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EPIDEMIOLOGICAL, CLINICAL AND ANATOMOPATHOLOGICAL ANALYSIS IN SARS_ COV_2 INFECTION IN ARAD

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Abstract: *Objective.* The year 2020 radically changed the therapeutic approach in patients of Romanian hospitals, and intensive care units (ICUs) were sometimes used over capacity; our goal was to establish the success rate in Covid 19 patients assisted in 2020 in Arad.

Material and methods. Were analyzed 450 Covid 19 cases hospitalized between 28.03-25.05.2020, regarding the demographic characteristics, symptomatology, comorbidities, hospitalisation length, treatment regimens and success rates.

Results. Patients ranged in age from 19 to 99 years, with a mean of 56.75 for women and 56.11 for men, with hospitalization averaging 12 days for women and 17 days for men, who had also a higher risk for severe forms compared to women (RR 1.4483, 95% CI 1.0012-2.095, P = 0.0493); Odds ratio OR in men was increased but without statistical validation (OR 1.5023, 95% CI 10.9175-2.46, P = 0.1057). The death rate was increased in cases with hypertension associated to diabetes (OR 26.6049, P = 0.0353). Isolation of associated bacterial agents was performed in only 5.55% of cases (n = 25) but antibiotic regimens targeted 54.66% (n = 246) of cases. Multidrug resistance of circulating strains in 2020 in this hospital has increased despite the decrease in the number of patients discharged and specimens, compared to 2019.

Conclusions. Antibiotic therapy in Covid 19 is not justified in the absence of a proven secondary bacterial infection, contributing to the increase in the selection of multidrug resistant bacterial strains, which is a fact already ascertained.

Keywords: Covid 19, paraclinical, antibacterial therapy.

INTRODUCTION

Pandemic means the introduction of new antigens in a naive population. Human immunity has two major defensive systems, humoral and cellular [1]. The humoral immune response, triggered by thymo-dependent antigens, involves cellular cooperation between: 1. Cells presenting antigen (CPA-macrophages, dendritic cells, lymphocytes) + 2. Immunoregulatory lymphocytes: helper T lymphocytes that amplify the cascade of reactions of the humoral immune response and suppressor T lymphocytes that diminish the intensity of immune reactions + 3. B lymphocytes, the final effector cells of the humoral immune response, which produce antibodies or immunoglobulins [2].

Cellular immune response, with cells: 1. Cytotoxic T lymphocytes having extracellular cytotoxicity through which they destroy non-self cells, have specific immunological recognition, the population of cytotoxic T lymphocytes being very diverse clonotypically + 2. Natural Killer NK cells, performing a nonspecific immunological recognition,

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being the first line of defense against viral infections (20% of all lymphocytes) + 3. Killer cells represent 5% of all lymphocytes being a subclass of NK cells, with nonspecific immunological recognition [3].

The emergence of SARS-CoV-2 at the end of 2019 was followed by 11 months of relatively stagnant evolution of viral strains and then a new set of mutations of "worrying variants" appeared, modifying transmissibility and antigenicity, possibly in response to changes in immune profile of the human population [4].

In SARS-CoV-2 there are paradoxical situations in which specific IgG and SARS-CoV-2 antigens can coexist for up to 50 days or SARS-CoV-2 antigens without specific IgG can be eliminated after 46 days, which shows that, without antibody-mediated immunity, solely the action of innate immunity it is efficient [5]. In SARS-CoV-2, the dissemination of the Delta variant is associated with escaping from neutralizing antibodies, which target both epitopes of the receptor binding domain (RBD) and those different from those of the receptor binding domain (non-RBD/ receptor- binding domain) on the spike protein [6].

Goal and objectives

The year 2020 radically changed the therapeutic approach in patients of Romanian hospitals, and intensive care units (ICUs) were sometimes used over capacity; Our goal was to establish the success of Covid -19 patients therapy, in 2020 in Arad, from the point of view of empirical antibimicrobial therapy in bacterial community-acquired pneumonia, regimens which are often prescribed to Covid 19 patients, although the incidence of reported bacterian co-infections is low. The objective of this study is to establish the impact of empirical antimicrobial therapy and the duration of this therapy in the COVID-19 patient.

MATERIAL AND METHODS

450 Covid 19 cases hospitalized between 28.03-25.05.2020, were analyzed regarding demographic characteristics, symptoms, comorbidities, length of hospital stay, treatment regimens and success rates. The variables were analyzed with MedCalc® 14, IBM® SPSS® Statistics version 24 and Excel Microsoft 365® as statistical software.

RESULTS

Incidence per 1000 residents of hospitalized cases: maximum incidence: 3.21 in aged 80 and over, minimum incidence: 0.03 in aged under 20. Incidence: over 2 per 1000 in aged over 60. Gender ratio F:M=1. 027 (Table 1). Resident ratio U:R=2,46, more than double in urban areas. Death rate: 17.33% (n=78), equal and slightly higher in rural areas. Length of hospital stay: 12 days.

Table 1. Demographic characteristics of Covid 19 patients

Item	Under 20	20-29	30-39	40-49	50-59	60-69	70-79	80 and over	Total
F	0	13	22	59	35	37	31	31	228
M	1	13	23	44	39	49	43	10	222
Total	1	26	45	103	74	86	74	41	450
Incidence	0.03	0.39	0.59	1.91	1.16	2.01	2.14	3.21	11.43

Table 2. Comorbidities and death rates in Covid 19 patients

Comorbidities	Frequency	Percent	Death cases	% Death
DM/HT/obesity	175	38.89	18	10.2857
CV	88	19.56	38	43.1818
Hemodialyzed	28	6.22	6	21.4286
Neoplasia	19	4.22	9	47.3684
COPD	19	4.22	8	42.1053
Neurodegenerative	18	4	5	27.7778
DM/obesity	17	3.78	1	5.88235
HT	16	3.56	15	93.75
Stroke sequel	16	3.56	4	25
Asthma	12	2.67	2	16.6667
Short and long term liver disease	12	2.67	7	58.3333
DM/HT	8	1.78	8	100
Obesity	7	1.56	5	71.4286
Autoimmune disease	6	1.33	0	0
DM	5	1.11	5	100

Legend: DM=diabetes mellitus,HT= hypertension, CV= cardiovascular disease, COPD= chronic obstructive pulmonary disease.

Table 3. Death Odds ratio for comorbidities *vs*. without comorbidities in Covid 19 patients

Death Odds ratio for comorbidities *vs*. without comorbidities	5.3293
95% CI	2.3935 to 11.8659
z statistic	4.097
Significance level	P < 0.0001

Legend: CI=confidence interval, P=p-value.

Table 4. Symptoms frequency in Covid 19 patients

Symptoms	Frequency	Percent
Fever	212	47.11
Dry cough	175	38.89
Dyspnea	165	36.67
Asthenia	159	35.33
Altered condition	62	13.78
Myalgia	56	12.44
Shiver	39	8.67
Anosmia	33	7.33
Chest pain	25	5.56
Nausea	21	4.67
Productive cough	16	3.56
Diarrhea	14	3.11
Vomitind	13	2.89
Rhinorrhea	6	1.33
Ageusia	4	0.89
Wheezing	3	0.67
Nasal obstruction	2	0.44
Nasal congestion	2	0.44
Arthralgia	1	0.22

Table 6. Biological alterations in moderate-severe Covid 19

Parameter	Alteration %
erythrocyte sedimentation rate (ESR)	85
fibrinogen synthesis rate	92
neutrophylia	78
lymphopenia	84
leukocytosis	78
monocytosis	75
low RBC	81
thrombocytopenia	61
low hemoglobin level	88
low hematocrit level	87
coagulation abnormalities	35
hyperglycemia	86
hepatocytolisis	91
high Blood Urea Nitrogen	95
high creatinine level	98
high amylase level	91
high procalcitonin level	60
increased ferritin concentration	100
increased D-dimer value	98
phospho creatin kinase PCK	60
C-reactive protein (CRP)	100

Comorbidities and death

Of 450 Covid 19 cases admitted between March 28-May 25.2020, 155 were without any comorbidities, death rate being 4.51% (n=7), 295 presented between 1-6 comorbidities, death rate being 24.06 % (n=77). Death

Table 5. Chest imaging in Covid 19 patients

Chest imaging	Survival	Death	Death %
Bilateral/double pneumonia	8	12	60
Right basal pneumonia	18	14	43.75
Left basal pneumonia	7	2	22.22222
Bronchopneumonia	72	17	19.10112
Interstitial pneumonia	192	32	14.28571
Total	297	77	20.58824

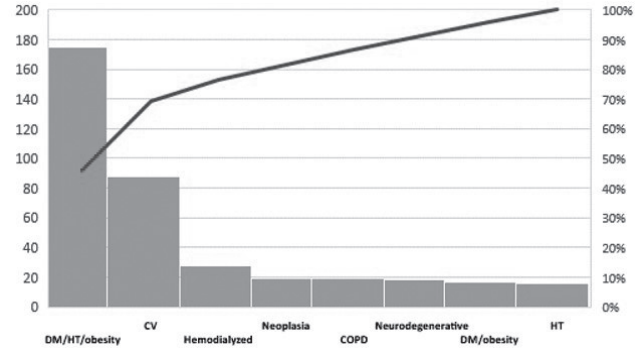

Figure 1. Pareto chart indicates the link between comorbidities and deaths. Legend: DM=diabetes mellitus,HT= hypertension, CV= cardiovascular disease, COPD= chronic obstructive pulmonary disease.

Odds ratio for Covid-19 patients with comorbidities *vs*. no comorbidities is 5.3293 (Tables 2-3, Fig. 1).

DM, HT and obesity prevalence, as a distinct pathology, affects 45% of Covid-19 patients. NB. For general population: HT prevalence in aged over 20 is 31.1%. (38% in Arad), DM prevalence in aged over 20 is 8.5% (9% in Arad), obesity prevalence in aged over 20 is 15.9% (11% in Arad).

Clinical severity and outcomes

Half of disease cases were mild, with no mortality. A third of disease cases were moderate, death rate being 0.76 (n=1). Under a quarter of disease cases were severe, death rate being 81%. (n=77). Pareto chart indicates the link between comorbidities and deaths.

Signs, symptoms and chest imaging

No signs and symptoms in 53 patients (11.77%), Symptoms were dominated by fever, dry cough, dyspnea, asthenia, altered condition (Table 4).

Chest imaging of pulmonary Covid 19 infection was performed in 374 cases (83%) (Table 5). Odds ratio for deaths in case of double pneumonia was 6.6692 (95% CI 2.6203 to 16.9744, z statistic 3.981, significance level P = 0.0001) compared to other pulmonary lesions.

Table 7. Microbial isolates in Covid 19 with 11 exitus cases

Number/deaths 11	Intubated	Tracheobronchial secretions	Death
1	1	*Klebsiella pneumoniae*	1
2	1	*Staphylococcus aureus*	1
3	0	*Staphylococcus aureus*	1
4	1	*Staphylococcus hominis+ Candida spp*	1
5	1	*Streptococcus viridans*	1
6	1	*Candida albicans*	1
7	1	*Candida albicans*	1
8	0	*Acinetobacter calcoaceticus*	0
9	0	*Staphylococcus hominis+Klebsiella pneumoniae*	1
10	0	*Enterococcus faecalis*	1
11	0	*Klebsiella pneumoniae*	0
12	1	*Staphylococcus capitis+Acinetobacter calcoaceticus*	1
13	1	*Klebsiella pneumoniae*	1

Table 8. Microbial isolates in Covid 19 with 11 exitus cases

Number/death 4	Death	Intubated	Tracheobronchial secretions	Uroculture
1	0	0	*0*	*Klebsiella pneumoniae*
2	0	0	*0*	*Enterococcus faecalis*
3	1	1	*Staphylococcus aureus*	*E coli*
4	1	1	*0*	*E coli*
5	0	0	*0*	*E coli*
6	0	0	*0*	*E coli*
7	0	0	*0*	*Enterococcus faecalis*
8	0	0	*Acinetobacter calcoaceticus*	*Pseudomonas aeruginosa*
9	0	0	*0*	*Enterococcus faecalis*
10	1	1	*0*	*Enterococcus faecium*
11	1	1	*0*	*Candida krusei*

Table 9. Microbial isolates in a survival Covid 19 patient, not intubated

Number	1
Death	0
Intubated	0
Tracheobronchial secretions	*Acinetobacter calcoaceticus*
Uroculture	*Pseudomonas aeruginosa*
Hemoculture	*Staphylococcus epidermidis*
Coproculture	*Candida albicans*

Biological alterations in moderate-severe clinical status

Biological alteration rate ranged between 35 - 100%. Patients displayed polymorphous images of biological alteration, most frequent being increased ferritin concentration, positive C-reactive protein (CRP), increased D-dimer value, high creatinine level, high fibrinogen synthesis rate, high Blood Urea Nitrogen, high amylase level; 35% of them presented also coagulation abnormalities (Table 6).

Survival in orothracheal intubation

Intubation and mechanical ventilation rate in literature: 5-88% [7]. Intubation and mechanical ventilation rate in our study: 12.44%. Study in literature stated that of the patients who died only ~ 25% received invasive mechanical ventilation (intubated) or ECMO. Our study showed that of the patients who died 72% were intubated.

Microbial isolates as co–infection

There were isolated and identificated only 29 strains in 25 patients, Gram negative 12, Gram positive 12 and 5 strains of Candida spp. (in only 5.55% of patients) (Tables 7-9).

Antimicrobial therapy

Of these 450 Covid-19 patients, 55% (n= 247) had antimicrobial regimens, although there were only 25 patients positive for microbial co-infections, (10.12% out of those with antimicrobial therapy) (Table 10).

There is no statistically significant correlation between antimicrobial regimens and survival in these patients. NB. Third-generation cephalosporins and quinolones regimens are increasing to an alarming extent, regarding Clostridium difficile infection.

Other therapies, without statistical significance in relation to favourable outcome, were antivirals designed for flu or HIV, corticoids, hydroxychloroquine and so on (Table 11).

Table 10. Antimicrobial and monoclonal antibody therapy in Covid 19 patients

Antimicrobial therapy	Survival	Fatality
IL-6 Monoclonal Antibody	5	0
Glycopeptides	3	3
Azoles	1	0
Carbapenems	8	6
Oxazolidinones	5	5
Antifungals	2	1
Nitrofurans	13	0
Quinolones	16	22
Polypeptides	2	2
Third-generation cephalosporins	24	23
Macrolides	154	38
Amynoglicosides	2	2
Beta lactamase inhibitor combinations	21	7
Total	256	109

Table 11. Therapies regimens without statistical significance in relation to favourable outcome

Therapy	**Survival**	**Death**
Antivirals	9	37
ARV	274	50
Glucocorticoids	43	32
Anticoagulants	104	47
Oxygen therapy	118	72
Hydroxychloroquine	201	43

Legend: ARV= antiretroviral therapy.

Pathology of lung lesions

There were not only morphological aspects of ARDS (acute respiratory distress syndrome), but particularly microvascular injury in small vessel with features of acute capillaritis in association with neutrophilic infiltration into the alveolar space and tracheal mucosa, results recorded by other authors as well [8].

Autopsy aspects in SARS-CoV-2 infections

Within the County Service of Forensic Medicine in Arad during the 2020-2021 pandemic period, there were a number of 18 medico-legal cases which were registered as been ordered by the investigation bodies and prosecutors with ordinance, in which we proceeded the medico-legal autopsy. These cases represented deaths associated with SARS-CoV-2 infection. Patients age ranged from 18 to 80, with mean age of 53.2 for men and 65.25 for women. The deaths occurred at home or in the Arad County Emergency Clinical Hospital, representing 7 cases (38.9%) and 11 cases (61.1%) respectively, of the total autopsies performed in cases of death associated with SARS-CoV-2 infection.

The causes of death were mostly represented by the initial morbid condition, namely Pneumonia associated with the SARS-Cov-2 virus infection and Bronchopneumonia, associated with SARS-Cov-2 virus infection which led to complication, the direct causes of death being considered Multiple Organ System Failure or Toxic Shock Syndrome. Out of the total number. Two of the cases (11.1%) were concluded as been violent deaths, in which a traumatic factor intervened before the pathological factor of SARS-CoV-2 virus infection, and 4 cases (22.2%) where the patients were autopsied because there were foreign citizens, necropsy being mandatory in these cases according to Order No. 1134/C-255 of May 25, 2000.

From a microscopic point of view at histopathological examination, in the investigated cases, fragments of organs were sampled and we found a series of changes especially in the pulmonary parenchyma as: Acute fibrinous pleurisy. Pneumonia with interstitial and alveolar component. "Hyaline membranes" in the lumen of terminal and respiratory bronchioles accompanied by atelectasis.

Visceral pleura – thickened, with the disappearance of mesothelial epithelial cells; amorphous eosinophilic material, extravasated red blood cells, infiltrated with lymphocytes, macrophages and granulocytes at the level of submesothelial connective tissue;

Bronchioles with areas of coagulation necrosis at the level of the epithelium and polymorphous leukocyte infiltrate at the level of the walls;

In the lumen of the terminal and respiratory bronchioles - homogeneous material densely eosinophilic arranged in the form of "strips", with an arched appearance in the mass of which a few red blood cells and leukocytes can be observed; the alveolar spaces are reduced in size, with a "slit" appearance.

In the case control study published by Bloom *et al.*, in 5 cases of post mortem examinations of COVID 19 infection the microscopic findings on the respirator system reveled multifocal to diffuse alveolar necrosis, bronchiolar respiratory epithelial necrosis and perivascular and peribronchiolar lymphoid infiltrates along with marked congestion. And in the severe cases of COVID 19 scattered fibroplasia were observed, with extension into alveolar spaces and thickening the alveolar septum. [9]. In Germany, in Hamburg, Carolin Edler *et al.*, analyzed the first 80 consecutive deaths at patients infected with SARS CoV2 infection. From the total cases, 57 (71%) had pneumonia. Out of ten cases (25%) of patients with pneumonia, seven cases presented with fatal fulminant pulmonary artery embolism while one case with aortic valve endocarditis. Seven cases of

total (10%) were considered with a competing cause of death in addition to COVID 19: aspiration pneumonia, pronounced emphysema without evidence of pneumonia or acute bronchitis. [10].

In conclusion, patients' age ranged from 19 to 99, with mean age of 56.75 for women and 56.11 for men, with average length of hospital stay 12 days for women and 17 days for men, men having also a higher RR relative risk for clinical severe forms compared to women (RR 1.4483, 95% CI 1.0012-2.095, P = 0.0493), Death Odds ratio OR in men was increased, without statistical validation (OR 1.5023, 95% CI 10.9175-2.46, P = 0.1057). The death rate was increased in the association of HT and DM (OR 26.6049, P = 0.0353). Comorbidities due to HT, DM, obesity, CV diseases, renal failure requiring dialysis, COPD and neoplasms have complicated the evolution of SARS_CoV_2 infection.

Isolation and identification of bacterial co-infection was performed in only 5.55% of cases (n = 25) but antimicrobial regimens targeted 54.66% (n = 247) of cases. Antibiotic therapy in Covid 19 is not justified in the absence of a proven secondary microbial infection, contributing to the increase in the selection of multidrug-resistant bacterial strains. The complexity of biological alterations induced by SARS_CoV_2 infection have expanded the therapeutic arsenal of these cases, by using glucocorticoids, antimalarials (hydroxychloroquine), antivirals, antiretrovirals, anticoagulants, symptomatic therapy, in addition to the medication of existent comorbidity. The small number of cases did not allow us to find a statistically significant causal link between the applied therapy and the outcome.

During the pandemic, anatomopathological autopsies have been limited in order to prevent the spread of COVID and to minimize the risk of exposing the medical personnel, due to the lack of level 4 protection in the pathological anatomy laboratory, as well as the uncertainties in the contagiousness and the ways of transmission of the virus. At the same time, an insufficiency was recorded regarding the supply of personnel protection materials in relation to the growing number of new cases. In conclusion, the few autopsies and anatomopathological aspects presented did not bring suggestive elements that differ from the rest of the medical literature.

Conflict of interest

The authors declare that they have no conflict of interest.

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