Clinical and diagnostic findings in COVID-19 patients: an original research from SG Moscati Hospital in Taranto Italy

M. Balzanelli[1*], P. Distratis[1*], O. Catucci[1], F. Amatulli[1], A. Cefalo[1], R. Lazzaro[1], K.S. Aityan[2], G. Dalagni[3], A. Nico[3], A. De Michele[4], E. Mazza[4], M. Tampoia[4], P. D'Errico[5], G. Pricolo[5], A. Prudenzano[5], E. D'Ettorre[6], C. Di Stasi[6], L.F.P. Morrone[7], KCD. Nguyen[8], HV. Pham[9], F. Inchingolo[10], D. Tomassone[11#], and C. Gargiulo Isacco[1,8#]

[1]SET-118, Department of Pre-hospital and Emergency, SG Giuseppe Moscati Hospital, Taranto, Italy; [2]Department of Multidisciplinary Research Centre, Lincoln University, Oakland CA, USA; [3]Department of Pneumology, SG Giuseppe Moscati Hospital, Taranto, Italy; [4]Department of Clinical Pathology, SS. Annunziata and SG Giuseppe Moscati Hospital, Taranto, Italy; [5]Department of Hematology, SS. Annunziata, Taranto, Italy; [6]Department of Diagnostic Imaging, SG Giuseppe Moscati Hospital, Taranto, Italy; [7]Department of Nephrology, SS. Annunziata, Taranto, Italy; [8]American Stem Cells Hospital Ho Chi Minh, Vietnam; [9]Phan Chau Trinh University of Medicine and Nam-Khoa Biotek, Ho Chi Minh, Vietnam; [10]"Aldo Moro", University of Bari, School of Medicine D.I.M. (Department of Interdisciplinary Medicine), Bari, Italy; [11]Foundation of Physics Research Center, Celico, Italy

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*Contributed equally as first authors

#Joint last authors

The coronavirus disease 2019 (COVID-19) pandemic is a worldwide medical challenge due to the scarcity of proper information and remedial resources. The ability to efficiently avoid a further SARS-CoV-2 pandemic will, therefore, depend on understanding several factors which include host immunity, virus behavior, prevention measures, and new therapies. This is a multi-phase observatory study conducted in the SG Moscati Hospital of Taranto in Italy that was converted into COVID-19 Special Care Unit for SARS-Co-V2 risk management. Patients were admitted to the 118 Emergency Pre-Hospital and Emergency Department based on two diagnostic criteria, the nasopharyngeal swab assessed by reverse-transcriptase–polymerase-chain-reaction (RT-PCR) and CT-scan image characterized by ground glass opacity. Patients were divided into four groups, positive-positive (ER-PP), negative-positive (ER-NP), negative-negative (ER-NN) and a group admitted to the ICU (ER-IC). A further control group was added when the T and B lymphocyte subsets were analyzed. Data included gender, age, vital signs, arterial blood gas analysis (ABG), extensive laboratory results with microbiology and bronchoalveolar lavage fluid (BALF) which were analyzed and compared. Fundamental differences were reported among the groups. Males were significantly higher in PP, ICU, and NP groups, from 2 to 4-fold higher than females, while in the NN group, the number of females was mildly higher than males; the PP patients showed a marked alkalotic, hypoxic, hypocapnia ABG profile with hyperventilation at the time of

Corresponding Author:
Dr C. Gargiulo Isacco,
SET-118, Department of Pre-hospital and Emergency,
SG Giuseppe Moscati Hospital,
Taranto, 74100, Italy
Tel.: +39 3273736805
e-mail: drciroisacco@gmail.com

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admission; finally, the laboratory and microbiology results showed lymphopenia, fibrinogen, ESR, CRP, and eGFR were markedly anomalous. The total number of $CD4^+$ and $CD8^+$ T cells was dramatically reduced in COVID-19 patients with levels lower than the normal range delimited by 400/μL and 800/μL, respectively, and were negatively correlated with blood inflammatory responses.

Key words: SG Moscati Hospital; 118 Pre-Hospital Medical System-Emergency Department; COVID-19, Sars-CoV-2; Arterial Blood Gas (ABG); bronchioalveolar lavage fluid (BALF)

COVID-19 as an atypical pneumonia infectious disease is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and shares 79.6% of its genome with SARS-CoV-1. COVID-19 as a disease was first acknowledged in isolated patients of Hubei Province in Wuhan City from China mainland and soon reached planetary proportions (1). SARS-CoV-2 belongs to the CoV phylogeny which includes two novel human coronaviruses, NL63 and HKU1, and recent findings have confirmed that it has evolved and tripled the number of full-length genome sequences. SARS-CoV-2 binds to host cell receptor-like Angiotensin-Converting Enzyme 2 receptor (ACE_2) thanks to its external spikes made of proteins composed of highly specific glycoprotein domain (possible therapeutic target) (1, 2).

Despite SARS-CoV-2 sharing the 3′–5′ exonuclease RNA proof-reading activity encrypted within nsp14 with other Coronavirus family members, it seems to be capable of "self-repairing", adjusting and adapting its RNA to environmental changes and during stress replicative process (3).

At present, what is known about COVID-19 disease is still based on data and outcomes coming from clinical experience. The transmission takes place via respiratory saliva droplets, and close contacts are considered the main mode of transmission. Clinically, the overall picture describes a blend of inflammatory and infectious patterns that may rapidly shift into typical systemic inflammatory response syndrome (SIRS) or into acute respiratory distress syndrome (ARDS), and eventually may conclude with multi-organ failure (MOF) and death (3-10).

Differences seen in disease incidence and severity are related to insufficiencies of both innate and adaptive immune responses also linked to definite gene polymorphisms (SNPs) that facilitate the virus binding capacity on cells receptors such as the ACEr. The presence of pre-existing comorbidities also plays a key role in enhancing viral invasion (10, 11).

Lymphopenia has been seen as an additional feature in the totality of screened COVID-19 patients and could be considered a determinant marker in cause-specific mortality among the general population. In COVID-19, $CD4^+$ helper T cells (Th) and $CD8^+$ cytotoxic T cells (CTLs) were markedly reduced, ascribed to the persistent viral stimulation that induce an earlier apoptosis of either $CD4^+$ or $CD8^+$ T-cells leading to a reduced immune modulatory responses (12).

Recent studies observed a decline of $CD4^+$ (70.56%), CD8+ T (95%) in a total of 499 COVID-19 patients hospitalized in non-ICU and ICU departments. These findings are attributed to the penetrating modality of the virus via lymphocyte ACE-$_2$r and, its direct to assaults on lymphatic organs, thymus, spleen and lymph nodes (9-14).

The presence of necrotic tissues assessed in COVID-19 deceased patients reflected a generalized amplification of inflammatory and infectious patterns as the main cause of multiple organ involvement. Microscopic autopsy confirmed massive micro-vascular thromboembolism and the presence of non-specific neutrophil and macrophage infiltrates, indicative of an auto-generating necroinflammatory loop arrangement. Conclusions that highlight the silent progression of the disease characterized by the "happy hypoxia" in which a patient's health degenerates silently, almost unnoticeably, up to the point of a sudden irreversible cardiorespiratory blockage (4, 16-20).

The current study focused on data collected from patients admitted to the 118 Emergency Department

of the SG Moscati Hospital in Taranto, Italy (Table I). The patients (n=181) were divided into four groups based on two main criteria, positivity to nasopharyngeal swab analyzed by RT-PCR and, positive to lung/thorax CT-scan imaging technique in compliance with the "typical" ground-glass opacity configuration (PP), patients negative to swab but positive to CT-scan, patients negative to both swab and CT-Scan (NN without "ground glass opacity" but affected by different diseases) and a COVID-19 patient group admitted into the ICU (PP-ICU). The average age of all patients admitted to the 118 Emergency Department and ICU was significantly higher than the average age of the general population of Taranto province (confidence level 95%). The overall outcomes suggested the elderly were more susceptible to COVID-19 disease and likely to have a poor prognosis (20). The age curve of ICU-PP and PP was marked from 60 to 79 years and the curve of NP was from 80 to 99 years, with a total peak that reached 80 to 99 years. Eventually, an alarming distribution rate was noted of the age for normal population of Taranto province; the peak was seen in the age group between 40 to 59 years (about 30% of the entire population); the younger generation (0-19, 20-39 years) showed a much lower percentage for the same age intervals which forecasted a decline of the general population, data confirmed by the general trend for Italy. The percentage of emergency patients grew exponentially with age. The relatively low number of emergency patients for the age group over 100 years was still the highest percentage age group adjusted for the age distribution of the population (Fig. 1 a,b; Fig. 2 a.b) (Table I). All confirmed COVID-19 patients admitted to our facility showed typical signs of respiratory distress with or without detectable comorbidities with an increase of alveolar-capillary permeability with interstitial edema of lung parenchyma, anomalies then confirmed by "ground-glass opacities" CT-scan. All patients showed an increase of classic inflammatory and infectious markers such as the IL-6, troponin, D-dimer, ESR, fibrinogen, and CRP.

MATERIALS AND METHODS

The patients admitted for COVID-19 diseases were informed, and consented to share clinical data for this study research that was conducted per the World Medical Association Declaration of Helsinki. From March 27th, 2020 to May 25th (the considered period), a total of 381 patients were screened at the 118 Emergency Department of the SG Moscati Hospital of Taranto, most of whom were male [n=22 (61%)], 19 deceased (2 in 118 Department, 5 in Pneumology Department, 5 in ICU and 7 in the Infectious Diseases Department [n=19 (5%)], over 80% of older age (mean age 72 years). The patients (n=181) enrolled in the study were divided into four groups, PP (n=46), NP (n=51), the NN (n=63), and PP-ICU (20).

Statistical analysis

Descriptive statistics were used to summarize the data; results are reported as distribution and correlation ranges and values as appropriate. Categorical variables were summarized as counts and percentages. Statistical analysis was supported by Student's *t*-test. The null hypothesis H0 was calculated by using t-statistics. The significance level, denoted as alpha (α) was used to indicate the probability of rejecting the "null hypothesis" when it was true. Confidence level=1-significance level; the p-value was calculated by using Student's *t*-Distribution, if p-value > alpha, the test failed to reject the null hypothesis (H0) and it was accepted (the result was considered not significant), if p-value < alpha it rejected the null hypothesis (the result was considered significant); the df or degree of freedom was 0.003671.

$$\text{t-score} = \frac{(\overline{X}_1 - \overline{X}_2) - (\mu_1 - \mu_2)}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

$$df = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)^2}{\frac{(S_1^2/n_1)^2}{n_1 - 1} + \frac{(S_2^2/n_2)^2}{n_2 - 1}}$$

$$\text{t-score} = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} \quad H0: \mu_1 = \mu_2 \quad HA: \mu_1 \neq \mu_2$$

RESULTS

The groups included both men and women; median age, 50 years (age range, 18-86 years). The results were then assessed and compared. The gender distribution between all patients COVID CT positive

(PP, NP, NN, ICU-PP) was statistically significant (with the significance level alpha = 0.05) from the total Table II; Figs 1, 2.

Age distribution including the total population of Taranto province

The average age of all four groups PP, NP, NN, and ICU-PP patients was significantly (confidence level 95%) higher than the average age of the general population of Taranto. The average age of PP and ICU-PP patients was statistically equal with the confidence level of 95%. The average age of NN and ICU patients was statistically equal with the confidence level of 95%. (A peculiar result. Possibly because ICU patients were suffering from general existing conditions as in the total population). The

Table I. *Population distribution - province of Taranto as of January 1 2019*

Age		Numbers			%		
From	**To**	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**
0	19	54,081	51,347	105,428	19.3%	17.3%	**18.3%**
20	39	66,337	64,777	131,114	23.7%	21.8%	**22.7%**
40	59	83,441	87,999	171,440	29.8%	29.6%	**29.7%**
60	79	61,410	69,769	131,179	22.0%	23.5%	**22.7%**
80	99	14,365	23,121	37,486	5.1%	7.8%	**6.5%**
100		22	87	109	0.0%	0.0%	**0.0%**
	Total:	**279,656**	**297,100**	**576,756**	**100%**	**100%**	**100%**

Table II. *Covid-19 patients admitted to 118 Emergency Department*

(PaO_2)	(PaO_2)	(PaO_2)	(PaO_2)
up to 60 mmHg	61 to 70 mmHg	71 to 90 mmHg	> 90 mmHg
Patients n. 59	Patients n. 64	Patients n. 132	Patients n. 55
11 Swab +	12 Swab +	17 Swab +	2 Swab +
48 Swab -	52 Swab -	115 Swab -	53 Swab -
CT scan	CT scan	CT scan	CT scan
27 CT +	20 CT +	22 CT +	5 CT +
32 CT -	44 CT -	110 CT -	50 CT -

Covid-19 patients (n=310) admitted to the 118 Emergency Department were screened for arterial blood gas (ABG) analysis, Swab Buffer (oral-nasal-pharynx) analyzed by RT-PCR and thoracic CT scan were then performed and results were compared. Notably, based on the oxygen saturation level (PaO2) the CT scan revealed, in line with different published data, a better degree of accuracy compared to swab-RT-PCR outcomes, the PaO2 up to 60 mmHg on a total of 59 patients the CT+ was 27 vs 11 swab+ (column 1); the pO2 from 61 to 70 mmHg, total 64 cases, 20 CT+ vs 12 swab+ (column 2); the PaO2 from 71 to 90 mmHg, total 132 cases, 22 CT+ vs 17 swab+ (column 3); the PaO2 90 mmHg, total 55 cases, 5 CT+ vs 2 swab+ (column 4).

mean ages for all other groups PP, NP and NN and ICU-PP categories were significantly different with the confidence level 95%. The percentage of patients admitted to the 118-Department grows exponentially with age. The relatively low number of emergency patients for the age group over 100 years was still the highest percentage age group adjusted for the age distribution of the population. The percentage of emergency PP and NP patients by age did not exceed the percentage of other patients, and the percentage of IC-PP by the age of the population was lower than the percentage of ER-PP.

Distribution of 118-Pre-Hospital Department the NN, NP, and PP patients based on the evaluation of the CT, RT-PCR and ABG values

An alarming distribution by age was seen for the normal population of Taranto, the peak was related to the age group 40-59 (about 30% of all population, the younger generation (0-19, 20-39) showed a much lower percentage for the same age intervals, which indicated that the population would decline in the future. The majority of PP patients showed an alkaline shift in pH over NP and NN patients (with the significance level alpha = 0.05; Confidence level

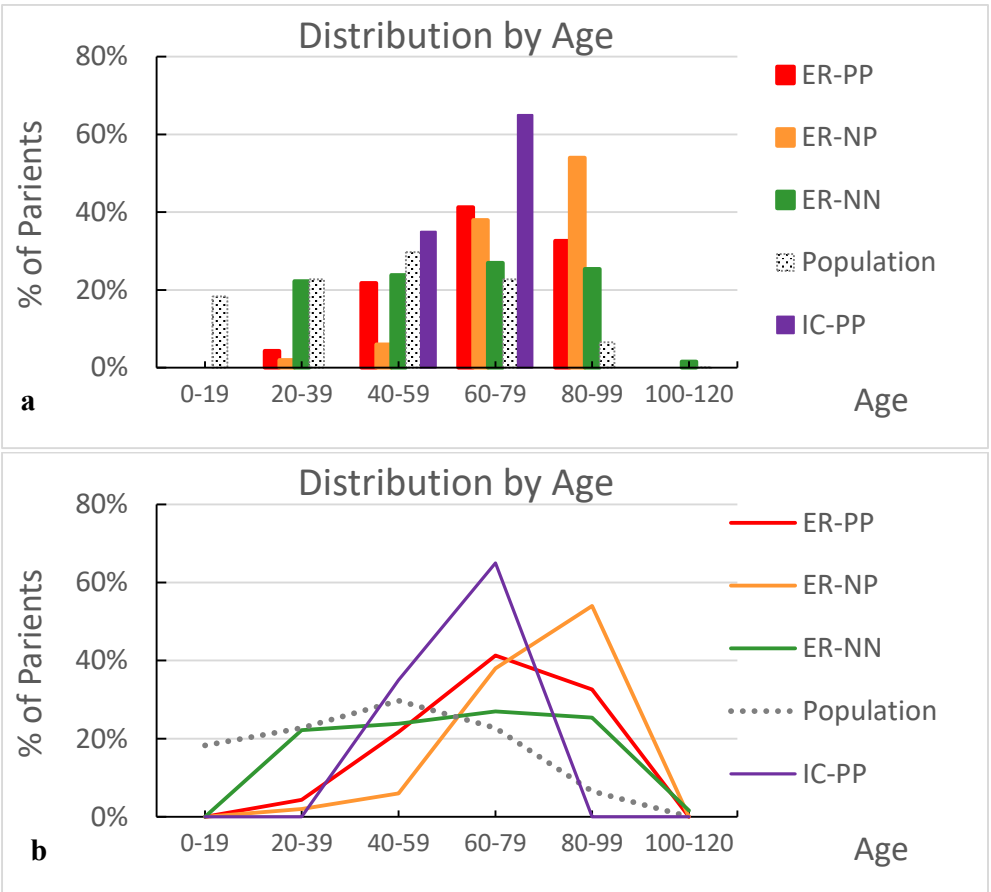

Fig. 1. *Number and percentage of emergency patients by age vs the age distribution of the Taranto population; (a) graph (b) histogram; the percentage of the elderly patients constitute the majority of the of patients admitted to 118 and then hospitalized. Younger patients with negative CT scan indicated that younger people handle COVID easier and call emergency for different health issues; an interesting fact, is the peak of PP: the ages varies between 60-79 and the peak of NP on 80-99 with total peak on 80-99, nevertheless, the age peak of ICU-PP of 60-79 results the same as for PP. The date revealed an alarming distribution by age for the general population of Taranto. It was found that the age group ranged between 40-59 (about 30% of all population) while the younger generation (0-19, 20-39) showed a much lower percentage for the same age intervals which indicates that the population is steadily declining.*

= 1-alpha =0.95). NP patients were more acidic than NN patients but this difference was statistically insignificant.

The PP (n=46) showed pH values alkalotic (>7,45) (n=33/72%), males were 26=59% and females 7=15%; the PaO2 value <75 mm/Hg (n=33/72%), males 27=59% and females 6=13%; the $PaCO_2$ value <75 mm/Hg (n=33/72%), males 26=57% and females 7=15%. The NP group (n=51) showed pH alkalotic (>7,45) (n=25/49%), males were 14=27% and females 11=22%; the PaO2 value <75 mm/Hg (n=35/69%), males 21=41% and females 14=27%; the $paCO_2$ value <75 mm/Hg (n=28/55%), males 20=39% and females 8=16%. The NN group (n=63) showed pH alkalotic (>7,45) (n=29/46%), males were 11=77% and females 18=29%; the PaO2 value <75 mm/Hg (n=27/43%), males 14=22% and females 13=21%; the $PaCO_2$ value <75 mm/Hg (n=32/51%), males 13=21% and females 19=30%.

Distribution by PaO_2 and $PaCO_2$. The oxygen level for PP and NP patients was similar (with the significance level alpha=0.05; Confidence level=1-alpha =0.95). The PaO_2 level in NN patients was significantly higher than the level of PP and NP patients (significance level 0.05). The $PaCO_2$ level for PP was significantly lower than the level for NN

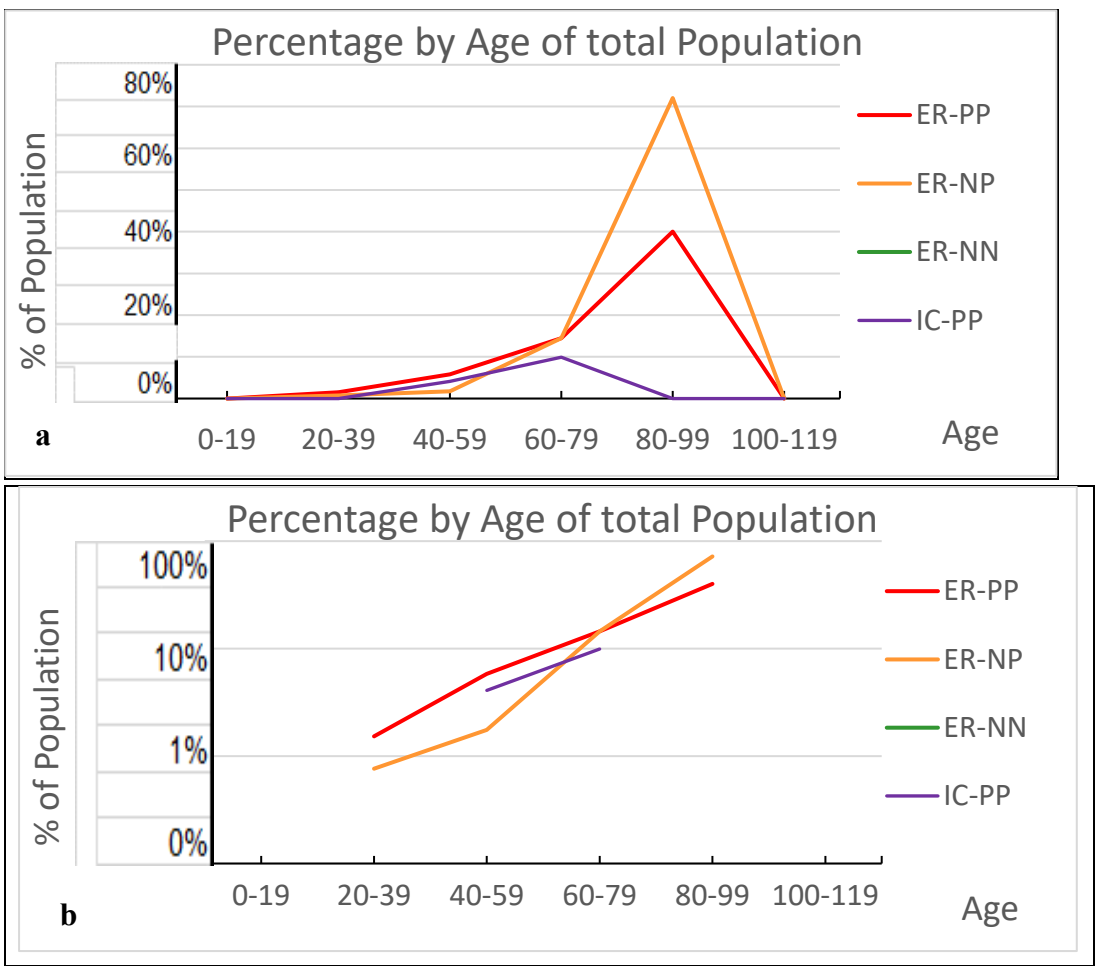

Fig. 2. *Percentage of patients of total population by age. a) Linear Graph; b) Logarithmic Graph. Percentage of emergency patients grows exponentially with age. The relatively low number of emergency patients for the age group over 100 years is still the highest percentage age group adjusted for the age distribution of the population. Percentage of emergency PP and NP patients by age does not exceed the percentage of other patients. Percentage of ICU-PP computed by the age of population is lower than that in PP.*

and NP patients (significance level 0.05); the CO_2 level for NP and NN patients are statistically the same (significance level 0.05). *Distribution by HCO_3.* There was no difference between HCO_3 levels for all 118 Emergency Department admitted patients including PP, NP, and NN (insignificance differences).

Distribution by BE. There was a significant difference between BE levels for PP and NP patients (significance level 0.05); there was no difference between BE levels for NN patients and NP patients (insignificance differences). *Distribution by FO_2HB.* There was a significant difference between FO_2Hb levels for PP and NP as well as for NP and NN patients (significance level 0.05); there was no difference between FO_2Hb levels for PP and NN patients (insignificant differences).

White blood cells, neutrophils, lymphocytes distribution significance for each group PP, NP and NN

Distribution by white blood cell count WBC. There was a significant difference in WBC levels for ICU-NP as well as for NP-NN patients (significance level 0.05); there was no difference in WBC levels for ICU-PP, ICU-NN, PP-NP, and PP-NN patients

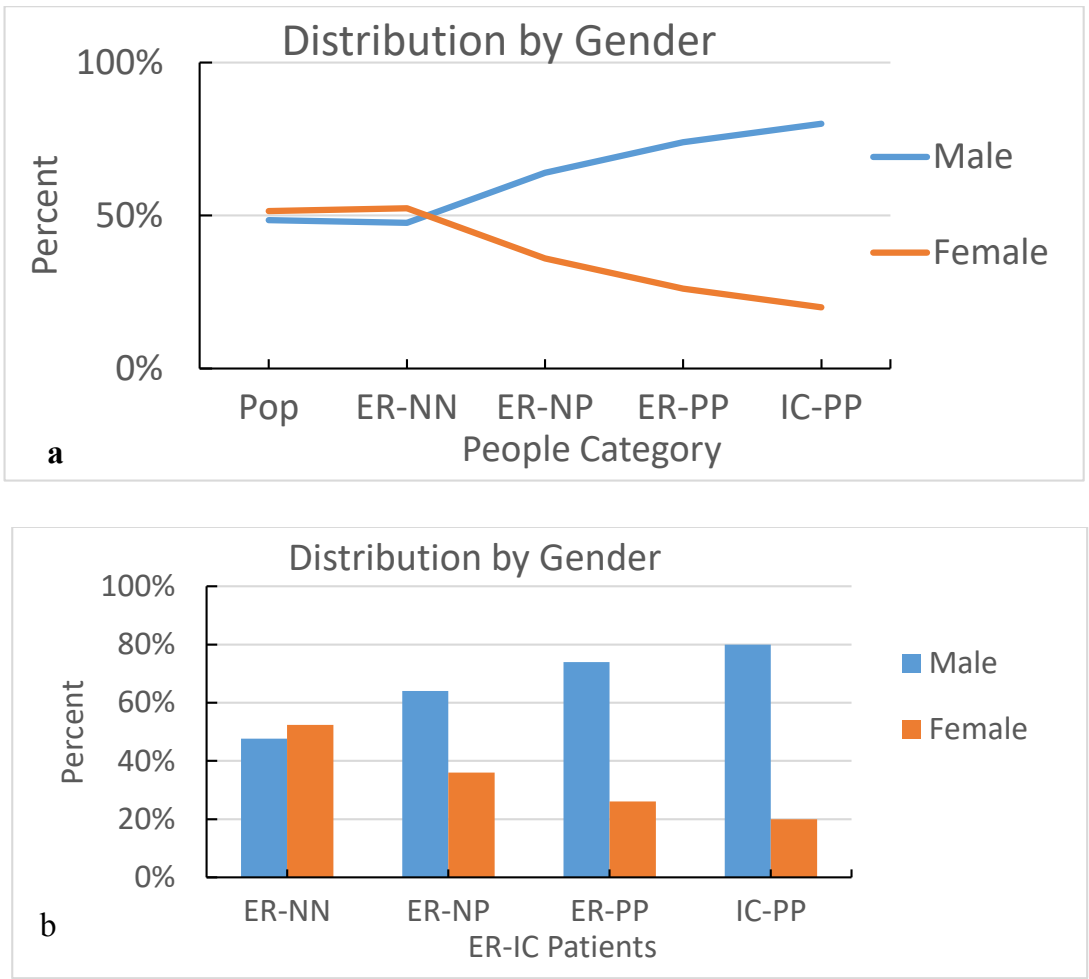

Fig. 3. ***a, b)*** *The gender distribution between the NN patients and the total population is statistically insignificant with the significance level alpha = 0.05. It means that all ER-NN patients are from the general population for different health reasons, a trait that has no impact on the gender differences; the gender distribution between all COVID CT positive patients (NP, PP, ICU-PP) is statistically significant (with the significance level alpha = 0.05) from the total population. Data clearly indicated that COVID-19 lung damage mostly impacts males compared to females. In addition, the number and percentage of emergency patients included in the totality of Taranto population show that the majority of affected patients are elderly. An interesting fact is that the peak of ICU-PP is between 60-79 the same as for PP (1-b).*

(insignificant differences). The WBC revealed the highest number in the NP group, 36 patients affected out of 51 (71%), 25males (49%) and 11 females (22%); the negative group had 29 patients with high WBC out of 63 (46%), 13 males (21%) and 16 females (25%); the PP was the less affected with only 16 patients out 46 (35%), 13 males (28%) and 3 females (6%).

Distribution by neutrophils. There was a significant difference in Neutrophils levels for PP- NP as well as for NP-NN patients (significance level 0.05); there was no difference in neutrophil levels for IC-PP, IC-NP, IC-NN, and PP-NN patients (insignificant differences level 0.05). An important presence was found of neutrophilia in the PP group, 27 patients out of 46 (59%) were found with a high neutrophil level, 21males (46%) and 6 females (13%); the NP group showed the highest presence of patients with high neutrophils 39 out of 51 (78%), 31 (61%) males and 13 (25%) females; the negative group showed the lowest patients affected by neutrophilia, 34 out of 63 (54%), 14 males (22%) and 20 females (32%). *Distribution by lymphocytes.* There was a significant difference in lymphocyte levels for PP-NP as well as for NP-NN patients (significance level 0.05); there was no difference in lymphocyte levels for IC-PP, IC-NP, IC-NN, and PP-NN patients (insignificant differences). Lymphopenia was the major value that characterized the PP group, 35 patients, out of 46 (76%) were detected with a marked low count of lymphocytes, 28 males (61%) and 7 females (15%); the NP lymphopenia was confirmed in 30 patients

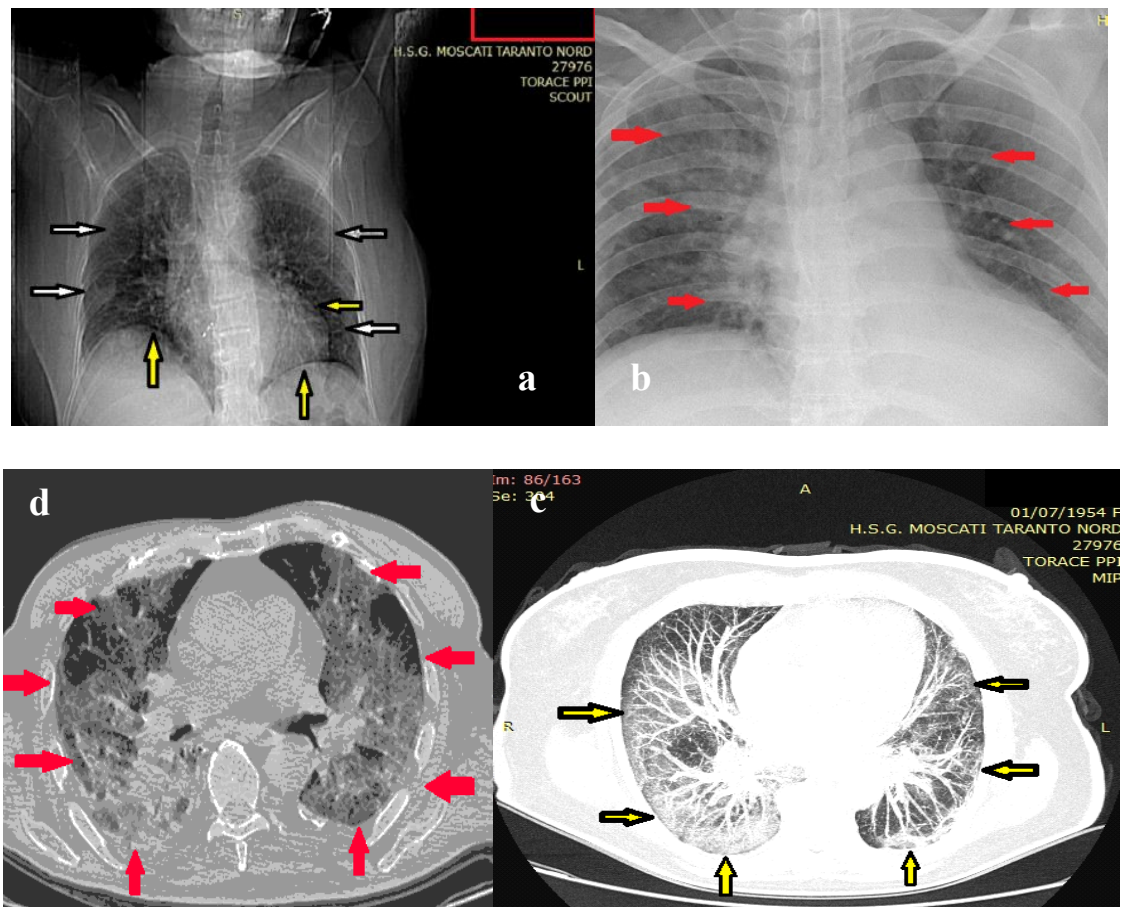

Fig. 4. *Chest CT of 38-year-old male with axial and coronal planes shows the progression of pneumonia with bilateral diffuse patchy mixed ground-glass opacities and linear opacities in the subpleural area (black/white arrow) associated with an anterior pericardial effusion (yellow arrows) (**a-b**); bilateral diffused ground-glass opacity (yellow arrows) from a 66-year-old man with predominant cardiomegaly (red arrows) (**c-d**).*

out 51 (60%), 3 1males (61%) and 14 females (27%); the lymphopenia was not a major problem in the NN- only 26 patients out of 63 (41%) were confirmed with the low level of lymphocytes, 20 males (32%) and 26 females (41%).

eGFR values and statistics analysis of the PP, NP and NN groups

There was a significant difference between eGFR levels for PP-NP as well as for NP-NN patients (significance level 0.05); there was no difference between eGFR levels for PP- NN patients (insignificant differences). The eGFR measurements were taken and divided into three different grades of severity, the eGFR % under 60 ml/min. (severe range), between 60-89 ml/min. (medium severity range) and over 90 ml/min. (mild range). The PP patients resulted to be the most affected in the medium severity range, 22 patients out of 46 (48%) 17 males (37%) and 5 females (11%); in the NP group 18 patients out of 51 were affected (36%), 14 males (28%) and 4 females (8%); in the NN group 19 patients were affected out 63 (30%), with 11 males and 8 females. In the lowest eGFR range (<60ml/min) the PP group had 14 patients out of 46 (31%) of which 10 males (21%) and 4 females (8%); the NP group had 25 patients out of 51 (50%) of which 15 males (30%) and 10 females (20%); the NN group had 21 patients out of 63 (34%) of which 10 males (8%) and 13 females (21%). In the mild range (>90 ml/min) the PP group had 10 patients out of 46 (21%) of which 8 males (17%) and 2 females (4%); the NP group had 8 patients out of 51 (16%) of which 4 males (8%) and 4 females (8%); the NN group had 23 patients out of 63 (36%) of which 11 males (17%) and 12 females (12%).

Statistical analysis and measurements of ESR, CRP, D-dimer, Fibrinogen and Troponin on PP, NP, and NN groups

There was a significant difference between ESR levels for PP-NN as well as for NP-NN patients (significance level 0.05); there was no difference between ESR levels for PP- NP patients (significance level 0.05). The values of erythrocyte sedimentation rate (ESR, >10 mm/h) in the PP were quite exclusive, patients with high ESR were 43 out of 46 (93%) of whom 32 males (70%) and 11 females (24%); in the NP group, patients with high ESR were 45 out of 51 (88%) of which 29 males (57%) and 16 females (31%); in the NN group patients with high ESR were 42 out of 63 (67%) of which 13 males (21%) and 29 females (46%).

There was a significant difference in CRP levels for all categories of ICU patients *vs* PP, NP, and NN, except for PP-NP patients (significance level 0.05); there was a significant difference in PP NN (significance level 0.05). The values of (CRP, >3.5 mg/L) in the PP were highly exclusive, patients with high CRP were 46 out of 46 (100%) of which 34 males (74%) and 12 females (26%); in the NP group patients with high CRP were 51 out of 51 (100%) of which 33 males (65%) and 18 females (35%); in the NN group patients with high CRP were 44 out of 63 (70%) of which 23 males (37%) and 21 females (33%).

There was a significant difference in D-dimer levels for PP-NP patients (significance level 0.05). The D-dimer values in the PP group did not show great relevance, 17 patients out 46 (37%) of which 11 males (24%) and 6 females (13 %); the D-dimer values in the NP group showed great relevance, 39 patients out 51 (76%) of which 27 males (53%) and 12 females (24 %); the D-dimer values in the NP group showed enough relevance, 43 patients out 63 (68%) of which 20 males (32%) and 23 females (37 %).

There was a significant difference in fibrinogen levels for PP-NN and NP-NN patients (significance level 0.05); there was no difference in fibrinogen levels for ICU patients with other patients and between PP-NP patients (insignificant differences). The values of fibrinogen (400 > mg/dl) in the PP were significant, patients with high fibrinogen were 44 out of 46 (96%) of which 33 males (72%) and 11 females (24%); in the NP group, patients with high fibrinogen were 45 out of 51 (88%) of which 31 males (61%) and 14 females (27%); in the NN group patients with high fibrinogen were 34 out of 63 (54%) of which 15 males (24%) and 19 females (30%).

There was a significant difference between Troponin levels for NP-NN patients (significance level 0.05); there was no difference between Troponin levels for PP-NP and PP-NN patients

(insignificant differences). The values of Troponin (14> ng/L) in the PP were less compared to the other groups, patients with high troponin were 14 out of 46 (30%) of which 11 males (24%) and 3 females (7%); in the NP group patients with high fibrinogen were 34 out of 51 (67%) of which 24 males (47%) and 10 females (20%); in the NN group patients with high troponin were 30 out of 63 (48%) of which 12 males (19%) and 18 females (29%).

Statistical analysis and measurements of T and B lymphocyte and their subsets in the Control, PP, NP, and NN groups

The T and B lymphocytes and their subsets were analyzed and compared among each group. There was a significant difference in levels for PP *vs* Control, NP, and NN patients (significance level 0.05). Lymphocytes (absolute count/ul) showed a significant difference of PP *vs* Control, NP and NN (significance level 0.05); both CD4 and CD8 (absolute count/ul) showed a significant difference of PP *vs* Control and NN (significance level 0.05) but not *vs* NP; B Lymphocytes (absolute count/ul) showed a significant difference of PP *vs* Control, NP and NN (significance level 0.05) but not *vs* NP; monocytes CD64++CD33++CD45++(absolute/ul) showed a significant difference of PP *vs* Control, NP and NN (significance level 0.05); T CD8+CD57- (cytotoxic CD8+%) showed a significant difference of PP *vs* NP and NN but not *vs* Control (significance level 0.05); CD4+CD45RA (T CD4+ naïve%) and CD8+CD45RA(T CD4+ naïve%) showed a significant difference of PP *vs* Control, NP and NN (significance level 0.05); CD8+CD38+DR+ (T CD8+ activated %) showed a significant difference of PP *vs* Control, NP and NN (significance level 0.05). The CD4+ and CD8+ T cells were negatively correlated with age and inflammatory responses (CRP, ESR, and fibrinogen) in both PP and ICU-PP groups *vs* Np and NN (coefficient rate of 0.70).

COVID-19 patients in ICU

A chest CT-scan was obtained in 20 patients (100%) at the time of ICU admission, and all the images showed bilateral pulmonary ground-glass opacities and pulmonary nodules. Seventeen pleural effusions were seen, BALF and blood microbiological analysis were obtained in 17 patients (96%); the whole 17 samples showed presence of different pathogens as follow, *Klebsiella spp* (n=7), *Candida albicans* (n=5), *Pseudomonas spp* (n=5), *Acinetobacter* (n=1), *Aspergillus* (n=1), *Staphylococcus aureus* (n=1), *Stenotrophomonas* (n=1); some patients (n=10) presented a positive blood culture for *Klebsiella spp* (n=5), *Candida albicans* (n=1), *Pseudomonas spp* (n=1), *Acinetobacter* (n=1), *Proteus spp* (n=1), *Staphylococcus hominis* (n=1), *Staphylococcus epidermis* (n=1).

DISCUSSION

The unpredictability of the SARS-CoV-2 could be related to the complexity of its inner structure, the virus possesses an envelope crucial for its pathogenicity as it also promotes viral assembly; it has four main structural proteins including spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, a nucleocapsid (N) protein, and is also equipped with accessory proteins that inhibit the host's innate immune response (2). The spiked glycoproteins are considered the most efficient weapons, composed of two subunits (S1 and S2), these homotrimers of the S proteins constitute the spears located on viral surface essential to dock on target host cells receptors (5, 6). In SARS-CoV-2, the S2 subunits contain a fusion peptide, a transmembrane domain, and a cytoplasmic domain, all highly conserved and could potentially be a target for antiviral therapy (anti-S2). Other structural elements on which research must necessarily focus are ORF3b, which has no homology with that of other members of the Corona family, and a secreted protein (coded by ORF8), which was also seen structurally different from those members (4-6). The wide presence of ACE2r located along the respiratory epithelium constitutes the main entrance for the Sars-CoV-2 body invasion and wide spectrum infection.

The over-expression of pro-inflammatory interleukins and cytokines led by IL-6 have been related to an uncontrolled neutrophil, T cell, and local polarized M1 macrophage activity in response to resilient virus stimuli. The excessive accumulation

of mucous within lung alveolar interstices, better known as neutrophil extracellular trap (NET), may certainly be considered one of the causes of bronchoalveolar blockage and the typical feature of "Ground glass opacity" seen in CT-scan images of patients affected by COVID-19 disease (Fig. 4 a-d) (21-23).

The arterial blood gas (ABG) analysis has remained a key marker in predicting COVID-19 from the very beginning. In general the majority of hospitalized patients showed acid-base anomalies, characterized by hypoxia (low O_2) and hypocapnia (low CO_2) with compensatory alkalosis (Table III). The age (>50 years), the gender (prevalently males) and the preexisting co-morbidities, such as chronic obstructive pulmonary diseases (COPD), chronic kidney failure, diabetes and cardiovascular diseases, have been proved to ease unfavorable course of COVID-19 (Figs. 1, 2). The low mortality rate (3-5% of the total of affected individuals), the speed, the unpredictability and the sudden aggravation of symptoms have been revealed to be the essential features of the tropism of SARS-CoV2, as patients have been seen to fall into a state of irreversible cardio respiratory arrest almost without warnings (11).

Pathologically, a secondary bacterial/mycotic infection could explain the full aggressiveness and inflating progression seen in many of the COVID-19-affected patients fully inherent to the rapidity of sepsis and multi-organ involvement. The BALF and blood specimen collected from PP-ICU patients seem to confirm the presence of several pathogens such as *Klebsiella spp*, *Candida albicans, Pseudomonas spp, Acinetobacter, Aspergillus, Staphylococcus aureus, Stenotrophomonas, Proteus spp, Staphylococcus hominis* and *Staphylococcus epidermis* (32-40).

The significantly reduced number of T-CD4+, T-CD8+, B cells and T-CD4 naïve with high level of activated T-CD8+ (CD8+CD38+DR+) has been seen a very peculiar immune dysfunction feature of COVID-19 as it was shown in the PP group, which makes this virus completely unique among the entire Corona family members. In addition, these outcomes lead us to hypothesize a certain affinity of sars-cov-2 with both HIV virus and severe influenza A virus (IAV) infection, either immunologically or genetically (41-53) (Table IV).

According to the latest Italian Health System guidelines, COVID-19 can be classified based on nasopharyngeal swab analyzed by RT-PCR as the main basis of disease confirmation, and other auxiliary examinations are used to distinguish the severity. Thoracic CT-scan, ABG and blood tests are easy, fast, and cost-effective. This study suggested that the ABG results and lymphocyte sub-sets can also be used as reliable indicators to classify and predict the moderate, severe, and critical course of the disease, independently of RT-PCR results. It follows that NP patients presenting with a COVID-like disease arrangement discharged as COVID-19 negative, which avoids the whole series of surveillance and controls, may eventually become a potential source of a larger scale infection in the future. To conclude, we are well aware that further studies are needed, mainly related to the scarcity of information regarding COVID-19.

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