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Clinical Studies

Coinfections among hospitalized patients with covid-19 in the first pandemic wave

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ARTICLE INFO

Article history:
Received 29 December 2020
Revised in revised form 19 April 2021
Accepted 24 April 2021
Available online 7 May 2021

Keywords:
microorganisms
coinfection
hospitalized patients
COVID-19

ABSTRACT

Background: COVID19 is the novel respiratory illness caused by SARS-CoV-2. The presence of other potentially pathogenic microorganisms could worsen the prognosis of these patients. Aim: The study aims to describe coinfections in COVID-19 patients and contrast it between standard ward and critical care patients at Hospital Central de la Defensa Gómez Ulla (HCDGU).

Methods: A retrospective study was carried out of patients with COVID-19 confirmed with RTPCR admitted to the HCDGU from March 5, 2020 to May 7 of 2020.

Findings: Of a total of 703 patients with COVID-19, 75 (10.7%) had other microbiologically confirmed infections: 9% (58/648) in standard ward patients and 31.5% (17/54) in critical care patients. In total 86 samples of the 75 patients presented some microorganism; clinically relevant bacteraemias, 50%, respiratory cultures, 32.6% and pneumococcal positive antigens, 17.4%.

Conclusions: We found a low frequency of microorganism coinfection in COVID-19 patients, however in critical care these coinfections increased considerably.

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1. Introduction

On December 2019, a novel coronavirus was discovered in the city of Wuhan, Hubei Province, China (Guan et al., 2020). It soon spread to other cities and countries and on 11 March 2020 was declared a pandemic by the World Health Organization. This novel virus, Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) caused a novel illness called coronavirus disease 2019 (COVID-19). Spain, with 3,387,022 cases and 76,756 deaths by 14 April 2021 (<Actualizacion_353_COVID-19.pdf> 2000), is considered one of the most affected countries in the European Union.

The clinical symptoms of most patients are a fever, sore throat, dry cough and shortness of breath (Jin et al., 2020). The principal microbiology diagnosis tends to be RT-PCR (Reverse Transcription Polymerase Chain Reaction), a molecular technique which detects the RNA of the virus (Jin et al., 2020, Liu et al., 2020).

Bacterial, viral, or fungal coinfections are common complications in patients with different types of pneumonia, especially in the most critical patients (Cawcutt & Kalil, 2017, Zhou et al., 2020). Therefore, the presence of other potentially pathogenic microorganisms could induce a more severe inflammatory and may worsen (Ruuskanen et al., 2011) the prognosis of patients infected by SARS-CoV-2, for this reason most COVID-19 patients were empirically treated with antibiotics.

Previous studies have shown that patients with COVID-19 can be coinfected with other microorganisms (Hazra et al., 2020, Hughes et al., 2020, Antinori et al., 2020, Lansbury et al., 2020, Zhang et al., 2020). The types of pathogen coinfections and the proportion of coinfection in SARS-CoV-2 positive hospitalized patients remain unclear. In this study we compare and describe coinfections in COVID-19 patients admitted to the standard ward and intensive care units (ICU).

2. Materials & methods

2.1. Study design

This study was carried out at HCDGU, a Spanish military tertiary hospital located in Madrid that is fully integrated in the Public Health System.

A retrospective observational study was carried out in all patients diagnosed with COVID-19 from 5 March 2020 to 7 May 2020 at the HCDGU. COVID-19 infection was confirmed in all patients by real-time reverse transcription RT-PCR (Allplex 2019-nCoV assay, Seegene, Seoul, South Korea) testing performed on nasopharyngeal swabs and
fulfilling with clinical diagnostic criteria provided during the pandemic peak of SARS-CoV-2. These clinical criteria comprised the presence of some of the following respiratory symptoms: sore throat, congestion, cough, dyspnoea, decrease or lost of taste and/or smell as well as uni/bilateral interstitial infiltrates on chest X-ray. (Jin et al., 2020)

COVID-19 patients were classified into two groups based on hospital admissions (patients admitted to the standard ward or patients admitted at ICU).

2.2. Data collection and outcomes

Patient demographics; gender, age, level of care (critical care or standard ward) and microbiology data. These data were collected from the clinical history of each patient.

2.3. Laboratory procedures

Investigation of common bacterial, Influenza virus A/B, Respiratory Syncytial Virus (RSV) and fungal pathogens were performed.

Respiratory, urinary and blood culture samples were obtained from COVID-19 patients and processed at the Microbiology Laboratory for routine diagnostic purposes.

The samples were processed in the usual way in the laboratory following the procedures of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC). Respiratory and/or blood samples were obtained for the identification of possible causative bacteria or fungi. Routine bacterial and fungal cultures were performed in accordance with the laboratory protocol (Lansbury et al., 2020, Zhang et al., 2020) following respiratory pathogenic microorganisms operating standards: the samples were seeded on bacteriologic media such as blood agar plate, chocolate agar plates and MacConkey agar plates using sterile wire loops and were incubated incubates at 30 °C the filamentous fungi and 37 °C the yeasts for 48 hours in a thermostatic incubator. Routine fungus cultures were inoculated on Sabouraud/glucose (4 %). The plates were incubated at 37 °C. Subsequently the dominant and potentially pathogenic colonies were picked for bacterial and fungus detection using the VITEK MS system. The percentage of patients with coinfection was determined using the chi-square test or Fisher’s exact test for contingency tables. In case of non-compliance with the assumption of normality, the non-parametric Wilcoxon-Mann-Whitney tests were used. The statistical software package STATA / IC version 13.1 (StataCorp, Texas, USA) was used.

2.7. Statistical analyses

The statistical significance of the comparison of proportions was determined using the chi-square test or Fisher’s exact test for contingency tables. In case of non-compliance with the assumption of normality, the non-parametric Wilcoxon-Mann-Whitney tests were used. The statistical software package STATA / IC version 13.1 (StataCorp, Texas, USA) was used.

3. Results

A total of 703 patients with confirmed SARS-CoV-2 were identified from 5 March 2020 to 7 May 2020. The media age of patients was 67.9 (IC95 % 66.6-69.2) and 61.2 % were male (95 % CI 57.5-64.8 %). Overall, 75 of 703 patients (10.7 %, 95 % CI 8.6 -13.2 %) had another potentially pathogenic microorganism.

A total of 648/703 (92.3 % IC 90.1-94.1 %) patients were admitted to the standard ward while 54/703 (7.7 % IC 5.9 - 9.9 %) were admitted to the critical care.

3.1. Coinfected patients

The median age of the coinfected patients was 70.9 years (95 % CI: 67.4-74.4). Of all the infected patients, 53.3 % (95 % CI: 41.8 - 64.5 %) were men.

In total there were 86 samples with 106 pathogenic microbiological isolate in 75 coinfected patients COVID-19. These samples were 32.6 % respiratory samples, 50 % blood cultures and 17.4 % pneumococcal urinary antigen positive. (Table 1)

3.2. Standard ward patients

Overall, 58 of 648 patients (9.0 %, 95 % CI 7.0 - 11.4 %) which were admitted to the standard ward had some other pathogenic isolates. These patients had more positive blood cultures with a significant microorganism than ICU patients (54.7 % vs 47 %) but there was no significant difference ($P = 0.4055$). Pneumococcal urinary antigen was higher in these patients (23.4 % while in ICU patients none of them presented a urinary antigen positive).

3.3. Intensive care units patients

A total of 17 of 54 patients (31.5 %, 95 % CI 20.2-45.4 %) which were admitted in critical care had presented another concomitant microorganism. The percentage of patients with coinfection was higher than standard ward patients (31.5 % vs 9.0 %) with significant

or anaerobes among others, the presence of growth in two sets of blood culture was considered a significant isolation (<seimc-procedimientomicrobiologia HEMOCULTIVOS.pdf> n.d., <seimc-procedimientomicrobiologia.pdf> n.d., Collazos-Blanco et al., 2019)

2.6. Urinary samples

Pneumococcal and Legionella pneumophila urinary antigen detection was carried out in all of our patients. Test used were The BinaxNOW™ Streptococcus pneumoniae/legionella Urinary Antigen Card. In this period, a protocol was established in the hospital in which it indicated to request urinary antigen from all COVID-19 patients.

2.8. Ethical approval

The study was conducted according to the ethical requirements established by the Declaration of Helsinki. The Ethics Committee of Hospital Central de la Defensa Gómez Ulla (Madrid) approved the study.
3.5. Blood culture samples outcomes

There are samples with more than one microorganism. Of the 22 samples obtained, 14 were from ICU patients. These isolates are likely to represent oropharyngeal thrust or normal flora rather than pulmonary candidiasis. Despite the fact that this microorganism was mainly only colonizing the respiratory tract, in our study he microorganism was mainly isolated in respiratory samples. *Pseudomonas aeruginosa* was more frequently isolated in standard ward patients than in standard ward patients (5/14 vs 3/14); while *Staphylococcus aureus* was more frequently isolated in standard ward patients than in critical care patients (3/14 vs 2/14). *Influenza* virus was only isolated in standard ward patients (4/14).

3.6. Blood culture samples outcomes

In blood cultures samples, CoNS (*Staphylococcus epidermidis* mainly), *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans* were the main microorganism isolates (*Table 2*).

*S. epidermidis* was more frequently isolated in standard ward patients than in critical care patients (14/35 vs 2/8 of blood cultures). Other CoNS were isolated in 13/35 of blood culture in standard ward patients but neither isolates in critical care patients. *S. aureus* were more frequently isolated in ICU patients than in standard wards patients (1/8 vs 4/35 of blood culture samples). Finally *E. faecalis* and *C. albicans* were more frequently isolates in critical care patients (3/8 vs 1/35 of blood culture) in both cases.

Frequently colonizing microorganisms, mainly CoNS found in a single blood draw for the study of possible sepsis were 19 of 703 patients. All these contaminations were found in the hospitalized group except in a single case that was found in an intensive care unit. (*Table 3*)

3.6. Urinary samples

Pneumococcal was detected in 15/15 positive urinary antigen. All of them in standard ward patients. No *Legionella* urine antigen was detected.

difference (*P < 0.0000*). Different patients presented several coinfactions simultaneously; therefore in total the number of microbiological samples was greater than the number of coinfected patients, in total 22 samples in 17 patients. The media age of patients was lower in critical care than standard ward patients (61.4 vs 73.7 years) with significant difference (*P < 0.0000*). The rates of positive respiratory samples (14/17, 82.4%, of which it was 7 bronchial aspirates and 7 endotracheal aspirates) was higher in patients from critical care admission than hospitalized (14/58, 21.9%, of which it was 3 nasopharyngeal exudates, 4 bronchial aspirates, 1 bronchoalveolar and 6 endotracheal aspirates) (*P < 0.0000*).

3.4. Respiratory samples outcomes

In respiratory samples, *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the main microorganism isolates.

In respiratory samples the main microorganism isolates were *Candida albicans* (6/14 respiratory samples standard ward patients and 6/14 respiratory samples critical care patients). These isolates are likely to represent oropharyngeal thrust or normal flora rather than pulmonary candidiasis. Despite the fact that this microorganism was mainly only colonizing the respiratory tract, in our study he microorganism was mainly isolated in respiratory samples. *Pseudomonas aeruginosa* was more frequently isolated in critical care patients than in standard ward patients (5/14 vs 3/14); while *Staphylococcus aureus* was more frequently isolated in standard ward patients than in critical care patients (3/14 vs 2/14). *Influenza* virus was only isolated in standard ward patients (4/14).

### Table 1

Characteristics and microbiologic isolates in COVID-19 hospitalized coinfected patients

| Characteristic | Total | Standard ward (n = 58) | Critical care patients (n = 17) | *P* |
|---------------|-------|-----------------------|-------------------------------|-----|
| Median Age    | 73.7  | 61.4                   | 0.0000                        |     |
| Gender        |       |                       |                               |     |
| Male          | 58 (9.0%) | 17 (31.5%)           | 0.0000                        |     |
| Female        | 28 (48.3%) | 12 (70.6%)          | 0.1049                        |     |
| Isolates      | 86    | 5 (29.4%)             | 0.1049                        |     |
| Blood cultures| 43 (50%)  | 8 (47%)              | 0.0000                        |     |
| Respiratory samples | 28 (32.6%)  | 14 (21.9%)         | 0.0000                        |     |
| Pneumococcal urine | 15 (17.4%)  | 15 (23.4%)         | 0.0190                        |     |

### Table 2

Microorganisms most frequently isolated in the different samples.

| Isolates                  | STANDARD WARD (65 samples in 58 patients) | Critical care patients (22 samples in 17 patients) |
|---------------------------|------------------------------------------|-----------------------------------------------|
| Blood cultures            | 35                                       | 8                                            |
|  *Staphylococcus aureus*  | 14 (40%)                                 | 2 (14.3%)                                    |
|  *Others CoNS*            | 13 (37.1%)                               | 0 (0%)                                       |
|  *Streptococcus pneumonia*| 4 (11.4%)                                | 1 (12.5%)                                    |
|  *Enterococcus faecalis*  | 1 (2.9%)                                 | 3 (37.5%)                                    |
|  *Candida albicans*       | 1 (2.9%)                                 | 3 (37.5%)                                    |
| Respiratory samples       | 14                                       | 14                                           |
|  *Candida albicans*       | 6 (42.9%)                                | 6 (42.9%)                                    |
|  *Pseudomonas aeruginosa* | 3 (21.4%)                                | 5 (35.7%)                                    |
|  *Streptococcus pneumonia*| 3 (21.4%)                                | 2 (14.3%)                                    |
|  *Influenza A/B*          | 4 (28.6%)                                | 0 (0%)                                       |
| Urinary antigens          | 15                                       | 0                                            |
|  *Pneumococcal*           | 15 (100%)                                | 0                                            |
|  *Legionella*             | 0                                        | 0                                            |

### Table 3

Frequency of microorganisms by type of samples in COVID-19 standard ward and critical care patients.

| Respiratory samples          | N = 28 (32.6%) |
|-----------------------------|---------------|
| *Candida albicans*          | 12 (37.5%)    |
| *Pseudomonas aeruginosa*    | 8 (28.6%)     |
| *Staphylococcus aureus*     | 5 (15.6%)     |
| *Klebsiella pneumoniae*     | 2 (6.3%)      |
| *Influenza A/B*             | 4 (12.5%)     |
| *Aspergillus fumigatus*     | 2 (6.3%)      |
| *Hafnia alvi*               | 2 (6.3%)      |
| *Stenotrophomonas maltophilia* | 1 (3.1%) |
| *Candida tropicallis*       | 1 (3.1%)      |
| *Candida norvegensis*       | 1 (3.1%)      |
| Blood cultures              | N = 43 (50%)  |
| *Staphylococcus epidermidis*| 16 (35.8%)    |
| *Others coagulase negative Staphylococci* | 13 (22.6%) |
| *Streptococcus pneumoniae*  | 5 (9.4%)      |
| *Enterococcus faecalis*     | 4 (9.4%)      |
| *Candida albicans*          | 4 (9.4%)      |
| *Enterococcus faecium*      | 3 (5.7%)      |
| *Hafnia alvi*               | 2 (6.3%)      |
| *Streptococcus pneumoniae*  | 2 (3.8%)      |
| *Pseudomonas aeruginosa*    | 1 (1.9%)      |
| *Morganella morganii*       | 1 (1.9%)      |
| *Klebsiella pneumonia*      | 1 (1.9%)      |
| *Serratia marcescens*       | 1 (1.9%)      |

There are samples with more than one microorganism.

4. Discussion

In our retrospective study, we studied coinfection in patients with confirmed SARS-CoV-2. We observed that overall 10.7% of
hospitalized COVID-19 patients had a coinfection, increasing to 31.5% in COVID-19 patients when they were admitted in ICU. The proportions of the coinfection had a significant statistical difference between ICU and standard ward patients.

COVID-19 coinfection was more likely to affect men that women (53.3% vs 46.7%), but in critical care patients this difference was higher (70.6% vs 29.4%). This coincides with the data already published by other studies (Chen et al., 2020). Patients with coinfections did not differ significantly in age from those infected with SARS-CoV-2 only as it has been proven in other studies (Kim et al., 2020). In this study, we also found no differences in ages among these two clinical classification groups.

Despite Candidas spp was mainly only colonizing the respiratory tract, in our study the microorganism was mainly isolated in respiratory samples. In other study, the microorganism has already been shown to be isolated quite frequently in respiratory samples from COVID patients (Chen et al., 2020). Coagulase-negative staphylococci was the main microorganism isolate in blood samples like other studies have previously published (Hughes et al., 2020). Even though previous studies suggested that patients with COVID-19 can also coinfect with high isolates of influenza A/B virus and respiratory syncytial virus, our results indicated that coinfections with Influenza A or Influenza B were not common in COVID-19 patients, since we only found four isolates of influenza virus in nasopharyngeal exudates (Zhu et al., 2020).

We only report two isolates of Aspergillus fumigatus in respiratory samples in patients without radiographic findings and we didn’t have other study mycological such as galactomannan or β-D-glucan to demonstrate a IPA (Chen et al., 2020). Some investigations suggest the possible occurrence of invasive pulmonary aspergillosis (IPA) in critical COVID-19 patients (Lai & Yu, 2021).

When we observe our series of patients studied, blood samples showed a higher number of isolates than respiratory samples. Previous studies have also observed the scheme (Hughes et al., 2020).

We know this study has some limitations. Firstly, the overestimation of coinfections due to the lack of established clinical criteria to be considered true infections, this would explain the higher rate of Candida albicans in respiratory samples, this was equal in both groups for respiratory samples (42.9%) if we eliminated the cases in which this microorganism was acting as isolated colonizers, the final result of coinfections would be lower. Secondly, the sample size, the study is being carried out during a certain period allowing a specific number of patients. A larger number of study populations would provide us with greater precision of the estimates. Thirdly, the absence of a search for respiratory viruses beyond influenza A and B viruses or RSV, such as Parainfluenza, Human Metapneumovirus, Adenovirus, Rho-inovirus and Bocavirus (Ruuskanen et al., 2011, Zhu et al., 2020). During the pandemic period, it was necessary to make the reagents available and to prioritize the performance of the SARS-CoV-2 RT-PCR outcomes, which prevented us from making some of our determinations. Others non-culturables bacterial such as Mycoplasma pneumoniae or Chlamydia pneumoniae neither were detected in our study.

More multicenter studies with a large number of patients would be necessary to know more about coinfected COVID-19 patients.

In conclusion, we find a low frequency of coinfections present in confirmed SARS-CoV-2 patients, which has increased in ICU patients. Blood cultures followed by respiratory samples were the main samples taken from COVID-19 patients suspected of having some other coinfection. Finally Streptococcus pneumoniae was the most common pathogens isolates respiratory infections and Staphylococcus epidermidis in bacteremia.

5. Ethical approval

The study was conducted according to the ethical requirements established by the Declaration of Helsinki. The Ethics Committee of Hospital Central de la Defensa Gómez Ulla (Madrid) approved the study.

Author contributions

Study concept and design: MZC, MSS and ACB. Clinical and microbiological data acquisition: MZC, MSS, DJL, JMN, TMR and JMS. Sample processing: MZC, MSS, DJL and MFRF. Statistical analysis and interpretation of data: MZC. Writing of the manuscript: MZC, MSS and ACB. Critical revision of the manuscript: MMM and MSS. All authors read and approved the final manuscript.

Acknowledgments

We thank all clinicians at the front line of COVID-19 epidemic.

Financial support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare no competing interest.

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