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Risk Factors of Fungal Growth in Sputum Culture from Severe and Critical COVID-19 Patients

Theresia Novi[1], B. Rina Aninda Sidharta[2], Heru Wijono[3]

[1] Laboratory Husada Utama Hospital, Surabaya, Indonesia. E-mail: thenovi.dr@gmail.com ORCID 0000-0002-5912-0205
[2] Department of Clinical Pathology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Indonesia
[3] Faculty of Medicine, University of Surabaya, Surabaya, Indonesia ORCID 0000-001-7406-3694

ABSTRACT

Patients infected with Coronavirus Disease (COVID)-19, especially those with comorbidities, immunosuppressive treatment, intubated, and on ventilators, are more likely to contract fungal infection.This study aimed to describe the profile of fungal growth in sputum culture from severe and critical COVID-19 patients, and to determine the association between the fungal growth with the patient's outcome and the association between several risk factors with the fungal growth. A retrospective case-control study was carried out at Husada Utama Hospital, Surabaya. This study samples were 119 patients; fungal growth on sputum culture was seen in 64 (53.8%) patients; the three types of fungi with the highest frequency were *Cryptococcus neoformans* found in 23 (19.3%) patients, *Candida tropicalis* found in 11 (9.2%) patients and *Candida dubliniensis* found in 10 (8.4%) patients. There was no significant association between fungal growth and patient mortality with a p-value of 0.940 ($p>0.05$), also between the patient's history of diabetes mellitus and fungal growth ($p=0.496$) and between corticosteroid treatment and fungal growth ($p=0.168$). Still, there was a significant association between the use of ventilators in patients and fungal growth with a p-value of 0.001($p<0.05$). A significant association was also found between IL-6 inhibitor (Tocilizumab) treatment and fungal growth with a p-value of 0.003 ($p<0.05$). The most common fungi found in this study with the highest frequency was *Cryptococcus neoformans*. There was an association between two risk factors (the use of a ventilator and tocilizumab treatment) and fungal growth.

Keywords: Fungi, COVID-19, sputum culture, ventilator, Tocilizumab

INTRODUCTION

Coronavirus Disease 19 (COVID-19) is an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus, resulting in mild to severe respiratory illness. Older people and those with underlying diseases like diabetes, cardiovascular disease, or cancer are more likely to develop severe illness. The virus is spreading from the mouth or nose of an infected person as small liquid particles, with sizes ranging from larger respiratory droplets to smaller aerosols.[1]

Patients infected with COVID-19 are prone to bacterial and fungal coinfection. Patients with comorbidities, immunosuppressive states, current intubation, and mechanical ventilation are more likely to contract fungal infections.[2]

Severe and critical COVID-19 treatment, such as mechanical ventilation, corticosteroid, and Tocilizumab treatment, can increase the risk of bacterial and fungal infection. Severe COVID-19 disease is associated with increased proinflammatory cytokines, interleukins (IL-1, IL-6), Tumor Necrosis Factor-alpha (TNF-alpha), decreased number of Cluster of Differentiation 4 (CD4) and CD8 T cells, which eventually lead to the increased susceptibility to fungal infections.[3-5]

This study aimed to describe the profile of fungal growth in sputum culture from severe and critical COVID-19 patients and to determine the association between the fungal growth and the patient's outcome and the association between several risk factors and fungal growth.

METHODS

A retrospective case-control study was carried out at Husada Utama Hospital, a private COVID-19 referral hospital in Surabaya. Data of patient characteristics (gender, age), comorbidities illness (diabetes, and other comorbidities), category of COVID-19 clinical disease, the use of mechanical ventilation, immunosuppressive treatment (corticosteroid), IL-6 inhibitor treatment (Tocilizumab), microbiological data from sputum culture, and the outcomes after hospitalization (mortality) were collected from medical records between January 2020 to December 2021.

All COVID-19 patients who underwent sputum cultures and were categorized as severely and critically ill were included. A Chi-Square test was used to measure the significance association between two categorical data groups at the significance level of 0.05. The odd ratio was also used to determine the probability of fungal growth in several risk factors (diabetes mellitus comorbidity, ventilator use, corticosteroid treatment, and Tocilizumab treatment).

The microbiological samples were sent and cultured in a Granostic Diagnostic Center, a referral laboratory in Surabaya. The sputum samples were collected by endotracheal aspiration in intubated patients and by oropharyngeal aspiration for non-intubated patients. Fungal identification and antifungal sensitivity tests were carried out on the VITEK2 Compact device.

The severity of COVID-19 was categorized based on The Guidelines for the Management of COVID-19 (Pedoman Tatalaksana COVID-19) published by Perhimpunan Dokter Paru Indonesia (PDPI), Perhimpunan Dokter Spesialis Kardiovaskular Indonesia (PERKI), Perhimpunan Dokter Spesialis Penyakit Dalam Indonesia (PAPDI), Perhimpunan Dokter Anestesiologi and Terapi Intensif Indonesia (PERDATIN), and Ikatan Dokter Anak Indonesia (IDAI), first, second and third edition.[6-8]

Ethical eligibility approval was obtained from the Health Research Ethics Commission, Faculty of Public Health Universitas Airlangga, No. 60/EA/KEPK/2022.

RESULTS AND DISCUSSIONS

This study collected data on 3513 COVID-19 patients from January 2020 to December 2021, consisting of 124 patients who underwent sputum cultures and 119 of the 124 patients classified as severe and critical COVID-19.These research subjects were 119 patients who underwent sputum cultures and were classified as severe and critical COVID-19.

Table 1 shows that from 119 patients who underwent sputum culture, 72 (60.5%) had comorbidities, and 71 (59.7%) weren't diabetics. One hundred two (85.7%) out of 119 patients in this study died, and only 17 (14.3%) patients recovered; 110 (92.4%) patients were on ventilators, and only 9 (7.6%) patients did not use ventilators. Of the patients in this study, 112 patients (94.1%) received corticosteroid treatment, 65 (54.6%) patients received Tocilizumab treatment, and 54 (45.4%) patients didn't receive Tocilizumab.

An overview of sputum culture results from 119 patients with severe and critical COVID-19 are listed in Table 2, showing that most patients had fungal growth. Fungal growth on sputum culture was observed in 64 (53.8%) patients; the most common three types of fungi with the highest frequency were *Cryptococcus neoformans* found in 23 (19.3%) patients, *Candida tropicalis* found in 11 (9.2%) patients and *Candida dubliniensis* found in 10 (8.4%) patients. The finding of the high frequency of *Cryptococcus neoformans* growth in the sputum culture of COVID-19 patients is fascinating; whether this *Cryptococcus neoformans* has caused pulmonary cryptococcosis, which can cause respiratory failure, as reported by Sharma *et al.* about a rare case of COVID-19-associated pulmonary cryptococcosis in January 2022.[9] About 50% of cases are asymptomatic in immunocompetent patients, but rarely asymptomatic in immunocompromised patients.

Table 1. Clinical characteristics of research subjects

Profile	Frequency	(%)
Comorbidities		
No	47	39.5
Yes	72	60.5
Diabetes Mellitus (DM)		
Non-DM	71	59.7
DM	48	40.3
Outcome		
Recovery	17	14.3
Death	102	85.7
The use of the ventilator		
No	9	7.6
Yes	110	92.4
Corticosteroid treatment		
No	7	5.9
Yes	112	94.1
Tocilizumab treatment		
No	54	45.4
Yes	65	54.6

Source: analyzed data (2023)

Table 2. Fungal growth in sputum culture

Sputum Culture Result	Frequency	(%)
Fungal growth		
None	55	46.2
Positive	64	53.8
Candida glabrata	8	6.7
Cryptococcus neoformans	23	19.3
Candida tropicalis	11	9.2
Candida albicans	5	4.2
Candida ciferrii	2	1.7
Candida dubliniensis	10	8.4
Candida guilliermondii	3	2.5
Candida parapsilosis	2	1.7

Source: analyzed data (2023)

Even 33% of them experienced acute respiratory failure.[10] Another data from hospital antifungal antibiogram 2020-2021 showed a very low (29.17%) sensitivity of *Cryptococcus neoformans* to Amphotericin B in the hospital in which the data in this study were collected.

Candida is an opportunistic pathogen that can cause superficial infections, (which include cutaneous and mucosal candidiasis) and invasive systemic infections. Although the definitive test of *Candida pneumonia* is lung biopsy with a very low incidence rate, Candida in respiratory specimens is usually regarded as colonization.[11-13] Hughes *et al.* mentioned that *Candida spp*. was found in 21.4% of respiratory sample cultures in the UK and suggested its possibility to represent oropharyngeal thrush or normal flora rather than pulmonary candidiasis, while other publications excluded the growth of *Candida spp*. in respiratory cultures as an indicator the presence of pneumonia.[14,15] Pendleton *et al*. mentioned publications about the synergistic effect between Candida species and bacterial pathogens, although the mechanism of how Candida can potentiate bacterial pneumonia remains incompletely understood. It was hypothesized that the presence of *C.albicans* in the airways can induce an immune response that inhibits the regular antibacterial activity of host immune cells, allowing bacterial pathogens to evade clearance and initiate infection.[16] Additional data in this study showed that 53 (47.9%) from 64 samples with fungal growth also had bacterial growth, and 36 (31.1%) from 55 samples with no fungal growth had bacterial growth, which was predominated by *Acinetobacter baumanii* (16 out of 36 samples).

Table 3 analysis shows no significant association between fungal growth in severe and critical COVID-19 patients undergoing sputum culture and patient mortality (p-value of 0.940 > 0.05). Although many publications mentioned that fungal infections increase mortality and can potentiate bacterial pathogens, this study found no significant association between fungal growth and patient mortality.[16]

Several risk factors associated with the presence of fungal growth were analyzed in this study. It was later found out that a decreased immune response might reduce the patient's immune system and lead to a greater risk of fungal growth. Although risk factors such as DM, ventilator use, corticosteroid, and Tocilizumab treatment were mentioned in this study, there was no significant association between DM and corticosteroid treatment with fungal growth. However, there was a significant association between ventilator use and Tocilizumab treatment, as seen in Tables 4, 5, 6, and 7.

Table 4 also shows no significant association between the patient's history of DM in severe and critical COVID-19 patients undergoing sputum culture and fungal growth (p-value of 0.496 > 0.05). Negm *et al.'s* study also showed that DM was not associated with fungal coinfection but rather with poor diabetic control.[17] Contrastingly, this study did not differentiate between uncontrolled and controlled DM.

Table 5 analysis shows a significant association between the use of ventilators in patients with severe and critical COVID-19 patients and fungal growth (p-value of 0.0007628 <0.05). Unfortunately, a cohort study was not performed to identify Ventilator Associated Pneumonia (VAP) since it was reported in a previous publication by Kubin *et al*. that Hospital Associated Infection (HAIs) occurred in 12% of COVID-19 cases and fungi caused 19% of them.[15] Rouyer *et al*. also mentioned that VAP occurred in

Table 3. Fungal growth and patient's outcome

Fungal growth	Outcome		Total n (%)
	Recovery n (%)	Death n (%)	
No fungal growth	8 (14.5%)	47 (85.5%)	55 (100%)
Fungal growth	9 (14.1%)	55 (85.9%)	64 (100%)
Total	17 (14.3%)	102 (85.7%)	119 (100%)

Note: Chi-Square = 0.006; p-value = 0.940; OR (95% CI) = 1.040 (0.372 – 2.910) Source: analyzed data (2023)

Table 4. Diabetes mellitus and fungal growth

Diabetes Mellitus	Fungal Growth		Total n (%)
	Yes n (%)	No n (%)	
With DM	24 (50%)	24 (50%)	48 (100%)
No DM	40 (56.3%)	31 (43.7%)	71 (100%
Total	64 (53.8%)	55 (46.2%)	119 (100%)

Note: Chi-Square = 0.463; p-value = 0.496; OR (95%CI) = 0.775 (0.372 – 1.616) Source: analyzed data (2023)

Table 5. The use of a ventilator and fungal growth

The Use of Ventilator	Fungal Growth		Total n (%)
	Yes n (%)	No n (%)	
With ventilator	64 (58.2%)	46 (41.8%)	110 (100%)
Without ventilator	0 (0.0%)	9 (100%)	9 (100%)
Total	64 (53.8%)	55 (46.2%)	119 (100%)

Note: Chi -Square = 11.33; p-value = 0.0007628; OR (95%CI) = can t be analyzed Source: analyzed data (2023)

Table 6. Corticosteroid treatment and fungal growth

Corticosteroid Treatment	Fungal Growth		Total n (%)
	Yes n (%)	No n (%)	
Yes	62 (55.4%)	50 (44.6%)	112 (100%)
No	2 (28.6%)	5 (71.4%)	7 (100%)
Total	64 (53.8%)	55 (46.2%)	119 (100%)

Note: Chi-Square =1.9016; p-value = 0.167896; OR (95%CI) =3.1 (0.5769-16.66) Source: analyzed data (2023)

Table 7. IL-6 inhibitor treatment and fungal growth

IL-6 inhibitor (Tocilizumab) Treatment	Fungal Growth		Total n (%)
	Yes n (%)	No n (%)	
Yes	43 (66.2%)	22 (33.8%)	65 (100%)
No	21 (38.9%)	33 (61.1%)	54 (100%)
Total	64 (53.8%)	55 (46.2%)	119 (100%)

Note: Chi -Square = 8.8211; p-value = 0.002978 OR (95%CI) = 3.071 (1.45– 6.505) Source: analyzed data (2023)

more than 50% of COVID-19 patients using mechanical ventilators.[18]

Table 6 shows no significant association between corticosteroid treatment and fungal growth in severe and critical COVID-19 patients undergoing sputum culture (p-value of 0.167896> 0.05).

A study by Li and Denning suggests that corticosteroid therapy is a risk factor for fungal infections in COVID-19 patients and can worsen outcomes, in contrast to this study.[19]

Table 7 analysis shows a significant association between IL-6 inhibitor (Tocilizumab) treatment and fungal growth in severe and critical COVID-19 patients undergoing sputum culture (p-value of 0.002978 < 0.05). The OR value was 3.071, which indicates that patients receiving IL-6 inhibitor (Tocilizumab) treatment are likely to have fungal growth in their sputum culture. This finding was by the previous publications, which mentioned that Tocilizumab therapy showed a significantly increased risk of fungal coinfections in COVID-19 patients.[20-22]

Tocilizumab is a recombinant humanized anti-IL-6 receptor monoclonal antibody approved by the Food and Drug Administration (FDA) for use in hospitalized adults with COVID-19 who require conventional oxygen, High-Flow Nasal Cannula (HFNC) oxygen, Non-Invasive Ventilation (NIV), or mechanical ventilation. Tocilizumab should be administered intravenously for the treatment of COVID-19. It belongs to the class of IL inhibitors and is used as immunosuppressants.[23,24]

CONCLUSIONS AND SUGGESTIONS

The highest frequency of fungal growth in sputum culture in severe and critical COVID-19 patients was *Cryptococcus neoformans*, an environmental fungus and opportunistic pathogen in immunocompromised patients. A total of 4 risk factors (DM, the use of ventilator, corticosteroid, and Tocilizumab treatment) were analyzed in this study, and a significant association was found between the use of ventilator and Tocilizumab treatment and fungal growth in severe and critical COVID-19 patients undergoing sputum culture.

Because some publications mentioned that candidiasis can potentiate bacterial pathogens, research was recommended to analyze the association between candidiasis and the presence of bacterial pathogens in respiratory sample cultures and the association between antifungal treatment and decreased susceptibility to bacterial pneumonia.

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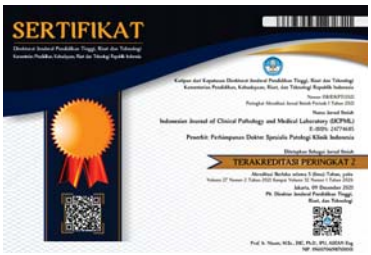

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Expertise: Senior Lecturer in Epidemiology, Statistics, and Research Methods

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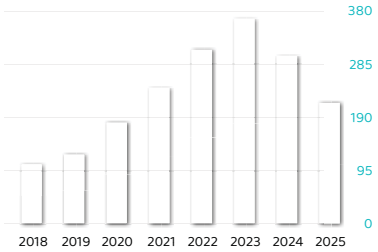

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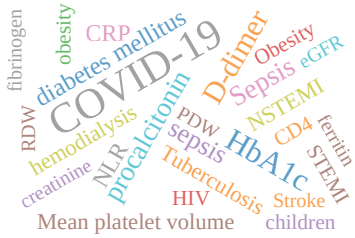

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Telp +6285733220600

Principal Contact :

Yulia Nadar Indrasari
Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga
Phone: +6285733220600
majalah.ijcp@yahoo.com

Support Contact :

Dian Wahyu Utami
Phone: +6285733220600
majalah.ijcp@yahoo.com
majalah.ijcp@gmail.com

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Articles

Performance Evaluation of Semi-quantitative Urine Albumin Creatinine Ratio Using Meditape UC-11A Strip Test

Elizabeth Sidhartha [1], Thoeng Ronald [2]

(1) Department of Biomedicine, Indonesia International Institute for Life Science, Jakarta Timur, DKI Jakarta, Indonesia ,

(2) Laboratory Department, Eka Hospital BSD, Tangerang Selatan, Banten, Indonesia

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Neha Garg [1], Chetan Jain [2], Karandeep Singh [3], Aditi Bagla [4]

(1) Department of Pathology, Maharaja Agrasen Medical College, Hisar, Haryana, India ,

(2) Department of Pathology, Maharaja Agrasen Medical College, Hisar, Haryana, India ,

(3) Department of Pathology, Maharaja Agrasen Medical College, Hisar, Haryana, India ,

(4) Department of Pathology, Maharaja Agrasen Medical College, Hisar, Haryana, India

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Distribution of SARS-CoV-2 Variants in West Java Based on Genomic Surveillance Data, 2021-2022

Cut Nur Cinthia Alamanda [1], Ida Parwati [2], Gusti Ayu Prani Pradani [3], Azzania Fibriani [4]
(1) West Java Health Laboratory, Bandung, West Java, Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, Padjadjaran University/Dr. Hasan Sadikin General Hospital Bandung, Indonesia ,
(3) West Java Health Laboratory, Bandung, West Java, Indonesia ,
(4) Bandung Institute of Technology, Bandung, West Java , Indonesia

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Carbapenem Susceptibility Rate Against Gram-Positive and Gram-Negative Bacteria and Its Correlation with Consumption

George Arthur Mantiri [1], Rheza Paleva Uyanto [2], Meita Hendrianingtyas [3]
(1) Medistra Hospital Jakarta, Laboratory and Blood Bank Unit, Jakarta, Indonesia ,
(2) Medistra Hospital Jakarta, Pharmacy Unit, Jakarta, Indonesia ,
(3) Department of Clinical Pathology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

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Analysis of Albumin to Globulin Ratio as A Prognostic Predictor in Lupus Nephritis Patients

St. Sandra Karyati Serel [1], Yuyun Widaningsih [2], Fitriani Mangarengi [3]
(1) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar/Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University Makassar/Hasanuddin University Hospital, Makassar, Indonesia ,
(3) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar/Syekh Yusuf Hospital, Gowa, Indonesia

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Susceptibility Pattern, Genotyping, and Mutations of Klebsiella pneumoniae at Dr. H. Abdul Moeloek General Hospital

Hidayat Hidayat [1], Ida Parwati [2], Eko Agus Srianto [3]
(1) Department of Clinical Pathology, Faculty of Medicine, Malahayati University, Lampung, Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia ,
(3) Disease Investigation Center, Bandar Lampung, Lampung, Indonesia

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Characteristics of Immunological Non-Responders in People Living with HIV at Abepura Hospital Papua

Justina Berry Sembiring [1], Agnes Rengga Indrati [2], Widya Amalia [3]

(1) Laboratory Installation of Abepura Hospital, Jayapura Papua, Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, Padjadjaran University/Hasan Sadikin Central General Hospital, Bandung, Indonesia ,
(3) VCT Outpatient Clinic at Abepura Hospital, Jayapura Papua, Indonesia

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Optimized Novel Antibacterial Production from Geobacillus kaustophilus Tm6T2 (a) as Treatment for Salmonella typhimurium

Akeyla Tabina Tawangalun [1], Ani Melani Maskoen [2], Emma Rachmawati [3], Candra Arumimaniyah [4], Shinta Asarina [5], Ratu Safitri [6], Tri Yuliana [7]

(1) Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung, Indonesia ,
(2) Department of Oral Biology, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia ,
(3) Department of Oral Biology, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia ,
(4) Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung, Indonesia ,
(5) Laboratory of Microbiology and Parasitology, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia ,
(6) Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung, Indonesia ,
(7) Department of Agroindustrial Technology, Faculty of Agroindustrial Technology, Padjadjaran University, Bandung, Indonesia

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Correlation between PLR and NLR with Tumor Size in Breast Cancer Patients

Sri Widyaningsih [1], Zelly Dia Rofinda [2]

(1) Department of Clinical Pathology, Faculty of Medicine, Muhammadiyah Surabaya University/Siti Khodijah Hospital, Sidoarjo, Indonesia ,
(2) Department of Clinical Pathology, Andalas University, Padang, Indonesia

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Cut-off Value of HSPG for Early Marker of Plasma Leakage in Adult Dengue Patient

Settrin Chenderawasi [1], Rahajuningsih Dharma [2], Herdiman T. Pohan [3], Beti Ernawati Dewi [4], Rianto Setiabudy [5], Suhendro Suhendro [6], Aryati Aryati [7], Hardi Darmawan [8], Leonard Nainggolan [9], Agustin Iskandar [10]
(1) Community-Based Dengue Study, Faculty of Medicine, University of Indonesia/Cipto Mangunkusumo Hospital, Jakarta, Indonesia ,
(2) Department of Clinical Pathology, University of Indonesia, Jakarta, Indonesia ,
(3) Department of Internal Medicine, University of Indonesia, Jakarta, Indonesia ,
(4) Community-Based Dengue Study, Faculty of Medicine, University of Indonesia/Cipto Mangunkusumo Hospital, Jakarta/Department of Microbiology, University of Indonesia, Jakarta, Indonesia ,
(5) Department of Pharmacology, University of Indonesia, Jakarta, Indonesia ,
(6) Department of Internal Medicine, University of Indonesia, Jakarta, Indonesia ,
(7) Department of Clinical Pathology, Airlangga University, Surabaya, Indonesia ,
(8) Department of Physiology, University of Sriwijaya, Palembang, Indonesia ,
(9) Community-Based Dengue Study, Faculty of Medicine, University of Indonesia/Cipto Mangunkusumo Hospital, Jakarta/Department of Internal Medicine, University of Indonesia, Jakarta, Indonesia ,
(10) Department of Clinical Pathology, Brawijaya University, Malang, Indonesia

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Comparison of Lateral-flow Nanoparticle Fluorescence Assay and ELISA Method for Interferon-γ Release Assay Test

Tandry Meriyanti [1], Maroloan Aruan [2], Diana Intan Lusiana Gabriella [3], Justina Andrea Renjaan [4]
(1) Faculty of Health Science, University of Pelita Harapan, Banten/Laboratory Department, Siloam Hospitals Lippo Village, Banten, Indonesia ,
(2) Faculty of Health Science, University of Pelita Harapan, Banten, Indonesia ,
(3) Faculty of Health Science, University of Pelita Harapan, Banten, Indonesia ,
(4) Faculty of Health Science, University of Pelita Harapan, Banten, Indonesia

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The Impact of Cycle Threshold Value in Influencing the Performance of COVID-19 Antigen

Rivaldi Febrian [1], July Kumalawati [2], Nina Dwi Putri [3], Linny Luciana [4], Aria Kekalih [5]
(1) Department of Clinical Pathology, Faculty of Medicine, University of Indonesia, Jakarta , Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia ,
(3) Department of Child Health, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia ,
(4) Department of Clinical Pathology, Faculty of Medicine, Krida Wacana Christian University, Jakarta, Indonesia ,
(5) Department of Community Medicine, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

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Risk Factors of Fungal Growth in Sputum Culture from Severe and Critical COVID-19 Patients

Theresia Novi [1], B. Rina Aninda Sidharta [2], Heru Wijono [3]
(1) Laboratory Husada Utama Hospital, Surabaya, Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Indonesia ,
(3) Faculty of Medicine, University of Surabaya, Surabaya, Indonesia

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Correlation Glucose, Uric Acid, and Cholesterol Levels Towards Health Conditions in the Highlands: POCT Approach

Arifa Mustika [1], Yetti Hernaningsih [2], Puspa Wardhani [3], Siti Khaerunnisa [4], Nastasya Nunki [5], Baiq Nasha Islaeli [6], Uli Mas'uliyah Indarwati [7], Musholli Himmatun Nabilah [8], Teguh Satrio [9], Alfino Validita Sidiq [10], Fita Triastuti [11], Mochammad Reza Desianto [12], Agil Saputra [13], Novi Ersanto [14], Widaninggar Rahma Putri [15], Desty Indah Sari [16]

(1) Master program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya/Department of Pharmacology, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo General Academic Teaching Hospital, Surabaya, Indonesia ,
(3) Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo General Academic Teaching Hospital, Surabaya, Indonesia ,
(4) Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(5) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(6) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(7) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(8) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(9) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(10) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(11) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(12) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(13) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(14) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(15) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia ,
(16) Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

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Correlation between VCAM-1 Level and Absolute Monocyte Count in Coronary Artery Disease

Anastasha Faustine [1], Andreas Arie Setiawan [2], Dwi Retnoningrum [3], Ariosta Ariosta [4]
(1) Student of Medical Faculty, Diponegoro University, Semarang, Indonesia ,
(2) Department of Internal Medicine, Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital, Semarang, Indonesia ,
(3) Department of Clinical Pathology, Faculty of Medicine, Diponegoro University/Diponegoro National Hospital, Semarang, Indonesia ,
(4) Department of Clinical Pathology, Faculty of Medicine, Diponegoro University/Diponegoro National Hospital, Semarang, Indonesia

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Literature Review

A Literature Review: Relationship between Interleukin-1 Beta and the Severity of COVID-19

Andi Ita Maghfirah [1], Tenri Esa [2], Uleng Bahrun [3]
(1) Department of Clinical Pathology, Faculty of Medicine, Alkhairaat University, Palu, Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia ,
(3) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

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Case Report

Ultra-Low Anti-Müllerian Hormone Levels in Recurrent Cystic Ovarian Neoplasm: A Case Report

Merci Monica br Pasaribu [1], Naufal Arkan Abiyyu Ibrahim [2], Dayu Satriani [3]
(1) Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia ,
(2) Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia ,
(3) Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

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Transient Abnormal Myelopoiesis in Down Syndrome Patients

Widya Pratiwi [1], Amaliyah T. Lopa [2], Darwati Muhadi [3], Mansyur Arif [4]
(1) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo, Makassar, Indonesia ,
(2) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo, Makassar/Tajudin Chalid Hospital, Makassar, Indonesia ,
(3) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo, Makassar, Indonesia ,
(4) Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo, Makassar, Indonesia

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Address:

Laboratorium Patologi Klinik RSUD Dr. Soetomo (Gedung Diagnostik Center, Lantai IV)
Jl. Mayjend. Prof. Dr. Moestopo 6-8 Surabaya, Indonesia
Telp +6285733220600

Principal Contact :

Yulia Nadar Indrasari
Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga
Phone: +6285733220600

majalah.ijcp@yahoo.com

Support Contact :

Dian Wahyu Utami
Phone: +6285733220600
majalah.ijcp@yahoo.com
majalah.ijcp@gmail.com

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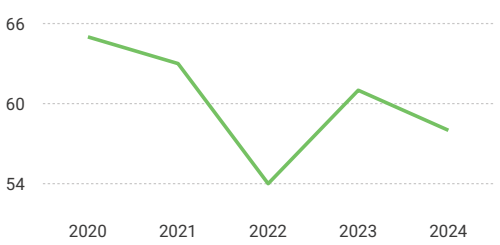

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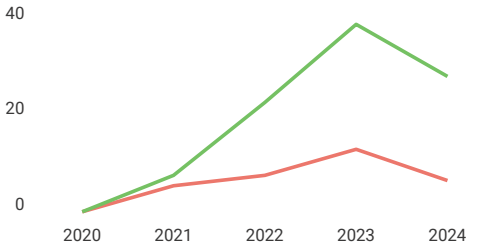

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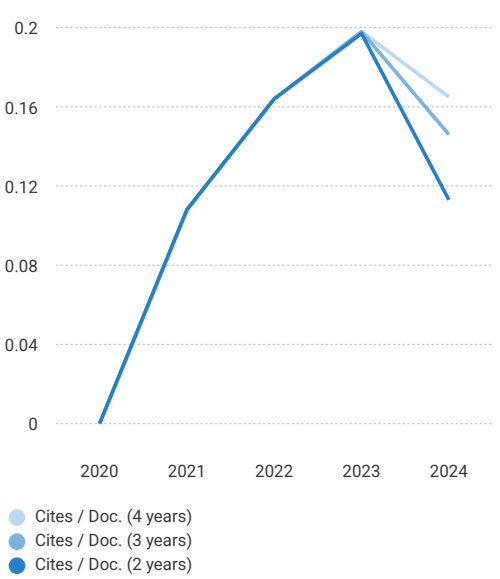

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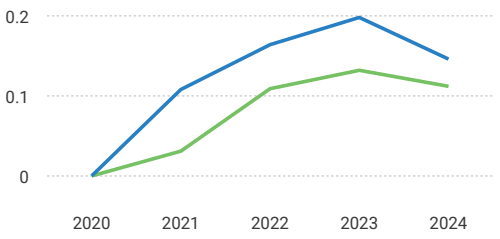

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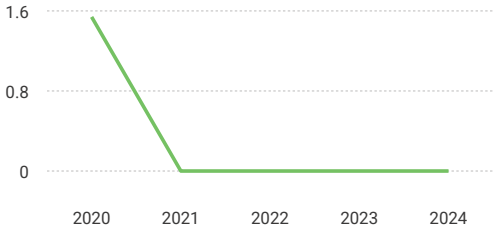

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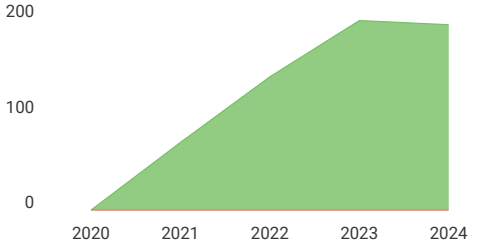

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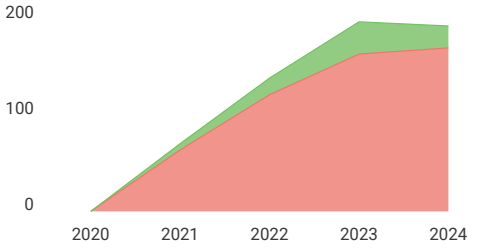

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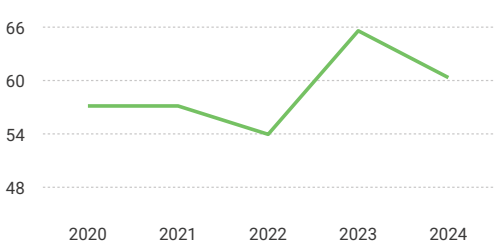

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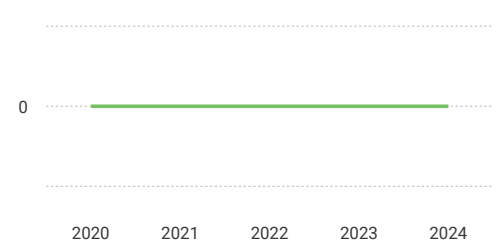

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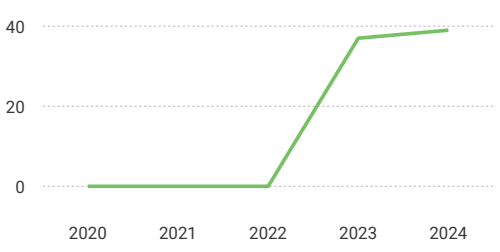

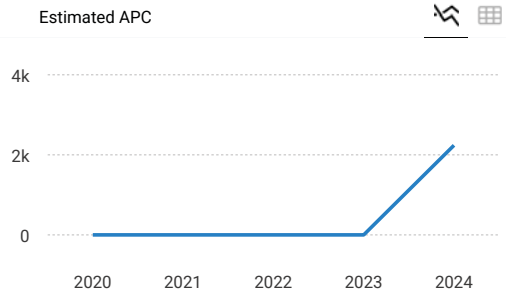

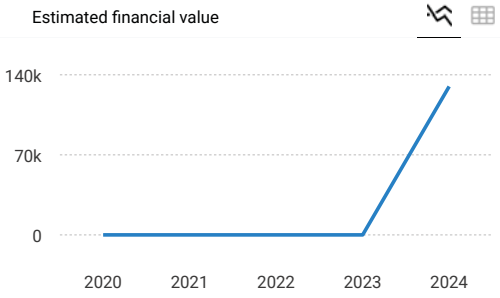

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