-08}**	2.16 10^{-01}	**1.30 10^{-03}**	**3.62 10^{-03}**
Worse P/F	**6.98 10^{-08}**	**2.14 10^{-06}**	**5.35 10^{-03}**	**6.55 10^{-11}**	**4.41 10^{-07}**
25-OH Vit D	1.94 10^{-01}	1.17 10^{-01}	9.73 10^{-01}	1.99 10^{-01}	2.68 10^{-01}
ALT	4.93 10^{-01}	**4.36 10^{-02}**	**4.48 10^{-03}**	**3.56 10^{-02}**	5.96 10^{-01}
AST			**2.03 10^{-02}**	4.42 10^{-01}	1.50 10^{-01}
Direct bilirubin	**4.08 10^{-02}**	**4.97 10^{-02}**	9.15 10^{-01}	7.08 10^{-01}	8.50 10^{-01}
Total bilirubin	**7.12 10^{-03}**	**3.70 10^{-02}**	5.02 10^{-01}	6.62 10^{-01}	1.00 10^{+00}
Calcium (Ca) total			7.41 10^{-01}	5.93 10^{-01}	8.45 10^{-01}
Max Creatinine	**3.46 10^{-06}**	**5.73 10^{-04}**	6.66 10^{-02}●	8.24 10^{-02}●	7.56 10^{-01}
D-dimer	**7.72 10^{-07}**	**4.79 10^{-05}**	**7.97 10^{-03}**	**2.52 10^{-02}**	7.39 10^{-01}
Ferritin	**4.66 10^{-02}**	**1.68 10^{-02}**	3.78 10^{-01}	1.84 10^{-01}	7.04 10^{-01}
Hb	9.60 10^{-01}	6.82 10^{-01}	6.63 10^{-01}	1.68 10^{-01}	4.48 10^{-01}
IL-6			1.88 10^{-01}	2.00 10^{-02}	4.80 10^{-03}
Potassium (K)	1.29 10^{-01}	**3.47 10^{-02}**	5.86 10^{-02}●	1.63 10^{-01}	6.05 10^{-01}
Lactate dehydrogenase (LDH)	**1.19 10^{-06}**	**2.54 10^{-05}**	6.23 10^{-02}●	**1.05 10^{-03}**	2.29 10^{-01}
Lymphocytes	**7.60 10^{-03}**	**8.89 10^{-03}**	4.74 10^{-01}	5.35 10^{-01}	2.52 10^{-01}
Sodium (Na)	1.12 10^{-01}	2.39 10^{-01}	8.82 10^{-01}	1.00 10^{+00}	8.25 10^{-01}
Neutrophils	**6.59 10^{-05}**	**1.33 10^{-04}**	9.56 10^{-02}●	**2.30 10^{-02}**	5.85 10^{-01}
C-reactive protein (CRP)	**6.01 10^{-03}**	**1.36 10^{-02}**	2.20 10^{-01}	**7.69 10^{-03}**	4.26 10^{-01}
Procalcitonin (PCT)			**3.77 10^{-02}**	**1.07 10^{-02}**	5.37 10^{-01}
Platelets (PLT)	3.35 10^{-01}	6.11 10^{-01}	7.38 10^{-01}	1.99 10^{-01}	2.11 10^{-01}
Urea.max			1.16 10^{-01}	**1.81 10^{-05}**	**2.58 10^{-03}**
White blood cells (WBC)	**1.57 10^{-03}**	**4.22 10^{-03}**	9.65 10^{-02}●	5.22 10^{-02}	6.33 10^{-01}

Bold values represent the significant variables. The symbol ● indicates variables that are not significant at the level 0.05, but these are slightly significant (P<0.1) and could have a partial effect on the group's discrimination.

be improved by considering P/F ratio, we have calculated *I* index in all survived patients. We subdivided them into Q1, Q2 and Q3 groups with the aim of better discriminating the disease severity of patients who were included in our study. The same quartile-based division in Q1, Q2 and Q3 classes was carried out also by considering the ACCI only (data not shown). However, in the latter case, the individuals' age had the main impact in classifying into Q1, Q2 and Q3 classes. As an example, most individuals under 60 years were included in Q1 group by ACCI, but five of them were grouped in Q2 using our *I* index due to the low P/F ratio. At the same time, most of the over-80 patients were grouped in Q3 by CCI, but some of them were grouped in Q2 by *I* index due to the high P/F ratio. Patients with lower P/F ratio had more severe forms of

Table 5. Prevalence of the biochemical parameters considered; patients are reported as entire dataset and classified as non-deceased patients (A), non-deceased patients older than 70 years (B), deceased patients (C), Q1, Q2 and Q3 groups, respectively. 1: ng/ml, 2: U/l, 3: mg/dl, 4: g/dl, 5: pg/ml, 6: mmol/l, 7: 10^9/l, 8: 10^{12}/l, 9: microg/dl, 10: mm.

	Entire dataset		Non-deceased				Age >70	
			All	Classified by *I*			Non-deceased	Deceased
			(A)	Q1	Q2	Q3	(B)	(C)
	Median	Range	Median values					
Worse P/F	224	36-459	258	295	260	111	250	89
25-OH Vit.D[1]	16.8	4-148	17.0	15.5	16.8	19.0	18.0	14.1
Alkaline phosphatase[2]	55	7-178	55	55.5	56	51	51	47
Alanine-transaminases (ALT)[2]	27	3-269	26.5	30.5	20.5	21.5	18.0	33.0
Aspartate transaminase (AST)[2]	34	11-132	34	37	30.5	34	34	23
Direct bilirubin[3]	0.16	0.05-0.8	0.15	0.14	0.17	0.16	0.18	0.30
Total bilirubin[3]	0.62	0.13 - 2.12	0.59	0.6	0.56	0.59	0.56	0.87
Calcium (Ca) total[3]	8.5	7.7-9.7	8.5	8.5	8.5	8.5	8.4	8.3
Max. creatinine[3]	0.87	0.42-4.41	0.84	0.78	0.88	0.875	0.89	1.43
Min. creatinine[3]	0.76	0.4-3.68	0.72	0.75	0.74	0.69	0.77	1.11
D-dimer[1]	0.88	0.19-28.1	0.78	0.61	0.90	0.93	0.93	2.58
Ferritin[1]	424	8-6238	393	339.5	426	590	313	784
Phosphorus (P)[3]	3.1	0-4.4	3.2	3.2	3.2	3	3.2	2.65
Gamma glutamyl transferase[2]	44	10-230	43	46	23	42.5	33	56
Hemoglobin (Hb)[4]	13.4	8.4-19.8	13.4	13.55	13.3	13.3	13.3	13.45
IL-6[5]	29	1.8-766	29.0	29.0	16.4	63.3	29.0	35.7
Potassium (K)[6]	4.1	2.3-5.1	4.1	4	4.2	4.1	4.2	3.9
Lactate dehydrogenase (LDH)[2]	331	152-759	302	280	324	371	290	488
Lymphocytes[7]	850	240-8530	880	880	930	820	880	640
Magnesium (Mg)[3]	2.1	1.4-2.9	2.1	2.1	2.1	2.2	2	2.4
Sodium (Na)[6]	138	122-147	137	137	137	138	138	139
Neutrophils[7]	3980	1290-55300	3765	3600	3765	4105	3560	6590
C-reactive protein (CRP)[3]	8.4	0.22-70	7.4	5.9	7.4	9.1	7.4	12.3
Procalcitonin (PCT)[1]	0.1	0.06-8.5	0.1	0.1	0.1	0.1	0.1	0.1
Platelets (PLT)[8]	186	18.5-507	186	196.5	200	173.5	178	160
Iron[9]	56	0-197	56	58	49	56	50.5	66.5
Urea.max[3]	41	12-237	39.5	35.5	38	55	48.5	64
Urea.min[3]	37	12-222	37	30	31	42	41.5	64
Erythrocyte sedimentation rate[10]	53	0-194	53	45	65	66	70.5	57.5
White blood cells (WBC)[7]	5440	2400-20840	5190	5130	5500	6075	5120	7845

the disease and, as expected, individuals in Q3 as well as patients who died had a significantly lower P/F rate than the one detectable in the other groups. Furthermore, these patients had more comorbidities and an older age. In particular, hypertension was observed in 60% of patients in Q3 group *vs*. 41% in Q2 and 28% in Q1, and in 62.5% of deceased individuals *vs*. 40% in non-deceased ones. Furthermore, diabetes was detected in 30% of individuals in Q3 group *vs*. 17% in Q2 and 7% in Q1 as well as in 44% of deceased subjects *vs*. 16% in non-deceased ones.

Discussion

Since its appearance at the end of 2019, SARS-CoV-2 has rapidly become a formidable public health problem worldwide, as this pathogen causes not only self-limiting- but also severe-forms of COVID-19, mainly in elderly people. Due to the important burden of morbidity and mortality, observed in the population worldwide, several efforts have been performed to clarify the mechanisms involved in the pathogenesis of SARS-CoV-2-related disease and the clinical course of the infection in symptomatic and asymptomatic individuals. Currently, a number of risk factors have been shown to be associated with more severe forms of COVID-19 and more adverse outcomes in several studies and meta-analyses, including old age, male sex, pre-existing comorbidities, and racial/ethnic disparities.[6,7] However, these risk factors may present differences in their distribution across different geographical areas and exert a distinct impact on the severity of COVID-19 even in different regions of the same country.[39]

We have analyzed the impact of clinical features, comorbidities and baseline laboratory parameters on the severity and prognosis of the disease in a subset population including 129 adult patients admitted to five IMWs in Emilia-Romagna.

Some data emerging from our study agree with those reported by trials performed in other IMWs, while other results seem to be different. This discrepancy may be due to various factors, such as the sample size, the enrolment period, the geographical area considered.

Presence of comorbidities and higher risk of death

Our results showing that diabetes, CRF and ARF, COPD and neoplasm are associated with a higher risk of death substantially confirm the studies of several authors,[39,40,44,55] with only slight differences. In particular, the comorbidities associated with a more elevated probability of exitus were represented by: i) cardiovascular disease, chronic heart failure (CHF), atrial fibrillation, hyperlipidemia, CRF, and dementia at univariate analysis and by CHF and COPD at multivariate analysis in SIMI study; [40] ii) CRF and active cancer with an upward trend of risk in subjects with a clinical history of myocardial infarction, COPD and obesity, whereas diabetes and hypertension were not in CORIST one;[39] iii) CHF, AMI, ARF, neurological disorders and fluid and electrolyte alterations in De Giorgi's one;[44] iv) hypertension, dyslipidemia, diabetes, COPD, atrial fibrillation, heart diseases, kidney diseases, cancers and stroke were the most common comorbidities in Biagi's one.[55]

Age and mortality rate

The death rate in our study was 12.4%, slightly lower than the one reported in other studies from IMWs: 18.3%,[39] 21.7%,[40] 19.9%,[44] and 30.5%[55] respectively. However, one trial included only patients who died during the in-hospital stay.[55] Furthermore, our trial confirms that deceased patients were older than non-deceased ones with median age was 83 years *vs*. 64 years respectively. Old age represents a known risk factor for mortality in patients with COVID-19.[41] Furthermore, our research showed that no patients under 70 years died, confirming the increasing of mortality rate in patients older than 70 years, as observed in previous studies.[39,40] In patients older than 70 years, the death rate ranged between 24.1% in the 71-80 decade (29 patients in total), 25.0% in the 81-90 decade (20 patients), and 42.8% for over 90 patients (7 in total). A death rate of 31.3% in the 71-80 decade, 47.5% in the 81-90 decade, and 64.4% in subjects older than 90 years is reported in.[40] Another study showed that the mortality rate ranged between 3.1 deaths × 1000 person-days and 9.4 deaths × 1000 person-days in the younger groups (18-64 years and 65-74 years respectively), but it rose to 22.1 deaths × 1000 person-days in older patients (aged ≥75 years).[39]

Length of in-hospital stay

The mean hospitalization length of patients in our trial was 10.5 days (median 9, range 3-34 days) for all patients, differing from one observed in other studies from other Italian Medicine Wards (Table 2). A slightly longer duration is reported in other studies, in particular, the mean duration of hospitalization for discharged patients was 14.6±12.3 days and 13.3±8.9 days.[40,44] Furthermore, the length of in-hospital stay in patients who died was 13.2 days (median 11, range 3-28 days) in our research and 7.6 days, [interquartile range (IQR): 5.0-11.5].[55] Several reasons may contribute to explain the discrepancies in the length of in-hospital stay observed between our trial and the others. In particular, the design of the studies, the sample size of enrolled subjects as well as the severity of their disease are all factors, causing the difference in the length of in-hospital stay among the various trials.

Laboratory parameters

In our population, median values of some laboratory parameters differ significantly between deceased and non-deceased patients. Deceased *vs*. non-deceased patients showed: i) more elevated WBC- (7845 *vs*. 5190/mmc) and neutrophils-count (6590 *vs*. 3765/mmc) and lower lymphocytes count (640 *vs*. 880/mmc); ii) higher values of total/direct bilirubin (0.59/0.15 *vs*. 0.87/0.3 mg/dl) and creatinine (maximum creatinine 1.4 *vs*. 0.84 mg/dl); iii) more elevated levels of some laboratory tests, generally associated with the inflammatory response, including CRP (12.25 *vs*. 7.4 mg/dl), LDH (488 *vs*. 302 mU/ml), ferritin (784 *vs*. 393 ng/ml) and D-dimer (2.58 *vs*. 0.78 ng/ml). Statistically significant differences in the same laboratory parameters were found when Q1, Q2 and Q3 groups were considered. In particular, the median value of the following parameters significantly differed between Q1 and Q3: CRP (5.1 *vs*. 9.1 mg/dl), LDH (280 *vs*. 371 mU/ml), IL-6 (29 *vs*. 63.3 pg/ml), D-dimer (0.61 *vs*. 0.93 ng/ml). Our results agree with the other trials carried out in IMWs. Some laboratory variables, indicating impaired renal and liver function, as well as elevated WBC count and elevated mediators of the inflammatory response (CRP, IL-6 and WBC count) have been associated with more severe forms and higher mortality risk in patients with COVID-19 in SIMI and in Biagi's studies.[40,55] However, the latter trial did not confirm the presence of kidney and liver injury in the enrolled patients. CRP has been the only serum parameter assessed in CORIST study as a potential risk factor for mortality. Elevated CRP levels correlated with higher mortality. De Giorgi has not included laboratory variables in his analysis.[44]

The following points may represent some innovative elements for the design and development of new studies:

i) CCI and then ACCI have been validated several years ago for estimating the risk of death in hospitalized patients with different pathological conditions in longitudinal studies.[52] In recent studies, both CCI and ACCI have been used in assessing the prognosis of patients with SARS-CoV-2 infection.[53] In particular, ACCI has been recently proposed as a reliable predictor for clinical outcome in hospitalized subjects with severe forms of COVID-19.[33] However, according to our knowledge, few authors have used CCI to assess the burden of the comorbidities in patients with COVID-19, who have been admitted to Italian IMWs, whereas no trials have classified these subjects, according to ACCI.[41] However, we believe that this Index may be a suitable tool for measuring disease severity also in patients with SARS-CoV-2 infection and it may allow the rapid screening and identification of individuals who are at risk of a poor outcome at an early stage of this pathological condition. Therefore, its use should be increased in clinical practice and its predictive ability should be possibly improved. So, we adopted the *I* index, which represents the direct expression of the ACCI and also includes the P/F ratio (this variable is computed independently from the ACCI criteria). *I* index rises both when the number of comorbidities increases and when the oxygenation of patient impairs. The incorporation of the worst P/F ratio, measurable during the in-hospital stay, into the *I* score links two well-known risk factors associated with the most important disease severity in these subjects: age and extent of respiratory impairment. Therefore, this new index may contribute to improve the predictive ability of ACCI in identifying individuals with a more compromised clinical status, among hospitalized patients with COVID-19 (Figure 3).[33] Unfortunately, due to a low number of subjects enrolled in our study, we were unable to validate this index, but further trials should be designed with this purpose. At the moment it can be observed that ACCI poorly distinguishes deceased patients with respect to non-deceased ones (Figure 3, left), whereas the *I* index provides a better discrimination (Figure 3, right);

ii) A coordinated innate immune response represents the first line of defense against viral infection, but a dysregulated and excessive inflammatory process may cause tissue injury in the host. The hypothesis of immunosenescence in elderly people with COVID-19 has been associated by some authors with frailty and higher mortality rates. The age-related impairment of immune system function involves both humoral (*i.e.*, antibody response) and cell-mediated arms of an immune response. Previously, it has been suggested that not all immune responses are protective, as antibody-dependent enhancement in humoral immunity may promote SARS-CoV-2 infection while Th17 response in cell-mediated immunity may contribute to the cytokine storm.[56] There are multiple studies reporting the association between the release of elevated levels of inflammatory molecules and severe forms of COVID-19.[8,10] In particular, severe infection due to SARS-CoV-2, has been associated with an exuberant inflammatory process known as "cytokine storm" similarly to the inflammatory process observed in the CRS, sepsis, or acute pancreatitis.[9,57] Elevated inflammatory cytokines, such as IL-6, IL-1, IL-2, IL-10, IL-12, TNF-α and IFN-γ have been associated with disease severity and poor outcome and death.[58-60] It has been formerly shown that more than 30 cytokines can be significantly elevated in patients with COVID-19.[61] Fourteen of these cytokines have been associated

with disease prognosis and prediction of severity.[61] Another study performed by Han demonstrated that levels of IL-6, IL-2, IL-10, IFN-γ, and TNF-α were higher in COVID-19 patients than in healthy subjects.[58] Among these cytokines, the authors found that IL-6 and IL-10 were independent factors for disease severity. Del Valle demonstrated that IL-6 and TNF-α were strong predictors of disease severity and independent factors associated with an increased risk of death.[60] In our study, we observed that the median level of IL-6 was 29 pg/ml (with levels ranging from 1.8 to 766.2 pg/ml). However, in patients with SARS-CoV-2 infection, the levels of these mediators are generally lower in comparison with those detectable in individuals with other pathological conditions, which are characterized by a significant inflammatory response, such as sepsis, CRS, acute respiratory disease syndrome non-COVID-19 and AP. In his meta-analysis, Leisman demonstrated that median levels of IL-6 in patients with these pathological conditions are about 10 to 100 times more elevated in comparison with the amounts detectable in individuals with severe COVID-19, with mean concentrations of IL-6 ranging from 983 pg/ml in patients with sepsis to 3110 pg/ml in patients with CRS.[16] Therefore, taking advantage of these observations, the presence of cytokine storm has been questioned.[16,62] However, interesting therapeutic perspectives have been shown about the potential use of immune-modulating drugs. In particular, anti-cytokine treatments, such as anti-IL-6 therapy with monoclonal antibodies against either IL-6 or IL-6 receptor are currently used in clinical trials.[62] Ferritin is associated with the acute response to inflammation in a large spectrum of acute infections, including both viral and bacterial pathogens. With the introduction of the hyperferritinemic syndrome connecting four severe pathological conditions such as adult-onset Still's disease, macrophage activation syndrome (MAS), catastrophic antiphospholipid syndrome, and septic shock, some authors have hypothesized another interesting aspect of ferritin, which could have a pathogenetic role rather than being an extremely elevated protein only.[63,64] However, in adult-onset Still's disease and MAS, levels of ferritin upwards of 30,000 ng/ml were reported to be a common finding, reaching even as much as 250,000 ng/ml in some studies for patients with adult-onset Still's disease.[65] In our population, we observed that ferritin levels increase in COVID-19 patients, mainly in individuals with severe forms of the disease, but the levels of ferritin do not reach the values observed in the above-mentioned syndromes. In particular, median levels of ferritin were 784 ng/ml in deceased patients *vs*. 393 ng/ml in non-deceased patients, 590 ng/ml in patients in Q3, 426 ng/ml in Q2 and 340 ng/ml in Q1. The entire dataset ranged from 8 to 6238 ng/ml. Nevertheless, several studies have explored ferritin as a potential target for the treatment of COVID-19 and,[66] due to the harmful effects of iron excess and the resultant high ferritin levels, iron-depleting therapy was suggested as a potential treatment in patients with COVID-19;[67]

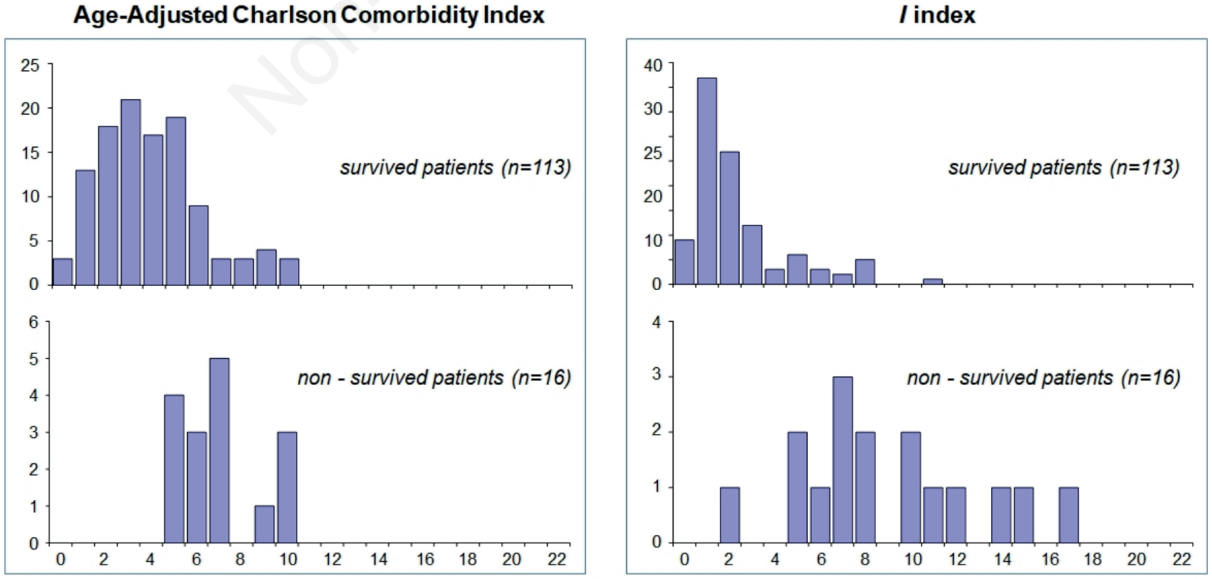

Figure 3. Distribution of non-deceased and deceased patients based on the Age-Adjusted Charlson Comorbidity Index and on the *I* index (ACCI*100/PF).

iii) In March 2020, since the earliest phases of SARS-CoV-2 pandemic outbreak, the rationale for a possible protective and therapeutic role of vitamin D and other micronutrients was proposed by Fiorino and other authors,[5,25,32,68] on the basis of the possible ability of some fat-soluble compounds, in modulating directly or indirectly the replication of SARS-CoV-2, in regulating host's immune activity against this pathogen and, probably, in improving his response to vaccination.[69] Since then, a large series of retrospective and prospective trials and systematic reviews have been carried out in patients suffering from COVID-19 with the aim to assess the potential association between their vitamin D levels and their disease outcome as well as the impact of vitamin D supplementation on their prognosis. These studies have been included in several meta-analyses. Overall, most of these statistical methods have shown that patients with vitamin D deficiency presented an increased risk of SARS-CoV2 infection and hospitalization.[70] Furthermore, individuals suffering from COVID-19 and with low levels of this micronutrient detected at hospital admission, had a more severe course of the disease, a more elevated probability of respiratory distress, a more need for ICU admission, requiring non-invasive or invasive ventilation through CPAP and/or endotracheal intubation, and a higher mortality in comparison with normal ones.[71-74] Furthermore, some meta-analyses have shown that vitamin D supplementation results in a decreased risk of death and ICU admission in patients with COVID-19.[75,76]

On the other hand, other meta-analyses have not confirmed these conclusions.[77,78] The heterogeneity of the studies included in some of the above-mentioned meta-analyses may have prevented the reaching of definitive conclusions on the possible protective activity of this micronutrient in patients with SARS-CoV-2. Some factors may be associated with a significant heterogeneity, such as timing of vitamin D testing, differences in definition of its deficiency/insufficiency as well as of severe COVID-19.[79] On the basis of our previous experience with the use of fat-soluble vitamins in the treatment of hepatitis viruses,[80,81] we have hypothesized a possible protective role of these micronutrients, mainly vitamin D, also in SARS-CoV-2 infection.[5] Therefore, in the current study, we have calculated median level of this compound in the different age groups of our patients. The median vitamin D levels did not significantly differ among the distinct classes of patients' age, but a high percentage of these subjects had vitamin D levels below 20 ng/ml (75 nmol/l). This value is generally accepted as a limit to discriminate between vitamin D sufficiency (>20 ng/ml) and insufficiency (<20 ng/ml).[82] Furthermore, in our study a trend of lower vitamin D values emerged in subjects who died in comparison with individuals who did not, but a significant correlation was not observed. A significant relationship between vitamin D values and death in our population was observed only in subjects with very low levels of vitamin D (≤7.5 ng/ml). The very small sample size of patients included in our study is probably one of the most important reasons, explaining the results emerging in our trial. However, our observations confirm that vitamin D deficiency represents an important public health problem worldwide, mainly in elderly people and even in high-income countries.[83] Vitamin D metabolism also may have a direct impact on this problem. Its absorption, transport to different tissues, uptake by tissue cells, activation and/or catabolism depend on the combined activities of different proteins (receptors, transporters and enzymes), interacting together in a very complex loop.[82] In particular, all these proteins bind vitamin D with variable degree of efficiency and with wide differences among the individuals, as these molecules are codified by genes, existing in several variants. Genetic vitamin D-related polymorphisms are currently under assessment by several authors, due to their potential impact on human biology. A recent study showed that polymorphisms in the vitamin D binding protein encoded by the GC gene in enrolled patients were significantly associated with: i) their vitamin D polygenic risk score; ii) their concentration of 25 (OH)-vitamin D; iii) infection severity; iv) their outcomes.[84] Therefore, the concentration of the biologically active forms of this micronutrient may widely vary among individuals. This is due to their ability in binding to their specific transporters to enter nucleus in human cells as well as to their specific motifs on promoter regions of DNA, modifying the transcriptional function of their target genes and the activities of epigenome and transcriptome in several human cells and tissues. Therefore, some authors have suggested that the efficiency of the molecular response to the supplementation with vitamin D and the final effects due to this compound depend on all these factors and have introduced the concept of the personal vitamin D response index. According to the current hypotheses, the need for vitamin D administration and the extent of the response to its supplementation appears to be due to the status of this micronutrient in association with the personal vitamin D response index of an individual rather than on the vitamin D status alone.[85] Further studies are needed to clarify this very interesting assumption.

Our trial has several limitations, such as: i) its retrospective design; ii) the low number of patients enrolled; iii) the lack of clinical parameters and vital signs (*e.g.*, respiratory and heart rate, consciousness status and blood pressure); iv) instead of d) the

short/medium term of follow-up, but it introduces some peculiar elements and points that may be the subject of discussion in future trials.

Conclusions

What this work adds to previous knowledge: i) a new score for the assessment of patients' COVID-19 severity, defined as *I* index is proposed. It incorporates ACCI and the worst pO_2/FiO_2 ratio measured during the in-hospital stay. *I* index links two well-known risk factors associated with the most important disease severity in subjects with SARS-CoV-2 infection: age and extent of respiratory impairment; ii) IL-6 levels detected in deceased patients are slightly higher in comparison with ones in non-deceased individuals. Nevertheless, its values, as well as ones of other mediators such as IL-8 and IFN-α, are generally lower in comparison with those detectable in subjects with other pathological conditions, which are characterized by a significant inflammatory response, such as sepsis, CRS, acute respiratory disease syndrome non-COVID-19 and severe AP. Median levels of IL-6 and IL-8 in adults with these syndromes are about 10 to 100 times higher in comparison with those observed in patients with severe COVID-19. Therefore, the results of our study confirm the conclusions of other authors, who disagree with the definition of "cytokine storm", applied to the pathogenetic process detectable in patients with COVID-19; iii) the impact of vitamin D deficiency on morbidity and mortality of patients with COVID-19 and the possible protective and therapeutic role of this micronutrient are widely discussed on the basis of the results reported by several meta-analyses and of data emerging from our report.

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