Iron metabolism and lymphocyte subpopulations during Covid-19 infection in ICU patients: an observational cohort study and a narrative review of clinical practice.

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Abstract

Background: Iron metabolism and immune response to SARS-CoV-2 have not been described yet in intensive care patients, although they are likely involved in Covid-19 pathogenesis. Little is known about clinical management of severe forms of Covid-19.

Methods: we performed an observational study during the peak of pandemic in our intensive care unit, serially dosing D-dimer, C-reactive protein, Troponin T, Lactate Dehydrogenase, Ferritin, Serum iron, Transferrin, Transferrin Saturation, Transferrin Soluble Receptor, Lymphocyte count and NK, CD3, CD4, CD8, B subgroups of 31 patients during the first two weeks of their ICU stay. Correlation with mortality and severity at the time of admission was tested with Spearman coefficient and Mann-Whitney test. Trend over time were tested with Kruskall-Wallis analysis.

Results: All patients show hyperferritinemia, and its dosage might be helpful in individuating patients developing hemophagocytic lymphohistiocytosis (we observed 1 case). Lymphopenia is severe and constant, with a nadir on day 2 of ICU stay (median 0.555 109/L; interquartile range (IQR) 0.450 109/L); all lymphocytic subgroups are dramatically reduced in critically ill patients, while CD4/CD8 ratio remains normal. Neither Ferritin nor lymphocyte count follow significant trends in ICU patients. Transferrin Saturation is extremely reduced at ICU admission (median 9%; IQR 7%), then significantly increases at day 3 to 6 (median 33%, IQR 26.5%, p-value 0.026). The same trend is observed with serum iron levels (median 25.5 µg/L, IQR 69 µg/L at admission; median 73 µg/L, IQR 56 µg/L on day 3 to 6) without reaching statistical significance. D-dimer is constantly elevated and progressively increases from admission (median 1319 µg/L; IQR 1285 µg/L) to day 3 to 6 (median 6820 µg/L; IQR 6619 µg/L), despite not reaching significant results. We describe trends of all the above mentioned parameters during ICU stay and provide a narrative review of our clinical experience about critical Covid-19 patients. D-dimer is constantly elevated in our ICU population and increases from admission to a maximum on day 3 to 6 of ICU stay (median 6820 µg/L; IQR 6619 µg/L)

Conclusions: iron metabolism and lymphopenia are key clinical features of Covid-19 patients in the ICU setting and have been specifically described in this paper. Keywords – MeSH repository (3-10): Iron, COVID-19, SARS-CoV-2, Coronavirus, Critical Care, Lymphocytes, Lymphopenia, Ferritins, Immunity, Coagulation.

Background

Covid-19 has been declared a pandemic by World Health Organization (WHO) on the 11th of March. The high contagiousness and the previously unknown clinical features of this new viral infection put under pressure healthcare systems and clinicians worldwide.

Early reports from the Chinese province of Hubei described some predictive biomarkers for the clinical outcome of hospitalised patients, namely lymphopenia and the elevation of D-dimer, ferritin, interleukin 6 (IL-6), troponin and myoglobin, C-reactive protein (CRP) and lactate dehydrogenase (LDH)[1,2]. LDH is a marker of parenchymal lung damage, troponin and myoglobin are markers of myocardial and muscular involvement, while the remaining molecules belong to the group of positive acute-phase proteins (APP).

Ferritin is a crucial component of iron metabolism, one of the most ancestral systems of host protection from pathogen infections[3]. Iron is a micronutrient necessary for both energy production at a mitochondrial level and nucleic acid replication at cytoplasmic and nuclear level. For its scarcity in the human body and the fundamental processes in which it is involved, pathogens (bacterial, viral or fungal) compete with the host for iron availability in order to guarantee their own replication. When innate immunity is activated and cytokine cascades start, IL-6 stimulates hepcidin expression in the liver, reducing iron bioavailability by decreasing its gut absorption and hiding it into ferritin, a shell-like molecule deposited in macrophages. These mechanisms have been extensively reviewed in the literature[4–7].

Lymphopenia and specific T-cell lineage affection are characteristic features of Covid-19[8]. In previous Coronaviruses outbreaks, such as SARS, the peak of viral load occurred 7 days after symptoms development, followed by elevation in IL-6 and IL-8, nadir lymphocyte count and successive pulmonary infiltrates. This description suggests that clinical symptoms are mediated by the immune system deregulation rather than direct viral damage[9]. Recently, a group described for the very first time the distribution of different subtypes of CD4 and CD8 T-cells in peripheral blood of symptomatic patients[10]. SARS Coronavirus type 2 (SARS-CoV-2)-induced hyperinflammation and cytokine storm have been suggested as possible triggers of hemophagocytic lymphohistiocytosis (HLH)[11], a frequently undiagnosed syndrome affecting patients suffering multiple-organ failure (MOF), exactly characterised by dramatically elevated levels of ferritinemia[12].

It has been reported that 5% of symptomatic patients require intensive care unit (ICU) treatments[13]. It is still not clear why some patients are more severely affected than others by SARS-CoV-2. These patients share some predisposing co-pathologies such as hypertension, diabetes, obesity and altered immune response is highly suspected to be responsible for their clinical deterioration: thus, several ongoing trials target immune response through different drugs.

Concluding, iron metabolism and immune system deregulation might be crucial to the progression of Covid-19. To date, no detailed description of biomarker trends exists specifically about those more severe patients requiring ICU admission.

With this observational study, we aim to provide one of the first complete and detailed descriptions of ICU Covid-19 populations, narratively explaining our approach, trying to share ideas and experiences about the management of this disease. We also aim to provide some hints about iron metabolism and immune system deregulations affecting these patients. It can lead to a better understanding of the underlying physiopathology of this unknown disease, foreseeing some possible alternative therapeutic targets.

Methods

Study population:

This is a single-centre retrospective observational cohort study. The first COVID-19 positive patient in our ICU has been admitted on the 5th of March 2020. Data collection goes from the 6th of March to the 6th of April 2020, following the local peak of epidemic. As standard practice of our unit, a protocol was established to determine the testing of predictive biomarkers. On the day of ICU admission and then twice-a-week (on Monday and Thursday) every ICU patient was tested for: ferritin, serum iron, transferrin and transferrin saturation (TfSat), soluble receptor of transferrin, CRP, D-dimer, LDH, troponin, lymphocyte count, characterisation of T cells (CD3, CD4 and CD8), B cells and NK cells. This allowed us to divide the measures in the following sub-categories: TI1-2 (first dosage made on ICU admission), TI3-6 (dosage between day 3 to 6 of ICU stay), TI 7-10 and TI 11-14. We observed their trends during the first 2 weeks of ICU stay. Following scientific focus, we tested some patients for Interleukin-6 (IL6) on the day of admission[14].

Inclusion criteria: every SARS-CoV-2 positive patient (oropharyngeal swab or bronchoalveolar lavage sample, PCR test) admitted to our ICU was automatically enrolled in the study. Overall, 31 patients entered in the final data analysis fulfilling inclusion criteria. Importantly, we count ICU length-of-stay (LOS) from the day of admission in the first ICU: this means that dosages of patients transferred to our unit from other ICUs (frequently for logistic reasons, being overwhelmed by the emergency) do not start from day 1.

Data collection ended with the following criteria: data collection was stopped after day 18 of ICU stay; iron-related data analysis was stopped (but not lymphocyte counts) if bacterial infection occurred, since we considered it a known confounding factor for inflammatory response; patients were discharged from our analysis on the date of extracorporeal membrane oxygenation (ECMO) start (3 patients) or death.

Exclusion criteria: all patients, admitted for strict clinical monitoring, discharged within 48 hours from ICU; all admitted patients who tested negative for SARS-CoV-2; no underaged patients were included. Overall, 6 patients were initially tested but then excluded from the final analysis for the above mentioned criteria.

Outcomes: the primary outcome was a mere description of iron metabolism and lymphocyte count in ICU patients. Then, as a secondary outcome, we tried to correlate iron metabolism parameters and lymphocyte count with mortality or severity at the moment of ICU admission. Severity at the moment of ICU admission was quantified with two parameters: mean PaO_2/FiO_2 ratio[15] during the first 24 hours (PFmed) and Platelet-to-Lymphocyte ratio[16] (PLR) on the day of ICU admission (data reported on Tabel 1).

Measurement technology: iron parameters were tested with Cobas analyser systems (Roche®), while lymphocyte subpopulations with the Navios EX flow cytometer (Beckman Coultrer®).

Statistical analysis:

Statistical analysis was performed using the software IBM SPSS 22.0. Data are reported as mean with standard deviation (std. dev.), median with interquartile range (IQR), number and percentage, depending on underlying distribution. Student's t-test, Mann-Whitney, Kruskal–Wallis, Spearman correlation, and x2 tests were used for statistical analysis.

Results

Results:

Table 1 summarises anthropometric features and ICU stay characteristics of our sample.

Table 1 Demographic and general sample description

Reported cases	Male (%)	Female (%)
Gender (M/F)	25 (81)	6 (19)
Variables	Male: median (IQ)	Female (median (IQ)
Age (year)	62 (57 - 67)	71 (62 - 74)
BMI (kg/m^2)	27.8 (25.3 - 31.1)	29.4 (29.3 - 30.8)
Symptoms-to-ICU (days)	9 (8 - 12)	7 (7 - 7)
In-hospital pre-ICU LOS (days)	2 (1 - 5)	2.5 (2 - 3)
ICU LOS	14 (9 - 19)	12.5 (11.2 - 16.7)
PFmed	195 (159.5 - 220)	165.5 (136.1 - 183.3)
PLR	436 (218 - 623)	305 (180 - 565)
CT scan (L:H)	6:15	2:2
Intubated patients (%)	22 of 25 (88)	6 of 6 (100)
Tracheostomy (%)	21 of 25 (84)	4 of 6 (67)
VAPs (%)	13 of 25 (52)	2 of 6 (33)
Pro-coagulative disorders (%)	3 of 25 (12)	0 of 6 (0)
Pnx (%)	7 of 25 (28)	1 of 6 (17)
CRRT (%)	10 of 25 (40)	1 of 6 (17)
Mortality (%)	7 of 21 (33) 4 men still admitted in our ICU	1 of 6 (17)

BMI = body-mass index; CRRT = continuous renal-replacement therapy; CT scan = computed tomography (only those with both images and radiologic report available - H stay for High-elastance pattern and L stays for Low-elastance pattern); ICU = intensive care unit; IQ = interquartile range; LOS = length-of-stay; Mortality: 21 men over 25 enrolled because 4 are still in our ICU; PFmed = mean PaO_2/FiO_2 ratio during the first 24 hours of ICU stay; PLR = platelet-to-lymphocyte ratio on the day of ICU admission; Pnx = pneumothorax; Pro-coagulative disorders: deep-vein thrombosis, ischaemic stroke or massive pulmonary embolism; VAP = ventilator-acquired pneumonia.

A significant modification of TfSat (%) occurs between the level during the first 48 hours of ICU admission and the following dosing between day 3 to 6 (Mann-Whitney, p-value = 0.026 - Figure 1). It is relevant to note that the formula we used cannot reliably estimate TfSat when Ferritin dosage is above 1,200 µg/L. No other significant trends and modifications of iron parameters and lymphocyte subpopulations were found during ICU stay. This is anyway the first description of these parameters in an ICU population of Covid-19 patients. Trends and modifications of iron parameters are reported in Figure 1, while trends and modifications of lymphocytes count and lymphocytic subgroups are reported in Figure 2.

Looking to secondary outcomes, neither iron parameters nor lymphocyte count correlate with mortality, PFmed or PLR. Their values are reported in Table 2.

Table 2a Iron parameters over time

	Day 1-2	Day 3-6	Day 7-10	Day 11-14	Day 15-18
Ferritin (µg/L)	1236.5 (1648)	1185 (1018)	964.5 (1251)	1470 (1866)	755 (172)
	10	22	12	9	2
Serum iron (µg/L)	25.5 (69)	73 (56)	58 (61)	55 (63)	45 (8)
	10	22	11	6	2
Transferrin (g/L)	1.67 (0.73)	1.82 (0.38)	1.56 (0.58)	1.88 (0.93)	2.06 (0.15)
	11	22	11	6	2
Transferrin Saturation (%)	9 (7)	33 (26.5)	29 (26.5)	22 (22)	16 (1)
	5	11	7	3	2
Transferrin Receptor (mg/L)	1.14 (0.40)	1.07 (0.49)	0.99 (0.47)	0.98 (0.58)	1.00 (0.11)
	5	17	9	5	2

Table 2b Lymphocyte subgroups over time

	Day 1-7	Day 8-14
NK (10^9/L)	44 (14.25)	57.5 (50.5)
	17	16
T - CD3 (10^9/L)	286 (284.75)	409.5 (422.5)
	17	16
T - CD4 (10^9/L)	246 (161)	331 (364)
	16	17
T - CD8 (10^9/L)	86.5 (121)	117.5 (117)
	18	16
B	116 (146)	102 (161)
	15	17

Table 2c Total lymphocyte count over time

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
Lympho (10^9/L)	0.630 (0.780)	0.555 (0.450)	0.610 (0.605)	0.600 (0.493)	0.670 (0.540)	0.645 (0.330)	0.655 (0.480)	0.680 (0.550)	0.840 (0.493)	0.830 (0.750)	0.780 (0.715)	0.850 (0.650)	0.860 (0.510)	0.800 (0.340
	14	18	20	21	22	22	22	22	19	21	21	18	17	15

Data are all expressed as median, interquartile range between brackets and number of measured cases in the second line.

Survival predictors in hospital population (LDH, troponin, CRP and D-dimer) are not significantly associated with outcome, PLR or PFmed in our ICU population and do not show significant trends. Data are reported in Table 3.

D-dimer shows a non-significant tendency to increase after ICU admission (Kruskal-Wallis, p-value = 0.108, Figure 3). IL6 elevation is lower than reported by other studies on critically ill Covid-19 patients[17]. We found a debile correlation between IL6 levels and lymphocyte count on the day of ICU admission (8 cases, Spearman rho 0.714, p-value = 0.047), but the analysis is limited by the low amount of cases.

Table 3 Clinical biomarkers over time

	Day 1-2	Day 3-6	Day 7-10	Day 11-14	Day 15-18
D-dimer (µg/L)	1319 (1285)	6820 (6619)	3718 (4631)	2959 (2923)	2391 (2693)
	11	19	14	12	10
LDH (U/L)	476 (226)	451 (205)	425 (218)	404 (134)	376 (223)
	13	26	22	19	9
CRP (mg/L)	116 (46)	192 (72)	52 (190)	129 (129)	111 (117)
	13	21	16	17	10
Troponin T (ng/L)	19 (15)	21 (10)	18 (10)	15 (18)	18 (12)
	7	19	15	13	7
IL6 (pg/mL)	42.5 (47.4)				
	9				

Discussion

A brief description of our sample (as reported in Table 1)

Due to the exiguity of our sample and the descriptive nature of our work, we intentionally did not test p-values for the clinical features reported in Table 1. It has already been described[18], and appears strikingly from our dataset, how men are more severely affected than women by Covid-19: they have been 81% of our admissions despite younger age, lower body-mass index (BMI) and lower PFmed. All of our patients were affected by one or more of the following co-pathologies: obesity, hypertension or diabetes (also one case of type 1 diabetes in a 52 year-old man). The interval between symptoms onset to ICU admission is 7-9 days, in the lower range of existing data reporting a median of 7 to 12 days[19,20]; the interval between in-hospital admission to ICU-admission is just 2 days. Our sample is likely to refer to a more severe population than those reported from China: 100% of patients transferred from other ICUs to relieve their workload arrived intubated, while 77% of local males and 100% of local females underwent intubation (other ICU reports remain below 30%)[1,19]. This is likely caused by the availability of sub-intensive units in our hospital dedicated to non-invasive ventilation.

We experienced 3 thromboembolic events (1 cerebral stroke, 1 massive and lethal pulmonary embolisms and 1 other pulmonary embolism of a patient within 48 hours from ICU discharge)[21–23], relatively high rates of ventilator-acquired pneumonias (VAP), pneumothorax and continuous renal replacement therapies (CRRT). All VAP occurred after 96h of mechanical ventilation.

We acted as a backup centre for other overwhelmed hospitals of our region, so 50% of our patients were primarily admitted in a different ICU and then transferred to us for logistic reasons. We experience a mortality rate of 30% to date (literature ranges from 20 to 45%)[19,24].

A narrative discussion of our demographic data and clinical experience

At the beginning of this pandemic in Italy, patients were admitted to hospitals only when ingravescent symptoms meant a severe progression and deterioration of their respiratory condition. This is testified by the elevated rate of High-elastance (H) pulmonary phenotypes observed at CT scans at the moment of hospital admission[25]: severe and frequently irreversible parenchymal damage already happened. During the last weeks, the strategy has been modified: hydroxychloroquine and enoxaparin treatments have been frequently provided early at home, tocilizumab or other available cytokine inhibitors have been administered at the moment of hospital admission. While waiting for scientific validation, it is reasonable to expect a significant improvement in Covid-19 management by these strategies.

Lacking strong evidences, we have adopted for our ICU patients the following treatment protocol: 7 days of Lopinavir/Ritonavir and Hydroxychloroquine; Valsartan 160 mg per day in patients not requiring aminic pressure support [26–28], Atorvastatin 40 mg per day [29] and 4 days of 50 mg/kg of vitamin C four times per day [30]. Methylprednisolone was only used as a rescue therapy in shocked patients facing MOF [31]. The active substances chosen depended mostly on what was made available from our hospital pharmacy.

In our centre, we have adopted a percutaneous tracheostomy strategy, explaining the high rate of tracheostomies performed. Knowing the median ICU LOS of about 2 weeks, we performed tracheostomy in 81% of our patients (median 7 days after intubation; IQR 5 days). We are aware of the debate about this choice [32] because of possible complications, risk of droplets diffusion, rehabilitation times and costs expected for these patients. Anyway, shorter cannuale allow lower ventilatory pressures (following Poiseuille law) [33]; more importantly, we had the chance to safely discharge to sub-intensive units “borderline” patients, still needing frequent aspirations or some hours-per-day of pressure support for lung recruitment, any time our ICU was close to exhaustion.

After the 2 dramatic pro-coagulative complications reported above, and following increasing literature evidences on the topic [34,35], we started an internal protocol of therapeutic anticoagulation with enoxaparin (twice daily, adjusted on body weight, renal and hepatic function), together with fibrinogen dosage at least twice per week. Since that moment, neither ischemic nor haemorrhagic clinically significant events have been observed. When minor bleeding was observed (usually minor epistaxis), a Rotem®-driven approach was successfully applied. All 31 patients were evaluated with compression ultrasounds (CUS) every 48 hours.

After careful consideration of the ongoing guidelines and recommendations[36,37], we decided not to apply prophylactic antibiotic regimens to newly admitted patients and to suspend the prophylaxis in those arriving from other centres. As reported by early autoptic reports, very few not intubated Covid-19 patients have signs of bacterial superinfections[38] and indiscriminate use can cause bacterial resistance (with long-lasting effects on the ICUs[39]), lower sensibility of diagnostic cultures and lower efficacy of empiric treatments. The elevated number of VAPs experienced is likely due to both the damaged pulmonary parenchyma and the difficulty of strictly respecting hygienic procedures while wearing personal protective equipment (PPE) for many hours. Our antibiotic-sparing approach, together with genotypic microarrays for rapid molecular diagnosis from bronchoaspirates or bronchoalveolar lavages (within 4-6 hours) and, if necessary, CT scans, lead to rapid and sensible VAP diagnosis and empiric treatments waiting for anti microbiogram-targeted therapies. VAPs are a major cause of clinical deterioration in Covid-19 patients, frequently leading to a dramatic reduction in pulmonary compliance and gas exchange. Thus, extreme alertness should be posed on their detection.

The high incidence of “H-lungs” [25] suffering reduced compliance might have caused the unusually high rate of pneumothoraces. On the other hand, the initial attempt to relief lungs from fluid overload (with frequent echographic B-line findings) and the presence of ACE2-viral receptor in the kidney might have caused renal damage explaining the frequent resort to CRRT.

Our mortality rate (30%) is in range with those reported by European literature. We just want to recall how, in our view, Covid-19 management is based on two key milestones: prepare for mass-casualty scenarios and work as a network. Despite our province has not been severely affected, since early March we have doubled the number of ICU beds, stopped ordinary operating room schedule and relocated workforce; contemporarily we acted as a backup hospital for other centres in our region (Emilia-Romagna). To date, 40% of our Covid-19 patients have been transferred from other overwhelmed ICUs. Recommended nurse-to-patient ratio was always respected. We believe that mortality rates should not be assessed per single centres, but per region: this is an estimate of network efficiency. With current empiric SARS-Cov-2 treatments still lacking strong scientific evidence, it is essential to apply normal standards-of-care to these

complex and fragile patients. Healthcare systems should implement strategies for rapid re-allocation of patients from the so-called “red zones” to every already existing ICU, trying to limit the recourse to extra-beds and the opening of brand-new emergencial ICUs.

Discussion about iron metabolism and lymphocyte counts in our sample:

Cytokines release hyper-express Hepcidin, leading to Ferroportin internalisation and reduced iron absorption and availability in body fluids [40,41]. Serum iron and TfSat are known to reduce early after infection, trying to block its onset by reducing iron availability to the pathogen, but then increase to almost-normal values within 7-10 days[42,43]. This is the same timing we observe in our patients: admitted to ICU around 7-9 days after symptoms onset still with extremely low levels of TfSat, then they present a statistically significant increase in its values. Serum iron does not show significant trends, but overall it follows the same distribution (it is part of the formula used to calculate TSat). Both TfSat and serum iron remain under the normal reference values during the whole infection.

Ferritin is a very early and non-specific indicator of inflammation. It resulted to be the first severely elevated biomarker together with lymphopenia[1]: it is reasonable to think that their early dosage in at-home symptomatic patients might be extremely useful in individuating those who can benefit of early hospital admission. After its initial rise, ferritin can take longer than a month to normalise after an infection[42]. Thus, it remains normally elevated in the ICU setting. Despite being apparently superficial, its dosage constitutes the key element to suspect sHLH. sHLH is a frequently misdiagnosed syndrome related to viral infections and thus of primary importance in this Covid-19 pandemics. We diagnosed at least one case in our centre.

Overall, despite unable of a more detailed description, we demonstrate that iron metabolism is deranged in Covid-19 and is likely to follow some already described patterns. We are not able to correlate it with immune response. These findings tell us that our actual ICU setting still focuses on very severe patients at an advanced state of disease. Referred to the early reports from Hubei, our patients possibly refer to the little subgroup of mechanically ventilated ones that experienced very poor survival rate[1]. Being such a specific subset of patients, we have not observed significant differences between survivors and non. Anyway, our work might be of specific interest for researchers involved in iron and immunity and for clinicians working in ICU.

Lymphocytes are constantly reduced in ICU Covid-19 patients with respect to reference values[44]. All the subsets are also dramatically reduced, more than reported by other recent publications referring to non-ICU populations [8,45]. We observe a conserved CD4/CD8 ratio. The nadir of lymphopenia is on day 2 of ICU stay; then, we observe a progressive tendency towards normalisation, more evident in patients experiencing positive outcome and ICU discharge. Similar timing of lymphocyte modifications were observed during Severe Acute Respiratory Disease (SARS) outbreak in 2004, despite with less dramatic reduction [46,47]. Referring to critically ill patients, this more severe reduction of all lymphocyte subgroups might be an indicator of severity: in fact, to date no other publications are available about ICU populations on lymphocytes subgroups. These data might be relevant to researchers for a better understanding of the altered immunologic response in severely affected patients.

The study is affected by some limitations. The sanitary emergency, the non-university nature of our hospital and the previously unknown characteristics of this disease led to many difficulties. Collect, analyse and communicate data has been complex and could only be performed during the scarce spare time left by healthcare assistance; during the initial phase, it has been difficult to create an effective protocol shared between the operators and some data have been missed. We could only rely on the commonly available tests present in our laboratories, without the chance to measure hepcidin and other crucial molecules that could have allowed a finer description of the investigated processes. Moreover, our centre is not located in the epicentre of the crisis and Covid-19 cases in our local community have been numerically limited; thus, we have frequently acted as a backup hospital to relieve other collapsed ICUs nearby: this means that, for a part of the reported cases, the first few days of ICU-stay occurred in a different unit and the dosage of relevant markers was missed during that initial phase. Being a monocentric study, the sample size remains limited and this could lead to the impossibility to detect and describe some more subtle physiologic processes. Finally, slightly different therapeutic approaches have been applied to patients following published findings and different complications (ventilator-acquired pneumonia, pro-coagulative states, renal and hepatic insufficiency) have affected patients’ evolution: it is not possible to quantify how these differences affected iron metabolism and lymphocyte count.

Conclusions

For the first time we describe iron metabolism, lymphocyte subgroups count and other biomarkers in ICU setting of Covid-19 patients. This might be relevant for ICU workers and provide further hints about the pathophysiology of this disease.

This descriptive work also shares our ICU experience and approach to Covid-19 patients, hoping to stimulate discussion and provide useful suggestions to other groups.

Abbreviations

APP: acute phase-proteins; CRP: C-reactive protein; CRRT: continuous renal replacement therapy; ECMO: extracorporeal membrane oxygenation; HLH: hemophagocytic lymphohistiocytosis; IL-6: interleukin 6; IQR: interquartile range; LDH: lactate dehydrogenase; LOS: length-of-stay; MOF: multiple organ failure; PCR: polymerase chain reaction; PFmed: mean PaO_2/FiO_2 ratio during the first 24 hours of ICU admission; PLR: platelet-to-lymphocyte ratio on the day of ICU admission; PPE: personal protective equipment; SARS: severe acute respiratory syndrome; SARS-CoV-2: SARS Coronavirus type 2; TfSat: Transferrin Saturation (%); VAP: ventilator-acquired pneumonia; WHO: World Health Organization.

Declarations

Ethics approval and consent to participate: protocol IR-COV, version 21st of April 2020, accepted by Romagna Ethical Committee, under final revision.

Consent for publication: all clinical data are completely anonymised, discharged patients are being telephonically contacted to get informed consent.

Availability of data and materials: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contribution: BG conceived the study project, wrote the paper and collected scientific literature; RE critically reviewed the study design, performed statistical analysis and contributed to drafting; GE, CA, MMCC, BE, VL and BL contributed to data collection, database curation and drafting; PV and AV supervised the project and critically reviewed the draft.

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Figures

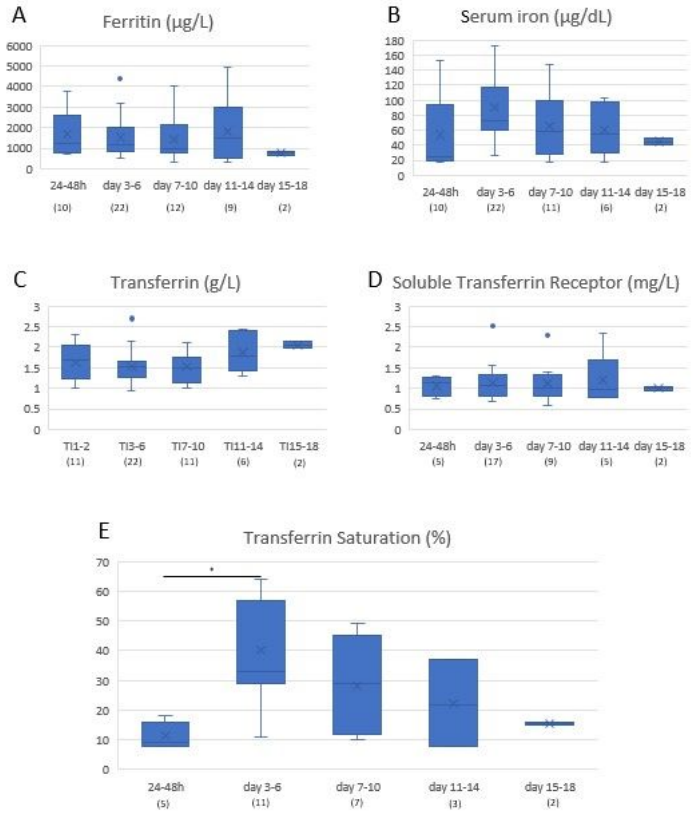

Figure 1

iron parameters Legend figure 1: distribution over time of the tested iron parameters with their units of measure. Box plots indicate median with interquartile range, dots indicate outliers. Groups specify the timing by which the dosage was effectuated form the moment of ICU admission, round brackets in the lower line indicate the number of patients tested for each group. In figure 1E, * indicates significant difference (p-value > 0.05).

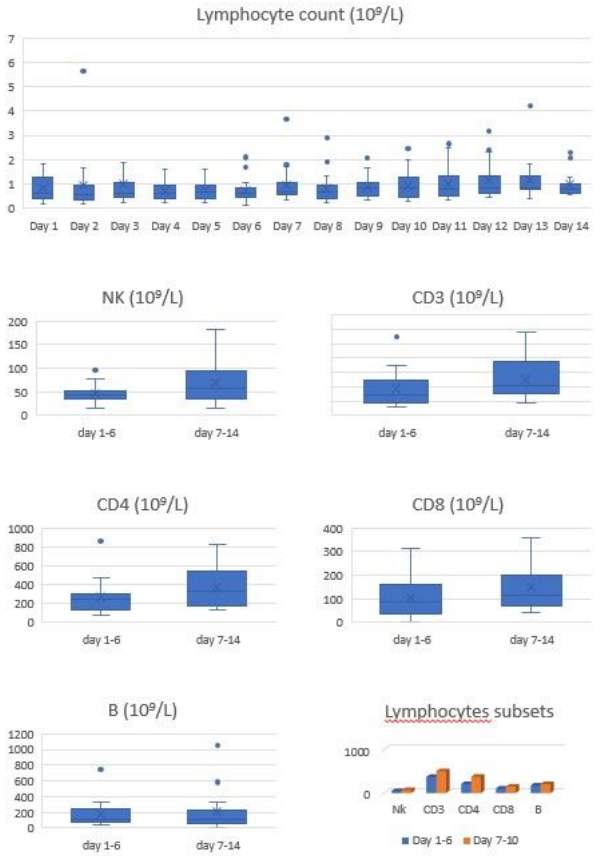

Figure 2

lymphocytes count Legend figure 2: distribution over time of lymphocytes and their tested subgroups, with their units of measure. Box plots indicate median with interquartile range, dots indicate outliers. Groups specify the timing by which the dosage was effectuated form the moment of ICU admission. The number of patients tested is reported in table 2b and 2c. Lymphocytes subsets merges all the subgroups to make their trend over time more readable.

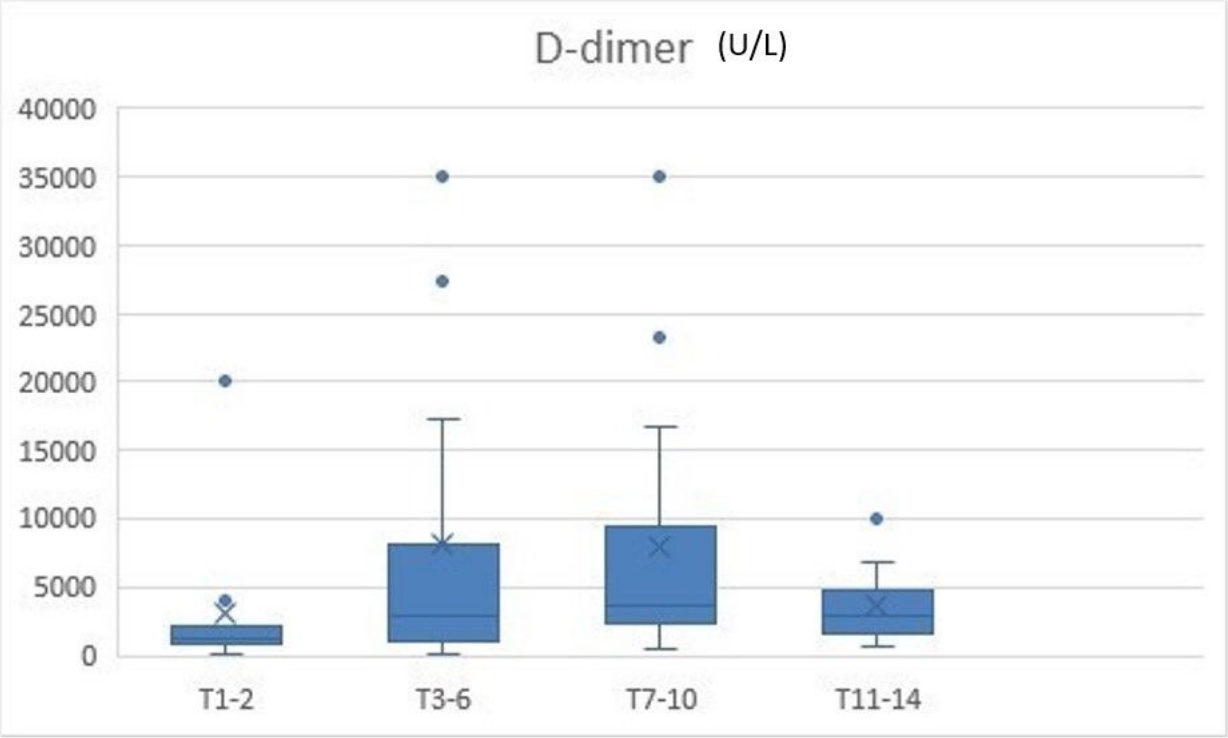

Figure 3

D-dimer levels Legend figure 3: D-dimer levels over time from the moment of ICU admission. It is observable a non-significant trend toward increase after the first 48h. It might be relevant to explain the increased tendency to hypercoagulability of Covid-19 patients. Box plots indicate median with interquartile range, dots indicate outliers. Groups specify the timing by which the dosage was effectuated form the moment of ICU admission.