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Circulating Tumour Cells and FOXP3 in Regulatory T-Cells as New Modalities in Cancer Diagnosis and Metastasis Location Prediction

*Ikhwan Rinaldi**

Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Prof. Ikhwan Rinaldi, MD., PhD. Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: ikhwanrinaldi@gmail.com.

Cancer is a complex group of diseases which arises from uncontrolled growth and spread of abnormal cells in the body. The pathophysiology of cancer involves a sequence of events at the cellular and molecular levels, often initiated by genetic mutations or alterations. These mutations can be acquired due to various factors like environmental exposures such as from carcinogens, lifestyle choices, or inherited genetic conditions. When a cell's DNA is damaged or mutated, it can disrupt the normal regulatory mechanisms that control cell division and apoptosis, leading to uncontrolled proliferation and cancer.

One of the important hallmarks of cancer is angiogenesis.[1,2] Angiogenesis plays a crucial role in cancer progression by providing the tumour cells with essential nutrients and oxygen, allowing it to grow and metastasize.

In cancer, angiogenesis becomes dysregulated. Tumour cells release signalling molecules, such as vascular endothelial growth factor (VEGF), that stimulate nearby blood vessels to sprout new branches toward the tumour, forming a network of abnormal blood vessels.[2,3] These vessels then supply the tumour with a constant flow of blood, facilitating its growth and also, enabling it to enter bloodstream and invade other locations (hematogenic spread).

Hematogenic spread, also known as hematogenous metastasis, is a key mechanism by which cancer cells travel through the bloodstream to establish secondary tumours in distant parts of the body.[4–6] Once inside the bloodstream, these circulating tumour cells (CTCs) travel throughout the body, carried along by the blood flow.[7,8] Although the majority of these cells perish in the bloodstream or fail to survive in distant tissues, some manage to evade the body's defence mechanisms and successfully establish secondary tumours in distant organs.

The shedding of CTC clusters from solid cancers is influenced by various biological factors that are not fully understood. Studies in breast cancer have shown that hypoxia contributes to the formation of CTC clusters, while normoxia is associated with single CTCs.[9–11]

CTCs have emerged as a significant area of study in cancer research, offering insights into cancer biology, prognosis, and treatment response.[12,13] They can be used as non-invasive biomarkers for diagnosis, monitoring disease progression and guiding treatment decisions.

CTCs is a non-invasive alternative to traditional tissue biopsies, which can be invasive and difficult to obtain.[7] This "liquid biopsy" is particularly beneficial for patients with tumours in difficult-to-reach locations or those undergoing frequent monitoring. Thus, the presence of CTCs in blood may be used to diagnose the type of cancers instead traditional biopsies.[14] Furthermore, CTCs can also be used to analyse

DNA mutations, epigenetic alterations, and metabolite profiling of cancer cells.[14–17]

Studies investigating the clinical utility of CTCs have also revealed their prognostic significance in different cancer types.[18–20] Elevated CTC counts have been associated with poor prognosis and increased metastatic potential, serving as an independent predictor of disease progression and survival outcomes. Higher CTC counts also often correlate with a more advanced stage of the disease and poorer prognosis.[21,22] Finally, a decrease in the number of CTCs following treatment often correlates with a positive response, indicating that the therapy is effectively targeting and reducing the tumour burden.[22–24] Conversely, an increase in CTC count during or after treatment might suggest resistance or disease progression.

Similarly, ctDNA, a type of DNA that is released into the bloodstream by cancer cells can also fulfill similar role of CTCs.[25,26] Nevertheless, detection and isolation of CTCs from blood samples represent a technical challenge due to their rarity amidst a vast number of blood cells.[12] It is very difficult to isolate CTCs but various innovative technologies have been developed to isolate and analyse CTCs such as immunomagnetic separation, microfluidics, and advanced imaging modalities.[12,27–31] These technologies continue to be improved with the aim of enhancing the sensitivity, specificity, and cost-effectiveness of CTCs detection.

Other than CTCs, Immune cells can also be used in cancer prognosis as immune cells have been shown to have influence on tumour development, progression, and response to treatment.[32,33] It is important to note that the immune system can either promote tumour growth or actively suppress it.

Certain immune cells, such as T cells, B cells, natural killer (NK) cells, and macrophages, infiltrate the tumour microenvironment.[34,35] Their presence and activity within the tumour hold prognostic value. In many cases, a higher density of certain immune cells within the tumour correlates with better clinical outcomes. For example, the presence of cytotoxic T cells (CD8+ T-cell) within the tumour has been associated with improved survival rates in several cancer types.[36–39]

Conversely, the presence of immune-suppressive cells like regulatory T cells (Tregs) or myeloid-derived suppressor cells (MDSCs) within the tumour microenvironment often indicates a poorer prognosis.[40,41] These cells can dampen the immune response against cancer cells, allowing tumours to evade destruction by the immune system and progress more aggressively.

Treg cells express FOXP3 biomarker. FOXP3, a transcription factor encoded by the FOXP3 gene, is considered the master regulator of Tregs.[42] It plays a fundamental role in the development, function, and stability of these specialized immune cells. Expression of FOXP3 is crucial for conferring suppressive function to Tregs, enabling them to regulate and modulate immune responses effectively.[42]

In cancer, FOXP3 has been shown to promote tumour growth and metastasis.[43–46] This is likely due to increased infiltration of FOXP3-expressing Tregs within the tumour microenvironment causes immunosuppression of immune cells. Thus, it can also be inferred that higher FOXP3 expression may relates to more aggressive cancer. All in all, many literatures have shown the role of immune cells as prognostic marker of many cancers, including in breast cancer.[47–52] Further researches in this area is needed to elucidate the complex roles of immune system in cancer.

Based on pathophysiology of cancer development which has been described, Acta Medica Journal publishes 2 articles. The first article is about CTCs assessment as a new diagnostic modality for colorectal cancer.[53] With new research such as this, it is hoped that in the future, analysis of cancer cells can be conducted through CTCs (liquid biopsy) instead of traditional biopsy to bring more convenience to patients, which may also indirectly increase the number of patients willing to take biopsy. Meanwhile, the second article is regarding the role of FOXP3 and T-cell in predicting metastasis location of triple negative breast cancer.[54] As described above, immune system has been shown to play a crucial role in cancer prognosis and management. We therefore can expect more

groundbreaking results from immuno-oncology field in the future.

Another article that is published by Acta Medica is about the role of unrefined extra virgin olive oil (EVOO) supplementation during capecitabine chemotherapy to reduce incidences of hand-foot syndrome (HFS).[55] Currently, HFS is one of the causes of treatment cessation in capecitabine chemotherapy and studies analysing the role of EVOO may help in reducing side effects of chemotherapy for patients.

In summary, the intricate interplay between genetic mutations, angiogenesis, hematogenic spread, CTCs, immune cells, and systemic cancer therapy defines the complex landscape of cancer progression and treatment. Understanding the role of immune cells, particularly Tregs marked by FOXP3, as prognostic markers in various cancers, alongside advancements in cancer diagnosis involving CTCs, holds promise in understanding cancer prognosis and improving cancer management. Moreover, ongoing research into alleviating chemotherapy-induced side effects, like HFS offer avenues for improving patient care and treatment outcomes in cancer management.

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The Association of Immune Cell Infiltration with Metastasis Location in De Novo Metastatic Triple Negative Breast Cancer: A Multicenter Cross-Sectional Study in Indonesia

Jeffry Beta Tenggara[1,2], Andhika Rachman[1,2], Joedo Prihartono[3], Lisnawati[4], Sonar Soni Panigoro[5], Didik Setyo Heriyanto[6], Ricci Steven[2], Kevin Tandarto[7], Samuel Juanputra[8], Aru Wisaksono Sudoyo[1,2]*

[1]Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.
[2]Division of Hematology and Medical Oncology, Department of Internal Medicine, MRCCC Siloam Hospital Jakarta, Jakarta, Indonesia.
[3]Department of Community Medicine, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.
[4]Department of Anatomical Pathology, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.
[5]Department of Surgical Oncology, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.
[6]Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada - Dr. Sardjito Hospital, Yogyakarta, Indonesia.
[7]Department of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Diponegoro University - Dr. Kariadi General Hospital, Semarang, Indonesia.
[8]Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.

Corresponding Author:
Jeffry Beta Tenggara, MD. Division of Hematology and Medical Oncology, Department of Internal Medicine Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro No. 71, Jakarta 10430, Indonesia. Email: jeffry.tenggara@yahoo.com.

ABSTRACT

Background: *Triple-negative breast cancer (TNBC) is an aggressive cancer subtype, with limited treatments and a high metastasis risk. The varying location of metastasis in TNBC patients often leads to in prognosis in breast cancer. Therefore, this study aimed to investigate the potential association between immune cells profiles in the tumor microenvironment and metastatic patterns.* ***Methods:*** *We conducted a multicenter cross-sectional study in 2022 to examine formalin-fixed paraffin-embedded (FFPE) and medical record data from 2015 to 2020 in de novo metastatic TNBC patients. The medical records provided crucial information about the sites of metastasis. Immunohistochemistry (IHC) analysis was carried out on primary breast tumor tissues to evaluate the expressions of cluster of differentiation (CD)4 T-cells, CD8 T-cells, CD163, FOXP3 Tregs, and programmed death-ligand 1 (PD-L1), along with immune cells ratios showing antitumor-to-protumor activity (CD4/FOXP3, CD8/FOXP3, CD4/CD163, CD8/CD163). Metastatic locations were grouped into bone-only, visceral, lung, liver, and brain metastasis.* ***Results:*** *A total of 120 metastatic TNBC patients were documented for their metastatic location and IHC report. The clinical and histopathological characteristics showed that the majority of the patients were within the 40-65 years old group, and 34.2% had standard body mass index (BMI). Furthermore, the majority (89.22%) of the patients showed No Special Type (NST), (56.7%) had histopathology*

grade III, high Ki-67 ≥20% (85.8%), and positive PD-L1 expression (30.8%), with visceral metastasis indicating the highest proportion of 75.8%. Patients with a high CD8/FOXP3 and CD4/FOXP3 ratio were significantly prone to have bone-only metastasis compared to visceral metastasis (p= 0.028 and p=0.024, respectively). ***Conclusion:*** *The ratio of antitumor to protumor T-lymphocytes had a significant relevance in the metastatic location patterns in TNBC.*

Keywords*: antitumor, immune cells, metastatic location, protumor, triple-negative breast cancer*

INTRODUCTION

Triple-negative breast cancer (TNBC) is breast carcinomas characterized by the absence of hormone receptors (estrogen and progesterone), and the human epidermal growth factor receptor 2 (HER2). TNBC accounts for 15–20% of all newly diagnosed breast cancer and is the most aggressive, posing a significant challenge for oncologists due to limited curative treatment and a high risk of distant metastasis.[1] Metastatic TNBC patients have the worst prognosis with a median survival of approximately 13.3 months (min 0.8 – max 99 months) and a 12-month mortality rate between 65–75% compared to other types, such as hormonal (HR+) type, which has approximately 37 months.[2–4] The poor prognosis characteristics and aggressive cancer nature can be associated with the tumor microenvironment of metastatic TNBC, which differs from other breast cancer.[5] Visceral metastasis carries a more unfavorable prognosis compared to bone metastasis. Bertho et al. stated that visceral metastasis had a more unfavorable prognosis than bone metastasis in breast cancer.[6]

The components within the microenvironment consist of cancer cells and non-cancerous elements such as stromal and immune cells, leading to the formation of complex interplay.[7] These interactions play a significant role in cancer progression and are essential factors in determining prognosis. Immune cells within the tumor microenvironment primarily comprise lymphocytes known as tumor-infiltrating lymphocytes (TILs) and macrophages, which are called tumor-associated macrophages (TAMs).[8] TILs are the main component of the adaptive immune system present in breast cancer tumor microenvironment, with varying levels across different types. The highest prevalence of lymphocyte-predominant breast cancer (LPBC) is found in TNBC at 30%, while macrophage infiltration reaches 50%. The HR+ type has the lowest LPBC prevalence at 13%.[9] Immunomodulation can be categorized into immunosuppressive and immunoreactive which is primarily mediated by cluster of differentiation (CD) 4 T-cells, and CD8 T-cells, including B-lymphocytes and natural killer (NK) cells. Meanwhile, immunosuppressive activity is majorly mediated by forkhead-box-P3 (FOXP3) regulatory T cells (Treg) and CD163 M2 macrophages, including myeloid-derived suppressor cells (MDSC).[10] The interaction between the immune system and cancer cells plays an important role in tumor development and progression. This role is characterized by a complex interplay of tumor cells and innate as well as adaptive immune system cells, which are influenced by chemical mediators such as cytokines and chemokines.[10]

A study investigating the influence of immune cells on prognosis has been widely conducted in non-metastatic TNBC. Widodo et al. found a significant association between stromal tumor-infiltrating lymphocytes (sTILs) and a higher grade or a lower stage of tumor in patients.[8] Park et al. also found that TILs provided valuable prognostic insights for early-stage non-metastatic TNBC patients who have not received systemic treatment.[11] However, there is no study regarding the influence of these immune cells on the prognosis of *de novo* metastatic TNBC, specifically in Indonesia. Previous studies also have indicated that differences in metastatic location yield distinct prognoses. Therefore, this study aimed to investigate the potential association between immune cells profiles in the tumor microenvironment and metastatic patterns influencing the prognosis of metastatic TNBC.

METHODS

A multicenter cross-sectional study was conducted at 7 hospitals in Indonesia. The population consisted of all patients diagnosed with *de novo* metastatic TNBC from January 2015 to December 2020. The inclusion criteria were subjects ≥18 years old and *de novo* metastatic TNBC, while exclusion criteria were incomplete medical record data and/or unsuitable fixed paraffin-embedded (FFPE) tissue samples for further examination.

TNBC was defined by immunohistochemistry (IHC) test from available formalin- FFPE tissue blocks with Estrogen Receptor (ER) and Progesterone Receptor (PR) <1% and HER-2 Receptors at 0, 1+, or 2+, and a non-amplified fluorescence in situ hybridization (FISH) test result, according to St. Gallen International Breast Cancer Conference definition and American Society of Clinical Oncology (ASCO) guidelines.[5] Metastatic sites were recorded from medical records and categorized into bone-only and visceral metastasis, as well as bone, lung, liver, and brain. Visceral metastasis was defined as any metastasis event with the lung, liver, or brain site. Bone-only metastasis was characterized by metastasis events affecting only the bone site. Meanwhile, bone, lung, liver, and brain metastasis was defined as an event occurring in the respective organ sites.

The expressions of CD4 T-cells, CD8 T-cells, FOXP3 Tregs, and CD163 were evaluated in immune cells in the invasive tumor area. Staining methods were used to detect CD8 T-cells and CD163, using specific antibodies for CD8 T-cells (Cells Marque, 108R-14) and CD163 (Biocare Medical, ACR353AK). FOXP3 Tregs and CD4 T-cells were evaluated by the double-staining method using antibodies for CD4 T-cells (Biocare Medical, ACI3148) and FOXP3 (Genetex, GTX107737). Subsequently, the MACH 2 Double Stain 2 (Biocare Medical) was used for incubation, where FOXP3 and CD4 T-cells were stained with Vulcan Fast Red (Biocare Medical). CD4 T-cells were defined by the expression of CD4 alone, while FOXP3-positive cells were defined by the co-expression of CD4 and FOXP3. Immune cells were evaluated by direct quantification under the microscope as well as by scanning the slides and subsequently analyzing them using the QuPath open-source software platform (version 0.4.1). The average of the total cell count from five fields with the highest concentration of TILs was quantified under 200x magnification. IHC staining of PD-L1 was performed using a mouse monoclonal primary anti-PD-L1 antibody (clone 22C3; Dako; Agilent Technologies, Inc.). The combined positive score (CPS) was used for evaluating the immunohistochemical expression of PD-L1. The CPS was determined as a ratio of the sum of tumor cells and immune cells in the tumor stroma stained with PD-L1 antibody to the total number of viable tumor cells and multiplied by 100. At least 100 viable tumor cells were observed in each section of the countable array core.

Ethics Approval

We received ethical approval from The Ethics Committee of The Faculty of Medicine, Universitas Indonesia (ethical approval number: KET-1209/UN2.F1/ETIK/PPM.00.02/2021). All principles used were in accordance with the Declaration of Helsinki.

Statistical Analysis

We analyzed the data using Statistical Package for the Social Sciences (SPSS) version 27. Descriptive categorical data of the baseline characteristics were shown in frequency (n) and percentage (%), while averages were presented in the median and its interquartile range (IQR). Subsequently, the Mann-Whitney test or independent T-test was used to compare numerical variables between subgroups. The Mann-Whitney test was used when the data distribution was not normal, while the independent T-test was carried out when the data followed a normal distribution. In cases where the data were not normally distributed, a logX transformation was performed to convert it into a normal distribution. All tests were considered statistically significant at p <0.05, with a 95% confidence interval (CI).

RESULTS

The baseline characteristics of 120 metastatic TNBC patients are presented in **Table 1**. All

patients were female, with a mean age of 51.3 years old and the majority ranged between 40 and 65 years old (71.7%), accounting for a mean age of 51.42 ± 11.94 years. Furthermore, all patients had a normal Asian body mass index (BMI) (34.2%), with a median BMI of 23.2 (IQR 20 – 25.9) kg/m^2. The histopathology characteristics showed that the majority of subjects had the No Special Type (NST) (89.2%), histopathology grade III (56.7%), and high Ki-67 (85.8%), and a median of 60 (IQR 30 – 80). Positive PD-L1 expression occurred in 30.8% of the subjects, with a median value of 0.00 (IQR 0.00 –1.10).

The majority of subjects had visceral metastasis (75.8%), consisting of the lungs as the prevalent site (56.7%). Bone-only metastasis occurred in 24.2% of the subjects, while 10% had brain metastasis, as presented in **Table 1**. IHC examination of immune cells showed that CD4 T-cells, CD8 T-cells, CD163, and FOXP3 had a median of 64.10 (IQR 31.35 – 121.75), 182.30 (IQR 118.15 – 304.25), 193.80 (IQR 148.80 – 281.80), and 6.10 (IQR 1.20 – 16.25), respectively. Examination of antitumor protumor ratio showed that the CD4/FOXP3, CD8/FOXP3, CD4/CD163, and CD8/CD163 ratio had a median of 8.76 (IQR 4.65 – 23.57), 26.73 (IQR 14.92 – 80,70), 0.31 (IQR 0.14 – 0.57), and 0.91 (IQR 0.56 – 1.48), respectively.

The comparison between immune cells and metastasis location was carried out using the Mann-Whitney test or independent T-test.

Table 1. Clinical and Histopathology Characteristics

Variables	n=120
Age (years), median (IQR)	51.42±11.94 years
Age (years), n (%)	
- 18-39	18 (15)
- 40-65	86 (71.7)
- > 65	16 (13.3)
BMI (kg/m^2), median (IQR)	23.2 (20 – 25.9)
BMI (kg/m^2), n (%)	
Underweight	11 (9.2)
Normal	41 (34.2)
- Overweight	31 (25.8)
- Obese I	21 (17.5)
- ·bese II	9 (7.5)
- Unknown	7 (5.8)
CD4 T-cells, median (IQR), cells/m^2	64.10 (31.35 – 121.75)
CD8 T-cells, median (IQR), cells/m^2	182.30 (118.15 – 304.25)
CD163, median (IQR), cells/m^2	193.80 (148.80 – 281.80)
CD4/FOXP3 Ratio, median (IQR), cells/m^2	8.76 (4.65 – 23.57)
CD8/FOXP3 Ratio, median (IQR), cells/m^2	26.73 (14.92 – 80,70)
CD4/CD163 Ratio, median (IQR), cells/m^2	0.31 (0.14 – 0.57)
CD8/CD163 Ratio, median (IQR), cells/m^2	0.91 (0.56 – 1.48)
FOXP3 Tregs, median (IQR), cells/m^2	6.10 (1.20 – 16.25)
Ki-67, median (IQR), %	60 (30 – 80)
Pathology, n (%)	
- NST/Ductal	108 (89.2)
- Lobular	5 (4.2)
- Other (Metaplastic, papillary, medullary)	8 (6.7)
Grade, n (%)	
- I	3 (2.5)
- II	36 (30)
- III	68 (56.7)
- Unknown	13 (10.8)
- < 20%	13 (10.8)
- ≥ 20%	103 (85.8)
- Unknown	4 (3.3)

PD-L1, median (IQR), %	0.00 (0.00 –1.10)
PD-L1, n (%)	
- Expressed (1%)	37 (30.8)
- Not Expressed (< 1%)	82 (68.3)
- N/A	1 (0.8)
Metastatic Site, n (%)	
- Bone Involvement	59 (49.2)
- Lung Involvement	68 (56.7)
- Liver Involvement	33 (27.5)
- Brain Involvement	12 (10)
- Others (Adrenal, soft tissue)	2 (1.7)
- Bone-only metastasis	29 (24.2)
- Visceral metastasis	91 (75.8)

When equal variance was assumed, FOXP3 Tregs showed a significant difference in average between cases with bone-only metastasis and visceral metastasis (p=0.047). Although a larger sample of TNBC patients with bone metastasis potentially yielded substantial outcomes, the results obtained in this study were not statistically significant (p=0.095). Subjects with bone-only metastasis had a higher average for the CD8/FOXP3 ratio compared to those with visceral metastasis (26.18 vs 82.50, p= 0.028). The CD4/FOXP3 ratio also had a significant average difference between the bone-only metastasis group and the visceral group (8.63 vs 16.50, p=0.024), as presented in **Table 2.**

Table 2. Difference of Immune Infiltration Cells in Bone-only and Visceral Metastasis Group

Immune Cells	Metastatic Group	N	Median [IQR]	p
CD8 T-cells	Visceral metastasis	91	187.20 [126.20 - 304.80]	0.975[a]
	Bone-only metastasis	29	157.20 [109.10 - 301.30]	
CD4 T-cells	Visceral metastasis	91	63.40 [32.00 - 121.00]	0.773[a]
	Bone-only metastasis	29	81.60 [24.50 - 148.00]	
FOXP3 Tregs	Visceral metastasis	91	7.00 [1.60 - 17.20]	0.095[a]
	Bone-only metastasis	29	3.20 [0.30 - 15.80]	0.047[c]
CD163	Visceral metastasis	91	194.80 [145.20 - 280.60]	0.957[b]
	Bone-only metastasis	29	188.00 [153.60 - 300.20]	
CD8/FOXP3 Ratio	Visceral metastasis	84	26.18 [14.79 - 49.93]	**0.028[a]**
	Bone-only metastasis	25	82.50 [15.93 - 262.50]	
CD4/FOXP3 Ratio	Visceral metastasis	84	8.63 [3.81 -22.47]	**0.024[a]**
	Bone-only metastasis	25	16.50 [5.83 - 44.75]	
CD8/CD163 Ratio	Visceral metastasis	91	0.92 [0.59 - 1.45]	0.946[a]
	Bone-only metastasis	29	0.93 [0.47 - 1.81]	
CD4/CD163 Ratio	Visceral metastasis	91	0.29 [0.15 - 0.58]	0.789[a]
	Bone-only metastasis	29	0.36 [0.11 - 0.58]	

[a] Analyzed using t-test after logX transformation.
[b] Analyzed using Mann-Whitney U test.
[c] If equal variances are assumed

DISCUSSION

The emergence of metastases in visceral organs due to breast carcinoma is strongly associated with an unfavorable prognosis, characterized by significantly diminished overall survival and reduced metastasis-free survival rates.[12] Recently, two studies have reported that bone-only metastatic patients exhibit improved outcomes in terms of overall survival (OS) and progression-free survival (PFS) compared to other subgroups, such as metastatic breast cancer patients with visceral disease.[13,14] Specific metastatic sites or the involved organs could predict the prognosis of breast cancer patients. Studies have revealed that breast cancer with bone-only metastasis had a better prognosis than the visceral one.[15] The median OS between bone-only and visceral metastasis is 38 and 21 months, respectively.[16,17] Among the visceral organ sites, brain metastasis had the poorest prognosis, followed by liver and lung. These results could improve clinical judgment involving the more unfavorable prognosis of visceral organ metastasis compared to bone-only metastasis. To the best of our knowledge, no current studies have examined the influence of immune cells on the prognosis of the metastatic TNBC population.

Cancer-immune cell interactions are critical in tumor progression and metastasis, yet their specific roles remain unclear. Immune surveillance and tolerance help maintain overall bodily balance but are disrupted in tumors, allowing unchecked growth, invasion, and metastasis.[18] Tumor microenvironments attract various immune cells, notably CD4 T-cells, CD8 T-cells, and FOXP3 Tregs. CD8 T-cells and FOXP3 Tregs play key roles in immune surveillance and tolerance, countering uncontrolled tumor growth.[18,19] This study, unique in the Indonesian population, compares immune cell infiltration in *de novo* metastatic TNBC with bone or visceral metastasis, advancing our understanding of these processes. This study reveals significant discrepancies in the CD8/FOXP3 ratio, favoring bone-only metastases over visceral ones. CD8 T-cells drive cancer cell apoptosis, while FOXP3 Tregs dampen CD8 T-cells activity, promoting tumor growth. Effective anti-tumor immunity hinges on CD4 T-cells and CD8 T-cells, with CD4 T-cells supporting CD8 T-cells proliferation and priming.[19,20] Early breast tumors are characterized by CD4 T-cells and CD8 T-cells predominance, potentially enhancing immunosurveillance. In contrast, advanced cancer exhibits an influx of CD4 T-cells, possibly fostering tumor progression. CD8 T-cells are pivotal for improved clinical outcomes, activated by CD4 T-cells derived IL-2, and producing tumor-suppressing cytokines like interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α).[20]

This study presented the clinical and histopathological characteristics of metastatic TNBC cases, with a mean age of subjects of 51.42 years, predominantly consisting of the 40 to 65 years old group. This report was different from several multinational studies indicating that TNBC was mostly diagnosed in the age group of < 40 years.[21,22] A previous TNBC study from Indonesia showed a similar mean age of the subjects (51.09 years).[8] These variations could determine racial differences, dissimilar health screening programs, and early diagnosis systems across various countries.[23] In this study, the majority of the subjects had normal and median BMI similar to a previous report in the Indonesian population, indicating low domination of hormonal stimulation from high adipose tissue in TNBC. Histopathological characteristics also showed a high proportion of NST/ductal carcinoma, elevated Ki-67, and high-grade tumors. Other studies also reported a high proportion of ductal type in TNBC, Ki-67 expression, and tumor-grade histopathology among TNBC subjects.[24] High Ki-67 levels and high-grade tumors indicated its aggressive nature, suggesting that tumor invasion and metastasis were progressing. The prevalence of the PD-L1 expression was similar to several other investigations of TNBC which ranged from 20%-59.5%.[25] This study showed a high proportion of visceral metastasis compared to the other sites, with the lung becoming the most common metastatic site and the brain being the least. Several studies of metastatic patterns in TNBC reported similar results, which consistently reported the lung, liver, bone, and brain as the most common metastatic sites.[16,17] These results presented the

distinctive characteristics of metastatic patterns in TNBC.[12,14]

This study indicates a significant difference in the average for the CD8/FOXP3 ratio between bone-only metastasis and visceral metastasis.[26] Patients with bone-only metastasis have a higher average for the CD8/FOXP3 ratio compared to patients with metastasis to visceral organs. Additionally, the CD4/FOXP3 ratio also shows a significant difference in average between the bone-only metastasis group and the metastasis to visceral organs group. CD8 T-cells induces the apoptosis of cancer cells by producing proteins including perforin and granzyme.[15,26] On the other hand, FOXP3 Treg cells are immunosuppressive cells that can induce the cytotoxic dysfunction of CD8 T-cells and NK cells through the production and expression of various factors, including β-galactosidase-binding protein (βGBP), PD-L1, and B7-H4, which increase tumor growth, invasion, and migration from the primary site.[27]

A previous study discovered that in most grade III breast cancer cases, the ratio of FOXP3 Tregs to CD8 T-cells consistently exceeds the observed in grade I/II breast cancer, indicating the well-established role of FOXP3 Tregs in facilitating the progression of breast cancer.[20] According to a recent investigation, the quantity of Tregs infiltrating tumors is associated with the initiation and advancement of breast cancer.[28] The average ratio of FOXP3 T regs gradually increases from 0.005 in normal breast tissues to 0.019 in ductal carcinoma in situ and 0.030 in invasive ductal cancer, mirroring their malignant progression.[29]

According to Liu et al., the correlation between Tregs and CD8 T-cells in 1270 cases of invasive breast carcinoma showed a significant impact on patient survival, histopathological characteristics, and molecular subtypes.[15] The results indicated that an increased presence of Tregs and T-cells within the tumor was related to adverse features, such as high histologic grade and negative ER and PR status. A high level of Treg infiltration was also connected to reduced OS and PFS. Meanwhile, a high ratio of T-cells to Tregs in the tissue surrounding the tumor was significantly related to improved OS and PFS. Miyan et al. assessed the expression of CD8, FOXP3, and CD3 in 177 patients with primary, invasive, unilateral early-stage breast cancer including all molecular subtypes.[30] The results indicated that T-cells infiltration was associated with hormone receptor-negative tumors, a high rate of proliferation, an elevated histological grade, and larger tumor sizes. Basal-like tumors showed the highest number of FOXP3 T-regs, which was associated with an unfavorable ratio compared to cytotoxic CD8 T-cells.[30]

This study also had a substantial number of samples despite the rarity of *de novo* metastatic TNBC cases. This study's limitation is that it only subjects to the *de novo* metastatic TNBC subjects. As such, it may not apply to progressive metastatic TNBC cases. The implication result of this study could assist clinicians in improving treatment judgment for metastatic TNBC cases.

CONCLUSION

In this study, the ratio of antitumor to protumor T-lymphocytes had a significant relevance with the metastatic location patterns in TNBC. This study shed a light on the immunopathological aspect of the metastatic TNBC, revealing the unique features in the Indonesian population.

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CONFLICT OF INTEREST

All authors have no conflict of interest.

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Assessment of Circulating Tumor Cells in Colorectal Cancer as an Adjunctive Non-invasive Diagnostic Method

Saskia Aziza Nursyirwan[1], Murdani Abdullah[1], Andri Sanityoso Sulaiman[2], Ikhwan Rinaldi[3], Dadang Makmun[1], Marcellus Simadibrata[1], Dimas Ramadhian Noor[4], Agustinus Wiraatmadja[1], Wifanto Saditya Jeo[5], Nur Rahadiani[6], Diah Rini Handjari[6], Hamzah Shatri[7]*

[1]Division of Gastroenterology, Pancreatobiliary, and Digestive Endoscopy, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2]Division of Hepatobilliary, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[3]Division of Hematology-Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[4]Human Cancer Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[5]Division of Digestive Surgery, Department of Surgery, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[6]Department of Anatomical Pathology, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[7]Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Saskia Aziza Nursyirwan, MD. Division of Gastroenterology, Pancreatobiliary, and Digestive Endoscopy, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: saskia.aziza@gmail.com; ORCID ID https://orcid.org/0000-0003-0166-7651

ABSTRACT

Background: *Colorectal cancer (CRC) is a significant contributor to cancer-related morbidity and mortality. Biopsy remains the gold standard for CRC diagnosis, but invasive testing may not be preferred as an initial diagnostic procedure. Therefore, alternative non-invasive approaches are needed. Circulating tumor cells (CTC) present in the bloodstream have great potential as a non-invasive diagnostic marker for CRC patients. This study aimed to assess the diagnostic potential of CTC in CRC as an adjunctive diagnostic method using a subjective manual identification method and laser capture microdissection at 40x magnification.* ***Methods:*** *A cross-sectional study was conducted on adult patients suspected to have CRC at Dr. Cipto Mangunkusumo National General Hospital, Jakarta, between November 2020 and March 2021. CTC analysis was performed using the negative selection immunomagnetic method with Easysep™ and the CD44 mesenchymal tumor marker. The identification and quantification of CTC were conducted manually and subjectively, with three repetitions of cell counting per field of view at 40x magnification.* ***Results:*** *Of 80 subjects, 77.5% were diagnosed with CRC, while 7.5% and 15% exhibited adenomatous polyps and inflammatory/hyperplastic polyps, respectively. The diagnostic analysis of CTC for detecting CRC (compared to polyps) using a CTC cutoff point of >1.5 cells/mL suggested sensitivity, specificity, and positive predictive value (PPV) of 50%, 88.89%, and 93.94%. Additionally, the negative predictive value (NPV), as well as the positive and negative likelihood ratio (PLR*

and NLR) were 34.04%, 4.5, and 0.56, respectively. The subjective manual identification and quantification of CTC were performed at 40x magnification using laser capture microdissection. ***Conclusion:*** *This study assessed the diagnostic potential of CTC examination in CRC as an adjunctive diagnostic method using the subjective manual identification method and laser capture microdissection at 40x magnification. Despite the limitations associated with subjective cell counting, the results showed 50% sensitivity and 88.89% specificity in diagnosing CRC. Further studies are needed to optimize the manual identification process and validate the clinical utility of CTC analysis in CRC patients.*

Keywords*: Circulating tumor cells; Colorectal cancer; Cancer; Adenoma; Diagnosis*

INTRODUCTION

Colorectal cancer (CRC) is a significant public health concern globally, contributing to a substantial morbidity and mortality rate.[1,2] Timely and accurate diagnosis is crucial for effective treatment and improved patient outcomes. The available diagnostic approaches primarily rely on the presence of CRC-related symptoms or abnormal screening results to initiate further investigations.[3–6] However, there is an increasing need for non-invasive and convenient methods to complement existing diagnostic strategies.[7–10]

Circulating Tumor Cells (CTC) have been reported as biomarkers with the potential to revolutionize cancer diagnosis and management, including CRC.[11–13] These tumor cells detach from the primary source or metastatic sites and enter the bloodstream.[14,15] CTC offers a unique opportunity for non-invasive assessment of tumor burden, molecular characterization, and monitoring treatment response.[16,17] Several studies showed their diagnostic value in various cancers, including CRC, providing insights into prognosis and treatment decision-making.[18–20]

Despite their tremendous potential, the use and standardization of CTC technologies in Indonesia remain in the nascent stage. Currently, there is a lack of established protocols and standardized approaches for CTC detection and characterization. This gap presents an opportunity to enhance the quality of investigations and explore the diagnostic potential in CRC patients.

This study aimed to assess the diagnostic potential of CTC in CRC using subjective manual identification and laser capture microdissection at 40x magnification. The results will contribute significantly to the advancement of CTC studies and the development of standardized protocols in Indonesia, ultimately enhancing patient outcomes.

METHODS

This cross-sectional study was conducted at Dr. Cipto Mangunkusumo National General Hospital in Jakarta from November 2020 to March 2021.

Ethics Statement

The study protocols adhered to the ethical principles outlined in the Declaration of Helsinki. Approval was also obtained from the Ethics Committee of Dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia (Approval No. LB.02/621/0014/2020).

Participants

The participants in this study were adult patients suspected to have CRC.

Circulating Tumor Cells (CTC) Analysis

CTC analysis was performed using the negative selection immunomagnetic method with Easysep™ and the CD44 mesenchymal tumor marker. Identification was carried out manually, while quantification was achieved through visual examination under a microscope, with three repetitions of cell counting per field of view at 40x magnification using laser capture microdissection. This method allowed for the selective isolation and enrichment of CTC from blood samples, followed by precise evaluation of their morphological and molecular characteristics. The selected method enabled the assessment of CTC presence and quantity, contributing to the understanding of their diagnostic potential in CRC.

Diagnostic Evaluation

Diagnostic study analysis was used to determine the cut-off point of CTC in detecting CRC. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were calculated using a CTC cut-off point of >1.5 cells/mL. Subsequently, a comparison was made between CRC and inflammatory/hyperplastic polyps, as well as adenomatous polyps.

Data Analysis

The demographic and clinical characteristics of the participants were summarized using descriptive statistics. The diagnostic performance measures were calculated to evaluate the accuracy of CTC examination for diagnosing CRC.

RESULTS

Clinical Characteristics and The Relevance to Circulating Tumor Cell (CTC) Analysis in Colorectal Cancer (CRC) Detection

This study aimed to assess the diagnostic potential of CTC in detecting CRC. To gain a comprehensive understanding, the clinical characteristics of the study population were analyzed. **Table 1** presents a detailed overview of these characteristics, providing detailed insight into their relevance to the investigation of CTC in CRC detection.

The study population consisted of 80 subjects, with a mean age of 56 ± 11 years. The gender distribution showed that 53.8% were male and 46.3% were female patients. Furthermore, 77.5% were diagnosed with CRC, 7.5% had adenomatous polyps, and 15% had inflammatory/hyperplastic polyps.

The tumoral location was also examined, with 77.5% of the cases found on the left side and 22.5% on the right side. The assessment of Carcinoembryonic Antigen (CEA) levels, a commonly used marker for CRC, showed that 50.8% of participants had CEA levels below 5, while 49.2% were recorded to have levels equal to or above 5.

Data on the smoking and alcohol drinking history of participants were also collected. Based on the results, 66.3% were non-smokers, and 33.7% were current or ex-smokers. Regarding alcohol consumption, 80% were non-drinkers, while 20% were current or ex-drinkers. Body Mass Index (BMI) analysis showed that 30% were underweight, 32.5% had normal weight, 13.8% were overweight, 18.8% fell into the Obese 1 category, and 5% were categorized as Obese 2. **Table 1** shows various parameters related to age, gender, diagnosis, tumoral location, CEA levels, smoking, and alcohol drinking history, as well as BMI.

CTC Characterization and Immunofluorescence Staining

The primary objective of the invention is to address the limitations of CTC investigations

Table 1. Clinical Characteristics of the Study Population

Clinical Characteristics	Amount (%)
Total, n	80
Age in years, mean (SD)	56 ± 11
Gender, n (%)	
Male	43 (53.8)
Female	37 (46.3)
Diagnosis, n (%)	
Inflammatory/hyperplastic polyps	12 (15)
Adenomatous polyps	6 (7.5)
Colorectal cancer	62 (77.5)
Tumoral location, n (%)	
Left-sided	62 (77.5)
Right-sided	18 (22.5)
Carcinoembryonic Antigen (CEA), n (%)	
<5	33 (50.8)
≥5	32 (49.2)
Smoking history, n (%)	
Non-smoker	53 (66.3)
Smoker/ex-smoker	27 (33.7)
Alcohol drinking history, n (%)	
Non-alcohol drinker	64 (80)
Alcohol/ex-alcohol drinker	16 (20)
Body Mass Index, n (%)	
Underweight	24 (30)
Normal weight	26 (32.5)
Overweight	11 (13.8)
Obese 1	15 (18.8)
Obese 2	4 (5)

in Indonesia. The majority of existing studies primarily focused on direct molecular marker measurement, with no reports on microscopic identification. The invention comprised a centrifugation method for CTC isolation, plating for cell adhesion, and microscopic evaluation techniques. It aims to facilitate CTC assessment, enhance investigation and publications in the field, as well as provide clinical benefits including improved diagnosis and disease monitoring. The use of CTC also has great potential for prognostic evaluation and predicting clinical outcomes after radiation therapy, chemotherapy, or surgery, thereby contributing to advancements in healthcare and clinical practice. **Figure 1** shows the post-isolation results of CTC, where immunofluorescence staining was utilized to enhance detection in the isolated samples.

The staining played a crucial role in improving CTC identification, given the challenges associated with identification within post-isolation samples. Representative images expressing strong CD44 intensity are shown in **Figure 1**. This strong expression was observed in patients with stages III and IV. A positive count of 2 CTC per field of view was considered indicative of CRC.

Figure 2 shows CTC that exhibit strong CD44 expression, appearing either as single cells or clustered together with other CD44-positive or non-specific cells. On the other hand, **Figure 3** shows isolates suspected to be CTC from patients with polyps, exhibiting weak or faint staining intensity. These samples were primarily found in patients with polyps and not in those diagnosed with CRC.

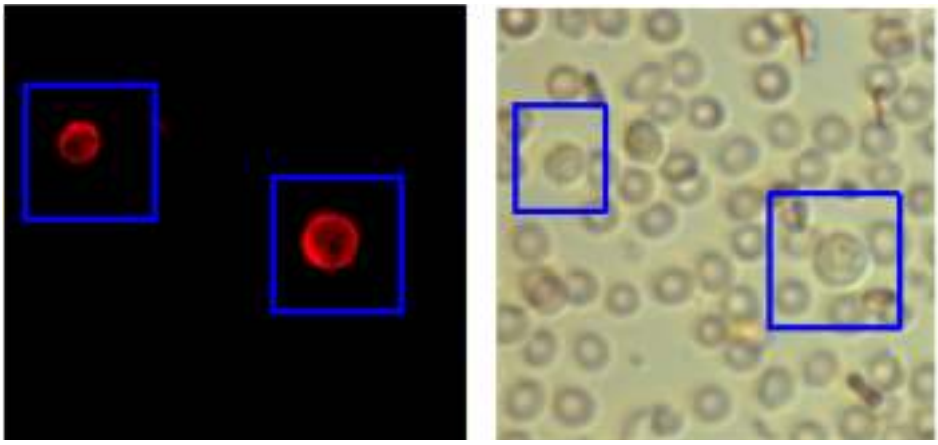

Figure 1. Immunofluorescence Staining Enhances CTC Detection in Isolated Samples: Post-Isolation Results

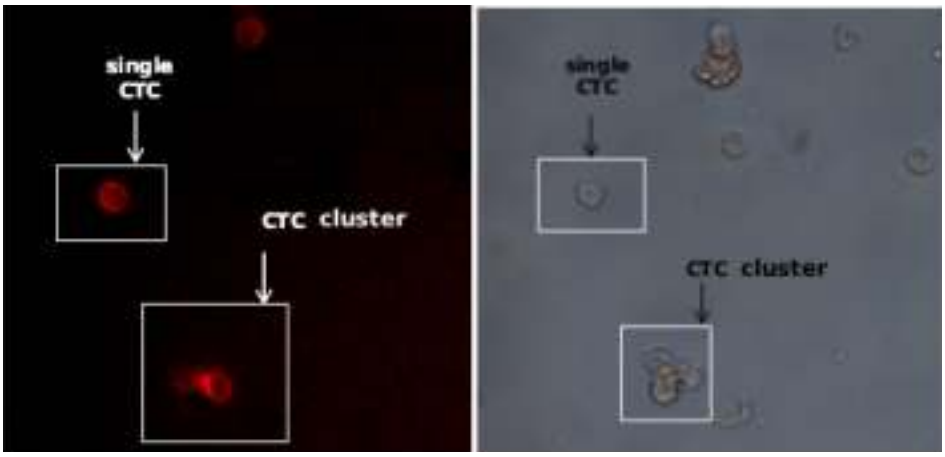

Figure 2. Representative Images of CTC with Strong CD44 Expression in Colorectal Cancer: Stage III and IV Patients

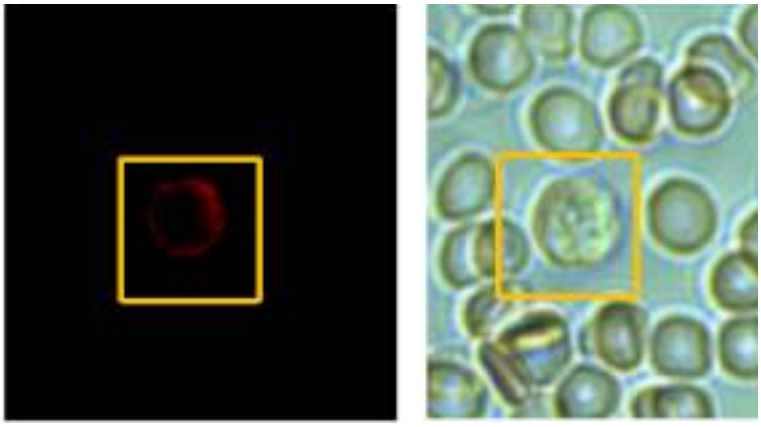

Figure 3. Differential CD44 Expression in CTC in Polyp Samples

Exploring the Diagnostic Potential of Circulating Tumor Cell (CTC) in Colorectal Cancer (CRC): Comparative Analysis and Cut-Off Point Evaluation

The diagnostic potential of CTC in detecting CRC was assessed by comparing with inflammatory/hyperplastic polyps and adenomatous polyps. The area under the curve (AUC) value for this comparison was determined to be 72.5%, indicating a moderate level of diagnostic accuracy. At a CTC cut-off point of 1.5 cells/mL, the sensitivity was 50%, meaning that CTC analysis correctly identified half of CRC cases. The specificity was 88.89%, suggesting a high rate of correctly identifying non-cancerous cases. Furthermore, the Positive Predictive Value (PPV) was 93.94%, implying a high probability of correctly identifying CRC cases among positive results. NPV was 34.04%, suggesting a moderate probability of correctly ruling out CRC among negative results. PLR of 4.5 indicated a moderate increase in the likelihood of CRC, while NLR of 0.56 implied a modest decrease in the likelihood of disease.

CTC analysis was performed to distinguish between inflammatory/hyperplastic polyps, adenomatous polyps, and CRC. The AUC value for this discrimination was found to be 66.7%, which was considered a fair level of diagnostic accuracy. At the same CTC cut-off point of 1.5 cells/mL, the sensitivity was 45.59%, implying CTC analysis correctly identified approximately 45.59% of colorectal neoplasms in the sample. A high rate of correctly identifying non-neoplastic cases was also observed according to the specificity value of 83.33%. PPV was 93.34%, suggesting a high probability of correctly identifying colorectal neoplasms among positive results. NPV was 21.28%, indicating a lower probability of correctly ruling out colorectal neoplasms among negative results. PLR of 2.74 suggested a small increase in the likelihood of colorectal neoplasms, while the NLR of 0.65 implied a modest decrease.

The cut-off point for CTC analysis in detecting CRC including inflammatory/hyperplastic polyps and adenomatous polyps compared with CRC, was determined using Receiver Operating Characteristic (ROC) curve analysis. The resulting AUC value was calculated as 72.5%, showing the overall diagnostic performance. The ROC curve, presented in **Figure 4**, visually represents the relationship between sensitivity and specificity at various cut-off points, aiding in the interpretation of the diagnostic potential of CTC analysis in CRC detection.

Circulating Tumor Cell (CTC) Analysis Shows Variations in Different Subject Groups

CTC analysis among various subject groups, including polyps and CRC provided valuable insights into the relationship between CTC presence and different pathological conditions. As shown in **Table 2**, there were detectable levels of CTC in both inflammatory/hyperplastic and adenomatous polyps. The mean number was higher in the inflammatory/hyperplastic (0.75 ± 0.96) compared to the adenomatous polyps group (0.33 ± 0.51), suggesting a potential association between CTC presence and the pathological characteristics of polyps.

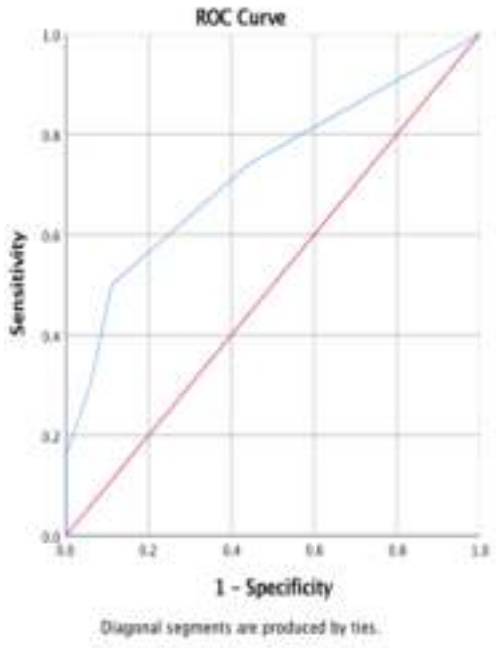

Figure 4. ROC Curve of CTC for Detecting CRC

CRC group exhibited a higher mean number of CTC (1.97 ± 2.1) compared to the polyp group. Furthermore, the non-metastatic CRC subgroup had a slightly lower mean number of CTC (1.63 ± 1.57). These varying levels of CTC indicate its potential as a diagnostic tool to distinguish between polyps and cancerous conditions.

The analysis explored the relationship between CTC and different stages of CRC. Stage I exhibited the lowest mean number of CTC (0.6 ± 0.89), while II and III showed higher mean values of 1.8 ± 1.9 and 1.76 ± 1.58, respectively. The metastatic CRC (Stage IV) subgroup had the highest mean number (2.74 ± 2.9). These results suggested a correlation between CTC levels and progression as well as the metastatic potential of CRC.

Association of Variables with Circulating Tumor Cell (CTC) Levels

The association between CTC levels and various variables was also analyzed. The distribution of individuals with CTC levels above or below 1.5 cells/mL and the corresponding p-values for each variable are presented in Table 3.

Age did not demonstrate a significant association with CTC levels (p = 0.617). Among participants aged ≤60, 23 (44.2%) had CTC levels above 1.5 cells/mL, while 29 (55.8%) exhibited levels below or equal the cut-off value. Similarly, in the group aged >60, 10 (35.7%) had CTC levels above 1.5 cells/mL, and 18 (64.3%) were found to have levels below or equal to the cut-off value.

The analysis showed no significant difference in CTC levels based on gender (p = 0.573). Among males, 16 (37.2%) had CTC levels above 1.5 cells/mL, while 27 (62.8%) exhibited levels below or equal to the cut-off value. Meanwhile, among females, 17 (45.9%) had CTC levels above 1.5 cells/mL, and 20 individuals (54.1%) showed levels below or equal to 1.5 cells/mL.

Smoking history also did not indicate a significant association with CTC levels (p = 0.81). Among non-smokers, 26 (49.1%) had CTC levels above 1.5 cells/mL, while 27 (50.9%) exhibited levels below or equal to the cut-off value. In the smoker/ex-smoker group, 7 individuals (25.9%) had CTC levels above 1.5 cells/mL, and 20 (74.1%) had levels below or equal to the cut-off value.

Table 2. CTC Values in Each Group of the Subjects

Subjects Groups	N	Mean ± SD	Range (Median)
Polyps	18	0.61 ± 0.85	0-3 (0)
Inflammatory/hyperplastic polyps	12	0.75 ± 0.96	0-3 (0.5)
Adenomatous polyps	6	0.33 ± 0.51	0-1 (0)
Colorectal cancer (CRC)	62*	1.97 ± 2.1	0-12 (1.5)
Non-metastatic CRC	43	1.63 ± 1.57	0-6 (1)
Stage I CRC	5	0.6 ± 0.89	0-2 (0)
Stage II CRC	5	1.8 ± 1.9	0-5 (1)
Stage III CRC	33	1.76 ± 1.58	0-6 (2)
Metastatic CRC (stage IV)	19	2.74 ± 2.9	0-12 (2)

*CTC level in CRC was significantly higher than in polyp/non-CRC group (p = 0.003)

Alcohol drinking history did not show a significant association with CTC levels (p = 0.23). Among non-alcohol drinkers, 29 individuals (45.3%) had CTC levels above 1.5 cells/mL, while 35 (54.7%) had levels below or equal to the cut-off value. In the alcohol/ex-alcohol drinkers group, 4 (25%) showed CTC levels above 1.5 cells/mL, and 12 (75%) had levels below or equal to the cut-off value.

Tumoral location analysis indicated no significant difference in CTC levels between left-sided and right-sided tumors (p = 0.967). Among individuals with left-sided tumors, 25 (40.3%) exhibited CTC levels above 1.5 cells/mL, while 37 (59.7%) had levels below or equal to the cut-off value. For those with right-sided tumors, 8 (44.4%) had CTC levels above 1.5 cells/mL, and 10 (55.6%) were found to have levels below or equal to the cut-off value.

CTC analysis levels in different stages of CRC did not indicate a significant association (p = 0.475). In stage I, 1 individual (3.2%) had CTC levels above 1.5 cells/mL, while 4 (12.9%) showed levels below or equal to the cut-off value. For stage II CRC, 2 individuals (6.5%) had CTC levels above 1.5 cells/mL, and 3 (9.7%) exhibited levels below or equal to 1.5 cells/mL. Furthermore, in stage III CRC, 17 individuals (54.8%) were found to have CTC levels above 1.5 cells/mL, and 16 (51.6%) showed levels

Table 3. Clinical Characteristics of the Subjects with CTC Value

Variables	CTC >1.5 cells/mL	CTC ≤1.5 cells/mL	P Value
Age, n (%)			0.617
≤60	23 (44.2)	29 (55.8)	
>60	10 (35.7)	18 (64.3)	
Gender, n (%)			0.573
Male	16 (37.2)	27 (62.8)	
Female	17 (45.9)	20 (54.1)	
Smoking history, n (%)			0.81
Non-smoker	26 (49.1)	27 (50.9)	
Smoker/ex-smoker	7 (25.9)	20 (74.1)	
Alcohol drinking history, n (%)			0.23
Non-alcohol drinker	29 (45.3)	35 (54.7)	
Alcohol/ex-alcohol drinker	4 (25)	12 (75)	
Tumoral location, n (%)			0.967
Left-sided	25 (40.3)	37 (59.7)	
Right-sided	8 (44.4)	10 (55.6)	
Colorectal cancer staging, n (%)			0.475
I	1 (3.2)	4 (12.9)	
II	2 (6.5)	3 (9.7)	
III	17 (54.8)	16 (51.6)	
IV	11 (35.5)	8 (25.8)	
Tumoral size, n (%)			0.86
<5 cm	9 (28.1)	23 (71.9)	
≥5 cm	24 (50)	24 (50)	
Cancer differentiation, n (%)			*0.005
Well-differentiated	19 (39.6)	29 (60.4)	
Poorly-differentiated	12 (85.7)	2 (14.3)	
CEA, n (%)			1
<5	15 (45.5)	18 (54.4)	
≥5	15 (46.9)	17 (53.1)	
Body Mass Index, n (%)			0.337
Underweight	13 (39.4)	11 (23.4)	
Normal weight	12 (36.4)	14 (29.8)	
Overweight	3 (9.1)	8 (17)	
Obese 1	4 (12.1)	11 (23.4)	
Obese 2	1 (3)	3 (6.4)	

*Statistical test using Chi-square showed that the cancer differentiation variable had a significant value (p<0.05) with CTC value.

below or equal to the cut-off value. For stage IV (metastatic) CRC, 11 (35.5%) had CTC levels above 1.5 cells/mL, and 8 (25.8%) exhibited levels below or equal to the cut-off value.

Tumoral size did not show a significant association with CTC levels (p = 0.86). Among individuals with tumors less than 5 cm, 9 (28.1%) were found to have CTC levels above the cut-off value, while 23 (71.9%) had levels below or equal to this value. For those with tumors measuring 5 cm or larger, 24 (50%) had CTC levels above 1.5 cells/mL, and 24 individuals (50%) exhibited levels below or equal to the cut-off value.

Cancer differentiation showed a significant association with CTC levels (p = 0.005). Among individuals with well-differentiated tumors, 19 (39.6%) had CTC levels above 1.5 cells/mL, while 29 (60.4%) showed levels below or equal to this value. In the group with poorly differentiated tumors, 12 individuals (85.7%) were found to have CTC levels above 1.5 cells/mL, and 2 (14.3%) exhibited levels below or equal to 1.5 cells/mL.

There was no significant association between CEA and CTC levels (p = 1). Among individuals with CEA levels below 5, 15 (45.5%) had CTC levels above 1.5 cells/mL, while 18 (54.4%) indicated levels below or equal to the cut-off value. For those with CEA levels of 5 or above, 15 (46.9%) had CTC levels above 1.5 cells/mL, and 17 (53.1%) exhibited levels below or equal to 1.5 cells/mL.

The BMI did not show a significant association with CTC levels (p = 0.337). Among individuals classified as underweight, 13 (39.4%) had CTC levels above 1.5 cells/mL, while 11 (23.4%) were found to have levels below or equal to 1.5 cells/mL. In the normal weight group, 12 individuals (36.4%) had CTC levels above 1.5 cells/mL, and 14 (29.8%) exhibited levels below or equal to the cut-off value. Among individuals classified as overweight, 3 (9.1%) had CTC levels above 1.5 cells/mL, and 8 (17%) showd levels below or equal to this value. For individuals classified as obese 1 or 2, the proportion of those with CTC levels above 1.5 cells/mL was 12.1% and 3%, respectively, while 23.4% and 6.4% exhibited levels below or equal to the cut-off value.

Patients who had poorly differentiated CRC also had a higher number of CTC (>1.5 cells/mL) compared with those with well-differentiated CRC. Other variables did not have a statistically significant value with CTC value.

DISCUSSION

Isolation and Identification of CTC

This study employed various methods for the isolation and identification of CTC in CRC patients. CTC isolation methods used were size-based filtration and immunomagnetic separation. These methods have been widely used due to their ability to isolate rare CTC from a complex background of blood cells. After isolation, immunofluorescence staining was used to enhance the detection in the isolated samples.

Immunofluorescence staining played a crucial role in improving the identification of CTC within post-isolation samples.[21] The focus was directed towards the expression of CD44, a cell surface marker associated with cancer stem cells and metastasis.[22,23] Representative images of CTC exhibiting strong CD44 expression were observed, particularly in patients with stage III and IV CRC. A positive count of 2 CTC per field of view was considered indicative of CRC, indicating the diagnostic significance of CD44 expression in CTC.

Diagnostic Potential of CTC

The results obtained regarding the diagnostic potential of CTC in CRC were consistent with previous investigations. ROC analysis also yielded an AUC value of 72.5%, categorizing CTC as a good diagnostic tool.

Tsai et al.[19] reported an AUC value of 75.5% for CTC in diagnosing CRC, which was consistent with the results in this study. The focus of the study was primarily on CRC-clinically suspected patients. Baek et al. achieved a higher AUC value of 90.6%, while Yu et al. reported a value of 90.4%.[18,24] The higher AUC values in these two studies were attributed to the recruitment of CRC-already-diagnosed patients compared to controls, which may have influenced the results.

CTC Cut-Off Values and Diagnostic Performance

This study used a cut-off value of >1.5 cells/mL, which provided valuable insights into the diagnostic performance of CTC in CRC. With this cut-off, the values obtained included 50% sensitivity, 88.89% specificity, 93.94% PPV, 34.04% NPV, 4.5 PLR, and 0.56 NLR.

Compared to other studies, Tsai et al. reported a sensitivity of 63% and a specificity of 82% using a CTC cut-off value of >2 cells/2 mL for diagnosing CRC.[19] Baek et al. also recorded higher values with a cut-off value of ≥5 cells/7.5 mL, including sensitivity, specificity, PPV, and NPV of 75%, 100%, 100%, and 58.5%, respectively.[24] Moreover, Haijiao et al. achieved a sensitivity of 83.05% and specificity of 100% with a cut-off of 2 CTC/3.2 mL, and the combination of CTC with CEA increased the sensitivity to 91.53%.[18] These comparisons demonstrated the variability in cut-off values and their impact on diagnostic performance, influenced by factors such as subject recruitment and CTC analysis techniques.

Significance of CTC Levels in CRC

The results showed significantly higher CTC levels in CRC-diagnosed patients compared to polyp or non-CRC groups ($p = 0.003$). The mean ± SD CTC level in the CRC group was 1.97 ± 2.1 cells/mL, with a range (median) of 0-12 (1.5) cells/mL. The metastatic CRC (stage IV) exhibited higher CTC levels compared to non-metastatic, potentially due to late-stage presentation, although the difference was not statistically significant ($p = 0.153$).

Consistent with other studies, the results indicated significantly higher CTC values in CRC compared to normal and polyp groups ($p<0.001$). The linear regression analysis of CTC values across the progression from the normal group to polyps, non-metastatic, and metastatic CRC showed a statistically significant increase ($p = 0.001$).[19,24] These results reinforced the clinical relevance of CTC levels in CRC and their potential as a biomarker for disease progression.

Association of CTC with Tumor Differentiation

The results showed that subjects with poorly differentiated CRC had higher CTC values (>1.5 cells/mL) compared to those with the well-differentiated type, as also reported by previous studies.[19,20,25] The association between cellular differentiation in CRC and tumoral metastasis into the bloodstream suggests the potential role of CTC in reflecting the aggressiveness of the disease.

In general, this study reinforced the diagnostic potential of CTC, the significance of specific levels in distinguishing CRC from other conditions, and the association between CTC and tumor differentiation. The results provided valuable insights and emphasized the need for further studies to optimize the diagnostic utility of CTC in CRC.

Limitation and Prospects

Although this study demonstrated the diagnostic potential of CTC in CRC, several limitations affected the results. The small sample size and potential selection bias may limit the generalizability, necessitating larger studies to validate the results. Additionally, the variability in CTC isolation methods and analysis techniques across studies necessitates the need for standardized protocols to ensure comparability and reliability.

The lack of a specific marker for CRC among CTC remains a challenge. CD44 expression as a potential biomarker provides valuable insights, but further investigation is required to identify more specific and sensitive markers for improved CTC detection and characterization in CRC.

Future studies should explore the combination of CTC analysis with established biomarkers, such as CEA, to enhance diagnostic accuracy and predictive value in CRC. Integration of advanced technologies, including next-generation sequencing and liquid biopsy platforms, has great potential for unraveling the genomic and molecular characteristics of CTC, facilitating personalized treatment strategies and better monitoring of disease progression. These efforts will contribute to the advancement of CRC diagnosis and management.

CONCLUSION

This study showed the diagnostic potential of CTC in differentiating CRC cases from

inflammatory/hyperplastic polyps and adenomas. CTC identification, particularly through CD44 expression, provided valuable insights into the metastatic potential of these cells and their association with advanced stages of CRC. Despite limitations in sample size and the lack of a specific marker, the results contributed to the growing body of evidence supporting the use of CTC as a non-invasive diagnostic tool for CRC. Further investigations with larger cohorts, standardization of methods, as well as the exploration of additional biomarkers and advanced technologies would advance the field and enhance the accuracy of CTC-based CRC diagnosis and prognosis.

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COMPETING INTEREST

The authors declare no competing interests relevant to the content of this article.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Saskia Aziza Nursyirwan, Murdani Abdullah, Dimas Ramadhian Noor, Agustinus Wiraatmadja, Wifanto Saditya Jeo, Nur Rahadiani, and Diah Rini Handjari. The first draft of the manuscript was written by Saskia Aziza Nursyirwan, Andri Sanityoso Sulaiman, Ikhwan Rinaldi, Dadang Makmun, Marcellus Simadibrata, Agustinus Wiraatmadja, and Hamzah Shatri. All authors commented on previous versions of the manuscript as well as read and approved the final manuscript.

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Effect of Extra-Virgin Olive Oil on Hand Foot Syndrome and hs-CRP in Patients Receiving Capecitabine: A Randomized Trial

Yenny Dian Andayani[1,*], Mgs. Irsan Saleh[2], Aru Wisaksono Sudoyo[3], Zulkhair Ali[4], Legiran Legiran[5], Radiyati Umi Partan[6], Eddy Yuristo[7], Fadhyl Zuhry Lubis[7]

[1] Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Sriwijaya - Mohammad Hoesin General Hospital, Palembang, Indonesia.
[2] Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.
[3] Division of Haematology and Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[4] Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine Universitas Sriwijaya - Mohammad Hoesin General Hospital, Palembang, Indonesia.
[5] Department of Anatomy, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.
[6] Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine Universitas Sriwijaya - Mohammad Hoesin Hospital, Palembang, Indonesia.
[7] Department of Internal Medicine, Faculty of Medicine Universitas Sriwijaya - Mohammad Hoesin General Hospital, Palembang, Indonesia.

Corresponding Author:
Yenny Dian Andayani, MD. Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Sriwijaya - Mohammad Hoesin General Hospital. Jl. Jendral Sudirman KM 3.5, Palembang, Indonesia. Email: yennydianandayani@fk.unsri.ac.id.

ABSTRACT

Background: *Hand Foot Syndrome (HFS) is a frequent adverse effect observed in patients undergoing capecitabine chemotherapy, often leading to treatment disruptions and dose adjustments. Elevated C-Reactive Protein (hs-CRP) levels have been associated with the development of HFS. This study aimed to assess the potential of unrefined Extra Virgin Olive Oil (EVOO) supplementation in mitigating HFS and hs-CRP elevation among individuals receiving capecitabine chemotherapy.* ***Methods:*** *Between November 2022 and May 2023, forty-five eligible participants were enrolled in this randomized trial. Patients with advanced colorectal or breast cancer were randomly allocated into three groups: an intervention group receiving unrefined EVOO supplementation (30 mL per day) alongside capecitabine, a placebo group receiving refined extra light olive oil (ELOO) supplementation (30 mL per day) alongside capecitabine, and a control group receiving capecitabine alone. The masking of both placebo and intervention groups was ensured through identical packaging and instructions, maintaining participant and physician blindness to the assigned treatments. Randomization, achieved via computer-generated sequences, ensured even distribution among the three groups.* ***Results:*** *HFS incidences were notably lower in the EVOO group (13.3%) compared to the placebo (66.7%) and control (80%) groups. Incidence of Grade 2 or more severe HFS were observed in 20% of placebo and 40% of control group patients. No cases of severe HFS were reported in the EVOO group. Moreover, EVOO supplementation led to a significant reduction in hs-CRP levels when contrasted with the placebo and control groups. These findings suggest that EVOO may serve as a preventive measure*

against HFS and exhibit anti-inflammatory effects in patients undergoing capecitabine chemotherapy. ***Conclusion:*** *This study demonstrates the potential benefits of incorporating unrefined EVOO into the regimen of patients undergoing capecitabine chemotherapy. EVOO supplementation was associated with lower incidences of HFS and a reduction in hs-CRP levels, indicating its possible role in preventing HFS development and mitigating inflammation.*

Keywords: *Hand Foot Syndrome, C-Reactive Protein, Extra Virgin Olive Oil, Capecitabine.*

INTRODUCTION

Hand Foot Syndrome (HFS), also known as palmar-plantar erythrodysesthesia, is a common and debilitating side effect in cancer patients taking capecitabine, an oral fluoropyrimidine chemotherapy agent. HFS symptoms include erythema, swelling, pain, tingling, and, in severe cases, blistering and ulceration, primarily affecting the palms of the hands and soles of the feet.[1] This syndrome can have a significant impact on patients' daily activities, resulting in reduced functional capacity and a lower quality of life.[2] Furthermore, HFS has been linked to treatment interruptions and dose reductions, potentially jeopardizing the efficacy of capecitabine-based chemotherapy regimens.[3]

C-Reactive Protein (CRP), an acute-phase reactant and marker of systemic inflammation, has been implicated in the pathogenesis of HFS.[4] CRP is synthesized by the liver in response to various inflammatory stimuli, and elevated levels of CRP have been observed in patients with severe HFS.[5] The presence of inflammation in HFS suggests a complex interplay between chemotherapy-induced tissue damage, inflammatory processes, and subsequent clinical manifestations.[6] Understanding the role of CRP in HFS could provide valuable insights into the underlying mechanisms and potential targets for therapeutic intervention.

Extra Virgin Olive Oil (EVOO) is a key component of the Mediterranean diet and has gained recognition for its numerous health benefits. It is derived from the pressing of olives without the use of heat or chemical treatments. EVOO is high in monounsaturated fatty acids like oleic acid, as well as bioactive compounds like oleocanthal, which have antioxidant and anti-inflammatory properties.[7] These properties have been linked to a variety of health benefits, including inflammatory pathway modulation.[8]

Despite EVOO's potential anti-inflammatory properties, its role in preventing HFS and modulating CRP levels in capecitabine-treated patients is largely unknown. The effect of EVOO supplementation on HFS and CRP levels in this patient population could provide important insights into the potential benefits of this dietary intervention in mitigating chemotherapy-induced side effects and reducing inflammation. Current study aims to evaluate the impact of EVOO supplementation on the incidence of HFS and high sensitivity CRP (hsCRP) levels in patients receiving capecitabine chemotherapy.

METHODS

This controlled clinical trial employed a randomized, three-arm design to assess the effect of extra virgin olive oil (EVOO) supplementation on the incidence of Hand Foot Syndrome (HFS) and C-Reactive Protein (CRP) levels in patients receiving capecitabine chemotherapy. The study was conducted from November 2022 to May 2023. The study included patients diagnosed with advanced colorectal or advanced/metastatic breast cancer who were scheduled to undergo capecitabine-containing chemotherapy regimens. Each participant of the trial was asked the informed consent and was willing to participate voluntarily. Ethical considerations were carefully addressed throughout the study, and the trial was conducted in compliance with relevant ethical guidelines and regulations. The study protocol was approved by The Ethics Committee of the Mohammad Hoesin General Hospital (ID 06/ XVII.5.11/RSMH/2022).

Forty-five eligible participants were enrolled and randomly allocated into three groups: the intervention group receiving unrefined EVOO supplementation (30 mL per day) alongside capecitabine, the placebo group receiving refined extra light olive oil (ELOO) supplementation

(30 mL per day) alongside capecitabine, and the control group receiving capecitabine alone, with both the placebo and intervention groups being masked through the use of identical packaging and instructions to ensure participant's and physician's unawareness of the assigned treatment. Randomization was performed using a computer-generated randomization sequence, ensuring an equal distribution of participants among the three groups.

The follow-up period for each participant extended over two cycles of chemotherapy (six weeks). HFS incidence and severity were evaluated using the standardized National Cancer Institute (NCI) grading criteria as shown in table 1. The presence of HFS-related symptoms, such as erythema, swelling, pain, and blistering, were carefully assessed by board certified medical oncologists. The follow-up period was terminated shortly in participants whom severe HFS was developed and thus standard management was delivered. Additionally, high-sensitivity C-Reactive Protein (hsCRP) levels were measured as a marker of inflammation. Blood samples were collected at baseline and after two cycles of chemotherapy. The hsCRP levels were quantified using the enzyme-linked immunosorbent assay (ELISA) method. The hsCRP assay was conducted according to established protocols and quality control measures to ensure accurate and reliable results.

Data collected from the study participants were analysed using *SPSS for windows 26 version*. Descriptive statistics were used to summarize the characteristics of the participant groups, including demographic data, baseline HFS incidence, and hsCRP levels. Comparative analyses, such as chi-square tests and analysis of variance (ANOVA), were employed to evaluate the differences in HFS incidence and severity between the intervention groups (EVOO and ELOO) and the control group. Changes in hsCRP levels within and between the groups were assessed using *Wilcoxon-tests* and *Mann-Whitney tests*, respectively.

RESULTS

Between November 2022 and May 2023, a total of 45 participants were enrolled in the study, and they were evenly distributed into three groups: EVOO, ELOO, and the control group, with 15 participants in each group. These participants constituted the full analysis set. The baseline demographic characteristics of the participants were found to be well balanced, as shown in Table 2. The median age of the participants was 55, 52, and 54 years in the groups with and without pyridoxine, respectively. Approximately half of the participants in each group had not received any previous chemotherapy for advanced or metastatic colorectal/breast cancer, while about one-third of the participants in each group had received first-line chemotherapy.

The incidence of Hand Foot Syndrome (HFS) was evaluated among the three groups, revealing distinct differences. In the EVOO supplementation group, HFS developed in 2 out of 15 participants (13.3%), demonstrating a significantly lower occurrence compared to the placebo group receiving olive oil (ELOO) supplementation, where HFS was observed in 11 out of 15 participants (73.3%), and the control group receiving capecitabine alone, where HFS occurred in 12 out of 15 participants (80%). Moreover, Grade 2 or worse HFS was seen in 3 participants (20%) in the ELOO group, while 6 participants (40%) in the control group experienced the same severity level. Notably, none of the participants in the EVOO group developed severe HFS.

Table 1. National Cancer Institute Grading Criteria of Hand Foot Syndrome[9]

Severity	Grade	Clinical Domain
Mild/Moderate	1	Dermatitis-related skin changes (e.g., erythema, peeling) with altered sensations (e.g., tingling, numbness, burning) that are unrelated to interruption in daily tasks.
	2	Skin alterations presented with mild pain that has minimal impact on daily activities, and the skin surface remains undamaged.
Severe	3	Ulcerative dermatitis or skin changes characterized by severe pain that significantly hinders daily activities; visible tissue breakdown is evident, such as peeling, blisters, bleeding, and oedema.

Table 2. Characteristics of The Participants

	EVOO Group (n=15)	ELOO Group (n=15)	Control Group (n=15)
Median age (years) (range)	55 (29-62)	52 (31-67)	54 (30-70)
Sex, Male, n (%)	9 (60)	8 (55.7)	6 (40)
Cancer Diagnosis, n (%)			
Colorectal	12 (80)	12 (80)	13 (87)
Breast	3 (20)	3 (80)	2 (13)
Stage, n (%)			
III	3 (20)	3 (80)	2 (13)
IV	12 (80)	12 (80)	13 (87)
Chemotherapy Regimen, n (%)			
Monotherapy Capecitabine	4 (26.7)	3 (20)	3 (20)
Capecitabine + Other Drug(s)	10 (66.7)	11 (73.3)	12 (80)
Concomitant Radiotherapy	1 (6.7%)	1 (6.7%)	0
History of Previous Chemotherapy, n (%)			
Naïve	10 (66.7)	9 (60)	9 (60)
Previous Chemotherapy	5 (33.3)	6 (40)	6 (40)
ECOG Performance Scale, n (%)			
0	10 (66.7)	11 (73.3)	8 (53.3)
1	2 (13.3)	2 (13.3)	4 (26.7)
2	3 (20)	2 (13.3)	3 (20)
Comorbidities, n (%)			
Type 2 Diabetes	1 (6.7)	2 (13.3)	3(20)
Hypertension	2 (13.3)	2 (13.3)	2 (13.3)

Table 3. Difference in Incidence of Hand Foot Syndrome

Severity	Grade	EVOO (n=15), n (%)	ELOO (n=15), n (%)	Control (n=15), n (%)	p[1]	OR[2] (95%CI)	OR[3] (95%CI)
Mild/ Moderate	1	2 (13.3)	8 (53.3)	6 (40)	0.219	4.0 (0.72-22.04)	3.0 (0.51-17.31)
Severe	2/3	0	3 (20)	6 (40)	0.089	7.0 (0.33-147.17)	13 (0.67-251.22)
Total		2 (13.3)	11 (73.3)	12 (80)	0.042	5.5 (1.03-29.15)	6.0 (1.14-31.53)

[1]Chi Square Test, [2]EVOO compared to Control, [3]EVOO compared to ELOO

In addition to evaluating Hand Foot Syndrome (HFS) incidence, the study also assessed the levels of high sensitivity C-Reactive Protein (hsCRP) among the three groups. The results revealed significant differences in hsCRP changes. In the EVOO supplementation group, hsCRP levels were significantly decreased after the intervention, indicating a reduction in systemic inflammation. In contrast, the placebo group receiving olive oil (ELOO) supplementation & control group showed significant increase in hsCRP levels.

Table 4. hsCRP Before and After Intervention among Groups

hsCRP	EVOO (n=15)	ELOO (n=15)	Control (n=15)	p
Baseline (mg/L)	9.45 (0.20, 126.90)	8.80 (0.90, 115.20)	9.60 (1.30, 80.70)	0,850[1]
After Chemotherapy (mg/L)	2.75 (0.18, 8.60)	21.00 (3.00, 60.00)	44.10 (9.70, 118.00)	<0,0001[1]
p'	0.005[2]	0.0021[2]	0.001[2]	

[1]Kruskall Wallis Test, [2]Wilcoxon Test

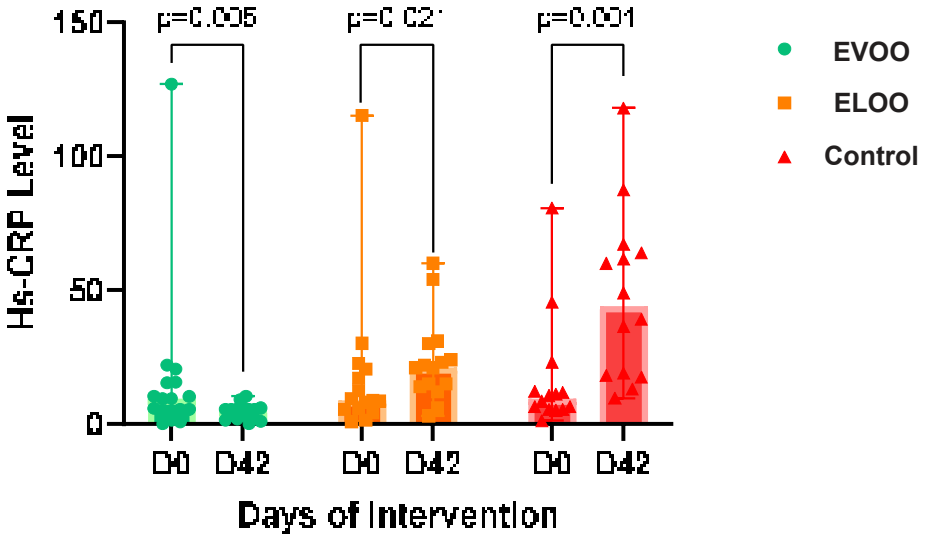

Figure 1. Bar Graph of serum Hs-CRP Level between intervention group.

DISCUSSION

The results of this clinical trial provide compelling evidence regarding the efficacy of extra virgin olive oil (EVOO) supplementation in reducing the risk of Hand Foot Syndrome (HFS) and modulating C-Reactive Protein (CRP) levels in patients receiving capecitabine chemotherapy. These findings contribute to our understanding of the potential benefits of EVOO as a dietary intervention in mitigating chemotherapy-induced side effects and inflammation.

The significant reduction in HFS incidence observed in the EVOO supplementation group compared to the refined olive oil (ELOO) supplementation group and the control group is noteworthy. HFS is a common and distressing adverse event associated with capecitabine, often leading to treatment interruptions and a decreased quality of life for patients.[10] Current study highlighted the potential preventive effect of EVOO supplementation on this side effect. Furthermore, the absence of severe HFS (Grade 2 or worse) in the EVOO group further supports the notion that EVOO may provide significant protection against the development of severe HFS.

The observed changes in CRP levels further reinforce the anti-inflammatory properties of EVOO. In the EVOO group, a significant decrease in hsCRP levels was observed after the intervention, indicating a reduction in systemic inflammation. In contrast, the ELOO group showed an insignificant increase in hsCRP levels, while the control group demonstrated a significant increase in hsCRP levels. The beneficial effects observed in the EVOO group can be attributed to the unique composition of EVOO, which is rich in monounsaturated fatty acids, particularly oleic acid, as well as bioactive compounds such as oleocanthal. Oleocanthal is a phenolic compound found in EVOO and is known for its potential health benefits.[11] It possesses strong anti-inflammatory properties and has been shown to exhibit similar effects to non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen.[12,13] Oleocanthal is believed to inhibit the activity of cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2, which are key enzymes involved in the production of inflammatory mediators called prostaglandins.[14,15] By blocking the activity of these enzymes, oleocanthal helps to reduce inflammation and alleviate related symptoms.

In the context of the present study, the potential presence of oleocanthal in the extra virgin olive oil used for supplementation could contri bute to the observed reduction in Hand Foot Syndrome (HFS) incidence and the modulation

of C-Reactive Protein (CRP) levels. However, more detailed investigations would be required to directly measure the levels of oleocanthal and its specific contribution to the outcomes of the trial. It is important to note that the ELOO supplementation group did not demonstrate significant benefits in preventing HFS or modulating CRP levels compared to the control group. This suggests that the specific components present in EVOO, such as oleocanthal, may play a crucial role in conferring the observed effects. The differences in outcomes between the EVOO and ELOO groups highlight the importance of using high-quality, unprocessed EVOO rather than other forms of olive oil in clinical interventions.

Despite the significant findings, several limitations should be considered. First, the sample size of this study was relatively small, which may limit the generalizability of the results. Further research with larger cohorts is needed to confirm the findings and assess the long-term effects of EVOO supplementation. Secondly, the mechanisms underlying the protective effects of EVOO on HFS and inflammation were not directly explored in this study. Further investigations are warranted to elucidate the specific pathways involved and to explore potential synergistic effects with capecitabine.

CONCLUSION

The study demonstrates that EVOO supplementation significantly reduces the incidence of HFS and decreases hs-CRP levels in patients receiving capecitabine chemotherapy.

CONTRIBUTION TO THE LITERATURE

This article significantly contributes to the current literature by being the first investigating the impact of non-refined Extra Virgin Olive Oil (EVOO) supplementation on Hand Foot Syndrome (HFS) and C-Reactive Protein (hs-CRP) levels in patients receiving capecitabine chemotherapy. The uniqueness lies in the focus on EVOO's potential preventive effect on HFS, a common and distressing side effect of capecitabine. Moreover, the study employs a randomized controlled clinical trial design, providing robust evidence for the potential benefits of EVOO supplementation. The observed reduction in hs-CRP levels further underscores EVOO's anti-inflammatory properties, which are crucial in the context of capecitabine-induced inflammation. Overall, this work enhances scholarship by offering valuable insights into a novel intervention that may improve patients' quality of life and treatment tolerability in the context of capecitabine chemotherapy.

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Risk Factors for Declined Functional Status within 30 days After Elective Surgeries in Elderly Patients: A Prospective Cohort Study

Yudo Murti Mupangati[1,2,3], Henry Setyawan[4], Czeresna Heriawan Soejono[5], Muhammad Hussein Gasem[6], Ignatius Riwanto[3,7]*

[1]Doctoral Study Program of Medical and Health Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.
[2]Division of Geriatric, Department of Internal Medicine, Dr. Kariadi Hospital, Semarang, Indonesia.
[3]Faculty of Medicine, Diponegoro University, Semarang, Indonesia.
[4]Epidemiology Master Study Program - School of Public Health, Diponegoro University, Semarang, Indonesia.
[5]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[6] Center for Tropical and Infectious Diseases, Dr. Kariadi Hospital and Diponegoro University, Semarang, Indonesia.
[7]Indonesian Digestive Surgeon Association, Indonesia.

Corresponding Author:
Yudo Murti Mupangati, MD. Division of Geriatric, Department of Internal Medicine, Faculty of Medicine, Diponegoro University - Dr. Kariadi Hospital, Semarang, Indonesia. Jl Dr Sutomo 18 Semarang 50244, Indonesia. Email yudomurtidisertasi@gmail.com. Orchid ID: 0000-0002-0163-2312

ABSTRACT

Background: *Older adults are at risk of decreasing functional status due to their condition and many factors. Although many studies have been conducted about declining in functional status, based on the author's knowledge, only this study that has conducted about functional status changes in the elderly involving the frailty status which undergoing surgery in Indonesia. There are many factor was postulated, some of that was checked routine and applicable in clinical practice. Furthermore, identification of these risk factors can be used a basis for decision making to perform surgeries in older adults because poor functional status causes declining quality of life in the elderly patients. The aim of this research was to determine the risk factors for declined functional status within 30 days after elective surgeries in elderly patients.* ***Methods:*** *We conducted a prospective cohort study from July 2021 to December 2021 at Dr. Kariadi Hospital, Semarang, Indonesia. We included patients aged 60 or older who underwent elective surgery under general anesthesia. We excluded those who underwent emergency surgery, day care surgery, or were unwillingness to participate. The functional status were assessed using the ADL (Activity of Daily Living) Barthel index. To identify risk factors of declined ADL scores, a logistic regression analysis was performed on the age variable, gender, body mass index, frailty status, postoperative complications, as well as haemoglobin, and albumin levels.* ***Results:*** *This study included 191 participants, with 97 women (50.79%) and 94 men (42.21%). Declined in functional status within 30-days after surgery occurred in 54 participants (28.2%). There was a significant changed of functional status before and after surgery. Multivariate analysis showed that independently significant variables for declined functional status were male sex (OR 4.48, p value < 0.001), hypoalbuminemia (OR 2.59, p value 0.02), preoperative functional status (OR 2.37; p value 0.05), and postoperative complications (OR 24.885; p value < 0.001).* ***Conclusion****: Risk factors for declined functional status within 30 days after elective surgery in older patients are postoperative complications, preoperative functional status, hypoalbuminemia, and male gender.*

Keywords*: ADL, Barthel index, older adults, surgery.*

INTRODUCTION

The growing number of older adults in a population is a public health concern, knowing that more older adults require surgical interventions.[1]According to the Indonesian Central Statistics Agency (BPS), life expectancy of Indonesian older population has increased from 70.1 years in 2010-2015 to 72.2 years in 2030-2035.[2] Increasing life expectancy rises several medical conditions that require surgeries in older patients such as arteriosclerosis, cancer, arthritis, prostatism, and fractures. Aging per se is a physiological process results in structural changes and decrement in functional capacities of organs over time.[3] In older age, there is increased use of physiologic reserves just to maintain homeostasis. Immune competence decline with advancing age, include immunosenescence, decreased production of naïve T cell , and inflamaging. When the body is stressed, fewer reserves are available to meet the challenge.[4]

Surgical procedures are considered as stressors for older patients. Healthy older adults have sufficient reserves that are needed in normal circumstances. However, if they have to undergo surgeries, their bodies must compensate by maximizing their current reserves (homeostasis).[5]

Older people are at risk of decreasing functional status due to their condition and many factors, which one of them is iatrogenesis caused by drugs used in anesthesia during surgery or drugs given after surgery.[6,7] Although many studies have been conducted about declining in functional status, based on the author's knowledge, only this study that has conducted about functional status changes in the older adults involving the frailty status which undergoing surgery in Indonesia. There are many factor was postulated, some of that was checked routine and applicable in clinical practice. Furthermore, identification of these risk factors can be used a basis for decision making to perform surgeries in the older patients because poor functional status causes declining quality of life in the older patients.[8] We can optimize the patient's condition before surgery so that we get better functional status after surgery.

The aim of this research was to identify the factors that affect declined functional status in older people after elective surgery.

METHODS

Study Participants

We conducted a prospective cohort study among 193 inpatients aged 60 years or older who underwent elective surgeries at Dr. Kariadi Hospital Semarang from June 2021 to December 2021. The sample was consecutively collected using inclusion criteria of age ≥ 60 years, admission for elective surgery, and being given general anestesia. Subjects, who were given local anesthesia or regional anesthesia or who were unwilling to participate, were excluded from the study. Two patients were dropped out because they had psychological problems. There were 191 participants who completed the study. (**Figure 1**) Potensial confounding factors were patient age and type of surgical.

Data Collection

Data on the patient's age, gender, occupation, education, and marital status was obtained from the medical record. Concurrently, two investigators collected preoperative functional status data at the same time. Two internal medicine specialists assessed functional status evaluation before elective surgery using the Barthel index. The Barthel index measures functions status as a parameter of dependency on daily functions. The Barthel index measures ten important functions for being independent, including: eating, bathing, self-care, dressing, urinating, defecating, using the toilet, transferring, mobilizing, and stairs climbing. Patients were followed up within 30 days after surgeries, and their functional status were reassessed by WhatsApp or phone calls to their family or caregivers. Patients who deceased were assumed as patients with high dependency status. We analyzed data functional status before and after surgery with wilcoxon test.

Patients who had decline functional status were analyzed based on the variables of age, gender, body mass index, frailty status, preoperative functional status, surgical complications, haemoglobin levels and albumin levels. Frailty status is defined as a multi-dimensional state resulted from decreased physiological reserves

resulting in reduced resilience, loss of adaptive capacity, and increased vulnerability to stressors. In this study, we used the Clinical Frailty Scale instrument which consists of nine components, then we divide the patients into two groups. Patients that are very fit, well, and managing well grouped into the non-frail group, and patients that are vulnerable, mildly frail, moderately frail, severely frail, and very severely frail components into the frail group. Post-operative complications are defined as undesirable and unexpected consequences that arise after an operation.[9] In this study, post operative complication was limited to whether or not complications that arose. Anemia is defined as a hemoglobin level below 11.1 G/dl, because in our laboratory we use a normal value of 11.1 G/dl and is based on the WHO definition that anemia is moderate if Hb < 11 G/dl in men or women.[10]

The ethical clearance of this study was approved by KEPK Dr. Kariadi Hospital Semarang (No. 842/EC/KEPK-RSDK/2021). Informed consents were obtained from patients who participated in this study. The confidentiality of patients was fully respected during data collection for manuscript preparation.

Statistical Analysis

Categorical variables are presented in numbers and percentages. Continuous variables are represented as mean and standard deviation, or median and minimum-maximum in non-normally distributed data. We used either Chi Square or Fisher Test to perform bivariate analysis among who experienced decline functional status. The bivariate analysis was carried out on the variable age, sex, BMI (Body Mass Index), vulnerable status, surgical complications, haemoglobin level, and albumin level. The bivariate analysis results with p-value < 0.25 were then included in the multivariate analysis using logistic regression. Multicollinearity analysis and variant inflation factor (VIF) were performed on the independent variables. All statistical analysis were carried out using SPSS. The p value less than 0.05 was considered statistically significant.

RESULTS

Among 191 study participants, 33 (17.3%) of them deceased within thirty days after surgeries. Participants who deceased were assumed to have high dependencies status. We compare ADL scores of participants, before and thirty days after surgeries. The number of participants who had preserved, decreased, and increased ADL scores were 113, 54, and 24 participants respectively. It was show in flowchart on **Figure 1**.

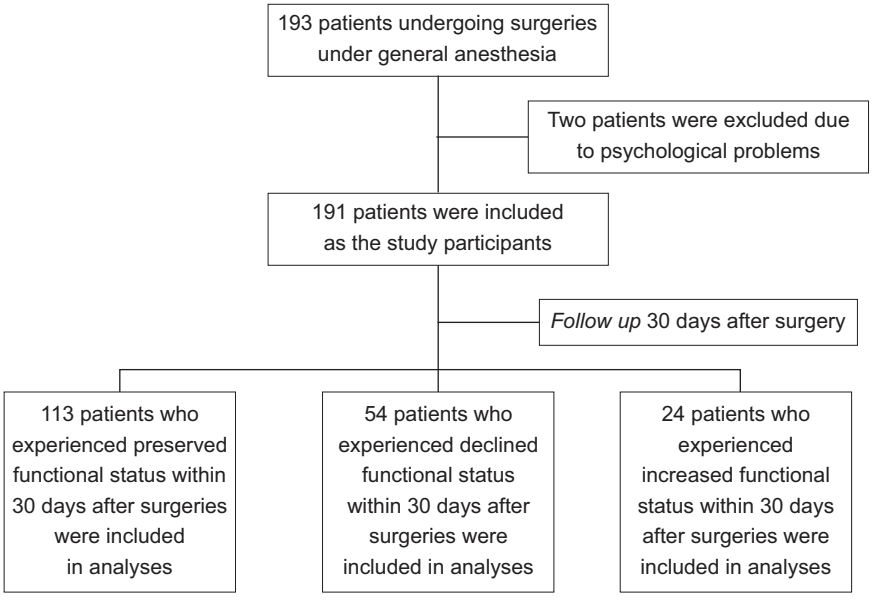

Figure 1. Flowchart from patient selection to analyses

There was a significant changed of functional status before surgery (high dependency 11 %; moderate dependency 14.1 %; mild dependency 15.2 %; independence 59.1 %) and after surgery (high dependency 25.7 %; moderate dependency 7.9 %; mild dependency 11 %; independent 55.5 %). Data before and after surgery were analyzed with wilcoxon test (p value < 0.001).

Table 1. Baseline Characteristics of Study Participants.

Characteristics	(n=191)
Age	
median (min-max)	66 (60-87)
BMI	
median (min-max)	21.64 (12.64-42.97)
Gender (n, %)	
Men	94 (49.2)
Women	97 (50.8)
Ethnic Group (n, %)	
Java	174 (91.1)
Others	17 (8.9)
Marital Status (n, %)	
Married	161(84.3)
Widow or widower	30 (15.7)
Education (n,%)	
Higher education	15 (7.9)
Senior High School	61 (31.9)
Junior High School	18 (9.4)
Elementary School	76 (39.8)
Not graduated from elementary school	3 (1.6)
No formal education	18 (9.4)
Employment (n, %)	
Government employees	9 (4.7)
Private employees	128 (67)
Unemployedes	54 (28.3)
Type Surgery (n, %)	
Uncomplicated head and neck	51 (26.7)
Endoscopic	44 (23)
Intra-abdominal	32 (16.8)
Related orthopedic	24 (12.6)
Extensive intra-abdominal or intrathoracic with large fluid or blood loss	15 (7.9)
Hip	6 (3.1)
Prostate	5 (2.6)
superficial	5 (2.6)
Major or peripheral vascular	5 (2.6)
Thoracic	4 (2.1)
Comorbidity component (n,%)	
Solid tumor	95 (49,7)
Diabetes mellitus	41 (21,5)
Congestive heart disease	14 (7,3)
Liver disease	12 (6,3)
Myocardial infarction	8 (4,2)
Cerebrovascular disease	6 (3,2)
Chronic kidney disease	4 (2,1)
Hemiplegi	3 (1,6)
Chronic obstuctive pulmonary disease	2 (1)
Peptic ulcer disease	2 (1)
Dementia	1 (0,5)
Peripheral vascular disease	1 (0,5)

Information: BMI (Body Mass Index); n, number of samples; min (minimum); max (maximum)

Table 2. Bivariate Analysis of the Variables Contributed to Declined Functional Status Participants

Variables	Declined Functional Status, n (%)	Preserved Functional Status, n (%)	p-value	OR (95% CI)
Age				
≥ 80 years	5 (71.4)	2 (28.6)	0.031*	6.88 (1.294-36.665)
< 80 years	49 (26.6)	135 (73.4)		
Gender				
Men	37 (39.4)	57 (60.6)	0.001*	3.055 (1.567-5.953)
Women	17 (17.5)	80 (82.5)		
BMI				
Underweight	10 (31.3)	22 (68.8)	0.846*	1.188 (0.521-2.709)
Non Underweight	44 (27.7)	115 (72.3)		
Frailty Status				
Frail	33 (28.7)	82 (71.3)	1.000*	1.054 (0.553-2.009)
Fit or pre-frail	21 (27.6)	55 (72.4)		
Having Postoperative Complication				
Yes	24 (82.8)	5 (17.2)	0.00*	21.12 (7.451-59.86)
No	30 (18.5)	132 (81.5)		
Haemoglobin level				
Anemia (<11,1 G/dl)	30 (34.5)	57 (65.5)	0.114*	1.754 (0.929-3.311)
No Anemia (≥ 11,1 G/dl)	24 (23.1)	80 (76.9)		
Albumin level				
< 3,5 G/dl	38 (39.2)	59 (60.8)	0.001*	3,140 (1.599-6.166)
≥ 3,5 G/dl	16 (17.0)	78 (83.0)		
Preoperative ADL scores				
Dependency (0-49)	23 (47.9)	25 (52.1)	0.001*	3.324(1.664-6.640)
Not Dependency	31 (21.7)	112(78.3)		

*Chi-square test, BMI (Body Mass Index)

Table 2 describes the multivariate analysis of this study. Variables that have p-values <0.25 were included. They were age, gender, postoperative complications, haemoglobin levels, albumin levels, and preoperative ADL score. **Table 3** shows the results of the multivariate analysis of variables which contributed to the declined functional status, as presented by lower ADL score.

DISCUSSION

In this study, 17.3% of the study participants deceased. This proportion is higher than the study conducted by Amemiya et.al, with a mortality rate of 0.4%. This disparity could be attributed to different characteristics of study participants, since Amemiya et.al conducted their study focused on inpatients with gastric and colorectal cancer, longer life expectancy in Japanese population than Indonesian, and there is a standard operating procedure for gastric and colorectal cancer surgery, namely resection of the primary tumor and involved lymphadenopathy in Japan.[11]

In this study, the functional outcome was worsened in 54 participants (28.27%) and better in 24 participants (12.57%) 30 days after surgery. There was a significant changed

Table 3. Multivariate Analysis of Variables Contributing to Decline.

	Coefficient	S.E	Wald	df	p-value	OR	95% CI	
							minimum	maximum
Having postoperative complication	3.088	.575	28.878	1	.000*	21.398	7.113	67.664
Preoperative ADL scores	0.862	.439	3.857	1	.050	2.369	1.002	5.601
Albumin < 3.5 G/dL	0.953	.436	4.772	1	.029*	2.593	1.103	6.097
Hb < 11.1 G/dl	.766	.421	3.314	1	.069	2.151	.943	4.906
Male gender	1.500	.439	11.678	1	.001*	4.480	1.896	10.590
Constant	-3.533	.564	39.270	1	.000*	.029		

of functional status before and after surgery. This findings are in line with a study conducted by Kwon et.al which also found a significant decline in functional status. Declined functional status is common in older adults who undergo surgeries.[12] The expected general functional outcome in this study is better post-operative ADL score compared to preoperative ADL score, or a return to normal functional status before having illness. Good functional status, allows the patients to be more independent. Measurement of functional outcomes plays a significant role in the management of older patients. To date, measurement of functional status is not routinely assessed in older patients who will undergo a surgery.[5,13]

In this study, preoperative ADL components were based on the Barthel index (eating, bathing, self-care, dressing, urinating, defecating, toileting, transferring, mobility, and stair climbing). One of the strengths of Barthel's Index compared to Katz's is that it describes disabilities more clearly and can detect functional changes in more detail.[14,15] The Barthel index is also a recommended functional capacity test in older patients.[16]

In this study, patients who had declined ADL scores appeared to be older, than those who did not have declined ADL scores. Old age is a physiologic process where structure and functional capacity of organ and tissue progressively degenerate over time. The human body has the capability to compesate for age-related changes to some extent, but older people have a limited physiologic reserve that can become decline ADL on application of stressor.[3]According to Hairi et al.'s research, old age is the variable associated with limitations in functional status. De Vasconcelos Torres' research also indicates an increase in dependency on ADL as one gets older.[14,17,18]

Data on the characteristics of participants showed women are less likely to have decreased ADL score compared to men participants (p-value <0.001). This might be caused by beneficial immunomodulatory effect of estrogen. Estrogen has been proved to regulate neutrophil number and function, and the production of chemokines such as monocyte chemoattractant protein (MCP)-a and cytokines including tumor necrosis factor (TNF)-α, interlukin (IL)-6 and IL-1β.[19,20] This aligns with research conducted by Nicole Veronese and friends regarding research to find association between gender and frailty in older patients.[21]

In this study, we found that male gender, hypoalbuminemia, anemia, postoperative complications, and dependence preoperative functional level are all variables that contribute to the declined functional status after surgery. In Kwon's study, male sex was likewise associated with declined functional status within one month after surgery, with an OR of 3.05 (95% CI 1.41-6.58).[12] Albumin level less than 3.5 g/dl has an effect on declined functional status after surgery with OR 2.59 (95% CI: 1.103-6.097) and p-value = 0.029. Research conducted by Hsu et.al also found that albumin level was associated with to functional status after surgery with p<0.001.[22] This study found that anemia affected the decline in functional status after surgery with OR 2.151 (95% CI 0.943-4.906). This is in accordance with a study conducted by Pennix et.al which showed that the decline in physical performance was higher in older patients with anemia compared to those without anemia, 2.3% vs 1.4% respectively (p=0.003).[23,24] Surgical complications were one of the causes of declined functional status with OR 21.39 (95% CI 7.113-67.664, p <0.001). The results are accordance with a study conducted by Zhang et.al on the decline in functional status thirty days after surgery, especially postoperative delirium with OR 2.20 (95% CI 1.60-3.02, P<0.001).[25] Preoperative ADL score lowered postoperative ADL score by OR 3.324 (95% CI 1.664-6.640, p < 0.001). These findings are remarkably similar to those of Finlayson's study in older patients undergoing colon cancer surgery, which found that preoperative functional status affected postoperative functional status. (ARR: 1.21, 95% CI 1.11-1.32).[26] Greer's study yielded the same results, with an RR of 0.55 (95% CI 0.36-0.74).[27]

Hypoalbumin plays important role in decreasing functional status after surgery because it is an indicator of malnutrition which plays a direct protective role through several biological mechanisms (such as molecular transport and the

main contributor in regulating colloid osmotic oncotic pressure), and plays an important role in the physiology of homeostasis.[28,29] Preoperative anemia plays a role in decreasing functional status through their influence on on increasing cardiac events, infectious complications, respiratory failure, kidneys, and central nervous system including the incidence of dilirium.[10,30] In this study, frailty was not significantly associated with decreased functional status. The possible explanation is frailty measured in an acute setting, so that the frailty score obtained may be higher due to effects that precede the disease (such as infection, new drugs).[31]

The age variable was significant in the bivariate analysis, but it was not significant in the multivariate analysis. There was no collinearity in the independent variables based on the tolerance test results for each variable which was larger than 0.4, and the VIF value was less than 10. Surgical complications are the most significant risk factor for declined ADL. This finding emphasizes the importance of joint action between geriatricians with the surgeons to identify the risk factors for surgical complications, improve the modifiable risk factors before surgeries, and warn the surgeons to perform the surgeries carefully to avoid these complications. The limitations of this study did not conduct analysis post-operative complications based on the severity of the complications and did not determine the time of decline in functional status. Future research needed to analize the time in declining functional status which can be used in survival analysis (Cox regression).

CONCLUSION

Functional status is a parameter that can be used in patients undergoing surgery. Patients who have independent or mild dependent status may be advised to undergo surgery, whereas patients with moderate and severely dependent functional status may be advised to choose palliative treatment. Before surgery, the optimization of albumin and haemoglobin levels was necessary in older patients. Coordination with surgeons who will perform surgeries in older patients is crucial in identifying and correcting risk factors for post-surgical complications and improving good surgical techniques to reduce the incidence of complications.

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DISCLOSURE STATEMENTS

The authors declare no conflict of interest.

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Effectiveness of Internet-Based Group Supportive Psychotherapy on Psychic and Somatic Symptoms, Neutrophil-Lymphocyte Ratio, and Heart Rate Variability in Post COVID-19 Syndrome Patients

Hamzah Shatri[1*], Dika Iyona Sinulingga[2], Cleopas Martin Rumende[3], Siti Setiati[4], Rudi Putranto[1], Eka Ginanjar[5], Iris Rengganis[6], Em Yunir[7], Raden Irawati Ismail[8], Petrin Redayani Lukman[8]

[1]Division of Pyschosomatic and Palliative, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2]Psychosomatic and Palliative Subspecialty Education Programs, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[3]Division of Respirology and Critical Care Medicine, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[4]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[5]Division of Cardiovascular, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[6]Division of Allergy and Immunology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[7]Division of Endocrinology and Metabolic, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[8]Department of Psychiatry, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding author:
Hamzah Shatri, MD, PhD. Division of Psychosomatic and Palliative, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl Diponegoro no. 71, Jakarta 10430, Indonesia. Email: hshatri@yahoo.com; psikosomatik.paliatif@gmail.com.

ABSTRACT

Background: *COVID-19 can have serious long term health consequences, which is called Post-COVID-19 Syndrome (PCS). Currently, the available evidence and understanding of PCS management is limited. Because one of the symptoms of PCS is associated to psychological symptoms, psychotherapy is believed to have a role in the management of PCS. This study aimed to identify the effectiveness of supportive psychotherapy in PCS patients at Cipto Mangunkusumo National General Hospital.* ***Methods:*** *This study was a single blind randomized clinical trial using a pre-and post-test with control group study design. Participants were randomly divided into two groups: a psychotherapy group with 40 participants and an education group with 37 participants. Each group was given internet-based psychotherapy or education three times a week in a form of group consisting of 6-8 participants. Symptom Checklist-90 questionnaire was used to evaluate somatic and psychological symptoms. Heart rate variability and neutrophil lymphocyte ratio were also investigated. Data analysis was performed using the independent T test.* ***Results:*** *An improvement in the SCL-90 score was found to be 17.51 (SD 30.52) in the psychotherapy group and 19.79 (SD 35.10) in the education group, although there was no significant*

difference between the two groups (p = 0.771). There was no significant difference between the two groups in decreasing NLR (p = 0.178) and improving HRV (p = 0.560). ***Conclusion:*** *Both internet-based group supportive psychotherapy and education improved psychological and somatic symptoms in PCS patients, although there was no significant difference between the two groups. There was no significant difference between the two groups in decreasing NLR and improving HRV. Suggestions for further research regarding adding frequency of internet-based group psychotherapy in PCS patients and held in the morning to achieve more optimal results.*

Keywords: *psychosomatic disorder, post COVID syndrome, internet-based group supportive psychotherapy, neutrophil lymphocyte ratio, heart rate variability.*

INTRODUCTION

COVID-19 caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) can have serious long term health consequences, which is called Post COVID-19 Syndrome (PCS) or Long COVID.[1] PCS is defined as disease that occurs in individuals with a confirmed or probable history of COVID-19, usually within 3 months of diagnosis, with symptoms and effects that last for at least 2 months and cannot be explained by other alternative diagnoses. Common symptoms include fatigue, shortness of breath, cognitive dysfunction, as well as other symptoms, which generally have impacts on daily functioning. Symptoms may be new-onset, after initial recovery from an acute COVID-19 episode, or persist from the onset of the illness. Symptoms may also fluctuate, or recur over time.[2] The incidence of PCS is estimated at 10-35%, while those who were hospitalized can reach to 85%.[1]

Patients with PCS experience persistence variety of physical and psychological symptoms.[3] Fatigue is the most commonly reported symptom in 1.5-72% of post COVID cases while psychological problems can affect up to 26%. Fatigue persisting for about 3 months post-infection appears to be associated with moderate to severe depression and a worsening quality of life.[4] Houbon Wilke et al also reported that 37.2% of patients with confirmed COVID-19 developed Post Traumatic Stress Disorder (PTSD) at 3 months follow-up, and remained high (26.8%) at 6 months follow-up.[5] Therefore PCS patients are often associated with psychosomatic disorders.

PCS must be managed appropriately, because in addition to disrupting the quality of life of patients and their families, PCS also burdens health system.[6] Various treatment methods have been developed to treat PCS. Currently, the available evidence and understanding of PCS management is limited. Because one of the symptoms of PCS is associated to psychological symptoms, psychotherapy as a psychological intervention is believed to have a role in the management of PCS.[7]

Internet-based psychotherapy is a new breakthrough that is increasingly being used in this pandemic era, because it is more efficient and saves time and is not constrained by distance. Internet-based group psychotherapy is still not widely employed, so further research is still needed. This study sought to evaluate the impact of supportive psychotherapy treatment delivered in the form of groups (three times per week for 1-2 hours) via internet-based teleconsultation in PCS cases. The impact was evaluated using a brief follow-up period of 1-2 days following the third session.

METHODS

This study was a single-blind randomized clinical study using a before-after intervention with control group to evaluate the effectiveness of supportive psychotherapy in PCS patients at Cipto Mangunkusumo National General Hospital during December 2022 to March 2023. The inclusion criteria were patients aged > 18 years; confirmed positive for SARS CoV-2 through molecular RT PCR examination 3 months before recruitment, with symptoms and effects that last for at least 2 months and cannot be explained by other alternative diagnoses; patients with or without comorbidities; patients can communicate and are willing to be interviewed, filling out questionnaires and psychotherapy. Exclusion criteria were psychotic patients and unable to access the internet. Subjects were divided into

2 groups: intervention group who received psychotherapy and control group who received education. The psychotherapy group consisted of 40 people, while the education group consisted of 37 people.

In this study, psychological and somatic symptoms were examined using the SCL-90 questionnaire. We also examined Neutrophil Lymphocyte Ratio (NLR) using blood tests, and Heart Rate Variability (HRV) using the Standard Deviation Normal to Normal (SDNN) index. Examinations were carried out 1-2 days before and after the intervention. In addition, demographic characteristics were also taken. Supportive psychotherapy and education were carried out in groups consisting of 6 – 8 people for 1-2 hours per session. Psychotherapy and education were carried out in 3 sessions in 1 week using zoom application. Psychotherapy and education were conducted by two different people with the same expertise, although no prior analysis was done. The psychotherapy provided was held by the researcher and also supervised by psychosomatic expert and psychiatrists. Supportive psychotherapy was given using a module whose Intellectual Property Rights has been registered with the number EC00202170140.

This study has received Ethical Clearance from the Faculty of Medicine – University of Indonesia Research Ethics Committee - RSCM with the number KET-1233/UN2.F1/ETIK/PPM.00.02/2022. This study was also registered in the clinical trials database registration at www.clinicaltrials.gov under the number: NCT05648123.

RESULTS

There were 96 subjects who were first recruited. After screening, 19 subjects were excluded because they refused to participate, could not communicate properly and could not access zoom application. The 77 subjects then were randomly divided into two groups: 40 subjects received psychotherapy and 37 subjects received education. Subjects who successfully completed the study to the end were 37 subjects in the psychotherapy group and 34 subjects in the education group. Three subjects in each group did not complete the study. This study used per protocol analysis.

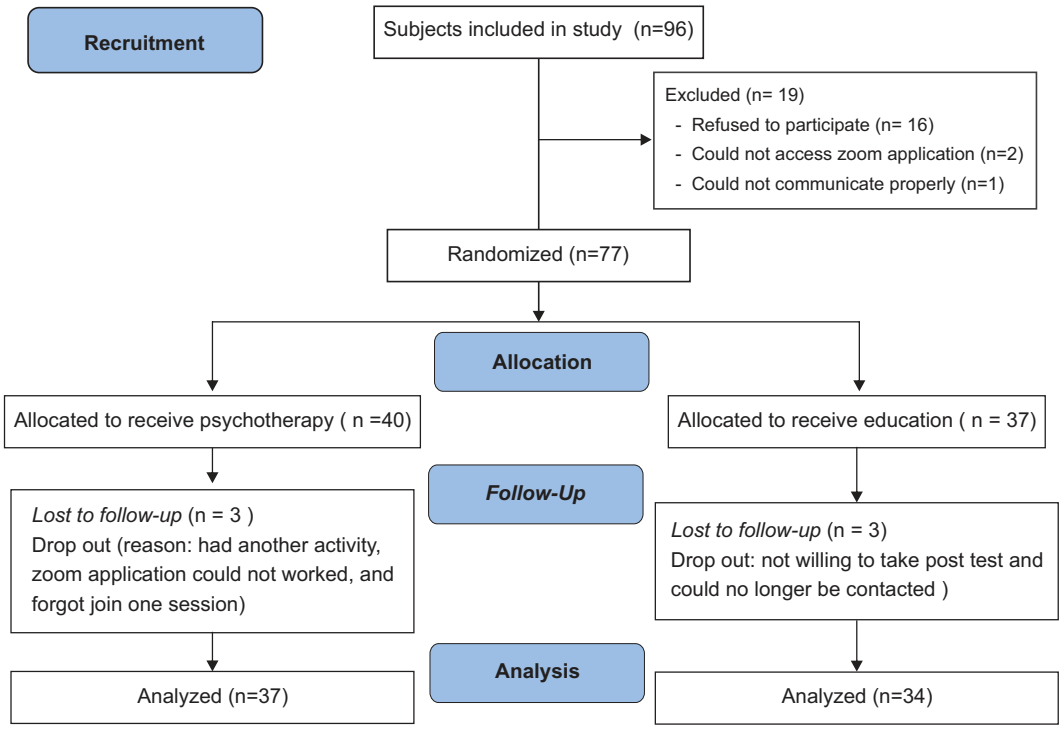

Figure 1. Flow of Subjects Recruitment.

The psychotherapy group has a median age of 43 years, whereas the education group has a median age of 33 years (**Table 1**). The study's participants are predominantly female, accounting for 76% of the sample overall. The majority of subjects work in health care (16.9%) or as housewives. The fourth vaccination showed the highest history rate at 46.8%. Furthermore, 77.9% of patients did not require hospitalization.

The psychotherapy and education groups had a median NLR of 2.06 and 1.825, respectively. The psychotherapy group had a median SCL-90 score of 76, while the education group had a median score of 99. The median HRV for the psychotherapy group was 29.88, whereas for the education group it was 40.66. Both groups had a high prevalence of 62.2% (psychotherapy), and 61.8% (education) of subjects with normal HRV. This research identified that the distribution of subjects was nearly equal between those with comorbidities and those without. Subjects with comorbidities accounted for 45.5%, and subjects without accounted for 54.5%. Hypertension was the most common comorbid condition, representing 26%. Mild severity of COVID-19 was the most frequently observed, accounting for 75.3%.

In the independent T-test carried out to assess the pre-test and post-test differences between both groups, an improvement in the SCL-90 score of 17.51 (SD 30.52) was observed in the psychotherapy group and 19.79 (SD 35.11) in the education group, with a p-value of 0.771 (**Table 2**). While the effect on NLR showed

Table 1. Basic Characteristic of Participants

Characteristic	Psychotherapy (n=40)	Education (n=37)
Age (year), median (IQR)	**43 (29.25 – 56.0)**	**33 (28 – 43.5)**
Gender, n (%)		
- Male	11 (27.5)	7 (18.9)
- Female	29 (72.5)	30 (81.1)
Occupation, n (%)		
- Housewives	9 (22.5)	4 (10.8)
- Healthcare workers	9 (22.5)	4 (10.8)
- Retired civil servants	6 (15)	2 (5.4)
- Pharmacists	5 (12.5)	7 (18.9)
- Private employees	4 (10)	8 (21.6)
- Honorary employees	3 (7.5)	2 (5.4)
- Entrepreneurs	0 (0)	7 (18.9)
- Government employees	1 (2.5)	3 (8.1)
- Not yet working	2 (5)	2 (5)
- Others	1 (2.5)	2 (5.4)
Vaccination History, n (%)		
- Not vaccinated	5 (12.5)	3 (8.1)
- First dose	0 (0.0)	1 (2.7)
- Second dose	3 (8.1)	6 (16.2)
- Third dose	12 (30.0)	11 (29.7)
- Fourth dose	20 (50.0)	16 (43.2)
Time symptoms persisted after COVID-19 infection, n (%)		
- > 3 months – 6 months	16 (40.0)	13 (35.1)
- > 6 months - < 1 year	9 (22.5)	8 (21.6)
- ≥ 1 year	15 (37.5)	16 (43.2)
Hospitalized, n (%)		
- Yes	9 (22.5)	8 (27.5)
- No	31 (77.5)	29 (78.4)
Neutrophil Lymphocyte Ratio (median, IQR)	2.06 (1.485 – 3.125)	1.825 (1.402 – 2.445)
SCL-90 score (median, IQR)	76 (48.25 – 105)	99 (69 – 133)
SCL-90 score, n (%)		
- ≥ 61	25 (62.5)	29 (78.4)
- < 61	15 (37.5)	8 (21.5)
Heart Rate Variability (median, IQR)	29,88 (22.27 – 60.49)	40.66 (21.44 – 49.82)
Heart Rate Variability, n (%)		
- Normal	23 (62.2)	21 (61.8)
- Low	14 (37.8)	13 (38.2)

Comorbid, n (%)		
- Yes	18 (45.0)	17 (45.9)
- No	22 (55.0)	20 (54.1)
Hipertension, n (%)		
- Yes	10 (25)	10 (27)
- No	30 (75)	27 (73)
Severity of COVID-19, n (%)		
- No symptom	3 (5.0)	1 (2.7)
- Mild	29 (72.5)	29 (78.4)
- Moderate	9 (22.5)	7 (18.9)
PCS symptoms, n (%)		
- Psychic symptoms		
• Anxiety	10 (25)	9 (24.3)
• Depression	2 (5)	1 (2.7)
- Somatic symptoms		
• Neuromusculoskeletal symptoms		
1. Fatigue	30 (75)	27 (73)
2. Headache/ vertigo	11 (27.5)	16 (43.2)
3. Memory loss/ impairment	15 (37.5)	11 (29.7)
4. Joint/ mucle/ nerve pain	10 (25)	15 (40.5)
5. Insomnia	8 (20)	11 (29.7)
• Respiratory symptoms		
1. Cough	9 (22.5)	13 (35.1)
2. Shortness of breath	6 (15)	9 (24.3)
• Cardiology symptoms		
1. Palpitation	6 (15)	6 (16.2)
2. Chest pain	1 (2.5)	6 (16.2)
• Gastrointestinal symptoms	8 (20)	14 (37.8)
• Other symptoms	20 (50)	17 (45.9)
Medication history, n (%)		
• No history of treatment	22 (55)	16 (43.2)
• Vitamin and supplement	11 (27.5)	8 (21.6)
• Antihypertensive	10 (25)	9 (24.3)
• Glucose lowering agent	3 (7.5)	2 (5.4)
• Pain killer	4 (10)	3 (8.1)
• GIT drugs (PPI, etc)	3 (7.5)	8 (21.6)
• Antihistamines	3 (7.5)	2 (5.4)
• Mucolytic	2 (5)	2 (5.4)

an improvement in the psychotherapy group to be 0.14 (SD 1.08), NLR in the education group increased by 0.22 (SD 1.23), with a p-value of 0.178. There was no improvement in HRV observed in either group. However, the psychotherapy group showed a reduction in HRV by 1.02 (SD 14.17), and the education group exhibited a decrease of 3.45 (SD 20.50), with p = 0.560.

Table 2. Effect of Internet-Based Group Supportive Psychotherapy and Education on SCL-90, NLR, HRV and VAS.

Variables	Group		CI (95%)	p
	Psychotherapy	Education		
SCL-90				
Pre test	82.98 (SD 51.95)	103.05 (SD 56.59)	-44.721 – 4.563	0.109
Post test	63.30 (SD 40.48)	85.03 (SD 56.17)	-4.776 – 1.312	0.064
Delta	-17.51 (SD 30.52)	-19.79 (SD 35.10)	-13.262 – 17.823	0.771
NLR				
Pre test	2.53 (SD 1.44)	1.99 (SD 0.78)	-0.024 – 1.087	0.061
Post test	2.46 (SD 1.15)	2.22 (SD 1.12)	-0.281 – 0.755	0.365
Delta	-0.14 (SD 1.08)	0.22 (SD 1.23)	-0.922 – 0.174	0.178
HRV				
Pre test	35.36 (SD 15.89)	38.62 (SD 19.37)	-11.625 – 5.099	0.439
Post test	33.34 (SD 15.14)	34.54 (SD 19.07)	-8.982 – 6.596	0.761
Delta	-1.02 (SD 14.17)	-3.45 (SD 20.50)	-5.856 – 10.723	0.560

Independent t-test

Independent T-test

Both supportive psychotherapy and education significantly improved SCL-90 ($p < 0.0001$ for psychotherapy and $p = 0.002$ for education). However, neither psychotherapy nor education reduced NLR or significantly increased HRV according to **Table 3**.

Dependent T-test

We investigated the impact of supportive psychotherapy and education on psychosomatic conditions using a visual analogue scale at each session. Both interventions had a significant positive influence on psychosomatic conditions throughout all the sessions (**Table 4**).

Table 3. Effect of Internet-Based Group Supportive Psychotherapy and Education on SCL-90, NLR and HRV.

Intervention	Variable	Pre Intervention	Post Intervention	CI (95%)	p
Psychotherapy					
	SCL-90, Mean (SD)	80.81 (SD 51.41)	63.30 (SD 40.48)	7.34 – 27.68	**<0.0001**
	NLR, Mean (SD)	2.53 (SD 1.44)	2.38 (SD 1.10)	-0.22 – 0.51	0.423
	HRV, Mean (SD)	35.36 (SD 15.89)	34.33 (SD 15.33)	-3.70 – 5.75	0.663
Education					
	SCL-90, Mean (SD)	104.82 (SD 52.27)	85.03 (SD 57.17)	7.54 – 32.04	**0.002**
	NLR, Mean (SD)	1.99 (SD 0.78)	2.22 (SD 1.16)	-0.66 – 0.20	0.285
	HRV, Mean (SD)	38.62 (SD 19.37)	35.16 (SD 19.21)	-3.69 – 10.61	0.332

Dependent T-test

Table 4. Effect on Supportive Psychotherapy and Education on Psychosomatic Conditions Based on A Visual Analog Scale in Each Session.

Intervention	Variable	Pre-Intervention	Post Intervention	CI (95%)	p
Psychotherapy					
- Session 1	Psychic, mean (SD)	7.24 (SD 1.68)	7.87 (SD 1.36)	-1.009 – (-0.254)	**0.002**
- Session 2	Psychic, mean (SD)	7.50 (SD 1.52)	8.11 (SD 1.27)	-0.943 (-0.267)	0.001
- Session 3	Psychic, mean (SD)	7.76 (SD 1.52)	8.59 (SD 0.93)	-1.257 – (-0.418)	**< 0.0001**
- Session 1	Somatic, mean (SD)	7.24 (SD 1.65)	7.87 (SD 1.39)	-0.951 – (-0.313)	**< 0.0001**
- Session 2	Somatic, mean (SD)	7.34 (SD 1.63)	7.89 (SD 1.39)	-0.900 – (-0.205)	**0.003**
- Session 3	Somatic, mean (SD)	7.62 (SD 1.53)	8.41 (SD 1.09)	-1.201 – (-0.367)	**0.001**
Education					
- Session 1	Psychic, mean (SD)	7.11 (SD 1.59)	7.63 (SD 1.70)	-0.769 – (-0.259)	**< 0.0001**
- Session 2	Psychic, mean (SD)	7.43 (SD 1.33)	8.09 (SD 1,31)	-0.892 – (-0.422)	**< 0.0001**
- Session 3	Psychic, mean (SD)	7.63 (SD 1.37)	8.29 (SD 1.87)	-1.246 – (-0.068)	**0.03**
- Session 1	Somatic, mean (SD)	7.11 (SD 1.39)	7.60 (SD 1.29)	-0.754 – (-0.217)	**0.001**
- Session 2	Somatic, mean (SD)	7.60 (SD 1.26)	8.09 (SD 1.22)	-0.741 – (-0.231)	**< 0.0001**
- Session 3	Somatic, mean (SD)	7.49 (SD 1.31)	8.17 (SD 1.42)	-1.074 – (-0.297)	0.001

Paired T-test

DISCUSSION

In this study, the psychotherapy group had a median age of 43 years, while the education group had a median age of 33 years. Though there was no statistical significance in age, clinically, the age difference of ten years between the groups could have impacted the results. In this study, it was observed that the majority of participants were female, comprising 76.6%. A prospective cohort study conducted by Bai et al demonstrated that female gender is associated with PCS.[8] This may also be brought on by psychological problems that are included as one of the symptoms of PCS, where risk factors for persistent psychological symptoms like anxiety and depression include female sex.[1] From the research, it was found that the most occupation of subjects were housewives and health care workers. There are no studies that assess the tendency of certain occupations to suffer from PCS. But PCS is associated the the possibility of not being able to work or being able to work full time. [9] Al Qaraibi et al are conducting a systematic review of the prevalence of PCS in health care workers, but to date the findings have not yet been published. [10] Majority of participants in this study (77.92%) had milder forms of COVID-19, and 75.3% were not hospitalized. This was consistent with a study by Mohamed Husein et al., that found PCS rose in patients who weren't hospitalized. Individuals with PCS who had previously been hospitalized experienced greater respiratory symptoms than non-hospitalized individuals, who experienced more neuropsychiatric symptoms.[11]

In this study, it was found that 46.8% of those who had received the fourth vaccination had a higher prevalence of PCS. The correlation between vaccination and PCS remains uncertain. This was also upheld by a systematic review conducted by Notarte et al, which indicated that there is a low level of evidence (level III, case-control and cohort studies) to suggest that vaccination prior to SARS-CoV-2 infection can reduce the risk of PCS. The effect of vaccination on PCS is subject to debate, as certain data suggest an amelioration of symptoms while others do not..[12]

In this study, fatigue was the most prevalent symptom, accounting for 74.03%, followed by headache/vertigo at 35.06% and memory loss/ impairment at 33.77%. Besides these primary symptoms, 15 diverse secondary symptoms were also noted. This finding is consistent with the WHO's 2021 Delphi consensus, which confirms the variation of PCS symptoms.[2] Aiyegbusi et al also found fatigue as the most common symptom.[3] The length of time suffering from PCS also varies, most are > 3 months – 6 months and ≥ 1 year. According to a retrospective cohort study carried out by Mizrahi et al in Israel, individuals with a mild history of COVID-19 will experience PCS symptoms for several months, which will gradually improve over the course of a year.[13] However, the NICE guidelines state that PCS usually presents with a variety of symptoms, often overlapping, which can fluctuate and change over time.[14]

This study found that the proportion of patients with and without comorbidities was nearly equal. Pavli et al. reported that over a third of PCS patients had additional conditions, with hypertension being the most prevalent, followed by diabetes mellitus, cardiovascular disease, lung disease and obesity.[1]

The psychotherapy group had a median SCL-90 score of 76, compared to 99 in the education group, suggesting a psychopathological condition. In the psychotherapy group, 62.5% of subjects had an SCL-90 score ≥ 61, whereas the education group had 78.4%. The 23-point difference in median scores between the two groups might have had a clinical impact on the intervention results, but there was no statistically significant difference. A study carried out by Clemente et al on long COVID patients assessed their psychological status using the SCL-90 questionnaire. The study discovered that during the initial 3 months following transmission, patients who recuperated from SARS-CoV-2 infection had a considerable probability of developing somatization, depression and anxiety.[15]

This study revealed median neutrophil-lymphocyte ratios of 2.06 in the psychotherapy group and 1.825 in the education group. Maamar et al.'s research indicated a correlation between elevated neutrophil-lymphocyte ratios in patients

with PCS.[16]

In this study, the psychotherapy group displayed a median heart rate variability of 29.88 ms, compared to 40.66 ms in the education group. In both groups, heart rate variability reached its highest at normal levels. The 10.78 ms HRV difference between the groups may have had a clinical impact on the results, although it did not prove statistically significant. A comparable finding was identified by Asarcikli et al, who noted parasympathetic activity and HRV increasing in PCS patients.[17]

From the results obtained, the average SCL-90 score decreased significantly in the psychotherapy group compared to the pretest value. The same effect was also observed in the control group. These findings suggest a positive impact of supportive and educational psychotherapy on the SCL-90 score. However, comparisons between the two groups showed no significant differences with a p-value of 0.771. Research on psychotherapy in PCS is still limited. There are no studies assessing the effects of psychotherapy in PCS patients on SCL-90 scores to date. Research conducted by Kuut et al on 114 patients using cognitive behavioral therapy (CBT) for 17 weeks is still ongoing and has not been published.[18] In the management of PCS, the NICE guidelines and several studies recommend psychotherapy in combination with rehabilitation therapy and other approaches. [14,19,11]

The therapeutic alliance comprises three components, of which two involve reaching an agreement on achievable goals and tasks in virtual groups. However, the quality of the relationship, which is essential in psychotherapy, remains uncertain. Due to the fact that interpersonal ties are crucial in psychotherapy, it is anticipated that the psychotherapy group's gains in psychological and physical symptoms will be less than those in the education group. The absence of direct interaction in virtual groups can be considered as the main obstacle in the transition from gathering in circles to screen forms. The absence of eye contact is also an obstacle for the therapist, where not all samples are willing to open the video. Presence is hard to achieve through on-screen relationships, due to numerous distractions. [20,21] In addition, the administration of psychotherapy carried out at night may have an effect on the results. Studies seem to show that early morning is the most effective time to take a therapeutic measure.[22]

This study revealed that supportive psychotherapy led to a decrease in NLR, whereas education had the opposite effect. Both the psychotherapy and education groups showed NLR within normal range in the pre- and post-test. No previous studies have explored the impact of supportive psychotherapy on NLR in PCS patients.

There was a decrease in HRV by 1.02 in the psychotherapy group and 3.45 in the education group, respectively. The statistical analysis showed no significant difference between pre-test and post-test values of both groups, with $p = 0.56$. This HRV measurement can produce different results as it is influenced by external factors, including age and gender, sampling time, nicotine use or caffeine consumption, physical activity, food and drink intake, tension during measurements, and the presence of coexisting illnesses.[23,24] HRV measurements that were taken 1-2 days after the participant completed the third session of psychotherapy may have affected the results. Additionally, the pre-intervention HRV values were clinically quite different between groups.

Both psychotherapy and education improve psychosomatic conditions in all sessions. Immediate improvements after the completion of each session were observed in both groups. It is worth considering that education was administered three times in this study, which is typical for psychotherapy, rather than just once. This may have contributed to the significant improvements observed in the education group.

All psychotherapy sessions in this study were conducted by the researchers themselves as therapists, which has the potential to introduce measurement bias. It would be better if future research is conducted with therapists who are not part of the research team. Furthermore, potential bias may also be present due to the fact that psychotherapy and education in both groups were conducted by two different people. This study did not identify appropriate participants

for group psychotherapy prior to the session. Moreover, a patient whose personality traits conflict with one group may be appropriate for another.[25] Selecting patients for placement in a particular group was challenging in this study due to its design as a randomized clinical trial. Therefore, we suggest that for future research, screening should be conducted beforehand to identify patients who may be suitable for inclusion in group psychotherapy. The following suggestion is to fulfill specific prerequisites before participating in an Internet-based group psychotherapy session. The primary requirement is that the participant can access videos during the psychotherapy session. This is intended to enhance the participant's concentration and focus, leading to improved therapeutic alliances with the therapist and interpersonal relationships between participants involved in psychotherapy. In addition, it is important that participants in psychotherapy are in a calm and conducive environment to fully engage in sessions. The management of PCS is a relatively new and continuously developing field. Research using different psychotherapy methods, including individual and group-based, in-person, and online, remains limited. Thus, further research is necessary to expand our understanding in this area.

The strength of this study is that there has not been any similar research related to psychotherapy for PCS in Indonesia Furthermore, few studies have examined the benefits of group-based internet psychotherapy, making this study valuable. The employment of RCT adds strength to the research design. However, this study's limitations are the lack of standardization for the duration and frequency of internet-based group psychotherapy. Psychotherapy conducted during nighttime with patients returning from work, feeling fatigued and lacking concentration, presents limitations that must be acknowledged. Furthermore, not all subjects are willing to open videos during psychotherapy sessions.

CONCLUSION

The study findings revealed that internet-based group supportive psychotherapy led to a significant improvement in both psychological and somatic symptoms, although no significant difference was observed compared to education. However, NLR and HRV showed no significant improvement. It was found that internet-based group supportive psychotherapy is less effective in patients with PCS. If internet-based group supportive psychotherapy is offered, it should be tailored to the individual.

Suggestion

Recommendations for further research regarding adding frequency of internet-based group psychotherapy in PCS patients and held in the morning to achieve more optimal results.

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AUTHORS CONTRIBUTIONS

This study was developed by HS, designed, directed, and coordinated by HS, DIS, CLM, and SS as the principal investigator, provided conceptual and technical guidance for all aspects of the project. RP, EG, IR and EY co-conceived the study, sample selection, and outcome parameters assessed. DIS conducted psychotherapy, supervised by HS, RII, and PRL. Data was analyzed by HS, DIS and SS. All authors participated in contributing to text and the content of the manuscript, including revisions and edits. All authors approve of the content of the manuscript.

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CONSENT TO PARTICIPATE

Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

COMPETING INTERESTS

The authors declare no competing interest.

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Sedentary Lifestyle of Older Adults and Its Associated Factors: A Multicentre Cross-Sectional Study During COVID-19 Pandemic in Indonesia

Siti Setiati[1], Kuntjoro Harimurti[1], Ika Fitriana[1], Noto Dwimartutie[1], Rahmi Istanti[1], Muhammad Khifzhon Azwar[1], I Gusti Putu Suka Aryana[2], Sri Sunarti[3], Agus Sudarso[4], Dina Aprillia Ariestine[5], Lazuardhi Dwipa[6], Novira Widajanti[7], Nur Riviati[8], Roza Mulyana[9], Rensa[10], Yudo Murti Mupangati[11], Fatichati Budiningsih[12]*

[1]Division of Geriatrics Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2]Department of Internal Medicine, Faculty of Medicine Universitas Udayana, Denpasar, Indonesia.
[3]Department of Internal Medicine, Faculty of Medicine Universitas Brawijaya, Malang, Indonesia.
[4]Department of Internal Medicine, Faculty of Medicine Universitas Hasanuddin, Makassar, Indonesia.
[5]Department of Internal Medicine, Faculty of Medicine Universitas Sumatera Utara, Medan, Indonesia.
[6]Department of Internal Medicine, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia.
[7]Department of Internal Medicine, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia.
[8]Department of Internal Medicine, Faculty of Medicine Universitas Sriwijaya, Palembang, Indonesia.
[9]Department of Internal Medicine, Faculty of Medicine Universitas Andalas, Padang, Indonesia.
[10]School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia.
[11]Department of Internal Medicine, Faculty of Medicine Universitas Diponegoro, Semarang, Indonesia.
[12]Department of Internal Medicine, Faculty of Medicine Universitas Sebelas Maret, Surakarta, Indonesia.

Corresponding Author:
Prof. Siti Setiati, MD., PhD. Division of Geriatrics, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia.
Email: s_setiati@yahoo.com.

ABSTRACT

Background: *COVID-19 is here to stay, and humans ought to decide how to adapt. We aimed to describe lifestyle changes during COVID-19 pandemic, and to determine the prevalence and factors associated with sedentary lifestyle among older adults.* ***Methods****: We obtained data from community-dwelling older adults aged ≥60 years. We presented the data descriptively and used multivariate analysis to assess the association between Physical Activity Scale for the Elderly (PASE) -based sedentary lifestyle and other variables in several tertiary geriatric centres.* ***Results****: Among 601 participants, 21.1% had sedentary lifestyle. Ethnic groups with the highest prevalence of sedentary lifestyle were Minang, Balinese, and Sundanese. Changes related to food intake, body weight, and physical activity were seen in a small proportion of older adults. Sun exposure habit was described. Sedentary lifestyle was associated with less consumption of food (OR 2.59, 95% CI 1.07-6.30), weight loss (OR 3.00, 95% CI 1.64-5.48), and higher intensity of snacking (OR 0.45, 95% CI 0.20-0.99).* ***Conclusion****: During COVID-19 pandemic, one out of five older adults had sedentary lifestyle, which was positively associated with less consumption of food and weight loss, and negatively associated with higher intensity of snacking. The*

prevalence of sedentary lifestyle varied across ethnic groups. Adequate and appropriate food intake may be crucial to keep older adults active, preventing them from entering vicious cycle of malnutrition, sarcopenia, and frailty.

Keywords: *sedentary behavior, life style, COVID-19, coronavirus, lifestyle.*

INTRODUCTION

The world population experienced a life-changing challenge due to COVID-19 pandemic.[1] At the same time, the world has been living with another pandemic for years, namely sedentary behaviour.[1–4] It is linked to increased risk of disability, chronic diseases and various poor health outcomes.[5] Unfortunately, COVID-19 is here to stay, and there could be outbreak of disease in the future with similar droplet transmission.

Studies showed contradictory results regarding physical activity of older adults during COVID-19 pandemic.[6–9] Although, a systematic review of observational studies recently concluded that the level of physical activity in older adults decreased during the quarantine period of COVID-19 worldwide.[10] The review included limited amount of studies representing the Asian population. Asian community is diverse, and a multicentre study involving several ethnic groups is warranted, e.g. Indonesian indigenous ethnic groups and people of Chinese descent.

Lifestyle changes during the pandemic may include changes in the pattern of food consumption and sun exposure habit. Sun exposure habit became more popular as a study published in early phase of COVID-19 pandemic showed that sunlight correlated significantly with recovered cases of COVID-19,[11] which might be linked to ultraviolet B (UVB) -induced gene downregulation.[12] It is crucial to understand the lifestyle changes of older adults during the pandemic before establishing evidence-based recommendations for the public.

We aimed to describe lifestyle changes, and to determine the prevalence and factors associated with sedentary lifestyle among older adults in a multi-ethnic Asian community. The findings can in turn be used to build strategies to improve health of older adults in the region.

METHODS

We conducted a cross-sectional observational study in 12 geriatric care centres in Indonesian archipelago from April to October 2022. The centres were Payangan Public Health Centre in Gianyar, Bali, Dr Soetomo General Hospital in Surabaya, Dr Wahidin Sudirohusodo General Hospital in Makassar, Haji Adam Malik General Hospital in Medan, Siti Khadijah Islamic Hospital in Palembang, Dr Mohammad Hoesin General Hospital in Palembang, Dr Moewardi General Hospital in Surakarta, Dr Kariadi General Hospital in Semarang, Dr Mohammad Djamil General Hospital in Padang, Dr Saiful Anwar General Hospital in Malang, Dr Hasan Sadikin General Hospital in Bandung, Atma Jaya Private Hospital in North Jakarta, and Cipto Mangunkusumo National General Hospital in Central Jakarta.

We obtained the data from multi-ethnic Asian adults aged 60 years and older through interviewer-administered questionnaire survey. The interviewers were trained physicians caring for older adults. We excluded patients with acute illness(es), acute exacerbation of chronic illness(es), and/or incomplete data.

We utilized Physical Activity Scale for the Elderly (PASE) to determine sedentary lifestyle of older adults, which was developed and evaluated by Washburn in 1990s.[13] PASE is a 12-item document with good test-retest reliability with an intraclass correlation coefficient of 0.75. We also chose PASE because it has been validated in other Asian population as well, e.g. in Hong Kong[14] and Malaysia.[15] We included additional questions in the interview related to lifestyle changes during COVID-19 pandemic. The questions were about difficulties in obtaining daily needs, difficulties in obtaining fresh food, less consumption of food, weight loss, weight gain, higher intensity of snacking, reduction in physical activity, reduction in exercise.

The sample size needed for the study was determined based on the formula for the sample size of the estimated proportion.[16] Since we analysed 12 independent variables in the bivariate analysis with a sample proportion of 36.3%,[17] the minimum sample size would be 331. Ethical approval was obtained from the Faculty of Medicine, Universitas Indonesia.

Data Collection

We collected primary data related to subject characteristics from history taking. Sex categories were documented as (1) Male and (2) Female. Age was classified as (1) 60-69 years and (2) ≥ 70 years old. The categories were made based on the cut-off point used by community-dwelling older adults studies in Southeast Asia, including a report in Indonesia[18] and Malaysia.[19] Ethnic groups were classified into Javanese, Chinese, Minang, Balinese, Malay, Sundanese, Batak, Betawi, Buginese, and Others. Malay ethnic group includes people native to the regions on the east coast of Sumatra island, such as Deli Malay and Palembang Malay. Current employment status was documented as (1) Working and (2) Not working. Smoking history was documented as (1) Never-smoker, (2) Former smoker, or (3) Current smoker. History of COVID-19 was documented as (1) No or (2) Yes.

Self-reported sun exposure habit was documented as (1) No sun exposure habit, (2) 1-3 time(s) a week, or (3) > 3 times a week. Following the Holick's rule,[20] we classified the time of sun exposure to (1) 9.00 a.m. onward or (2) before 9.00 a.m.

We calculated total PASE score by multiplying the amount of time spent in each activity (hours per day over the past seven-day period) by the respective weights and summing up the scores of all activities. The PASE score ranges from 0 to 793, with higher scores indicating greater physical activity.[13,21] We classified the score into (1) Sedentary lifestyle for the total score of <40,[22] and (2) Non-sedentary lifestyle for the total score of 40 or higher. Self-reported responses to additional questions in the interview related to lifestyle changes were documented as (1) No or (2) Yes.

Statistical Analysis

We analysed the data with SPSS version 21 (IBM, Armonk, New York, USA). We provided descriptive data regarding lifestyle changes of older adults. Afterwards, we used Chi-square test to perform the bivariate analysis utilizing data from independent variables related to physical activity, food intake, weight changes, work and history of COVID-19. Variables with p value < 0.25 in bivariate analysis were included for multivariate analysis with multiple logistic regression method to assess the association between sedentary lifestyle and the independent variables. A p value < 0.05 was considered significant.

RESULTS

A total of 601 older adults were included in the study. The demographic characteristics of the older adults are presented in Table 1. 56.7% were female and 47.4% were aged 70 years and older. The ethnic distribution was 25.8% Javanese, 17.8% Chinese, 13.5% Minang, 12.0% Balinese, 10.3% Malay, 9.3% Sundanese, 4.8%

Table 1. Subject Characteristics (n=601).

Characteristics	Total (n=601)
	N (%)
Sex	
- Male	260 (43.3)
- Female	341 (56.7)
Age	
- 60 – 69 years old	316 (52.6)
- ≥ 70 years old	285 (47.4)
Ethnic group	
- Javanese	155 (25.8)
- Chinese	107 (17.8)
- Minang	81 (13.5)
- Balinese	72 (12.0)
- Malay	62 (10.3)
- Sundanese	56 (9.3)
- Batak	29 (4.8)
- Betawi	11 (2.8)
- Buginese	6 (1.0)
- Others	20 (3.3)
Employment status	
- Working	92 (15.3)
- Not working	509 (84.7)
Smoking history	
- Never-smoker	423 (70.4)
- Former smoker	146 (24.3)
- Current smoker	32 (5.3)
History of COVID-19	
- No	563 (93.7)
- Yes	38 (6.3)

Table 2. Sun exposure and lifestyle changes of older adults during the pandemic.

Variables	Total (n=601)
	N (%)
Sun exposure	
- No sun exposure habit	4 (0.7)
- 1-3 time(s) a week	338 (56.2)
- > 3 times a week	259 (43.1)
Time of sun exposure	
- 9.00 a.m. onward	279 (46.4)
- Before 9.00 a.m.	322 (53.6)
Sedentary lifestyle	
- No	474 (78.9)
- Yes	127 (21.1)
Difficulties in obtaining daily needs	
- No	528 (87.9)
- Yes	73 (12.1)
Difficulties in obtaining fresh food	
- No	561 (93.3)
- Yes	40(6.7)
Less consumption of food	
- No	570 (94.8)
- Yes	31 (5.2)
Weight loss	
- No	536 (89.2)
- Yes	65 (10.8)
Weight gain	
- No	541 (90.0)
- Yes	60 (10.0)
Higher intensity of snacking	
- No	521 (86.7)
- Yes	80 (13.3)
Reduction in physical activity	
- No	433 (72.0)
- Yes	168 (28.0)
Reduction in exercise	
- No	431 (71.7)
- Yes	170 (28.3)

Batak, 2.8% Betawi, 1.0% Buginese, and 3.3% others. Only 15.3% of them were still working during the data collection. 5.3% of participants were current smokers and 6.4% had history of COVID-19.

The data related sun exposure and lifestyle changes of older adults during the pandemic are presented in Table 2. 0.7% of older adults had no sun exposure habit, whereas 56.2% of the participants reported 1-3 time(s) a week of sun exposure. 43.1% of older adults reported more than 3 times of sun exposure per week.

Based on PASE score, 21.1% of older adults had sedentary lifestyle (PASE score of < 40). The prevalence of sedentary lifestyle in Javanese, Chinese, Minang, Balinese, Malay, Sundanese, Batak, Betawi and Buginese cohort was 18.7%, 2.8%, 55.6%, 27.8%, 14.5%, 26.8%, 6.9%, 0.0%, and 16.7%, respectively. On the other hand, the prevalence of sedentary lifestyle in other ethnic minority groups combined was 15%. (**Figure 1**)

Lifestyle in Different Ethnic Groups

During COVID-19 pandemic, 12.1% of all older adults in this study reported difficulties in obtaining daily needs, whereas 6.7% reported difficulties in obtaining fresh food. 5.2% of older adults reported less consumption of food, 10.8% reported weight loss, whereas 10.0% reported weight gain. 13.3% of older adults had higher intensity of snacking during the pandemic.

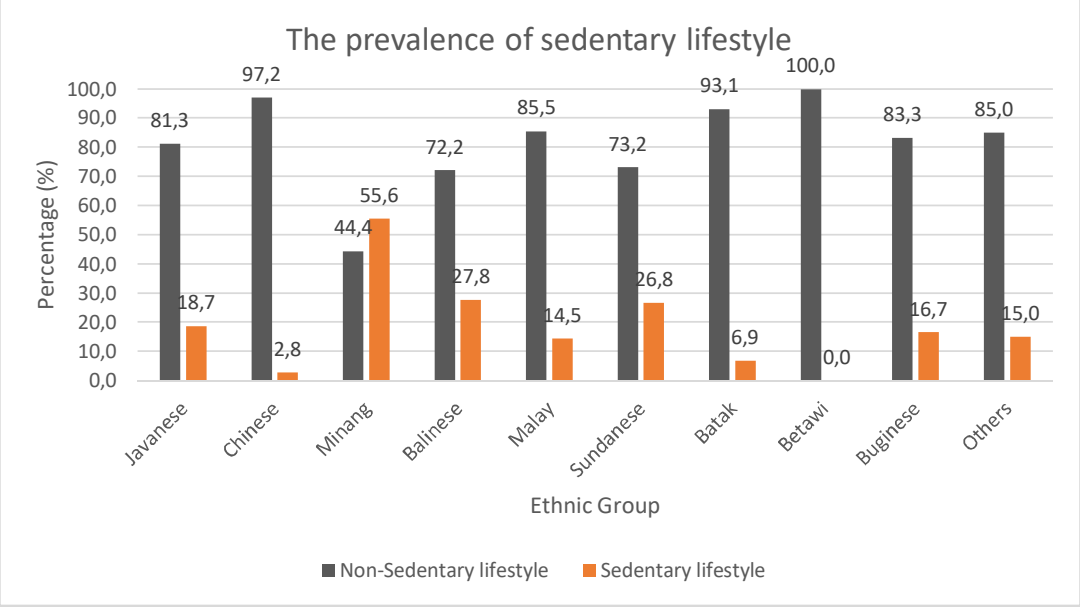

Figure 1. The prevalence of sedentary

Reduction in physical activity and exercise were reported by 28.0% and 28.3% of older adults, respectively. Our data suggested unremarkable differences in reduction of physical activity and exercise between those who had sedentary lifestyle and those who had non-sedentary lifestyle.

Bivariate analysis results presented in **Table 3** suggested several variables had p value of below 0.25, which were female sex, age 70 years or older, not working, difficulties in obtaining fresh food, less consumption of food, weight loss, weight gain, higher intensity of snacking, and history of COVID-19. Of all older adults with sedentary lifestyle (n=127), 52% were female, 53.5% were aged 70 years and older, 89.8% were not working, 11.8% reported difficulties in obtaining daily needs, 9.4% reported difficulties in obtaining fresh food, 12.6% reported less consumption of food, 22.8% reported weight loss, 7.1% reported weight gain, 9.4% reported higher intensity of snacking, 26.8% reported reduced physical activity, and 27.6% reported reduced exercise during COVID-19 pandemic.

Multivariate analysis results presented in **Table 4** suggested that sedentary lifestyle in

Table 3. Bivariate analysis results

Variables	Sedentary lifestyle		Crude OR (95% CI)	p-value
	No; [n (%)]	Yes; [n (%)]		
Sex				
- Male	199 (42.0)	61 (48.0)	1	
- Female	275 (58.0)	66 (52.0)	0.78 (0.53-1.16)	0.222
Age				
- 60 – 69 years old	257 (54.2)	59 (46.5)	1	
- ≥ 70 years old	217 (45.8)	68(53.5)	1.37 (0.92-2.02)	0.120
Employment status				
- Working	79 (16.7)	13 (10.2)	1	
- Not working	395 (83.3)	114 (89.8)	1.75 (0.94-3.27)	0.074
Difficulties in obtaining daily needs				
- No	416 (87.8)	112 (88.2)	1	
- Yes	58 (12.2)	15 (11.8)	0.96 (0.53-1.76)	0.896
Difficulties in obtaining fresh food				
- No	446 (94.1)	115 (90.6)	1	
- Yes	28 (5.9)	12 (9.4)	1.66 (0.82-3.37)	0.155
Less consumption of food				
- No	459 (96.8)	111 (87.4)	1	
- Yes	15 (3.2)	16 (12.6)	4.41 (2.12-9.19)	<0.001
Weight loss				
- No	438 (92.4)	98 (77.2)	1	
- Yes	36 (7.6)	29 (22.8)	3.60 (2.11-6.15)	<0.001
Weight gain				
- No	423 (89.2)	118 (92.9)	1	
- Yes	51 (10.8)	9 (7.1)	0.63 (0.30-1.32)	0.220
Higher intensity of snacking				
- No	406 (85.7)	115 (90.6)	1	
- Yes	68 (14.3)	12 (9.4)	0.62 (0.33-1.19)	0.149
Reduction in physical activity				
- No	340 (71.7)	93 (73.2)	1	
- Yes	134 (28.3)	34 (26.8)	0.93 (0.60-1.44)	0.738
Reduction in exercise				
- No	339 (71.5)	92 (72.4)	1	
- Yes	135 (28.5)	35 (27.6)	0.96 (0.62-1.48)	0.838
History of COVID-19				
- No	447 (94.3)	116 (91.3)	1	
- Yes	27 (5.7)	11 (8.7)	1.57 (0.76-3.26)	0.223

Table 4. Multivariate analysis results

Factors	Coefficient B	Standard error	p-value	OR (95% CI)
Female sex	-0.225	0.213	0.289	0.80 (0.53-1.21)
Age 70 years or older	0.302	0.212	0.154	1.35 (0.89-2.05)
Not working	0.491	0.330	0.137	1.63 (0.86-3.12)
Difficulties in obtaining fresh food	0.167	0.418	0.689	1.18 (0.52-2.68)
Less consumption of food	0.952	0.453	0.036	2.59 (1.07-6.30)
Weight loss	1.097	0.308	<0.001	3.00 (1.64-5.48)
Weight gain	0.067	0.447	0.880	1.07 (0.45-2.57)
Higher intensity of snacking	-0.806	0.409	0.048	0.45 (0.20-0.99)
History of COVID-19	0.491	0.388	0.206	1.63 (0.76-3.50)

older adults during the pandemic was associated with less consumption of food (odds ratio [OR] 2.59, 95% confidence interval [CI] 1.07-6.30) and weight loss (OR 3.00, 95% CI 1.64-5.48). Sedentary lifestyle was negatively associated with higher intensity of snacking (OR 0.45, 95% CI 0.20-0.99).

DISCUSSION

Our study suggested that less consumption of food and weight loss to be associated with sedentary lifestyle in Indonesian older adults. In contrast, higher intensity of snacking during the pandemic was inversely associated with sedentary lifestyle.

Our study cohort was multiethnic older adults from different geriatric care centres in Indonesia (**Table 1)**. Of all older adults, only 6.3% had prior documented history of COVID-19.

Our study suggested that 99.3% of Indonesian older adults had sun exposure habit. This means that only 0.7% of older adults did not have sun exposure habit, unlike the proportion suggested in a study before the pandemic suggesting a proportion as high as 8.1%.

Unfortunately, more than half of the older adults in our study (53.6%) had a habit of obtaining sun exposure before 9.00 a.m. This was not in accordance with the Holick's rule suggesting that exposure to sunlight at the face and both arms 3 times a week for 25 min at 9.00 a.m. should maintain adequate vitamin D status.[20] A study from the region also suggested the highest intensity of UVB occurred at 11.00 a.m. to 1.00 p.m.[23]

As stated above, evidence suggested sun exposure was linked to more COVID-19 cases of recovery.[11] This might be better explained by the findings of previous study suggesting the role of UVB-irradiation exposed to melanocytes using UVB lamps in causing significant downregulation of DPP9, CCR2, IFNAR2, TYK2, OAS1, and HSPA1L. DPP9 was known to encode for dipeptidyl peptidases involved in immune regulation,[12,24] whereas CCR2, IFNAR2, TYK2 are the key regulators for immune cell-mediated excessive inflammatory response in various viral infections. There was association between both OAS1 and HSPA1L and increased susceptibility to SARS-CoV-2 infection through enhanced viral replication and survival in host cells. In vitro study also showed that SARS-CoV-2 virus lost its viability after sunlight exposure stimulation for 107 minutes in mucus and 37 minutes in culture media.[25]

The prevalence of sedentary lifestyle in older adults in this study was 21.1%. Thus, approximately 1 of 5 older adults Indonesia had sedentary lifestyle. PASE-subscores used in this study may reflect work, leisure and domestic life. Higher total PASE score signifies more physical activity during the previous week. Prolonged sedentary time appears to be harmful in older adults.[26] Breaking up sedentary time was associated with improved health outcomes.[26,27] Once sedentary lifestyle is detected by PASE score, PASE can be used to monitor habitual physical inactivity and to identify motivations to optimize health promotion.[28]

Ethnic groups with the highest prevalence of sedentary lifestyle in our study were Minang, Balinese, and Sundanese. On the other hand, ethnic groups with the lowest prevalence of sedentary lifestyle were Betawi, Chinese, and

Batak (**Figure 1**). We should therefore encourage strategies to maintain physical condition with physical exercises that meet the needs of older adults in the current pandemic scenario.[10] Strategies should also be developed for similar disease outbreak in the future. Since different ethnic groups speak different languages and have distinctive customs, healthy lifestyle promotion might need to be prepared in a multilingual manner, especially in older adults in rural areas who may not understand official languages such as English, Chinese and Indonesian.

A small proportion of older adults in this study reported difficulties in obtaining daily needs (12.1%), difficulties in obtaining fresh food (6.7%), or less consumption of food (5.2%). During the pandemic 13.3% of older adults also reported higher intensity of snacking. Some community-dwelling older adults across the world also reported increased snacking during the pandemic.[29,30]

Of 10 older adults, approximately one reported weight loss and one reported weight gain. Nearly a quarter of older adults reported reduced physical activity. Likewise, approximately a quarter of older adults also reported reduced exercise during the pandemic. Our findings showed that only a minority of older adults experienced change(s) in physical activity during the pandemic. Evidence suggested that among all age groups including young adults, older adults had the smallest decrease in physical activity during the pandemic,[8] and were less likely to have changed their physical activity levels during the pandemic.[9]

Our bivariate and multivariate analyses results suggested that sedentary lifestyle was positively associated with less consumption of food and weight loss. In the absence of acute illness or acute exacerbation of chronic illness, weight loss in older adults occurred commonly due to a reduction in food consumption.[31] Decreased nutrition intake or anorexia of ageing may act as the culprit of sarcopenia and be linked with nutritional frailty.[32] Low daily energy intake of 21 kcals/kg or lower is significantly associated with frailty of older adults.[33] Experts have already warned the vicious cycle of malnutrition, sarcopenia, frailty, falls, illness, hospitalisation and death.[32]

A low body mass index (BMI) may in turn increase the risk of disability[34] and mortality.[31,35] Our study suggested that PASE score of <40 was linked to weight loss. Weight loss, sarcopenia, and nutritional frailty may occur independently of each other. However, Curcio, et al. found that PASE score was significantly lower in noninstitutionalized older adults with sarcopenia. Lower PASE score was also related to muscle mass and strength.[22] The findings may help explain why weight loss is related to sedentary lifestyle in our study.

In the opposite manner, higher intensity of snacking was negatively associated with sedentary lifestyle. Snacking patterns throughout the life span may be beneficial for maintaining health.[36] Good nutrition may slow physical deterioration of older adults.[37] However, food consumption recommendation should be provided cautiously and be made specific, as inappropriate snacking may lead to overnutrition.[30]

After understanding the lifestyle changes of older adults during the pandemic, key health messages for this population include: (1) Adequate and appropriate food intake is crucial to keep older adults active, preventing them from entering vicious cycle of malnutrition, sarcopenia, and frailty (2) older adults living in locations with intense UVB radiation.

To the best of our knowledge, this cross-sectional study was the first multicentre study to examine the changes in lifestyle among older adults during COVID-19 pandemic. Our study proved that only a small proportion of older adults changed their lifestyle during the pandemic. However, small changes might have important consequences. The result of this study may help doctors and the Ministry of Health provide lifestyle recommendations in future outbreak of disease with droplet transmission requiring large-scale social restriction. We realized the limitation of cross-sectional study design and thus causal relationship between statistically significant study variables could not be established.

CONCLUSION

One out of five older adults had sedentary lifestyle during COVID-19 pandemic. Nearly all older adults had sun exposure behaviour, but more than half of the older adults had inappropriate timing of sun exposure. Sedentary lifestyle was positively associated with less consumption of food and weight loss, and negatively associated with higher intensity of snacking. The prevalence of sedentary lifestyle varied across ethnic groups. Adequate and appropriate food intake may be of paramount importance to keep older adults active, preventing them from entering vicious cycle of malnutrition, sarcopenia, and frailty.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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The Profile of Multidrug Tuberculosis Regimen and Treatment Outcomes in Pulmonary MDR-TB Patients at the Tertiary Referral Hospital Dr. Soetomo, East Java, Indonesia: A Seven-Year Retrospective Study on Bedaquiline

Muhamad Frendy Setyawan[1], Ni Made Mertaniasih[2,4,5], Soedarsono[3,4,5]*

[1] Doctoral Program of Medical Science, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia.
[2] Department of Medical Microbiology, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia.
[3] Divison of Pulmonology, Department of Internal Medicine, Faculty of Medicine Hang Tuah University, Surabaya, Indonesia.
[4] Dr. Soetomo Hospital, Surabaya, Indonesia.
[5] Tuberculosis Laboratory, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.

Corresponding Author:
Prof. Ni Made Mertaniasih, MD., PhD. Department of Medical Microbiology, Faculty of Medicine Universitas Airlangga. Jl. Mayjen. Prof. Dr. Moestopo No. 47, Surabaya 60131, Indonesia. Email: ni-made-m@fk.unair.ac.id.

ABSTRACT

Background: *The use of bedaquiline has been reported to minimize the number of lost to follow-up and fewer rejections from the patients. This study is the first to depict the use of bedaquiline. It aims to provide information related to the profile of the MDR-TB drug regimen in the last 7 years with the treatment outcomes of pulmonary MDR-TB patients at a tertiary referral hospital in East Java.* ***Methods:*** *This study was a retrospective, descriptive, and data analysis on 1053 pulmonary MDR-TB patients in tertiary referral hospital Dr Soetomo, East Java, Indonesia, with the SPSS software version 25 and Microsoft Excel 2021.* ***Results:*** *The study analyzed the MDR-TB treatment regimen following the latest guidelines from WHO (2020) at a tertiary referral hospital in East Java. This study shows that a bedaquiline-containing regimen started in January 2015 to July 2022 with the percentage of distribution (1, 3, 11, 4, 18, 13, 29, 21)% consecutively in the regimen. The treatment outcome profile of MDR-TB patients shows the average percentage of cured (15%), died (12%), lost-to-follow-up cases (27%), moved to an individualized regimen or a different health facility (42%), and currently in the evaluation stage (4%). Overall from January 2017 to July 2022, the number of LTFU cases decreased (42, 46, 29, 19, 8, 4)%. However, the cured case fluctuated between 2017-2022 (16, 28, 26, 32)% respectively after Bdq started to be included in the regimen regularly for treating RR/MDR-TB.* ***Conclusion:*** *After seven years of study, we revealed an association between adding bedaquiline to the regimen and the treatment success and decreasing lost-to-follow-up cases.*

Keywords: *MDR-TB treatment regimen, bedaquiline, pulmonary TB, treatment outcomes.*

INTRODUCTION

The WHO consolidated guidelines on drug-resistant (DR) and multi-drug-resistant (MDR) TB treatment in 2020 and recommended two categories of treatment. The shorter and the longer treatment (individualized) consists of a combination of three groups of medicines. The use of Bedaquiline (Bdq) in the MDR-TB drug regimen started in 2015 in Indonesia also in Dr Soetomo Hospital as a tertiary referral hospital, after being recommended by WHO. All MDR-TB drug regimens are used with patient consent.

The treatment dose is adjusted to the guidelines from the WHO in 2020 based on the patient's body weight.[1–6] Despite the availability of treatment guidelines from WHO, it has never been reported the data regarding a combination of drugs in the patient's regimen.[3,7–9] This study is important to provide actual data on drugs prescribed in the regimen and the very first to show the use of Bdq in 7 years of study. This study shows more data on the clinical use of Bdq and patients' outcomes to descriptively evaluate the safety and effectiveness of the drug in MDR-TB patients. The study also shows that Bdq could be safely given to patients with a disease comorbid.

Bedaquiline has been known to cause a prolonged QT interval in previous reports, however, the composition of the regimen and the metabolic differences could also determine the occurrence of this Adverse event in MDR-TB patients. In other studies, it is reported if Bdq is safe to be administered in MDR-TB patients with HIV comorbid.[10,11] Bedaquiline-containing regimens help to decrease the treatment period, faster culture conversion rate, and reduce mortality among HIV/AIDS patients infected with MDR-TB. The major adverse events have been reported in previous studies and thus create alarm of fear in the increase of loss to follow-up (LTFU) cases, this article has a clinical significance in reporting the update on the association between receiving a bedaquiline regimen with treatment success and LTFU case and the AE events in MDR-TB patients. Moreover, the inappropriate composition of the drug regimen will subsequently affect a patient's treatment outcome. Patients with a history of treatment or relapse and new drug-resistant TB patients are receiving bedaquiline-containing regimens.

The use of Bdq has been reported to minimize the number of lost to follow-up patients and less rejection from the patients, lower risk of resistance, faster bacterial culture conversion, and fewer side effects.[12,13] It is important to measure the level of efficacy of the updated regimen, especially in Dr Soetomo, the tertiary referral hospital in East Java, a leading medical center for treating drug-resistant tuberculosis. Medical reports such as patient cured, dead, and lost to follow-up status are important in monitoring regimen composition efficacy.

METHODS

This study is an observational retrospective. This study used data sources for MDR-TB treatment regimens at a tertiary referral hospital, Dr Soetomo, East Java, Indonesia. Data were collected from January 2015 to July 2022, and a number of 1053 MDR-TB patients' data were analyzed. The data was obtained from the medical record. This research has undergone ethical approval with certificate No. 0456/KEPK/VIII/2022 from the Ethical Committee of Dr Soetomo Hospital.

Data Processing and Analysis

Analysis was performed using SPSS software version 25 and Microsoft Excel 2021. The study is descriptive quantitative by analyzing patient demographics in the form of frequency and percentage of variables. Any incomplete data is removed from the analysis. Furthermore, the data is represented in Figures and Tables.

RESULTS

Demographic Characteristics

This research aimed to study the MDR-TB treatment regimen profile and the administration of Bedaquiline in the WHO-recommended treatment regimen in a tertiary referral hospital in East Java, Indonesia. A total of 1053 data of pulmonary MDR-TB adult patients were evaluated, and it was observed that men dominated multidrug-resistant pulmonary TB patients. From 2015 to 2022, the number of new and relapsed MDR-TB patients fluctuated, with 2017 recording the highest number of cases. The MDR polyclinic began categorizing new and relapsed pulmonary TB patients in 2018, with the most relapsed patients in 2019. The incidence of relapse was observed to be higher in women. From 2015 to 2020, the number of MDR-TB patients grew as new cases rose. The most significant recurrence prevalence was recorded in the group weighing 33-50 Kg. **Table 1** shows the data on the MDR-TB patient's characteristics registered between 2015 and 2022.

Table 1. Characteristics of a total of 1053 Patients with MDR-TB in the tertiary referral Hospital Dr Soetomo, East Java, Indonesia, January 2015 to July 2022

Characteristics	Number	Frequency (%)
Sex		
Male	608	57.7
Female	445	42.3
Age (years)		
≤ 50	628	59.6
≥ 50	425	40.4
Body weight		
<33 Kg	41	3.9
33-50 Kg	529	50.2
>50 – 70 Kg	382	36.3
>70 Kg	53	5.0
NR	48	4.6
Case		
Relapse	368	34.9
New	684	65.0
NR	1	0.1
Type of regimens		
Shorter	641	60.9
Individualized	409	38.8
NR	3	0.3
Comorbid		
Extrapulmonary and others	12	11.9
Chronic Renal Disease	2	2.0
HIV	5	5.0
Type II DM	82	81.2

Clinical Characteristics

The analysis of comorbid from the pulmonary MDR-TB patients acquired data on up to 101 patients with type II DM higher among other types including HIV, renal diseases, extrapulmonary, and others. Based on a statistical evaluation of the profile of the treatment regimen offered to patients from 2015 to July 2022, it was revealed that 15 different anti-TB drugs for MDR-TB patients were utilized and available at the East Java tertiary referral hospital, as shown in **Figure 1** shows an increase in the use of bedaquiline containing regimen was started to be regularly added to the regimen in 2017-2022. Based on the 2020 WHO recommendation, which proposes a treatment combination for MDR-TB that does not comprise Km and Cm, such medications will no longer be prescribed to MDR-TB patients beginning in 2021.[14–16] Patients with comorbid such as chronic renal disease, HIV, and diabetes type II were treated with Bdq-containing regimens.

Profile of Drug Regimen in Multidrug Tuberculosis Patients

The latest WHO recommendations in 2020 stated kanamycin and capreomycin are no longer included in long-term or individualized regimens. There has been a change in the shorter drug regimen guidelines from the 2016 WHO

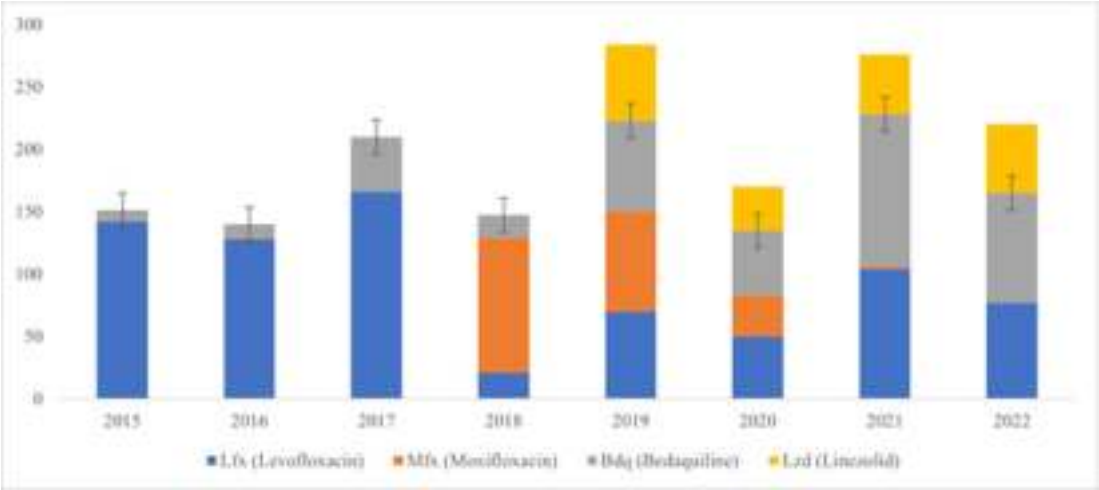

Figure 1. The profile of main Group A anti-TB drugs prescribed to pulmonary MDR-TB patients in the tertiary referral hospital Dr. Soetomo from January 2017- July 2022.

guideline.[3,14,17] The shorter regimen is given in two stages, namely the initial stage and the advanced stage, with high doses of isoniazid, the duration of the initial stage ranging from 4-6 months, and a follow-up stage of 5 months. Analysis of the type of treatment regimen in **Table 2** shows that standardized shorter treatment regimens have a greater frequency of being given to patients. However, if conditions such as drug allergies and side effects cannot be tolerated, then treatment is continued with a long-term regimen, individualized with a duration of 18-20 months, with the length of therapy after conversion being 15 months.[18,19] The previously reported outcomes of treatment with a Bdq-containing regimen in MDR-TB patients across nations are summarized in **Table 3**.

Profile of Multidrug-Resistant Tuberculosis Treatment Outcomes

The treatment outcome profile of MDR-TB patients can be seen in **Figure 2** categorized into cured, died, Lost To Follow-up (LTFU), moved to an individualized regimen or a different health facility, and currently in the evaluation stage. Newly enrolled patients in this study were categorized in the evaluation stage of administering a regimen tailored to the patient's condition. The number of patients who recovered and died both fluctuated, before Bedaquiline was used, the number of patients who died increased from 2015-2020, and after Bedaquiline was endorsed by WHO, it seems patients who died decreased in 2021-2022, and the lowest occurred in 2022 after Bedaquiline started to be prescribed regularly to treat RR/MDR-TB cases.

Table 2. Profile of drugs administered in patients with MDR-TB.

Regimen	Drugs name	Number (%)	
		Short/Standardized Regimen (n=641)	Individualized Regimen (n=410)
Category A	Levofloxacin (Lfx)	562 (86)	197 (48)
	Moxifloxacin (Mfx)	52 (8)	173 (42)
	Bedaquiline (Bdq)	191 (30)	225 (55)
	Linezolid (Lzd)	74 (12)	125 (30)
Category B	Clofazimine (Cfz)	217 (34)	367 (90)
	Cycloserine (Cs)	526 (82)	93 (23)
	Terizidone (Trd)	-	-
Category C	Ethambutol (E)	567 (88)	287 (70)
	Delamanid (Dlm)	5 (1)	12 (3)
	Pyrazinamide (Z)	560 (87)	314 (77)
	Imipenem-cilastatin (Ipm-Cln)	-	-
	Meropenem (Mpm)	-	-
	Amikacin (Am)	-	-
	Streptomycin (S)	-	-
	Ethionamide (Eto)	552 (86)	256 (62)
	Prothionamide (Pto)	-	-
	p-aminosalicylic acid (PAS)	37 (6)	-
Other drugs in the WHO-endorsed regimen (2020-2022)	High dose Isoniazid (H)	124 (19)	237 (58)
	Kanamycin (Km)	263 (41)	99 (24)
	Capreomycin (Cm)	245 (38)	21 (3)

Table 3. Previous studies on the clinical significance of Bedaquiline-containing regimen in the treatment of MDR-TB.

Study Location	Study period	Total number of patients	Regimen	Treatment duration	Outcome	Clinical significance	Reference
South Africa	2015-2017	5981 MDR-TB	A bedaquiline-containing or non-bedaquiline-containing regimen	< 6 months	Success rate - Bedaquiline-treated patients (66.9%) - Non-bedaquiline-treated patients (49.4%) Death rate - Bedaquiline treated patient (15.4%) - Non-bedaquiline treated patient (25.6%)	Bedaquiline-containing regimen was associated with higher survival and effectiveness benefit. Few patients reported bedaquiline-related adverse events (1.8%), and discontinuations (1.4%); QTcF prolongation >500 ms (2.5%) was observed during treatment.	(20)
East China	2016-2020	Patients with refractory RR/MDR/XDR-TB; Total of 202 patients; BDQ group, n = 102) and BDQ-free regimens (non-BDQ group, n = 100).	A bedaquiline-containing or non-bedaquiline-containing regimen.	18-20 months.	Culture conversion rates in the BDQ group at month 3, month 6, month 9, and month 12 (94.1% vs 65.0%) were all significantly higher than those in a non-BDQ group ($p < 0.001$).	- BDQ-containing regimen has a better clinical outcome and similar safety compared to the regular non-bedaquiline-treated patients - Adverse Effects (AEs) were similarly reported in 26.5% of patients in the BDQ group and 19.0% in the non-BDQ group ($p = 0.2$).	(21)
India	2019-2021	A total of 165 patients with pre-XDR-TB; 158 had MDR-TBFQ+.	Bedaquiline, Delamanid, Linezolid, and Clofazimine	6-9 months	139 (91%) patients had a successful treatment; 14 (9%) patients had unfavorable outcomes: 4 deaths, 7 treatment changes, 2 bacteriological failures, and 1 withdrawal.	Bedaquiline-containing regimen has a minimum cardiotoxicity and myelosuppression.	(22)

Table 3. Previous studies on the clinical significance of Bedaquiline-containing regimen in the treatment of MDR-TB.

Study Location	Study period	Total number of patients	Regimen	Treatment duration	Outcome	Clinical significance	Reference
Multicenter (16 countries)	2015-2018	A total of 2296 patients of MDR-TB.	Regimens Containing Bedaquiline and	16.5 months	Treatment success was not reported	- Clinically relevant QT interval prolongation was reported in a small proportion of patients (3%) 2.6 times per 1000 patient-months of bedaquiline, even when 96% of them received at least 1 QT-prolonging anti-TB drug (Mfx, Lfx, or Cfz).	(23)
			Delamanid; injectable; linezolid.			- Arrhythmia possibly related to QT prolongation is a potential cause in the Bedaquiline-containing regimen group.	
South Korea	2014-2020	Of 1998 patients; 315 (15.8%) and 292 (14.6%) received bedaquiline and delamanid respectively.	Regimens containing bedaquiline and regimens containing delamanid.	Approx. 24 months	Bedaquiline and delamanid did not increase the risk of all-cause death at 24 months (HR 0.73 and 0.89).	Bedaquiline-containing regimen increased the risk of acute liver injury (1.76[Ci:1.31-2.36]).	(24)
						Safety concerns associated with hepatic-related AEs for bedaquiline received little attention.	

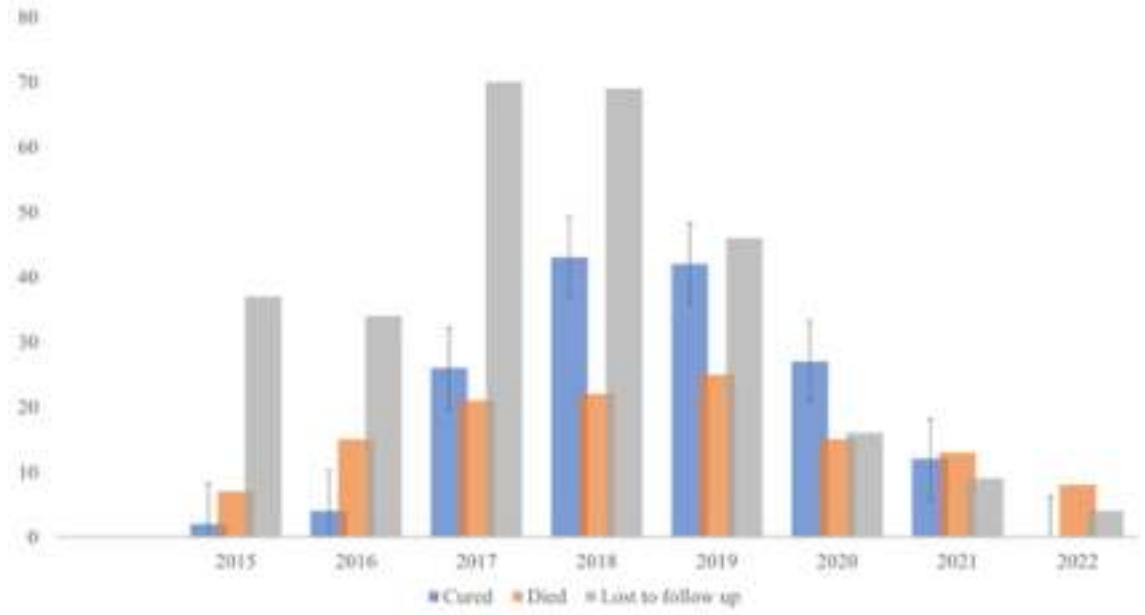

Figure 2. Distribution of the treatment outcomes of pulmonary MDR-TB patients in the tertiary referral hospital Dr Soetomo from January 2015 to July 2022.

DISCUSSION

In this study, the use of bedaquiline increases the chance of a cure and reduces the risk of death in MDR-TB patients.[7,25–27] The use of Bdq takes 12 months and requires evaluation; hence individuals who have side effects should be re-evaluated.[18,28] The statistical use of Bedaquiline in shorter and long-term regimens supports therapeutic success in treating MDR-TB, Bedaquiline combined with other drugs has the potential to significantly increase therapeutic efficacy, especially with Levofloxacin, Clofazimine, and Linezolid. No significant drug interaction was found between Bedaquiline and Moxifloxacin. The use of Moxifloxacin has reduced significantly in 2021 at hospitals, thus can be due to distribution issues, and the efficacy of the drugs based on clinical evaluation such as higher rejection and more side effects.[29] In the study, we found a small number of patients who reported a prolonged QT interval and some patients reported minor AEs such as skin itchy, and numbness have been reported but did not discontinue the treatment. Patients with a history of treatment or relapse and new DR-TB patients received a Bdq-containing regimen. Previously relapse patients received non-Bdq-containing regimens which reported to have many side effects and a higher risk of resulting in LTFU cases.

The clinical outcomes of pulmonary MDR-TB patients are lower in the lost-to-follow-up cases; the inclusion of Bedaquiline in the regimen seems an increase the number of cured and decrease the number of patients who died and lost to follow-up. In 2019, a similar study was performed by Borisov using the same research design in Italy, the data collected between January 2007 and March 2015 found that Bdq in surgery cases can be safely and effectively combined with appropriate drugs and result in a higher percentage of treatment success, and only a few had unfavorable outcomes including LTFU cases with fewer side effects such as complication.[30] Another study conducted by Korala in 2015 worldwide, unfortunately, Indonesia is not one of them, discovered a great potency of Bedaquiline-containing regimen for treating MDR-TB cases showed by higher percentage (75%) of treatment success and a lower chance of treatment failure (25%).[31] From our study, however, we revealed additional information based on our findings showed more patients are in either individually based regimens or moved to other health care facilities due to mild syndrome, and demographic reasons are higher than in previous years when Bdq is not yet started in the regimen. This report

is supported by evidence of the potential of Bdq as a therapeutic agent in MDR-TB patients has been widely reported to have faster culture conversion times, less toxicity, high efficacy and cure rates, and low mortality rates. Bdq kills active and dormant bacteria by binding to ATPase and blocking the energy production of bacteria, the affinity of this drug is 20.000 times more in Mycobacteria than in humans became the main reason for the safety and specificity of the drug's pharmacokinetics.[30–33] Bdq is given in a dose of 400 mg during the initial 1-2 weeks, followed by lower 200 mg doses in weeks 3-24. In the clinical setting, it has a steady killing activity after 7 days with a steady-state plasm concentration of 600 ng/mL. Furthermore, Bedaquiline can penetrate the alveoli tissue in mild-severe cases of MDR-TB. However, Bedaquiline has also been reported to cause prolonged QT intervals to >500 ms.[30,32]

The action of Bdq affects several pathophysiologies of patients including oxidative metabolism through the CYP3A4 pathway, with the formation of N-mono-desmethyl metabolite (M2), and the use of this drug should not be co-administered with the CYP3A4 inducer such as Rifampicin which could cause liver toxicity. The regimen for MDR-TB is made from the combination of groups A, B, and C of drug categories within WHO consolidated guidelines, without the use of rifampicin. Co-administration with Rifampicin decreased the effectiveness of Bdq, therefore, these two drugs should not be prescribed together.[32] However previous study reported that Bdq is safe to use in patients with diabetes mellitus, HIV, renal complications, and extra-pulmonary TB with strict evaluation from clinicians. This study finds no adverse effects in the patients such as previously reported peripheral neuropathy, hyperuricemia, nausea, arthralgia, liver injury, and prolonged QT. However, this study reveals the treatment also depends on the host metabolism capability to compensate for the action of Bdq. Some patients have mild reactions that do not lead to discontinuance. Treatment regimens including Linezolid, Clofazimine, and Moxifloxacin have also been reported to help the compensation mechanism of these drugs to achieve fewer side effects and faster culture conversion.[34–37] Further study needs to evaluate the efficacy and safety of Bedaquiline when combined with Pretonamid, Linezolid, and Delamanid in the treatment of DR-TB.

The clinical benefit from this research is showing evidence based on the treatment outcomes from different regiments to treat drug-resistant tuberculosis which includes the efficacy of Bedaquiline-containing regimen yields in all-oral, better treatment success rate, fewer rejections, LTFU cases, and lower risk of resistance when combined with appropriate drugs. This research is the first to investigate the profile of a drug-resistant treatment regimen and the efficacy of the Bedaquiline-containing regimen after seven years of use, hence giving a new perspective in a clinical care setting. Furthermore, in a clinical care setting other factors might contribute to the patient's treatment outcomes such as nutrition and immunity, demographic characteristics, diagnosis, drugs and logistics, patient support, integrated record system, management system, also financial support.[38] Patients with comorbid have been reported to be safely treated with a Bdq-containing regimen and have a similar success rate with those with no comorbids. The use of a Bdq-containing regimen seems to have no antagonist action against the antiretroviral treatment and blood glucose-lowering drugs for diabetes.

Finally, from this study, we believe that future multicentre studies need to be performed to broadly define the efficacy of Bedaquiline in MDR-TB drug regimen for MDR-TB patients and advance discovering more effective, fewer drug compositions and side effects, and also a faster conversion rate treatment including Bedaquiline-containing regimen.

CONCLUSION

Our study revealed a relation between adding Bedaquiline to the regimen and the decrease in lost-to-follow-up cases. Although the treatment success of the patient fluctuated, the drug Bedaquiline has a positive response to patient health improvement, fewer rejections due to tolerable side effects, fewer cases of resistance, and a faster smear and bacterial culture conversion after seven years of evaluation.

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A Mislocation of Double-lumen Catheter Guidewire in Right Atrium Successfully Retrieved with Loop-wire Snaring: A Case Report

Dono Antono*

Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Dono Antono, MD.. Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia.
Email: dantonos@yahoo.com.

ABSTRACT

The increasing rate of central vascular access use especially for hemodialysis access in Indonesia carries risk of retention of the guidewire to the heart resulting in a condition known as heart foreign bodies. We described a case of mislocation of double-lumen catheter guidewire to the right atrium in a patient planned to perform hemodialysis. The patient complained of dyspnea and swelling of extremities but the symptoms had already appeared before the insertion of the catheter due to the patient's underlying kidney disease arising conclusion that the foreign bodies itself are asymptomatic. The wire was found on chest x-ray and then confirmed on fluoroscopy during the retrieval procedure. Loop-wire was used to snare the guidewire. The wire was successfully evacuated and the patient was stable. The rare nature of the condition could become a challenge in recognizing the condition. Percutaneous retrieval is the preferred management of the condition.

Keywords: *heart foreign bodies, double-lumen catheter guidewire, right atrium, snaring.*

INTRODUCTION

The rate of central vascular access use for either central venous line or hemodialysis is always on the rise. In the United States, almost 150 million central venous catheters are used every year.[1] In Indonesia, there is no available data yet. Moreover, Indonesia Renal Registry recorded 132.142 patients actively performing hemodialysis until 2018, which part of the number received central access for the short-term hemodialysis.[2] However, the use of central vascular access carries risk of dislodged catheters or wires to the heart which lead to complications putting the patients into fatal outcomes.

Here, we report a case of double-lumen catheter guidewire, originally intended to give access for short-term hemodialysis, dislodged to the right atrium which then was successfully retrieved with the percutaneous loop-wire snaring. The aim of this report is to report the case with rare nature to give readers a new perspective.

CASE ILLUSTRATION

A 62 year-old man was referred by fellow anesthesiologist from RS Adhyaksa to the RS Cipto Mangunkusumo emergency department (ER) with the plan to evacuate a double-lumen catheter guidewire that was being misplaced in the right atrium. Previously, the patient presented to RS Adhyaksa with severe dyspnea since the day before. Since the beginning of the week, he reported dyspnea that was exacerbated by moderate activity. He also had a productive

cough, yet fever was denied. He reported swelling of arms and legs without redness and pain. The day before admission to hospital, he complained that the swelling had increased and he had not urinated yet for the day. At Adhyaksa Hospital, furosemide was administered and the urine production was 1.1 liters for twelve hours, nonetheless the dyspnea were getting severe. Therefore, the internist in charge consulted the patient to anesthesiologist to perform hemodialysis access. The anesthesiologist inserted the double-lumen catheter, but the guidewire jumped in deeper in the vessel and in radiograph evaluation, the wire was displaced in the right atrium. No symptoms such as chest pain were reported. The patient had never performed hemodialysis before. He had a history of type 2 diabetes mellitus 19 years earlier and routinely consumed gliquidone and acarbose. Hypertension was diagnosed one year earlier, coinciding with the patient's declining kidney function. No history of cardiac catheterization reported.

On physical examination, the patient was alert. Blood pressure was 147/74 mmHg, heart rate 78 bpm, respiratory rate 23 times/min and pulse oximeter 99% saturation on 10 lpm simple mask oxygen supplementation. Conjunctiva were anemic, rales were heard on the lung bilaterally, and both upper and lower extremities were edema bilaterally. Other remaining physical examinations were normal. Laboratory results revealed anemia (Hb 6.8 mg/dL), left shift of white blood cells, marked decreasing kidney function (blood urea nitrogen 224 mg/dL and serum creatinine 5.4 mg/dL), hypoalbuminemia (albumin 2.4 g/dL) and electrolytes imbalance including hyponatremia (sodium 130 mEq/L), hypercalcemia (total calcium 7.7 mEq/L; ionized 1.14 mEq/L), hyperphosphatemia (phosphorus 4.9 mEq/L), and hypermagnesemia (magnesium 2.88 mEq/L). Electrocardiogram revealed sinus rhythm, heart rate 75 bpm, left axis deviation, PR interval 0.12 s, QRS complex 0.08 s, inverted T wave on V5-V6, no ST segment changes, no ventricular hypertrophy, and no branch blocks. Chest x-ray showed cardiomegaly, suspected pericardial effusion, pulmonary congestion, and minimal bilateral pleural effusion as shown in **Figure 1**. The suspicion of pneumonia could not be ruled out from radiography. However, the radiologist failed to recognize the foreign body in their expertise' report. The patient then was being referred to us for identification of the foreign body, its location, and plan to evacuate it.

The patient was given oxygen supplementation, electrolyte correction solutions, blood transfusion, diuretics, anti-hypertension, and antibiotics while waiting for the scheduled evacuation in the ER. The next day, the evacuation was done in the cath lab. The patient was locally anesthetized within the area of the access near the right jugular vein with 200 mg lidocaine 2%. Then, a fluoroscopy was performed to confirm the exact location of the guidewire. The wire was found to be lying from the jugular vein to the right atrium. The procedure of the extraction as shown in **Figure 2A** began with the insertion of sheath in the right jugular vein. 6-French Judkins Right 3.5 catheter then inserted and MemoPartTM Snare -20 entered the catheter lumen to perform the

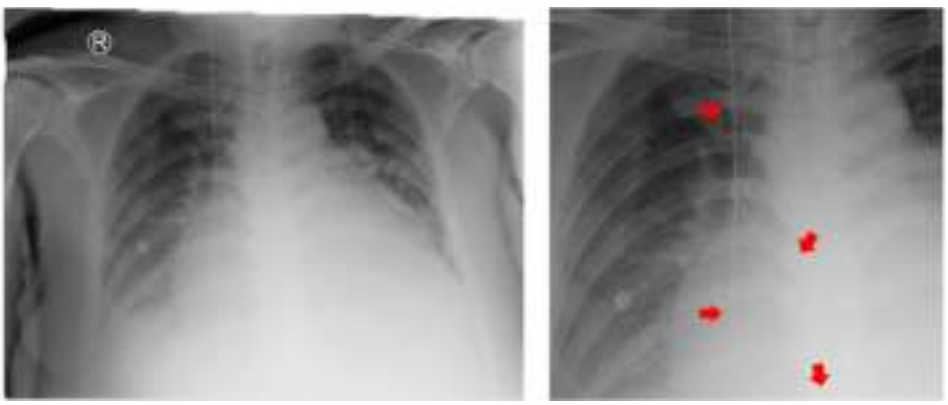

Figure 1. Chest x-ray of the patient

Figure 2. A. Procedure of the guidewire extraction, B. Snaring of the guidewire with MemoPart™ Snare -20, C. The undamaged extracted guidewire, D. Post-procedure chest x-ray.

snaring. The MemoPartTM Snare -20 could locate the guidewire without difficulty as shown in **Figure 2B**. The wire then was successfully snared and extracted. The extracted wire was intact as shown in **Figure 2C**. A long-term double-lumen catheter then was tunneled from jugular vein to superior vena cava. The patient was stable after the procedure, no complication occurred except minimal bleeding on the site of the access. Chest x-ray was done to evaluate the patient post-procedure and revealed normal condition as shown in **Figure 2D**. Subsequently, heparin lock was administered through the newly established catheter access. Finally, emergent hemodialysis was performed.

DISCUSSION

The cases of foreign bodies in the heart are rare, however no data currently available on the prevalence of heart foreign bodies. The most common foreign bodies encountered are different catheters and wires (16%), followed by vena cava filters (14%) and stents (11%).[3] The increasing use of these devices nowadays provides risks of the increasing rate of the iatrogenic retention of heart foreign bodies. A retrospective study with around 5000 participants who received intracardiac catheters for treatment modalities reported 0,07% incidence on iatrogenic retention.[4] The approximate number for the retention or misplacement of wires, specifically double-lumen hemodialysis catheter guidewires as presented in this paper is unknown, though several cases were reported.[5,6] Other commonly encountered foreign bodies are ventriculo-atrial shunts, VP shunts, pacemaker leads, and non-iatrogenic objects namely bullets, needles, tattoos, staples, and pieces of metal.[3]

Regardless of the limited cases, heart foreign bodies impose major risk for complications related to the heart. Several complications reported caused by these objects are fatal notably thromboemboli, infective endocarditis, valve regurgitation, sepsis, arrhythmia, and even cardiopulmonary arrest[3,7–9] with mortality rate ranging from 24 to 60 percent[10], while another study reported mortality of 4 percent.[3] Except for a small number of cases with the complications, heart foreign bodies are generally asymptomatic. Therefore, it becomes a challenge to identify heart foreign bodies from clinical appearance. Radiograph evaluation like chest x-ray is usually required. Symptoms associated with heart foreign bodies are dyspnea, arrhythmia, chest pain, infection, and tamponade or pericardial effusion.[3] However, in the case presented, the symptoms complained by the patient namely dyspnea and swelling of extremities are likely due to the underlying condition of the patient which is the chronic kidney disease. In fact, after the insertion of the double-lumen hemodialysis catheter and the displacement of the guidewire, the patient did not report worsening dyspnea nor swelling. Hence, the patient was asymptomatic in relation to the heart foreign bodies.

The most frequent location of heart foreign bodies is in the right ventricle, while the right atrium as in the patient in this report is the second most frequent location.[3] Looking at the common objects to be the foreign bodies, it came to no surprise that the right side of the heart is the common site. Catheters and their following guidewires are usually inserted at the peripheral vein including jugular vein and subclavian vein that lead to superior vena cava and finally to the right atrium and ventricle. Likewise, filters are

inserted in the vena cava that leads to the right atrium. Other frequent locations are pulmonary artery (18%), left ventricle (7%), pericardium (7%), and left atrium (3%).[3]

The patient in this report is managed with percutaneous retrieval with loop-wire snaring technique. The loop-wire was inserted through the sheath to the location of the mislocated guidewire and snared it before it was pulled out. Percutaneous endovascular approaches are now the preferred management for the heart foreign bodies, especially those with the form of catheters and wires. It is considered to be effective and safe compared to the previously adapted open surgery, specifically thoracotomy. The successful rate of percutaneous approach in retrieving foreign bodies is above 90 percent, while no injury to the vascular or heart wall is recorded.[10] Moreover, the other study reported the successful rate up to 100 percent with no complications being recorded.[11] The varied devices to be used to retrieve the foreign bodies is also an advantage. Some of the forms of the snare are goose-snare, dormia basket, forceps, pigtail catheter, and balloon.[10] On the other hand, thoracotomy possesses a risk of post surgery mortality up to 4 percent. In addition, the patients need antibiotics for 10 to 14 days after the surgery. The open surgery is now only performed in the foreign bodies located in the peripheral vein.[12,13]

CONCLUSION

Foreign bodies in the heart such as double-lumen hemodialysis catheter guidewire retained in the right atrium which presented in this report is a rare case. Furthermore, the condition is asymptomatic in most of the cases. However, it carries risk of fatal complications. Therefore, rapid recognition of the condition from the available data including chest x-ray is important. The object is preferably retrieved with the percutaneous technique, in this case, loop-wire snaring.

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Chlorpromazine-Induced Severe Hyponatremia in 66 Years Old Patient

Sri Soenarti[*], *Muchammad Kamal Hadi*

Division of Geriatric and Medical Gerontology, Department of Internal Medicine, Faculty of Medicine Universitas Brawijaya - Dr. Saiful Anwar Hospital, Malang, Indonesia.

**** Corresponding Author:***
Sri Soenarti, MD., PhD. Division of Geriatric and Medical Gerontology, Department of Internal Medicine, Faculty of Medicine Universitas Brawijaya - Dr. Saiful Anwar Hospital. Jl. Veteran, Malang 65145, Indonesia. Email: sri_sunarti.fk@ub.ac.id.

ABSTRACT

Hyponatremia is a common clinical problem in older people. The aging process is usually accompanied by various maladaptations to stress in different organs and physiologic functions. Medications are often the cause of hyponatremia such as thiazide diuretics, antidepressants, antiepileptic and antipsychotics. Antipsychotics can lead to severe hyponatremia by the mechanism of the development of the syndrome of inappropriate antidiuretic hormone secretion (SIADH). We report a patient who presented with severe hyponatremia due to Chlorpromazine and improved after receiving corrective hyponatremia.

Keywords*: Hyponatremia, aged, chlorpromazine, syndroma of inappropriate antidiuretic hormone.*

INTRODUCTION

Hyponatremia in older adults is a common clinical problem.[1] There are many causes of hyponatremia, including various medications. Medications that are often the cause of hyponatremia include thiazide diuretics, antidepressants, antiepileptic and antipsychotics.[2,3] Chlorpromazine, an antipsychotic approved by the Food and Drug Administration (FDA) for use in the treatment of hiccups (singultus), has a side effect of hyponatremia, by the syndrome of inappropriate antidiuretic hormone secretion (SIADH) mechanism.[3,4] Severe hyponatremia that occurs rapidly can be fatal as a result of cerebral edema.[1] Hyponatremia is independently associated with a 55% increase in the risk of mortality, substantial hospital resource utilization, and costs. Treatment of hyponatremia due to SIADH involves managing the underlying cause, cessation of the suspected drug, restriction of water intake and correcting serum sodium level.[5] Although it is easy to detect sodium levels from serum electrolyte tests and easy to provide sodium correction with fluid restriction or correction with saline, the treatment of hyponatremia is a challenge for doctors because the symptoms that appear are very varied and various underlying diseases, so a careful examination needs to be done to prevent the mortality.

We describe a patient who developed severe hyponatremia due to chlorpromazine administration, and who improved after receiving correction of hyponatremia in this case report.

CASE ILLUSTRATION

A 66-year-old male patient was brought by his family to the ER, Dr. Saiful Anwar with altered mental status, accompanied by weakness, difficulty communicating, confused and a history of repeatedly falling at home since 2 days prior

to admission. History of taking chlorpromazine 3 days before hospital admission, complaining of nausea, flatulence and hiccups, 25 mg b.i.d. for two days.

The patient has a history of diabetes mellitus since 30 years ago, with regular monitoring and routine administration of metformin 500 mg p.o. t.i.d., gliquidone 30 mg p.o. b.i.d., and acarbose 50 mg p.o. b.i.d. He also has history of hypertension since 10 years ago, treated with candesartan 16 mg p.o. o.d. No history of allergies. No patient's family who suffers from the same complaint.

The physical examination when he came to the Emergency Department Dr. Saiful Anwar Hospital showed; decreased consciousness (GCS 345), the patient opened his eyes with verbal stimulation, he was still disorientated and was able to localize painful stimulation. The patient's blood pressure was 179/110 mmHg, pulse is 106 beats per minute, and the others found no abnormalities.

The laboratory finding, was found severe hyponatremia 107 mmol/L with serum osmolality 231 mOsm/kg (hypoosmolar), hypokalemia 2.8 mmol/L and hyperglycemia with a random blood sugar 237 mg/dL.

From the chest x-ray examination, it was concluded an aortic sclerosis, and CT scan of the head, was shown there were subacute infarction of the left corona radiata, chronic infarction of the left lentiform nucleus, pons, senile brain atrophy, and right maxillary sinusitis.

We make hypothesis as delirium, hypertension, type 2 diabetes mellitus, severe hypoosmolar hyponatremia due to chlorpromazine induced SIADH, moderate hypokalemia and geriatric problems (innanition, instability). The patient received initial therapy to correct hyponatremia with 3% saline 41 ml/hour over 24 hours (total sodium deficit 585 mEq ~ 1,140 ml 3% saline). Correction of hypokalemia with KCl 25 mEq in NS 500 ml for 4 hours repeated for 3 times, citicoline 250 mg i.v. b.i.d., metoclopramide 10 mg i.v. t.i.d., candesartan 16 mg p.o. o.d., metformin 500 mg p.o. t.i.d., gliquidone 30 mg p.o. b.i.d., and acarbose 50 mg p.o. b.i.d.

Outcome and Follow Up

The patient was transferred to the High Care Unit for further evaluation and management. On the second day of treatment, the patient still complained of weakness, consciousness had begun to improve but still fluctuated from GCS 345 to 456. Blood pressure was 146/81 mmHg, and other vital signs were within normal limits. From the results of serum electrolytes, serum sodium level increased to 110 mmol/L, potassium level became 3.6 mmol/L and blood sugar level was 182 mg/dL. The patient was treated for correction of hyponatremia with 33 ml/hour 3% saline for 15 hours (total sodium deficit 487 mEq ~ 950 ml 3% saline).

On the third day of treatment, the patient still complained of feeling weak, no longer nauseous, and was able to eat by spending 1 portion. Mental status started to improve but remained fluctuating. Vital signs were within normal range. From the results of serum electrolytes, it was found that blood sodium levels were 119 mmol/L and blood glucose levels were 144 mg/dL. The patient received therapy for correction of hyponatremia with 31 ml/hour 3% saline for 12 hours (total sodium deficit 195 mEq ~ 380 ml 3% saline) and received salt capsules 1000 mg 3 times daily.

On the fourth day of treatment, the patient was fully conscious, there was no disorientation, the limp body had improved. The sodium level has increased to 126 mmol/L. The patient received maintenance fluid 0.9% saline 1,500 ml/24 hours and was transferred to the chronic ward.

On the fifth day of treatment, the patient felt improvement in his weakness. Vital signs were within normal limits, and the patient was allowed to go home that day.

Barthel ADL index assessment result before he was discharged suggested 16 points as opposed to the previous score of only 7 points, the patient experienced an improvement from a condition of severe dependence to mild dependence in carrying out daily activities. The subsequent outpatient follow-up visit showed that the Barthel ADL index was 20 points suggestive of functional independence. From the nutritional status of malnutrition screening (MNA) a score of 11 points was obtained as opposed to the previous 7 points, patients with nutritional status are at risk of malnutrition but have improved.

DISCUSSION

Hyponatremia is the most common electrolyte disturbance affecting 15–30% of hospitalized patients. Symptoms range from mild, nonspecific symptoms such as lethargy, agitation and confusion to severe life-threatening symptoms such as seizures, coma and ultimately death due to brain oedema. Medications are often the cause of hyponatremia resulting in hospitalization. Thiazide diuretics, antidepressants and antiepileptic drugs are the most frequent culprits. In addition, antipsychotics can occasionally lead to severe hyponatremia. Since decreased sodium values can result in symptoms resembling those seen in psychiatric conditions, including dementia, hyponatremia may be difficult to recognize.[2]

First-generation (typical) antipsychotics (FGAs) have been available for over 6o years and produce a combination of strong D2-antagonism together with anticholinergic and antihistaminergic effects. Antipsychotic drugs have been associated with hyponatremia in schizophrenia and other severe mental illnesses.[2]

One of the first generation antipsychotics is chlorpromazine. Apart from being an antipsychotic, chlorpromazine is the Food and Drug Administration (FDA)-approved treatment for persistent singultus, a medical problem where hiccupping can last for more than 48 hours. Persistent singultus can be treated by giving 25 to 50 mg of chlorpromazine orally every 6 to 8 hours. If hiccups persist even after oral treatment of 2 to 3 days, chlorpromazine is given as an intramuscular or intravenous dose.[4]

Antipsychotics have been hypothesized to promote ADH release through inducing hypersensitivity of the D2 receptor, hypotension related baroreflex stimulation, and serotonin-mediated effects (involving the 5-HT1A and 5-HT2 receptors). Patients with SIADH from psychotropic medications will have normal volume status, low serum osmolality (<285 mOsm/kg), and urine osmolality >100 mOsm/kg.[5]

Table 1 lists established risk factors and potential causes of SIADH. Patients over age 60 are at a higher risk of developing hyponatremia. The increased risk in the elderly is multifactorial in etiology and is likely to be secondary to lower total body mass and lower volume of distribution, which amplifies the impact of ADH-dysregulation, decreased glomerular filtration rate as well as increased rates of polypharmacy and medical comorbidity. Females have a higher risk of psychotropic medication-induced SIADH

Table 1. Risk factors and potential causes of SIADH[5].

Risk factors and potential causes of SIADH.		
Age	≥60 years old	
Sex	Female gender	
Nutritional status	Low Body Weight (BMI <18.5 kg/m2)	
Baseline Na	History of hyponatremia ([Na+] <135 mEq/L)	
Medications	CNS Active Drugs	Antipsychotics, Antidepressants, Amphetamines, Carbamazepine, Oxcarbazepine, Valproate, Opiates, Barbiturates, Nicotine, Bromocriptine
	Other Drugs	Amiodarone, Ciprofloxacin, Vincristine, Vinorelbine, Vinblastine, Cisplatin, Cyclophosphamide, Ifosfamide, Sulfonylureas, Interferons-alpha/gamma, NSAIDs, Methotrexate
CNS disorders	CVA, infection, TBI, hemorrhagea, MS, lupus cerebritis, epilepsy, hydrocephalus, encephalitis, meningitis	
Malignancies	Lung (especially small cell carcinoma), gastrointestinal, head & neck, genitourinary, sarcomas, lymphomas and neuroblastomas	
Pulmonary Disorders	Bacterial/viral infection, bronchial asthma, atelectasis, acute respiratory failure, pneumothorax, positive pressure ventilation, tuberculosis, aspergillosis, COPD, pulmonary fibrosis, sarcoidosis	
Major surgery	Abdominal, thoracic, brain	
Hormone administration or deficiency	Vasopressin, desmopressin, oxytocin	
Other	AIDS, Malaria, Rocky Mountain Fever, Hereditary SIADH, smoking	

than males. Underweight patients as well as those with a history of hyponatremia are also at an increased risk. Many non-psychotropic medications and medical conditions are associated with SIADH and may add to the risk of psychotropic-induced hyponatremia. Smoking is considered a risk factor for hyponatremia in the context of psychiatric illness and nicotine was found to stimulate ADH in humans.[5]

In this case, the patient was a 66-year-old man, with a history of taking the antipsychotic drug chlorpromazine on the indication of persistent hiccups for 2 days. There was no baseline data on the patient's previous sodium levels, no other risk factors.

The most common classification system for hyponatremia is based on volume status: hypovolemic (decreased total body water with greater decrease in sodium level), euvolemic (increased total body water with normal sodium level), and hypervolemic (increased total body water compared with sodium).[6,7]

Symptoms of hyponatremia depend on its severity and on the rate of sodium decline. Gradual decreases in sodium usually result in minimal symptoms, whereas rapid decreases can result in severe symptoms. Polydipsia, muscle cramps, headaches, falls, confusion, altered mental status, obtundation, coma, and status epilepticus may indicate the need for acute intervention. Most patients with hyponatremia are asymptomatic, and hyponatremia is noted incidentally.[6]

Euvolemic hyponatremia is most commonly caused by SIADH, but can also be caused by hypothyroidism and glucocorticoid deficiency. Euvolemia is diagnosed by findings from the history and physical examination, low serum uric acid levels, a normal blood urea nitrogen–to-creatinine ratio, and spot urinary sodium greater than 20 mEq per L. Diuretic therapy can artificially elevate urinary sodium, whereas a low-salt diet can artificially lower urinary sodium, thus clouding the diagnosis of hypovolemia versus euvolemia. Treatment generally consists of fluid restriction and correcting the underlying cause. Fluid restriction should be limited to 500 mL less than the daily urinary volume. Salt and protein intake should not be restricted. Predictors of failure with Salt and protein intake should not be restricted. Predictors of failure with fluid restriction include

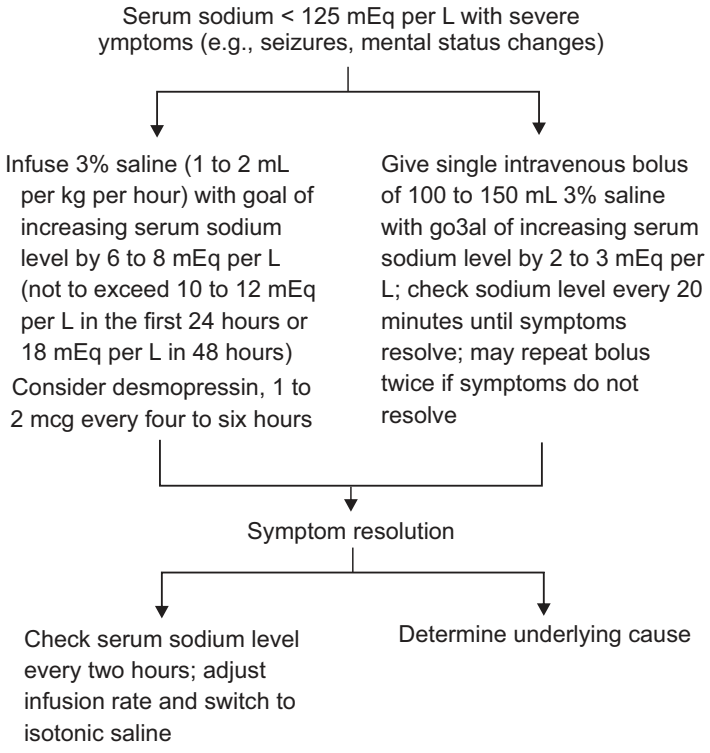

Figure 1. Algorithm for the treatment of severe symptomatic hyponatremia[6]

urinary osmolality greater than 500 mOsm per kg, 24-hour urinary volume less than 1.5 L, an increase in the serum sodium level of less than 2 mEq per L within 24 to 48 hours, and a serum sodium level less than the sum of the urinary sodium and potassium levels.[13] Volume status can be difficult to determine; therefore, a trial of intravenous fluids may be warranted.[6]

Severe symptomatic hyponatremia occurs when sodium levels decrease over less than 24 hours. Severe symptoms (e.g., coma, seizures) typically occur when the sodium level falls below 120 mEq per L, but can occur at less than 125 mEq per L. Severe symptomatic hyponatremia must be corrected promptly because it can lead to cerebral edema, irreversible neurologic damage, respiratory arrest, brainstem herniation, and death. Treatment includes the use of hypertonic 3% saline infused at a rate of 0.5 to 2 mL per kg per hour until symptoms resolve. The rate of sodium correction should be 6 to 12 mEq per L in the first 24 hours and 18 mEq per L or less in 48 hours. An increase of 4 to 6 mEq per L is usually sufficient to reduce symptoms of acute hyponatremia.[6] Oral salt tablets at 6–9 g per day in two to three divided doses is typically used to correct this level of hyponatremia. Correction calculations using salt tablets can be made knowing that a 1 g oral salt tablet is the equivalent to about 35 ml of a 3% saline solution.[5]

From this case, patient symptoms were decreased consciousness, lethargy, difficulty communicating, confused and a history of fall. From the laboratory examination, it was found that severe hyponatremia was 107 mmol/L with a serum osmolality of 231 mOsm/kg (hypoosmolar). The patient was treated for correction of hyponatremia with 3% saline 41 ml/hour for 24 hours. (total sodium deficit 585 mEq ~ 1,140 ml 3% saline).

CONCLUSION

Severe hyponatremia is associated with high morbidity and mortality. In order to implement correct treatment, an accurate clinical assessment must be made, focusing on potential etiology, chronicity and fluid status. It is crucial that a thorough investigation is made to secure the correct diagnosis.

A case has been reported, a 66-year-old man with a history of chlorpromazine treatment for the indication of singultus and a patient who fell into severe hyponatremia with complaints of loss of consciousness, weakness, difficulty communicating, confusion and a history of repeated falls. The patient received corrective therapy for hyponatremia with intravenous sodium and oral saline and the patient experienced improvement in both clinical and laboratory conditions.

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Hypokalemia Related to Distal Renal Tubular Acidosis as an Initial Presentation of Primary Sjogren's Syndrome

I Gede Yasa Asmara[1,2], Maruhum Bonar Hasiholan Marbun[3]*

[1] Department of Internal Medicine, Faculty of Medicine, University of Mataram - West Nusa Tenggara General Hospital, Mataram, Indonesia.
[2] Fellow of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.
[3] Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.

Corresponding Author:
I Gede Yasa Asmara, MD, MMed, FINASIM. Department of Internal Medicine, Faculty of Medicine, University of Mataram - West Nusa Tenggara General Hospital. Jl. Pendidikan No. 37, Mataram 83125, Indonesia.
Email: yasa.asmara@unram.ac.id.

ABSTRACT

Hypokalemia due to loss of potassium through the kidneys can be caused by distal Renal Tubular Acidosis (dRTA). The etiology of dRTA can be primary due to genetic defects or secondary to autoimmune diseases, especially Sjogren's syndrome (SS). The occurrence of dRTA in SS patients is low, at only 5% of cases. This case was interesting because dRTA was the initial clinical manifestation that led to the diagnosis of SS in the patient. A 48-year-old woman came with complaints of recurrent weakness. The patient was routinely hospitalized with severe hypokalemia and received potassium supplementation. The diagnosis of dRTA was based on repeated weakness, normal blood pressure, severe and recurrent hypokalemia, high urinary potassium, alkaline urine, low plasma bicarbonate, and normal anion gap metabolic acidosis. The diagnosis of SS in this patient was confirmed based on dry eyes, dry mouth, positive Schirmer's test, and positive autoantibodies to SS-A and Ro-52. There was a delay in the diagnosis of SS for two years in this patient because the complaints were initially subtle and non-specific. The hypokalemia in this patient was secondary to dRTA associated with primary SS. The possibility of an underlying autoimmune disorder should be considered in a patient presenting with recurrent severe hypokalemia. dRTA, as the etiology of hypokalemia, can be a gateway to the diagnosis of SS. In this patient, complaints related to dRTA appeared before the onset of sicca symptoms, and the diagnosis of SS was established.

Keywords: *Distal Renal Tubular Acidosis, Hypokalemia, Sjogren's Syndrome.*

INTRODUCTION

Distal renal tubular acidosis (dRTA) is a condition in which the kidneys cannot process urine acidification in the presence of systemic acidosis. The primary disturbance of dRTA lies in a defect in the basolateral $HCO3^-/Cl^-$ exchanger or H^+ ATPase subunit. The main features of dRTA are alkaline urine (pH >5.5) and hypokalemia (<3 mmol/L).[1] The etiology of dRTA is observed to be primary if a genetic defect is found, and secondary if it is caused by an autoimmune disease, drug, or other disorder. Secondary dRTA usually occurs in adults, the most common cause being an autoimmune disease, such as Sjogren's syndrome (SS).[2] If not appropriately treated, dRTA can develop into chronic kidney disease. Renal involvement in SS has been observed to be approximately

10%; notably, dRTA was found in 5% of patients with SS.[1,3] SS is a relatively rare autoimmune disease resulting from chronic inflammation of the exocrine glands, the salivary and lacrimal glands, with general symptoms of dry eyes and mouth.[4] Kidney disorders in SS can manifest into tubulointerstitial inflammation, such as dRTA, Fanconi syndrome, nephrogenic diabetes insipidus, hypokalemia, and glomerulopathy.[5] Here, we reported patients with dRTA who, after being investigated, were also accompanied by SS. This case was notable because dRTA was the initial clinical manifestation leading to the diagnosis of SS in the patient.

CASE ILLUSTRATION

A 48-year-old woman visited the internal medicine clinic of West Nusa Tenggara General Hospital on January 12, 2022, with the chief complaint of weakness throughout the body. The patient was treated with oral potassium supplementation at the District Hospital for incidental detection of hypokalemia of unknown etiology for the last two years. The patient was referred from East Lombok District Hospital for further investigation. Initially, in January 2020, the patient complained of weakness after strenuous exercise accompanied by cramping from the thigh to the leg. Subsequently, the patient felt weakness in both legs, so the patient could only lie down. The patient was treated for the first time at the East Lombok District Hospital for two days. The complaints improved after being administered an infusion and the patient was subsequently allowed to go home. The patient occasionally complained of dizziness and lightheadedness when their body felt weak. In July 2021, the patient complained of epigastric pain and a decreased appetite. The patient did not receive regular oral potassium supplementation because of dyspepsia syndrome. In addition, the patient complained of frequent urination, either day or night. Even at night, the patient reported passing urine of 3–4 times. The patient presumed that it was because she consumed a lot of water.

The patient denied other complaints, such as a history of palpitations, excessive sweating, hearing loss, intolerance to heat, weight loss, tremor, fever, vomiting, coughing, shortness of breath, or fainting. Complaints of hair loss, joint pain and swelling, bone pain, rash, and bruising on the skin, and mouth ulcers were also ruled out by the patient. The patient had no history of hypertension, diabetes mellitus, asthma, heart disease, autoimmune disease, kidney disease, and liver disease. The patient had no drug allergies. None of the patient's families had the same illness. The patient denied a family history of hypertension, diabetes mellitus, asthma, heart disease, kidney disease, or liver disease. The patient was a teacher who was married with two children.

On physical examination, the following were observed: blood pressure of 110/70 mmHg, pulse rate of 86 bpm, respiration rate of 18 breaths/minute, temperature of 36.5 °C, body weight of 52 kg, height of 150 cm, and body mass index of 23.2 kg/m^2. Examination of the head, neck, chest, and abdomen revealed normal findings. Examination of the extremities revealed that both lower extremities were warm, there was no visible edema, and there was decreased muscle strength (4/5) in both legs; however, there was no other significant neurological deficit.

Laboratory investigation revealed a white blood cell counts of 7570 /µL, hemoglobin value of 13.1 g/dL, thrombocyte count of 212000 / µL, urea level of 25 mg/dL, creatinine level of 1.0 mg/dL, estimated glomerular filtration rate value of 66.6 ml/minute/1.73 m^2, glucose level of 107 mg/dL, aspartate aminotransferase value of 61 U/l, alanine aminotransferase value of 47 U/l, albumin value of 3.3 mg/dL, sodium value of 138 mmol/L, potassium value of 1.9 mmol/L, and chloride value of 111 mmol/L. The thyroid function test showed a TSH value of 2.42 uIU/mL and free T4 value of 15.12 Pmol/L. Urinalysis revealed a specific gravity of 1.020, a urine pH of 7.0, negative proteinuria, and urine sediment within normal limits. Blood gas analysis showed a pH of 7.32, pCO_2 of 22.0 mmHg, pO_2 of 119 mmHg, base excess of -12.2 mmol/L, HCO_3 value of 11.8 mmol/L, and SO_2 value of 97%. Chest radiography revealed no abnormal findings. Electrocardiographic examination showed a sinus rhythm of 86 beats/minute and a normal axis. Abdominal ultrasonography showed

no nephrolithiasis, nephrocalcinosis, or other abnormalities.

The patient was diagnosed with severe hypokalemia due to dRTA and dyspepsia. The patient's 24-h urine collection was subsequently sent for potassium examination. In addition to potassium correction, lansoprazole was administered intravenously. After being treated in the hospital for two days, the patient was discharged with a final electrolyte level of 141 mmol/L of sodium, 3.1 mmol/L of potassium, and 118 mmol/L of chloride. The patient was administered oral potassium, KSR 600 mg twice daily and lansoprazole 30 mg twice daily. Oral sodium bicarbonate was not available when the patient was discharged from the hospital.

The patient was observed to feel better at the first outpatient visit after hospitalization on January 17, 2022. An electrolyte examination revealed a sodium level of 137 mmol/L, potassium level of 3.8 mmol/L, and chloride level of 120 mmol/L. Examination of 24-h urine potassium showed an increase of 48 mmol/24 hours, fasting glucose of 83 mg/dL, and glycated hemoglobin level of 5.1 percent. Oral sodium bicarbonate (500 mg three times daily) was administered. On further questioning at the second visit on January 29, 2022, the patient complained of dry eyes and mouth for several months. This may have caused the patient to feel thirst, drink, and urinate frequently. The patient denied any inflammation of the eye or use of artificial tears. Because SS was suspected, an antinuclear antibody (ANA) profile test and consultation with the ophthalmology department were performed.

At the third visit on January 31, 2022, the patient showed a positive ANA profile test for autoantibodies against SS-A, Ro-52, and AMA-M2. The serological tests for rheumatoid factor, hepatitis B, and hepatitis C were negative. The ophthalmologist's consultation confirmed sicca symptoms using an objective test (Schirmer's test, 0 mm/5 minutes in each eye). The patient was administered artificial eye drops six times daily. The patient was unwilling to undergo salivary gland biopsy, and salivary gland scintigraphy was unavailable. A diagnosis of SS was established, and the patient was started on methylprednisolone 8 mg twice daily.

On follow-up after 6 months, the patient has had no significant complaints and has never been hospitalized again. The last therapy was KSR 600 mg twice daily, lansoprazole 30 mg once daily, sodium bicarbonate 500 mg three times daily, and methylprednisolone 8 mg once daily.

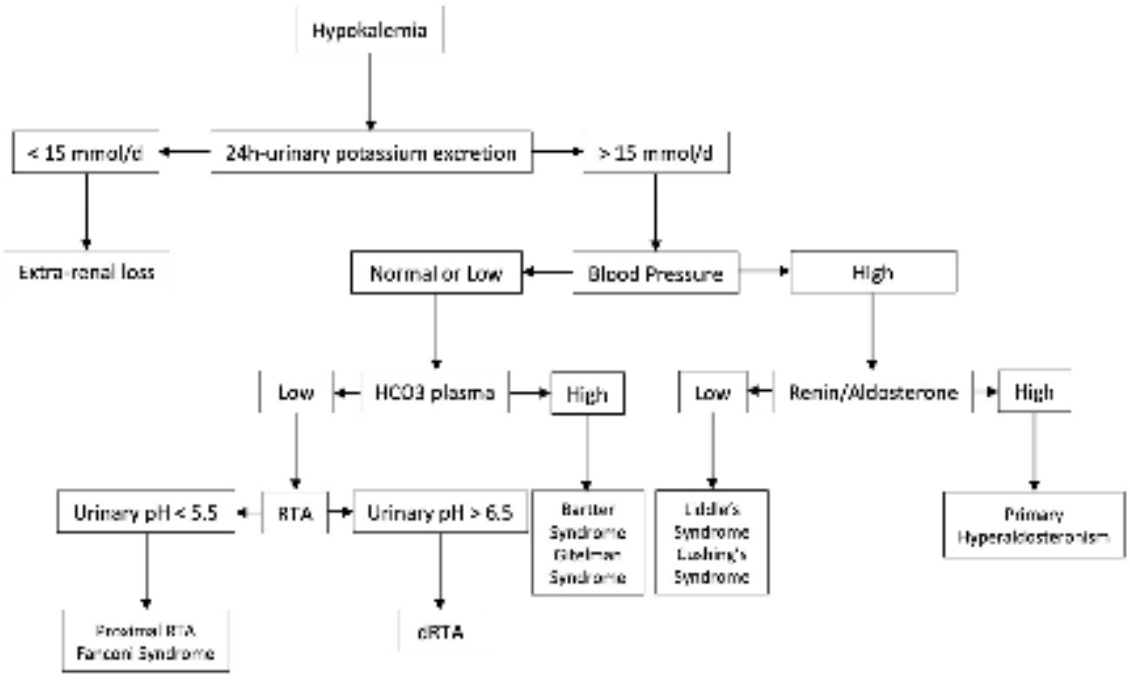

Figure 1. Simplified approach to diagnose patients with hypokalemia (Adapted from [2] and [6]).

Subsequently, the patient requested to be referred back to the East Lombok District Hospital because the hospital was closer to her family so that she could adhere more to the treatment.

The authors obtained all appropriate consent forms from the patients to publish this case report.

DISCUSSION

In general, the causes of hypokalemia are divided into three categories: low potassium intake, excess potassium loss, and changes in the distribution of intra- and extra-cellular potassium.[6] Endocrine diseases with renal potassium loss can be caused by primary hyperaldosteronism, Liddle syndrome, renal tubular acidosis (RTA), and Bartter syndrome. Renal tubular acidosis is a collection of disorders due to the inability of various segments of the renal tubules to reabsorb bicarbonate and secrete acid, causing acid-base disorders. There are four types of RTA based on their pathophysiology and location. Type 1 RTA, also known as dRTA, is the inability of the distal nephron to excrete protons maximally under conditions of metabolic acidosis. dRTA is the most common type.[7] RTA type 2 is a disorder due to the inability of the proximal tubule to reabsorb bicarbonate. Type 3 RTA is a mixture of type 1 and type 2 RTA. Type 4 RTA is caused by either aldosterone deficiency or renal tubular resistance to aldosterone.[2] The diagnosis of dRTA in this patient was based on recurrent weakness, normal blood pressure, severe hypokalemia, high urinary potassium, alkaline urine, low plasma bicarbonate, and normal anion gap metabolic acidosis (Figure 1).

There are two primary components in the urine acidification process in the kidneys. HCO_3^- reabsorption occurs mainly in the proximal tubule and in the thick ascending limb of loop of Henle. This complete absorption process causes urine pH to decrease to approximately 6. Second, proton secretion occurs in the distal collecting tubules of the nephron. In healthy adults, this process can cause a decrease in the pH of urine to 4.5–5.5. If the urine acidification process fails to fall below 5.5 in the state of acidosis, it will lead to the diagnosis of a distal acidification disorder.[8] Nephrocalcinosis or urolithiasis occurs in approximately 65% of patients with dRTA. The primary inhibitor of urinary calcium precipitation is citrate. In acidosis, excess protons are buffered by bone apatite, resulting in the release of calcium in the blood and causes hypercalciuria. In addition, the proximal convoluted tubule increases citrate reabsorption, leading to hypocitraturia. The combination of hypercalciuria and hypocitraturia in dRTA increases calcium precipitation.[8]

According to the European Rare Kidney Disease Reference Network and inherited kidney diseases of the European Society for Pediatric Nephrology guidelines, dRTA is a treatable disease. The etiology of dRTA is divided into two categories: primary, which occurs in children, and secondary, which occurs in adults. Secondary dRTA is most commonly associated with autoimmune diseases, such as SS, systemic lupus erythematosus, primary biliary cirrhosis, autoimmune hepatitis, and autoimmune thyroiditis.[8] Other differential diagnoses that need to be considered in secondary dRTA include drugs (ifosfamide, amphotericin B, lithium carbonate, and ibuprofen), hypercalciuric conditions (hyperparathyroidism, vitamin D intoxication, and sarcoidosis), and inherited disorders (medullary sponge kidney and Wilson's disease).[4]

SS is an autoimmune exocrinopathy with systemic involvement caused by monolymphocytic infiltration.[7,9] SS often affects middle-aged women aged 30–50 years with an incidence of 2.2–10.3 per 10,000 population.[4,10] The systemic disorders of SS may include QT prolongation, pulmonary disease, neurologic involvement, and renal tubulointerstitial disease (tubulointerstitial nephritis, RTA, Fanconi syndrome, and glomerulonephritis). However, the mechanism of action of dRTA in SS remains unknown. It is thought to be due to damage to the ATPase hydrogen pump in type A intercalated cells mediated by the immune system and the presence of autoantibodies to the carbonic anhydrase II enzyme.[5,9] The anatomic pathologies of SS in the kidneys are tubulointerstitial nephritis and glomerulonephritis.[4] Tubulointerstitial nephritis that occurs in SS has a pathological picture of predominantly lymphocytic infiltration of CD4

T cells, few CD8 T cells, and only 10% B cells, similar to that of salivary glands.[11]

The biochemical characteristic of dRTA is metabolic acidosis without an anion gap. The normal urine pH ranges from 6.7 to 7.4, and the serum bicarbonate level is approximately 5–20 mmol/L with an equivalent increase in chloride, such that the anion gap remains normal. Upon laboratory examination, it is possible to find auto-antibodies against type-A intercalated cell antigens.[8] The characteristic symptoms of SS are xerosis (dry eyes) and xerostomia (dry mouth). Approximately 70–80% of patients with SS complain of dry mouth. Other organ disorders include skin, lung, gastrointestinal, and kidney disorders.[4,7] The extra-glandular symptoms of SS include arthralgia, arthritis, Raynaud's phenomenon, myalgia, pulmonary disease, gastrointestinal disease, leukopenia, anemia, lymphadenopathy, neuropathy, vasculitis, and lymphoma.[1] The diagnosis of SS is often delayed, with a delay of approximately four years since disease onset was reported in one study.[3] The symptoms and signs associated with dRTA, even in a patient without sicca symptoms, can be indicative of SS cases.[4,12]

The criteria for the diagnosis of SS follow the American-European Consensus Group guidelines.[10] Patients must meet at least four of the six criteria (Table 1). The diagnostic test for SS finds a homogeneous or speckled pattern of ANA with positive anti-Ro/SSA and anti-La/SSB.[1] The prevalence of positive SSA-52 positive is approximately 63.2% in SS. The presence of positive anti-Ro/SSA indicates that the patient with SS is at an early stage accompanied by extraglandular involvement.[9] The diagnosis of SS in this patient was based on complaints of dry eyes, dry mouth, positive Schirmer's test, and positive autoantibodies to SS-A and Ro-52. Histopathological examination of the salivary glands and salivary scintigraphy were not performed due to limited diagnostic facilities. The patient had complaints related to dRTA since two years ago and complaints related to SS, which had also been around for a long time, but were only realized six months ago. Severe undiagnosed hypokalemia may cause muscle weakness that may precede sicca symptoms for months or even years before the diagnosis of primary SS, as in this case. There was a delay in the diagnosis of SS because the complaints were subtle and non-specific. Hypokalemia in this patient was secondary to dRTA associated with primary SS.

European guidelines recommend alkaline supplementation for dRTA therapy.[8] The mechanism of hypokalemia is due to excessive

Table 1. Revised International classification criteria for Sjogren's Syndrome[10]

I	Ocular symptoms: a positive response to at least one of the following questions:
	1 Have you had daily, persistent, troublesome dry eyes for more than 3 months?
	2 Do you have a recurrent sensation of sand or gravel in the eyes?
	3 Do you use tear substitutes more than 3 times a day?
II	Oral symptoms: a positive response to at least one of the following questions:
	1 Have you had a daily feeling of dry mouth for more than 3 months?
	2 Have you had recurrently or persistently swollen salivary glands as an adult?
	3 Do you frequently drink liquids to aid in swallowing dry food?
III	Ocular signs—that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests:
	1 Schirmer's I test, performed without anaesthesia (≤5 mm in 5 minutes)
	2 Rose bengal score or other ocular dye score (≥4 according to van Bijsterveld's scoring system)
IV	Histopathology: In minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score ≥1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm^2 of glandular tissue
V	Salivary gland involvement: objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests:
	1 Unstimulated whole salivary flow (≤1.5 ml in 15 minutes)
	2 Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts
	3 Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer
VI	Autoantibodies: presence in the serum of the following autoantibodies:
	1 Antibodies to Ro(SSA) or La(SSB) antigens, or both

sodium reabsorption in the collecting duct; therefore, excess potassium is secreted as a substitute for sodium. Sodium base salt supplementation is expected to increase the blood potassium levels. However, oral potassium formulations often cause dyspepsia. Moreover, it must be consumed 3–4 times per day. The liquid form of potassium aspartate is tolerable because it contains additional bases. Patients on a vegetarian diet may need lesser alkaline supplementation than those on a high animal protein diet.[8] SS with or without renal involvement adequately responds to steroid treatment.[1,9,13,14] Patients with early-stage SS with renal involvement usually have a poor prognosis.[3] This patient was given a moderate dose of corticosteroids and responded well. Potassium supplementation was continued while monitoring the serum potassium levels. The patient's prognosis was good as she has never been hospitalized again and was able to return to her daily activities as a teacher and housewife.

CONCLUSION

Patients with recurrent hypokalemia should be investigated for the cause, one of which is dRTA. The symptoms and signs of SS are often nonspecific and underdiagnosed. The dRTA can serve as a gateway for the diagnosis of SS. In this patient, complaints related to dRTA appeared before the onset of the sicca symptoms, and a diagnosis of SS was established. Both diseases can be managed simultaneously, even though the patient has to take medication for a long time. Education regarding treatment compliance is a crucial aspect of management.

COMPETING INTERESTS

The authors have no conflicts of interest to declare.

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None.

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Myocarditis Presenting as Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA) in a Young Man: A Case Report

Giovanni Reynaldo[1], Rachmat Hamonangan[2], Princella Monica[1]*

[1]St. Carolus Hospital, Jakarta, Indonesia.
[2]Division of Cardiovascular, Department of Internal Medicine, St. Carolus Hospital, Jakarta, Indonesia.

Corresponding Author:
Rachmat Hamonangan, MD. Division of Cardiovascular, Department of Internal Medicine, St. Carolus Hospital. Jl. Salemba Raya no. 41, Jakarta 10440, Indonesia. Email: rachmath1415@gmail.com; gioreynaldo@yahoo.com.

ABSTRACT

Myocardial infarction with non-obstructive coronary arteries (MINOCA) is a unique disorder that manifests as an acute myocardial infarction clinically without overt coronary arteries obstruction on angiography. Herein, we report a 17-year-old male presented with a chest pain occurring 3 hours before admission and fever lasting for 2 days. Electrocardiogram examination showed ST elevation in lead II, III, aVF and V3-V6. Laboratory tests results showed a normal leukocyte level of 9850/µL, an elevated troponin of 3.55 ng/mL and an elevated quantitative CRP of 46 mg/L. Coronary angiography performed, indicating 20-30% stenosis of the left anterior descending artery, left circumflex artery and right coronary artery, whereas in typical acute myocardial injury, angiography shows >50% coronary stenosis. Additional cardiac MRI examination showed a fulfillment of Lake Louis Criteria for myocarditis, with further findings of acute myocardial edema in the lateral wall of left ventricle, with left ventricle ejection fraction of 59.73%. As researchers are still working on the definition of MINOCA, present knowledge of the causes, pathophysiology, clinical features, or specific phenotypes of MINOCA is also limited. A stepwise diagnostic approach is needed to diagnose MINOCA, with subsequent differential diagnosis exclusion.

Keywords : *MINOCA, Myocarditis, Myocardial infarction, Angiography, Chest Pain, Electrocardiogra.*

INTRODUCTION

Myocardial infarction with non-obstructive coronary arteries (MINOCA) is a unique clinical entity that demonstrates acute myocardial infarction (AMI) clinically without overt coronary artery obstruction on angiography. As researchers are still working on the definition of MINOCA, present knowledge of the causes, pathophysiology, clinical features, or specific phenotypes of MINOCA is also limited. Furthermore, the diagnosis and management of these patients are yet to be improved. Thus, this case report is expected to advance the current understanding of MINOCA, especially those caused by myocarditis.

CASE ILLUSTRATION

A 17-year-old male without any significant medical history presented to the emergency department with chest pain occurring 3 hours before admission. He underwent similar chest pain 1 day earlier, lasting for 2 hours. He also got a fever in the last 2 days. The patient reported no history of smoking, diabetes mellitus, hypertension, or prior heart disease. He had already been vaccinated for COVID-19 with Sinovac, the second dose being 3 months earlier. There was no history of heart diseases nor autoimmune diseases in his family.

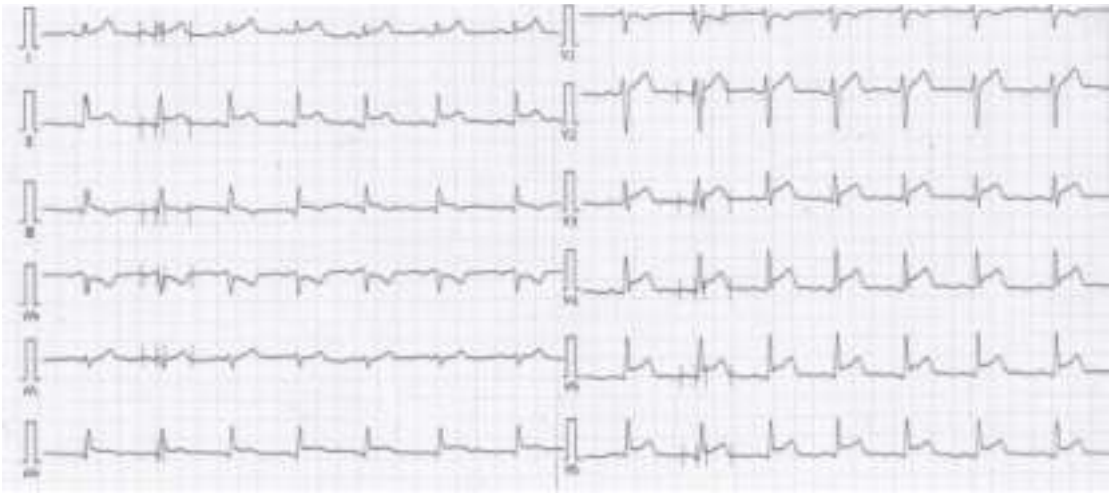

Figure 1. ECG show ST Segment elevation in lead II, III, AvF, V4-6.

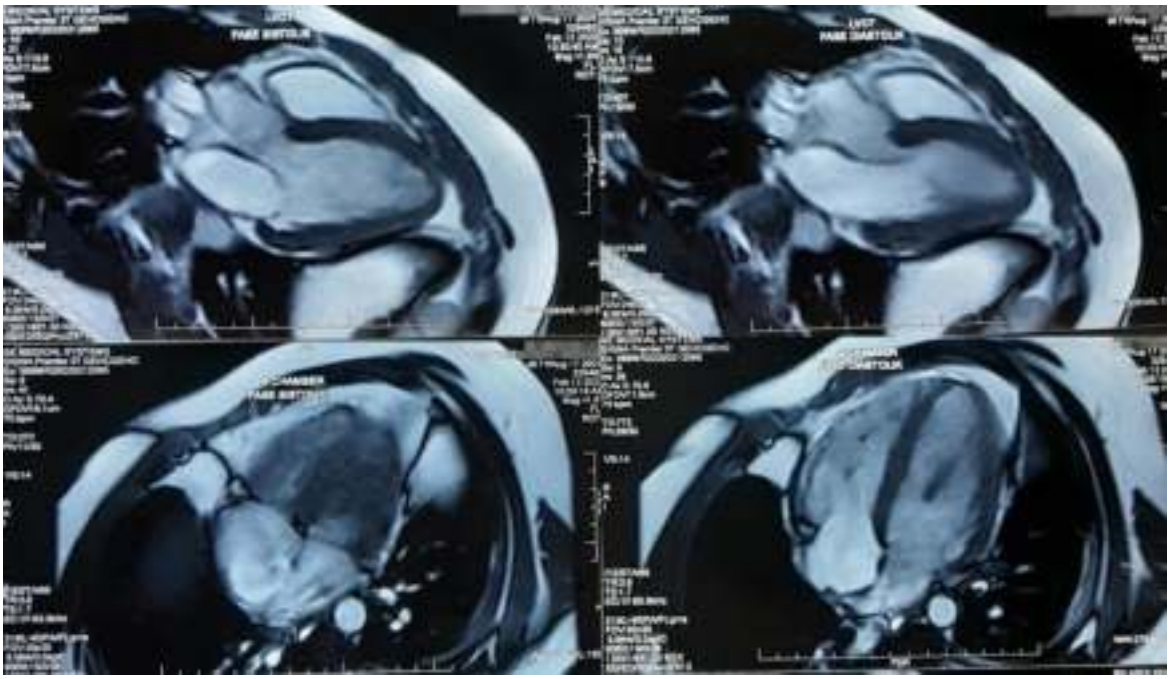

Figure 2. Cardiac MRI show hyperintensity of the mid myocardium in lateral wall of left.

Electrocardiogram (ECG) examination showed ST elevation in lead II, III, aVF and V4-V6. Laboratory tests results showed a normal leukocyte level of 9850/µL, elevated troponin of 3.55 ng/mL, elevated quantitative CRP of 46 mg/L and a negative SARS-CoV-2 PCR test. Further coronary angiography was performed, indicating 20-30% stenosis of the left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCA). The following laboratory tests showed ASTO of 400 IU/mL, TSH of 1.895 µIU/mL, anti-ds-DNA of <10 IU/mL, HbA1C of 6.8%, LDL cholesterol of 99 mg/dL, triglyceride of 156 mg/dL, and uric acid of 8.2 mg/dL. ANA profile showed positive results with SSA native (69 kDa) being +3, and anti-CMV IgG showed a positive result of 1.1 AU/mL. Anti-CMV IgM, anti-HSV, anti-HIV, HBsAg and anti-HCV were negative. Additional cardiac MRI examination showed a fulfillment of Lake Louis Criteria for myocarditis, with further findings of acute myocardial edema in the lateral wall of left ventricle, with left ventricle ejection fraction of 59.73%.

Afterwards, the patient was admitted to the High Care Unit. He was given subcutaneous fondaparinux 1x2.5 mg, intravenous pantoprazole 1 x 40 mg, oral acetylsalicylic acid 1x80 mg, nebivolol 1x2.5 mg, sacubitril valsartan 2x50 mg, rosuvastatin 1x40 mg, trimetazidine 1x80 mg and colchicine 2x0.5 mg. Later, the chest pain subsided and there was improvement in ECG examination as well as troponin test (0.547 ng/mL). The patient was discharged and further follow-up after 1 month showed no notable clinical findings. Then, the patient was scheduled for a follow-up cardiac MRI examination in 3 months, which showed improvement of myocardial edema.

DISCUSSION

Myocardial infarction with non-obstructive coronary arteries (MINOCA) encompasses approximately 6-15% of those presenting clinically with acute myocardial injury (AMI) without angiography showing typical >50% coronary stenosis.[1] Compared to obstructive AMI, the demographic and clinical manifestations of MINOCA slightly differ. Patients diagnosed with MINOCA are barely younger, with an average age of 58 years for MINOCA and 61 years for obstructive AMI.[2] Women are twice as likely to have MINOCA than men, as opposed to the obstructive AMI more commonly associated with men.[3] MINOCA has also been featured with less cardiovascular risk factors such as hypertension, metabolic disorders and other conventional risk factors.[2,4] Prognostic outcomes of MINOCA are still unclear, with studies showing overall results of no difference for better prognosis in MINOCA than obstructive coronary artery disease.[5-7]

The Fourth Universal Definition of Myocardial Infarction defines MINOCA as a group of patients with myocardial infarction with no angiographic obstructive CAD (≥50% diameter stenosis in the major epicardial vessel). The myocyte injury, like the diagnosis of MI, should be indicated by an ischemic mechanism. Myocarditis without any ischemic mechanism should be excluded from MINOCA.[8] The following statement from the American Heart Association (AHA) defines the diagnostic criteria for MINOCA as a patient with (1) Acute myocardial infarction (modified from the "Fourth Universal Definition of Myocardial Infarction" Criteria), (2) Non-obstructive coronary arteries on angiography, and (3) No specific alternate diagnosis for the clinical presentation. Myocarditis can cause a clinically subtle non-ischemic myocyte injury mimicking myocardial infarction (excluded from MINOCA) or presents as part of MINOCA (with the mechanism stated below).[3]

Several specific causes of MINOCA have been identified, but the pathophysiology of MINOCA is far yet to be explored. One mechanism involves atherosclerosis caused by plaque disruption in the coronary artery. Any plaque rupture, erosion, or calcific nodules can trigger thrombus formation leading to distal artery embolization, spasm, or complete transient thrombosis with spontaneous thrombolysis, ending with acute myocardial injury. Another mechanism involves nonatherosclerotic causes, including epicardial coronary vasospasm, coronary microvascular dysfunction, coronary thrombosis/embolism, spontaneous coronary artery dissection (SCAD), and a supply-demand mismatch. Myocarditis is one specific etiology that can cause MINOCA, in which pathophysiology comprises coronary microvascular dysfunction caused by myocardial edema with subsequent ischemic myocardial injury.[3] A previous meta-analysis by Tornvall et al. showed myocarditis itself is common in MINOCA, with a prevalence of 33%.[9] It should be remembered that myocarditis itself could occur without any myocardial infarction and mimic the clinical findings in MINOCA.[10]

A stepwise diagnostic approach to MINOCA is made by defining the presence of acute myocardial injury, clarifying the working diagnosis of acute myocardial infarction, confirming the finding of non-obstructive CAD, further diagnostic workup for the existence of ischemia (MINOCA), and ruling out other non-ischemic etiology (not MINOCA).[1] A cardiac troponin test showing an acute rise or fall in cardiac troponin (cTn) above the 99th percentile upper reference indicates an acute myocardial injury.[3] The clinical evidence of infarction can be seen as at least 1 of the following: (1) Symptoms of myocardial ischemia, (2) New ischemic

electrocardiographic changes, (3) Development of pathological Q waves, (4) Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic cause, or (5) Identification of a coronary thrombus by angiography or autopsy.[3] The next step is to detect using invasive coronary angiography whether obstructive CAD is present, followed by the appropriate management guidelines, or non-obstructive CAD, defined as the absence of obstructive disease on angiography in any major epicardial vessel, including normal coronary arteries (no angiographic stenosis), mild luminal irregularities (angiographic stenosis <30%) and moderate coronary atherosclerotic lesions (stenosis >30% but<50%). Also consider clinically overt differential diagnosis such as sepsis, pulmonary embolism, cardiac contusion, heart failure, stroke, critical illness, acute toxicity, strenuous exercise and burns.[1,3]

If all the criteria above are met, a working diagnosis of MINOCA can be made, which is confirmed by subsequent investigations. A coronary angiogram should be re-evaluated for the possibility of side branch occlusion, missed obstructive CAD, or spontaneous coronary artery dissection. Cardiac ventriculography can also be considered if there is a possibility of Takotsubo syndrome. The last step is a preference based on clinical and angiographic presentation and considering local availability and expertise, which includes a non-invasive pathway with cardiac magnetic resonance (CMR) and an invasive pathway with optical computed tomography (OCT). CMR could detect any acute myocardial infarction, Takotsubo syndrome, acute myocarditis and other cardiomyopathies, while OCT can help detect any epicardial spasm, plaque-induced event and SCAD.[1]

The diagnostic criteria for MINOCA were fulfilled in this patient, with symptoms of myocardial ischemia, ST-elevation indicating ischemic ECG changes, an elevated troponin of 3.55 ng/mL and 20-30% stenosis found in coronary artery angiography. However, subsequent cardiac MRI examination indicated fulfillment of Lake Louis Criteria for the diagnosis of myocarditis, with findings of lateral and left ventricle myocardial edema suggestive of parvovirus infection. Currently, there is limited evidence of the simultaneous occurrence of acute myocarditis and MINOCA, with a possibility of either acute myocarditis mimicking MINOCA or acute myocarditis followed by MINOCA through coronary microvascular dysfunction.[1,3,9-11] The use of anti-inflammatory drug colchicine inhibits NLRP3 activity. Furthermore, anti-fibrotic effects of colchicine have also been reported.[12] It is recommended to be use as a first line therapy for treatment of acute pericarditis, which is similar to myocarditis.[12] Further supports the hypothesis that colchicine could also be useful in the treatment of myocarditis.[12] The fact that colchicine is inexpensive and its use worldwide is established, colchicine could be an ideal candidate for the treatment of potentially all forms of virus-induced myocarditis, including SARS-CoV2-related myocarditis syndromes.[12]

CONCLUSION

Patients with MINOCA usually present with clinical manifestations of acute myocardial infarction but with distinct demographic presentations. A stepwise diagnostic approach is needed to diagnose MINOCA, with subsequent differential diagnosis exclusion. Further studies are required to explain the coexistence of MINOCA and myocarditis.

CONFLICT OF INTEREST

All authors declare no conflict of interest related to this study.

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A Mixed Gonadal Dysgenesis in an 19 Year Old Girl with Ambigous Genitalia: A Case Report

Amanda T. Hardigaloeh[1], Tri Juli E. Tarigan[1], Em Yunir[1*], Kanadi Sumapradja[2], Feranindhya Agiananda[3], Yulia Ariani[4], Nanis S. Marzuki[5], Irfan Wahyudi[6], Lisnawati[7]

[1]Division of Endocrinology, Metabolism, and Diabetes, Departement of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2]Department of Obstetric Gynecologist, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[3]Department of Physiciatris, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[4]Department of Biology, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[5]Eijkman Institute for Molecular Biology, Jakarta, Indonesia.
[6]Department of Urologist, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[7]Department of Pathology, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Em Yunir, MD, PhD. Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: e.yunir@ui.ac.id.

ABSTRACT

A 19-year-old girl was referred with delayed puberty and ambiguous genitalia. She had short stature with high blood pressure and Turner's stigmata with external genitalia Prader Score 4. Ultrasound revealed hypoplastic uterus with no gonad. Follicle stimulating hormone, luteinizing hormone and testosterone level were increased (51.29 mIU/mL, 23.66 mIU/mL and 742 ng/dl). Karyotyping revealed 46 XY with Fluorescence in situ hybridization cytogenetic study based on 300 cells showed mosaic chromosome, monosomy X (17%) and XY (83%). Laparascopic gonadectomy was done and showed that testes were only in the right inguinal canal. Then patient had external genitalia reconstruction and received estrogen replacement therapy.

Keywords*: mixed gonadal dysgenesis, ambiguous genitalia*

INTRODUCTION

Disorders of sexual development (DSD) are a congenital condition in which the development of the chromosomal, gonadal, or anatomic sex is atypical.[1] These are divided into 46 XX DSD, 46 XY DSD, and sex chromosome DSD.[2] Gonadal dysgenesis is part of DSD and characterized by incomplete or defective gonadal development as a result of either structural or numerical anomalies in the sex chromosomes or mutation in the genes involved.[3] Mixed gonadal dysgenesis (MGD) belongs to sex chromosome DSD, which has a prototypical karyotype of 45,X/46,XY, and many variations in clinical manifestation. We

herein report 19 years old female with MGD presenting with ambiguous genitalia and delayed puberty.

CASE ILLUSTRATION

A 19-year-old "girl", was referred to our hospital by Abdoel Moelek Hospital in Lampung Province, with a suspect of Turner Syndrome. The chief complaint of the patient were delayed menstruation and breast development. Her medical history revealed 38 weeks' gestation with a weight of 2700 gr and length of 48 cm. At birth, atypical "clitoris" as female externa genitalia was found. She was raised as female and had short stature with normal motoric development. The girl had not pubarche and thelarche but had virilization sign as voice deepening at puberty and clitoris become bigger (clitoromegaly). She was also a quite type of person. At presentation aged 19 years, physical examination revealed a height of 135 cm, a weight of 38 kg, body mass index was normal (20.9 kg/m2) and with a potential genetic height of 136.5-156.5 cm. Blood pressure was 145/98 mmHg. She had low hairline, strabismus, hirsutism and wide neck. She showed a normal cardiopulmonary and abdominal examination; no intra-abdominal or inguinal masses were palpable. External genitalia inspection revealed a marked clitoromegaly, labia enlargement resemble scrotum with no testes and introitus vaginal. The Tanner stage was pubic hair II, breast I, axillary hair I. The Prader Stage was 4. The karyotyping examined based on 20 cells showed 46 XY. Testosterone level was 742 ng/dl (8.4-48.1 ng/dl) and DHEA level was 95.3 ug/dl (61.2-493.6). Luteinizing Hormone (LH) was 23.66 mIU/mL (2.4-12.6), Follicle Stimulating Hormone (FSH) was 51.29 mIU/mL (3.5-12.5) with normally 17 OH progesterone and cortisol (2.68 ng/ml and 15.6 ug/dl) and decrease of Anti Mullerian Hormone (AMH) (1.64 ng/ml). Renal function (eGFR) was decreased (33.6 mL/min/1.73 ml). Bone age revealed average 18 years old girl. Abdominal CT revealed undescended testes only in right inguinal canal and hypoplasia uterus. There was no left testes and ovarium founded. Abdominal doppler vascular ultrasound revealed suspicious of proximal renalis artery stenosis. Then patient was referred for Fluorescence in situ hybridization (FISH) test and showed mosaic chromosome, monosomy X (17%), and XY (83%) from 300 cells. Rekaryotyping after FISH examination revealed mos 45,X[2]/46,XY[38]. We make the diagnosis of this patient became mixed gonadal dysgenesis (MGD). The parents were informed about this disorder then referred to a psychiatrist. Gender identity revealed that the patient was adopted as female because she has raised as a girl and has been comfortable with it. She also had moderate mental retardation that made the decision depend on her mother. The patient then undergone the right gonadectomy and external genitalia reconstruction. The biopsy result was atrophic testicular cell without spermatogenesis. Now she receives the estrogen replacement therapy. Psychosocial support was started as soon as the diagnosis was established.

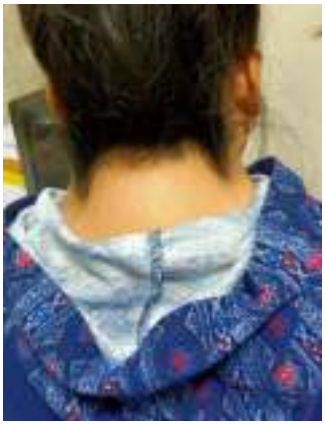

Figure 1. Low Hairline and Web.

Figure 2. Ambiguous Genitalia.

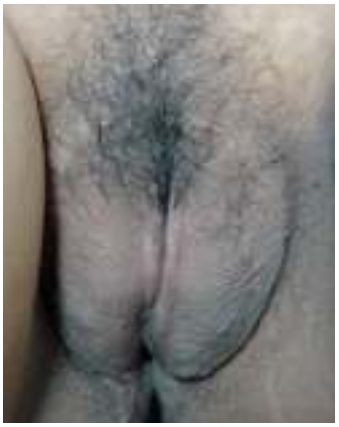

Figure 3. Post Genitalia Reconstruction.

Figure 4. Second Karyotype.

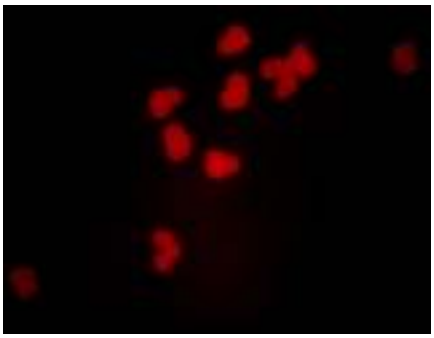

Figure 5. FISH.

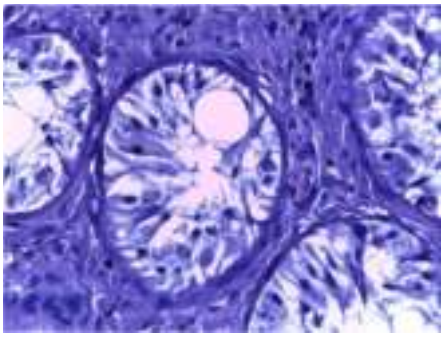

Figure 6. Abundant Leydig Cell Without Spermatogenesis.

DISCUSSION

Ambiguous genitalia is a common term that is used to describe atypical internal or external genitalia. This condition is one of clinical manifestation of DSD. DSD can be defined where the genital is disconcordant with chromosomes or gonads. DSD have 3 categories, 46 XX DSD, 46 XY DSD, sex chromosome DSD.[2]

Our patient had turner's syndrome phenotype with ambiguous genital. Classical Turner's Syndrome never present with ambiguous genital so Congenital Adrenal Hyperplasia (CAH) was first our consideration. The first step that we did was to look genotype of the patient by chromosomal analysis. Standard chromosomal analysis from 20 cells examined revealed 46 XY. The 17 OH Progesterone and cortisol level showed in the normal limit, made CAH could be excluded. Because of a discrepancy between phenotype, biochemical results and imaging, we decided to do second karyotyping with more number of cell and FISH (fluorescence in situ hybridization) to look particular DNA sequence. FISH cytogenetics revealed the presence of XY chromosome in 83% cell and one X chromosome in 17% of cells studied (300 cells). The rekaryotyping was 45,X[2]/46,XY[38], suggestive mixed gonadal dysgenesis (MGD). In this case, the level of mosaicism 45,X/46,XY was low, that could not be detected by standard karyotyping procedure. Analysis of FISH on large numbers of cells helped us to confirm the diagnosis by looking the particular of DNA sequence.[4]

MGD is a rare disorder of sexual differentiation which occurs due to asymmetrical gonadal development and belongs to sex chromosome DSD.[5] MGD commonly has a combination between XO and XY genotype in a cell which showed by karyotype of 45,X/46,XY. The 45,X/46, XY mosaicism is estimated detection rate of 1.7 per 10.000 newborns.[6] It usually happens because of non-disjunction in mitotic phase post zygotic. 45 X appears because of chromosome Y rearrangements (commonly dicentric and ring Y chromosome). Partial testicular dysgenesis on 45,X/46,XY can be happened because of partial expression of the SRY gene made a disturbance of testosterone synthesis with undervirilization. SRY gene provides instruction for making sex determining region Y protein. The presence of Y chromosome in dysgenetic gonad renders the child at high risk to gonadal malignancy (gonadoblastoma) and dosage loss of the SHOX (Short Stature Homeobox) gene that made short stature phenotype. The other features in our patient was uterus existence, which is determined by lack of AMH level which was responsible with the regression of the mullerian ducts, fallopian tubes and uterus.[7]

The clinical symptoms of MGD are combination between XO gonadal dysgenesis and the normal XY male.[5] It varies depends on ratio of 45, X/46, XY fragment among the tissues, with those having a higher ratio being more likely to exhibit the turner syndrome phenotype. We could find a wide spectrum of phenotypes, from turner syndrome to phenotypically normal males. In our patient, 45 X cell (monosomy X) made the presence of the somatic stigmata of turner syndrome's such as short stature, low hairline, strabismus, wide neck, anomaly of renal until mental retardation. MGD patients sometimes have overlapping features of classical Turner syndrome.[8] There were 10-12% cases of Turner Syndrome has 45,X/46,XY mosaicism.[9] Furthermore, high level of testosterone in our patient represent the function of XY cell. The mosaicism ratio in gonadal tissue and blood may explain the variability in the phenotypes.[8] Turner syndrome phenotype in this patient may occur because of the higher ratio of 45,X/46,XY fragment and consequent lack of Y chromosome in her gonadal tissue. Unfortunately, we just measure the ratio only in peripheral lymphocyte. Wu Q et al reported 16 Chinese patients with 45,X/46,XY mosaicism demonstrated that most patients had persistent Mullerian structures with a streak or unidentified gonads which present as an infantile or rudimentary uterus with 2 patients having a normal sized uterus. These findings are consistent with our patient. [10] Anomaly renal also happened in our patient. She has secondary hypertension with chronic kidney failure stage IIIa with suspection of renalis artery stenosis based on doppler vascular abdominal ultrasound. The last, the Turner's mosaicism also explained the hypergonadotropic in this patient.[9]

The average age of onset of puberty in MGD, was from 11 to 13 years, which is within the normal range. It consistently appears with our patient.[7] The genital appearance of MGD is characterized by the asymmetrical testis with unilateral testis dysgenesis, a streak gonad on contralateral side and persistent Mullerian structures.[11] First time, we found clitoromegaly resemble phallic enlargement without visualization of testes by physical examination and ultrasound. So, we conducted abdominal CT and found single testis in dextra canalis inguinalis, uterus hypoplasia and no ovarium. The biopsy showed that there were much leydig cell, but without spermatogenesis process. This explained why our patient had high level testosterone and male sex secondary sign likes deepening voice. Imaging is generally used to evaluate presence localized gonads and detect any malignanty features. However, small tumours or dysgenetic gonads may be missed due to the heterogenecity of their size and appearance.

The multidisciplinary approach, involving endocrinologist, psychiatrist, gynecologist, urologist and biological scientist, is the corner stone for DSD. We conducted a careful review of physical and psychologic features, hormonal evaluation, karyotyping, and imaging. The psychological evaluation revealed female gender identity. We found little difficulties in this process, because this patient had moderate mental retardation. So the decision depends on her mother. This explained why she was so quite when we did some questions.

In the early process, we much considered the balance of risk and benefit of surgery. Our patient was phenotypically dominance of female and has been raised as a female for her whole life. In case of the decision to convert to female gender, testis structures should be removed. So we decided to do laparoscopy gonadectomy and external genitalia reconstruction. The surgical approach also considered about future surveillance and risk of malignancy because dysgenetic gonad. The risk of malignancy particularly gonadoblastoma which caused by the presence of Y chromosome is 7-10% in one study. [9] The risk of developing a malignancy was noted to be highest in individuals with ambiguous phenotype, 52% compared to 2.2% in females without signs of virilization.[12] The histopathological examination revealed the testis contained abundant mature leydig cells and tubules with only sertoli cells but without spermatogenesis process, i.e. the picture of germinal cell aplasia. Post operative, the level of testosterone becomes normal and estrogen replacement therapy was started to stimulate secondary sexual development and uterine growth and prevent bone loss. Follow-up for

the patient should also encompass monitoring of health performance and hormonal replacement. Full of informed consent should be given in family because it can address some stigma in the future.

CONCLUSION

We present a rare disorder of mixed gonadal dysgenesis(45,X/46 XY) with turner's phenotype, ambiguous genital with gender identity of female. This case needs comprehensive management of the rare endocrine condition.

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Effectivity of Bromocriptine Administration Towards Prolactin Positive Breast Cancer Receiving Anthracycline-Based Chemotherapy: A Literature Review

Muhammad Yadi Permana[1], Sarwanti[1], Siti Fauziah[2]*

[1] Fatmawati Hospital, Jakarta, Indonesia.
[2] Faculty of Medicine, Syarif Hidayatullah National Islamic University, Jakarta, Indonesia.

*** Corresponding Author:**
Muhammad Yadi Permana, MD. Fatmawati Hospital. Jl. RS Fatmawati Raya No. 4, Jakarta 12430, Indonesia. Email: yadionko@gmail.com.

ABSTRACT

Breast cancer is among the deadliest gynecology cancers in the world. However, the management of advanced-stage breast cancer is often harder as a result of chemoresistance. This review aimed to discover the effect of bromocriptine on prolactin-positive breast cancer patients who received anthracycline-based chemotherapy. It is known that anthracycline works by inhibiting topoisomerase IIα (TOP2A), forming free radicals, binding DNA, and altering cell homeostasis, hence stopping the cell cycle and inducing cell death. However, reduction of TOP2A expression and increased glutathione s-transferase (GST) and ATP-binding cassette (ATP) membrane activity increase anthracycline efflux from the cell membrane, hence reducing its effectivity. Prolactin is one of the most common chemoresistance agents whose complex with its receptor will induce JAK/STAT pathway to increase GST. The regulation of Bcl-2 and ERK was also determined by prolactin. Bromocriptine is an agonist of the D2 dopamine receptor that inhibits adenyl cyclase and a D1 dopamine weak antagonist. Bromocriptine could reduce prolactin serum and receptors in various cases. Some studies have found that bromocriptine could improve the effectiveness of chemotherapy regimens, including cancer-related hyperprolactinemia, breast cancer that underwent cisplatin, and taxanes. Therefore, bromocriptine offers potential as it could improve outcomes and reduce resistance in prolactin-positive breast cancer patients who are administered anthracycline-based neoadjuvant chemotherapy.

Keywords: *breast cancer, bromocriptine, chemotherapy, prolactin, receptor.*

INTRODUCTION

Breast cancer is the most common gynecological malignancy in the world. Data from the International Agency for Research Cancer reported that the incidence of breast cancer was as high as 11.6% in 2018. Breast cancer contributes to the most death by cancer, above lung and colorectal cancer.[1,2] About 60 to 70 per cent of breast cancer patients are diagnosed in late stages (III or IV).[3] Breast cancer treatment involves various modalities, including neoadjuvant chemotherapy. Neoadjuvant chemotherapy is aimed at making tumors likelier to be resected, hence improving the chance of breast-conserving operations in some cases.[4] Neoadjuvant chemotherapy is also beneficial in eradicating micrometastasis and reducing surgical morbidity in patients with axillary node involvement.[4–6]

There are various chemotherapy regiments for tumor eradication, including anthracycline, which consists of doxorubicin, epirubicin, idarubicin, and daunorubicin. Anthracycline can cause irreversible defects in cancer cells

by inhibiting the topoisomerase IIα (TOP2A) enzyme in the cell nucleus. This process could stop the DNA replication process and started apoptosis.[7,8] There is some evidence of anthracycline-based chemotherapy. Nakajima reported that breast cancer stage II to III patients showed a positive response toward anthracycline-based chemotherapy, with an overall survival rate of 83.4%.[9] Another study suggested that response rates in triple-negative, HER2-positive, luminal B, and luminal A were 87.5%, 80%, 61.2%, and 55.2%, respectively.[10]

Chemoresistance is one of the biggest burdens of cancer therapy. Chemoresistance was found in various chemotherapy regimens, including anthracycline. Chemoresistance toward anthracycline is developed through reduction of TOP2A expression, elevation of GST, and elevation of ATP binding cassette (ABS) membrane transporter's expression.[11,12] Another neglected chemoresistance mechanism is complex between prolactin and prolactin receptor.[13] Prolactin physiologically helps secretion, differentiation, stimulation, and proliferation of breast cells. On the other hand, prolactin was linked to aggressivity of tumour.[13–15] A study in Indonesia found that 62.5% of advanced stage breast cancer patients showed prolactin receptor expression.[16] Another study found that more than 90 per cent of pre-menopausal women with breast cancer expressed prolactin receptor.[17]

Bromocriptine is an alkaloid ergot with high selectivity toward the D2 dopamine receptor, which could reduce prolactin secretion in the anterior hypophysis.[18,19] A study reported that low-dose bromocriptine (2.5 mg) could normalize prolactin levels in breast cancer and prostate cancer patients for 24 hours. The same dose is also considered beneficial as supplementary drug to endocrine therapy or chemotherapy.[20] Another study suggested that there was synergy between bromocriptine and doxorubicin or paclixatel in inhibiting the growth of leukemic cell CEM/ADR5000.[21] Therefore, we conducted a literature review to review chemoresistance in prolactin-positive breast cancer receiving anthracycline-based chemotherapy and bromocriptine administration effectivity in the population.

BASIC KNOWLEDGE OF BREAST CANCER

Breast cancer is one of the most common types of women's cancers in the world. Data from Global Cancer Statistics (Globocan) stated that there were 2,088,849 incidences of breast cancer in 2018.[1] About 43.6% of these were found in Asia. The data also estimated that 1 out of 4 women who was diagnosed with cancer was diagnosed with breast cancer. Breast cancer was ranked as the fifth most fatal cancer and the most fatal woman cancer.[1,2] Breast cancer could be caused by various factors, which are categorized into demographic, reproductive, hormonal, hereditary, lifestyle, and other factors. These factors are grouped as intrinsic and extrinsic based on their nature of origin. Intrinsic factors included age, gender, race, and genetic predisposition. These intrinsic factors are independent parameters that cannot be modified. Extrinsic factors are modifiable and are commonly targeted in breast cancer prevention strategies.[22,23]

Based on molecular subtype, breast cancer is divided into luminal A, luminal B, HER2+, and triple-negative/basal-like which is categorized based on estrogen receptor, progesterone receptor, and HER2 expression on immunohistochemistry examination.[24] A study in Indonesia showed that luminal B was the most common subtype found (43.9%), followed by HER2-positive (14.6%), luminal A (14.0%), and basal-like (11.3%). The same study also found a significant relationship between tumor stage and TOP2A expression with molecular subtypes.[25] Another study conducted in Indonesia showed that luminal A was the most common type found, followed with triple-negative/basal-like, HER2-positive, and luminal B, with proportions of 38.1%, 25.0%, 20.2%, and 16.7%, respectively. The study stated significant differences in age, histological gradation, stage, and lymph nodes' status between subtypes.[26] A nationwide study in Indonesia showed that luminal A was the most common type found, followed by triple-negative, HER2-positive, and luminal B. Luminal B was the most common type found in the age group below 40 years old. HER2-positive was the most common type found

in the age group older than 50 years. Larger tumor size was observed in triple-negative/basal-like tumour.[27]

ANTHRACYCLINE-BASED NEOADJUVANT THERAPY

Anthracycline is one of the agents used for neoadjuvant chemotherapy, which aims to change an inoperable state into an operable state and to downgrade mastectomy to a more conservative surgical option.[28] It is extracted from *Streptomyces peucetius* var. *caesius*. Anthracycline covers large therapy options, including doxorubicin, epirubicin, idarubicin, and daunorubicin, which differ in chemical structure and cell activity. Doxorubicin and epirubicin are used for solid tumors, whereas daunorubicin and idarubicin are mainly used for acute leukemia. Anthracycline is given through circulation and extensively metabolized in the liver, 50% of which is excreted through biliary excretion. An anthracycline therapeutic effect is reached after being converted into intermediate alcohol form. Anthracycline is distributed rapidly into various organs but cannot pass the blood-brain barrier.[7,8]

Anthracycline works through four main processes. Anthracycline inhibits TOP2A, forms free radicals, which are semiquinone and iron-dependent reactive oxidative species (ROS), binds DNA through an intercalation process that blocks DNA and RNA synthesis, and impairs ion transport and fluid metabolism through its binding toward the cell membrane.[8,29] Anthracycline active metabolites passively diffuse into the cell and bind proteosomes. Anthracycline is then translocated from the cell nucleus cytoplasm through the help of ATP, further binding with DNA. Anthracycline works by blocking catalytic enzymes, which stabilize DNA chain separation in a covalent way. Anthracycline causes irreversible DNA damage, which is mediated by TOP2A in actively proliferating cancer cells. Anthracycline also works by inhibiting the TOP2A enzyme in the cell nucleus and inhibiting double-bond DNA formation; hence, DNA replication stops, and apoptosis is induced as a result of the inability to repair or replicate DNA. Anthracycline also works as a DNA intercalator that could insert a planar ring within the DNA base, thus preventing replication and transcription of DNA.[29] In addition, binding between anthracycline and the cell membrane could impair fluid balance and ion transport. This phenomenon could induce the production of free radicals within cells. The quinone component of anthracycline reacts with oxygen and produces radical anion superoxide. This causes peroxidase and superoxide production, which could impair DNA and cause DNA base oxidation, leading to apoptosis. The formation of these free radicals is significantly mediated by the interaction of anthracycline and the iron component.[8]

Anthracycline-based chemotherapy has been proven effective in various studies. A study showed that good responses were found in luminal A, luminal B, HER2, and triple-negative breast cancers that received anthracycline-based chemotherapy with response rates of 55.2%, 61.2%, 80.0%, and 87.5%, respectively.[10] Another study showed that anthracycline was effective in breast cancers with negative estrogen receptors, a high S-phase fraction ratio, and a high Ki-67 level.[30] It was reported that TOP2A was one predictive effect of the factor. However, a recent study did not find a correlation between TOP2A expression and breast cancer response or survival. The study also found that a positive response to breast cancer was independently associated with TOP2A expression.[31]

CHEMORESISTANCE TOWARD ANTHRACYCLINE-BASED NEOADJUVANT CHEMOTHERAPY

Chemoresistance toward anthracycline-based regiments results from various mechanisms, including reduction of TOP2A expression, increase of Glutathione S-transferase activity, or increase of ABC membrane transporter expression, all of which could reduce chemotherapy toxicity.[32] Topoisomerase II alpha (TOP2A) enzyme is the main target of anthracycline-based chemotherapy. Therefore, downregulation of TOP2A is suspected to cause resistance in anthracycline-based regiment.[33] Suppression of TOP2A could induce chemoresistance toward doxorubicin. A study suggested that cells that express TOP2A at a low

level tend to be more resistant to chemotherapy regimens. This was explained by the reduction of the TOP2-DNA complex, which could cause less tumor cell DNA that experiences impairment due to anthracycline administration. Therefore, it was reported that patients with TOP2A deletion were less responsive to doxorubicin administration.[31,33]

Another mechanism that could explain chemoresistance is inactivation by glutathione S-transferase (GST), which is a phase II detoxification enzyme whose function is to protect macromolecules by forming electrophilic components. GST catalyzes glutathione conjugation toward the chemotherapy regiment's metabolite, which could fasten the effective duration of medication within cells, hence reducing the effectivity of the chemotherapy regiment.[34,35] ABC component also accounted for anthracycline-based chemotherapy chemoresistance. ABC is found to cause medicine metabolite efflux through MDRP1, which codes P-gp. A study mentioned that anthracycline uptake was found to be reduced substantially alongside an increase in ABS transporter expression (ABCb1, ABCc1, ABCc2).[36,37]

The expression of the superfamily aldo-keto reductase (AKRs) enzyme is another factor in chemoresistance to this regimen. AKRs change ketones and aldehydes into primary and secondary alcohols. AKR expression is regulated by osmotic pressure, AP-1 transcription factor, and anthracycline-generated reactive oxygen species (ROS).[38,39] AKR1C metabolizes various chemotherapy agents, including doxorubicin. AKR1A1 and AKR1C2 could converse the anti-tumor agent doxorubicin into doxorubicinol, which is less toxic. Another substance called carbonyl reductase and quinone oxidoreductase-1 (NQO1) could metabolize doxorubicin into doxorubicinol. This conversion could cause a change of medicine localization into lysosome, which impairs the ability to penetrate the cell nucleus.[38]

One of the effects of anthracycline administration is increasing free radicals and reactive oxidative species through an enzyme-mediated process as a result of anthracycline toxicity. This phenomenon induces a protective mechanism to reduce free radicals, called a glutathione-dependent protective mechanism, which is also related to doxorubicin chemoresistance.[40,41] Change of p53

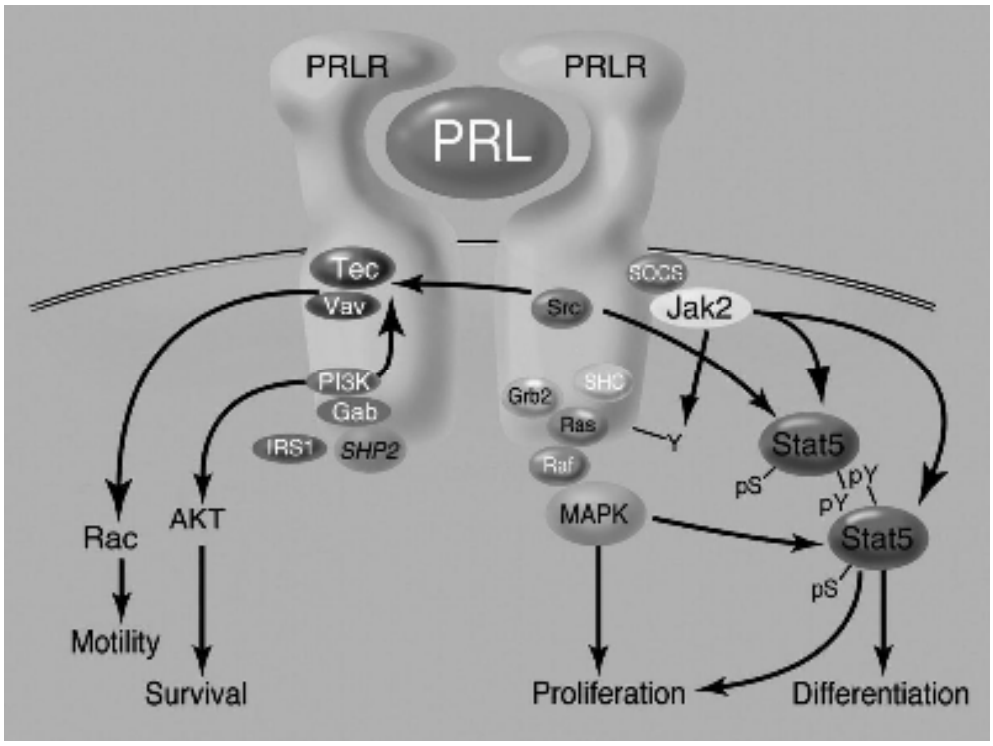

Figure 1. Complex of ligand PRL-RP, JAK/STAT, and MAP kinase.[46]

is also related to doxorubicin administration, which could reduce the apoptosis effect of chemotherapy. Another study suggested a positive relationship between transmembrane TNF-α and anthracycline resistance. It was found that transmembrane TNF-α was present in more than 50% of patients' breasts, which could impair breast cancer cells' sensitivity toward doxorubicin.[42]

PROLACTIN ROLE IN BREAST CANCER AND CHEMORESISTANCE

Prolactin is a 23-kDa-sized hormone that is mainly produced by the hypophysis, with the breast as its main target. Prolactin is categorized as a lactogen protein that consists of a homologous structure.[43,44] Prolactin could promote malignant cells' proliferation and differentiation (**Figure 1**). This was proven by various studies that linked high prolactin levels with tumor cell progression, both in vitro and in vivo.[45] Prolactin was also linked with the failure of chemotherapy and management. Prolactin binds with the prolactin receptor to induce the signaling cascade and the Janus kinase-signal transducer and activator of transcription (JAK-STAT) cascade. Prolactin also induces other cascades, such as Ras-Raf-MAPK, ERK1/2, and c-Jun N-terminal kinase.[13]

The prolactin receptor is a cytokine receptor that serves as a single-pass membrane. The prolactin receptor isoform weighs 80 kDa. The prolactin receptor is mainly mediated through JAK2/STAT5 pathway, but also through other signaling pathways.[47] Prolactin receptor activation needs ligand binding in two places where one place should have higher affinity. This bond activates the ternary complex, which consists of one molecule hormone and a homodimer receptor.[48] This receptor could also bind to placental lactogens and growth hormones. Unlike estrogen, which could bind to classic and non-classic receptors, prolactin only has one specific receptor).[45,49]However, prolactin could form various isoforms that could be processed by various signaling cascades.[49] There is a relationship between prolactin receptors and classic estrogen receptors on various levels.[50]

Increased expression of prolactin receptors and prolactin levels are associated with the risk of tumor progression, invasion, and metastasis.[51] Various studies have reported the majority of breast cancers on human-expressed prolactin. Prolactin receptor expression is found positive in 80 to 90 per cent of breast cancer.[13] A prospective study demonstrated that prolactin and prolactin receptors were found in up to 95% and 60% of breast cancer cases in women and men, respectively.[52,53] A large-scale study in Poland showed that almost 83% of breast cancer cases on 142 pre-menopausal women and 594 post-menopausal women showed prolactin receptor expression.[54] A study in Indonesia showed that 62.5% receptor samples showed prolactin receptor expression.[16]

Prolactin is an important component of breast cancer chemoresistance, but it is often neglected. An epidemiology study mentioned that chemoresistance and lower survival rates were related to higher prolactin levels. It was also mentioned that the prolactin and prolactin receptor complex improved the expression and activity of GST, which was mainly mediated through JAK/STAT.[13] Besides JAK/STAT, this complex was mediated by MAPK. A study found that there was an antagonist effect of prolactin toward cisplatin-induced apoptosis.[55] Binding between prolactin and its receptor induces JAK/STAT and Ras-Raf-MAPK pathway activation. These phenomena activate GST, which conjugates cisplatin into glutathione.[13] Glutathione activation increases efflux and minimalizes DNA impairments. GST activates regiments that are based on platinum, doxorubicin, cyclophosphamide, and etoposide, but not in microtubule-based regiment.[55,56] Chemoresistance mechanism which was not linked with GST, could involve alterations of Bcl-2 protein (**Figure 2**).[13]

Other than JAK/STAT pathway, prolactin could synergize with IGF-I and EGF-family ligand to activate MAPKs and AKT. This pathway contributes to gene expression, which is related to proliferation, survival, and invasiveness. Furthermore, this activation is linked to chemoresistance toward endocrine therapy, molecular therapy, chemotherapy, and radiotherapy.[13] ERK1/2 (MAPK) and

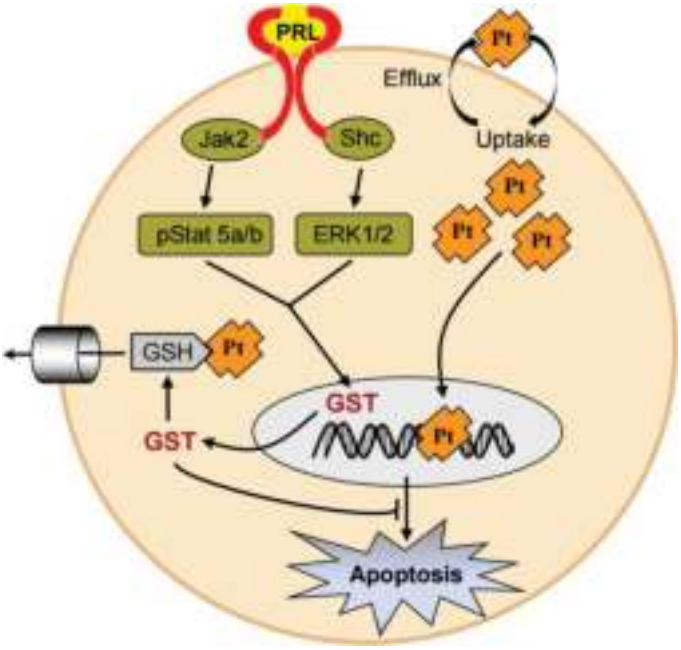

Figure 2. Mechanism of cisplatin chemoresistance by prolactin.[56]

AKT activation could also induce estrogen and prolactin receptors, which could increase transcription and degradation activity. The prolactin pathway through JAK2/STAT5 will increase estrogen receptor expression, which could increase estrogen sensitivity and estrogen-targeted gene expression, including the prolactin receptor.[57] A study found that there was a prolactin and hormone/cytokine role in activating the STAT5 pathway and intervening in cell cycle function, which was regulated by BRCA1 wild type.[58]

Endogenous prolactin's ability to reduce chemotherapy regiment efficacy was also observed in an in vitro study that used ceramide metabolites to mediate apoptosis induction as a result of stress stimulation and receptor death through gamma irradiation. It was observed that breast cancer cells that produced prolactin were more resistant to ceramide-induced apoptosis than non-prolactin-producing cells.[59]

BROMOCRIPTINE ADMINISTRATION TOWARD BREAST CANCER

Bromocriptine is a dopamine agonist that can inhibit prolactin release, which is triggered by the prolactin-releasing hormone (PRH). Bromocriptine specifically works as an agonist in the D2 dopamine receptor and inhibits adenylyl cyclase. Bromocriptine is a weak antagonist of the D1 dopamine receptor and an alkaloid ergot, which is extracted from the *Claviceps purpurea* fungus. This fungus produces histamine, acetylcholine, tyramine, and other active biological substances. Bromocriptine has various effects on receptors, including the adrenoreceptor, serotonin receptor, 5-HT receptor, and dopamine receptor. Bromocriptine and other ergot derivates have high selectivity toward hypophyseal dopamine receptors, thus directly suppressing prolactin secretion from hypophyseal cells through the regulation of dopamine receptor.[19,20]

Bromocriptine has been used on various occasions. Mostly, bromocriptine is used for parkinsonism therapy through the inhibition of dopamine receptors in the nigrostriatal tract. Bromocriptine is also used for hyperprolactinemia and acromegaly through inhibition of the tuberoinfundibular tract.[60] Other newer studies suggested that bromocriptine was useful against diabetes mellitus and other diseases.[19,20,61] Bromocriptine was also found to be useful against hypophyseal tumors and other tumours.[19,20]

Bromocriptine is usually administered orally, which has good absorption. However,

bromocriptine is extensively extracted and metabolized in the liver; hence, only 7% of absorbed materials can reach systemic circulation with a half-life of 2–8 hours.[20] Bromocriptine has already been available in a slow-release dose, which could reduce the frequency of medicine consumption. Other than oral, bromocriptine could be administered through the intravaginal method, which provides fewer gastrointestinal adverse effects.[19,20] Bromocriptine is metabolized extensively in the liver through the help of cytochrome P450 oxygenase before returning to circulation. Therefore, medications that interfere with the same metabolic pathway could reduce bromocriptine degradation; hence, dose adjustment is needed.[62]

Bromocriptine is commonly consumed twice to thrice daily at a 2.5 mg dose. The same dose is used to suppress physiological lactation in some conditions, even though it was reported to be linked with postpartum cardiotoxicity.[20] In order to avoid toxicity, research suggested that bromocriptine should be given from 1,25 mg low dose at night, then increased to twice a day 2.5 mg dose or once a day 5 mg dose after one week or according to patient's tolerance.[19] A study showed that bromocriptine administration significantly reduced breast milk production after 7 days of giving birth.[62]

Bromocriptine is a Food and Drug Administration (FDA)-approved medication, as it is considered safe to use for parkinsonism. Today, bromocriptine usage is being developed, which is being studied in relation to diabetes. Bromocriptine repurposing is also being developed toward cancer therapy.[21] Recent study showed that low dose bromocriptine administration could normalize prolactin levels on cancer-related hyperprolactinemia through a 24-hour period. This dose was considered beneficial for endocrine therapy or chemotherapy, including in metastatic breast cancer thrapy.[20]

A recent study showed that there was an increase in high-dose cisplatin cytotoxic effects toward T47D cells that were given additional therapy with the hPRL G129 antagonist. This showed that endogenous prolactin protected tumor cells from chemotherapy regiment's cytotoxic effects.[63] Another study suggested that there was improvement in cancer response when bromocriptine was added to the therapy regiment.[64] An in vitro study found that combination of bromocriptine and doxorubicin/paclitaxel gave synergistic inhibition effects toward CEM/ADR5000 leukemia cells' growth which was measured using resazurin assay.[21] These findings reciprocated other findings which reported that bromocriptine showed cytotoxic effect on a broad range of cancer cells, including those that were multi-resistance toward common treatment and expressing P-glycoprotein. A previous study also found that bromocriptine administration of 1.25–2.50 mg/day for 5 days showed a significant decrease in prolactin levels and tumor cells in the synthesis phase of the cell cycle.[21,65]

Previous studies have not successfully determined the benefits of bromocriptine in lowering prolactin levels in breast cancer patients receiving tamoxifen. This failure was explained by the fact that hypophyseal and extra-hypophyseal prolactin expression were regulated by different regulators under various lactogen effectors. Therefore, inhibition of the prolactin receptor is not guaranteed to be beneficial in breast cancer therapy. These facts support the idea that prolactin receptor inhibition and suppression are considered more beneficial and effective in increasing the response to chemotherapy by administering bromocriptine, although there is still yet to be done.[66]

CONCLUSION

Neoadjuvant chemotherapy is required in advanced-stage breast cancer to increase operability, quality of life, and survival. Anthracycline is a potential chemotherapy agent. However, chemoresistance is a challenging factor in chemotherapy, which is commonly caused by prolactin and prolactin receptor expression. Amid chemotherapy resistance, bromocriptine offers potential, as bromocriptine could improve outcomes and reduce resistance in prolactin-positive breast cancer patients who are administered anthracycline-based neoadjuvant chemotherapy.

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Success Treatment of Severe and Active Graves' Orbitopathy with Tocilizumab After Thyroidectomy and Maximum Dose of Intravenous Methylprednisolone

Laurentius A. Pramono,[1,2] Darmayanti Siswoyo,[2] Yunia Irawati,[2] Alia Arianti,[2] Erwin D. Julian[3]*

[1] Department of Public Health and Nutrition, School of Medicine and Health Sciences Atma Jaya Catholic University of Indonesia – Department of Internal Medicine St Carolus Hospital, Jakarta, Indonesia.
[2] Thyroid Eye Center, Jakarta Eye Center Menteng, Jakarta, Indonesia.
[3] Department of Oncology Surgery St Carolus Hospital, Jakarta, Indonesia.

**** Corresponding Author:***
Laurentius Aswin Pramono, MD. School of Medicine and Health Sciences Atma Jaya Catholic University of Indonesia. Jl. Pluit Selatan Raya No.19-21, Jakarta 14440, Indonesia. Email: aswin.pramono@atmajaya.ac.id; L.aswin.pramono@gmail.com.

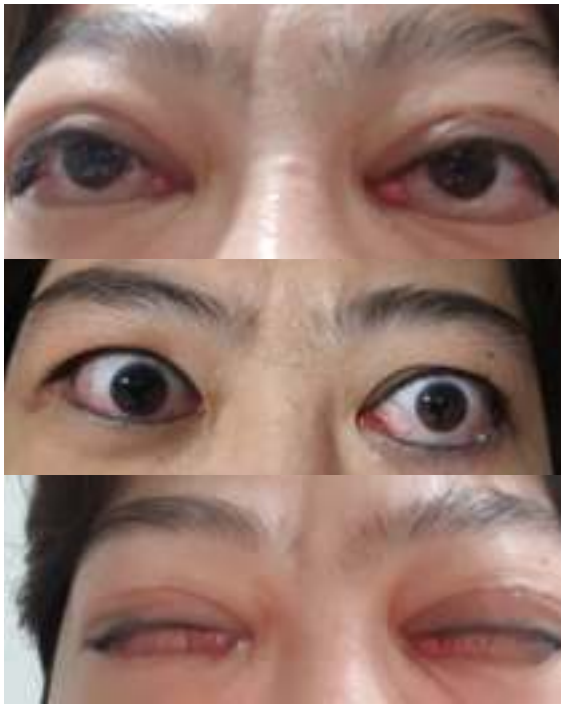

Figure 1. Patient's eye before tocilizumab infusion.

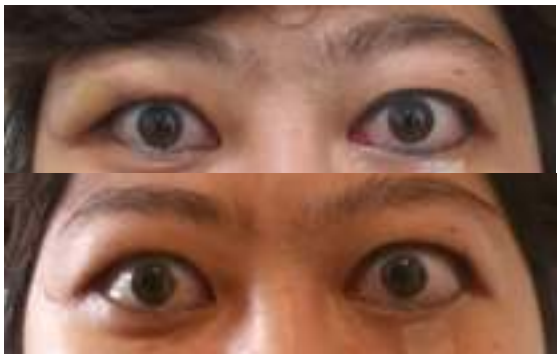

Figure 2. Patient's eye after first (upper) and second (lower) tocilizumab infusion

Figure 3. Patient's eye after third tocilizumab infusion and orbital decompresion surgery (1 week).

Figure 4. Patient's eye after third tocilizumab infusion and orbital decompresion surgery (3 weeks).

Graves' orbitopathy or thyroid eye disease is the most prevalent and difficult-to-treat extrathyroidal comorbidities in patients with Graves' disease.[1] Moderate to severe and active Graves' orbitopathy treated with intravenous methylprednisolone 0.5 g weekly/ 6 weeks combine with mycophenolate sodium 0.72 g daily/ 6 weeks. If there is only partial response, guidelines recommend another 0.25 g weekly intravenous methylprednisolone for 6 weeks combine with mycophenolate sodium 0.72 g daily/ 18 weeks. If no response, we should move to second-line treatment.[2]

The latest, 2021 EuGOGO (European Group on Graves' Orbitopathy) Clinical Practice Guidelines mention several second-line treatment options after failure with maximum dose of intravenous methylprednisolone. These options are second course of intravenous methylprednisolone (total dose 7.5 g), oral prednisolone or prednisone with oral immunosupressants such as cyclosporin or azathioprine, orbital radiotherapy with oral or intravenous glucocorticoids, teprotumumab, rituximab, or tocilizumab.[2] These second-line treatments rarely use in our patients since there are not many dedicated thyroid eye center or clinic in Indonesia.

The use of teprotumumab in severe and active Graves' orbitopathy patients never been done in Indonesia since teprotumumab not yet marketed in Indonesia. Whereas, teprotumumab has very good efficacy for treating difficult cases of Graves' orbitopathy.[3] Likewise, the use of tocilizumab (anti-interleukin 6 or anti-IL6) also never been reported in Indonesia. Not many trial and case report published the outcome after treatment using tocilizumab in severe-active Graves' orbitopathy patients. The use of teprotumumab and tocilizumab itself has not been stated in the Indonesian practical guidelines management of Graves' opthalmopathy published in 2019.[4] This is the first case report in Indonesia evaluating the use of tocilizumab for treatment of difficult case of Graves' orbitopathy.

In this medical illustration, we report a woman, 45th year old with Graves' disease treated with anti-thyroid drug (thiamazole). She came to our clinic with severe and active Graves' orbitopathy. We treated her with high dose intravenous methylprednisolone weekly (0.5 g weekly/ 6 weeks) and mycophenolate sodium 0.72 g daily/ 6 weeks. Because of her longterm consumption but not successful to achieve remission of anti-thyroid drugs and the size of her goiter, we decided to do total thyroidectomy. Only one week after thyroidectomy, her eye inflammation grade was reduced, but still bulging. We continue with the intravenous methylprednisolone weekly. Because of the partial response, we continue with another dose of methylprednisolone (0.25 g weekly for another 6 weeks).

After 12 weeks of intravenous methylprednisolone (maximum dose for 1st course 4.5 g), there is a partial response make it to moderate to severe grade but still active inflammation. Our team decided to give her second-line treatment and we give her intravenous tocilizumab monthly for 4 weeks. This scheme is based on the study of tocilizumanb for treatment of steroid-resistant Graves' orbitopathy.[5] At the time we decided to give tocilizumab, the thyroid function was normal with daily levothyroxine, FT4 1.46 ng/dL (normal 0.92-1.68 ng/dL), TSHs 3.92 uIU/mL (normal 0.27-4.2 uIU/mL). TSH Receptor Antibody (TRAb) was 5.46 IU/L (normal < 1.75 IU/L) and Interleukin 6 (IL-6) was very high 17.9 pg/mL (normal < 7 pg/mL).

After three tocilizumab infusion, the inflammation is reduced remarkably. Her overall appearance is getting better. But, because of her sight was not improved much as the inflammation reduced, we done orbital MRI and we decided to do another intravenous methylprednisolone 1 g for three days followed by orbital decompresion surgery. Shortly after the orbital decompresion, her sight was improved very well. She can now doing activities she can do previously. After recovery, we plan to give her the fourth (last) tocilizumab infusion. Overall, tocilizumab improves clinical outcome in patient with active corticosteroid-resistant moderate to severe Graves' orbitopathy.6 Patient's quality of life also improved.

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Diagnostic Accuracy of Emergency Ultrasonography Compression by Non-Radiologists or Cardiologists for Diagnosis of Deep Vein Thrombosis in Lower Extremity: An Evidence-Based Case Report

Ayers Gilberth Ivano Kalaij[1], Mohamad Syahrir Azizi[2], Angga Pramudita[2]*

[1] Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[2] Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

[*] ***Corresponding Author:***
Mohamad Syahrir Azizi, MD. Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: dr.syahrir@gmail.com.

ABSTRACT

Background: *Deep vein thrombosis (DVT) is a medical condition with dangerous complications including lung thromboembolism which can cause death. However, the disease is often neglected, leading to delays in diagnosis and treatment. Patients with lower extremity DVT clinical signs and symptoms usually cause diagnostic dilemmas, specifically for general practitioners (GP). Various diagnostic strategies have been proposed to diagnose DVT although they still have several limitations. Therefore, emergency compression US by non-radiologists or cardiologists needs to be further considered as a fast and accurate alternative. This study is aimed to analyze the potency of emergency compression US by non-radiologists or cardiologists to diagnose DVT in the lower extremity.* ***Methods:*** *A comprehensive literature search was conducted through PubMed, Scopus, and Cochrane Library. The articles were screened based on predetermined inclusion and exclusion criteria with the keywords emergency, general practitioners, compression US, and DVT. Critical appraisal was performed using the Oxford CEEBM Critical Appraisal Tools for Diagnostic studies criteria.* ***Results:*** *This study analyzed a total of five cross-sectional studies and one prospective cohort. The emergency compression US performed by general practitioners and emergency physicians had a sensitivity of 86-93% and specificity of 90-97.1%. This analysis produced reliable results for diagnosing DVT in bedside settings compared to compression or doppler US performed by experts.* ***Conclusion:*** *Emergency compression US performed by general practitioners and emergency physicians had great potential to be a fast and accurate method for diagnosing and excluding DVT in lower extremities. However, standardized training is necessary to produce the highest diagnostic accuracy.*

Keywords: *Compression US, emergency physician, diagnosis, deep vein thrombosis, Lower extremity.*

INTRODUCTION

Deep vein thrombosis (DVT) is a frequently neglected disease, with life-threatening effects such as lung embolization, which can cause death among patients. Cao et al[1] (2021) have revealed that among 25 hospitals, 10 out of 100 patients admitted to the hospital will have lower extremities DVT. Another study has also shown that DVT can cause death and disabilities among outpatients.[2] Currently, DVT affects one out of 1000 people worldwide,[3] although there are no specific data in Indonesia. A study

conducted at Cipto Mangunkusumo General Hospital in 2008 has shown that DVT prevalence in Indonesia in post-gynecological surgery patients is 33.3%.[4] Specifically, almost 200.000 outpatients are diagnosed with DVT each year, without including many potential outpatients who are undiagnosed.[3,5] When left untreated, one out of three patients with DVT will progress into lung embolization significantly, which can cause death in more than 20% of these patients.[3,6] Therefore, rapid and accurate diagnosis, as well as management of DVT, are urgently needed.

DVT symptoms are usually unspecified, which can make diagnosis difficult. Furthermore, patients with lower extremities DVT signs and symptoms commonly possess diagnostic dilemmas, specifically for general practitioners. Diagnosis based on only clinical findings can cause misdiagnosis, unnecessary exposure to anticoagulant therapies, and even more additional costs. Therefore, rapid, and accurate diagnosis of DVT is needed to start anticoagulant therapies administration and reduce the risk of lung embolization that possesses a fatal prognosis among patients.[6-8]

An optimal diagnostic strategy has been proposed for diagnosing DVT. This includes the Wells score, which is assessed as not adequately accurate to be used in primary care settings, while d-dimer tests are not always available.[9,10] Studies have also shown that the d-dimer test only excludes DVT in less than half of patients with DVT suspicion, and cannot confirm the diagnosis. Venous ultrasonography (US) using doppler is still recommended as the main modality in evaluating DVT comprehensively. However, it consumes more time in transferring the patients to the radiology department and depends on the availability of experts in examining and interpreting this modality.[11]

Prospective studies have shown the potency of compression US as an alternative method in confirming or excluding DVT diagnosis.[12] This technique has several benefits including universal availability among many settings, the ability to be performed by various operators, and using any kind of US machine, making it suitable in primary care settings. This method is usually performed by experts, radiologists, and cardiologists. However, the availability of experts, radiologists, and cardiologists in primary care settings often possess problems in diagnosing DVT. Studies have shown that general practitioners in primary care settings also have the potential to diagnose DVT rapidly and accurately. This can increase DVT management and prevent inpatient evaluation, laboratory, or the use of any scoring in emergency settings.[13] However, the diagnostic accuracy of the emergency compression US method perfomed by non-radiologists or cardiologists is still unclear. Thus, this is the first evidence-based case report that aims to analyze the diagnostic accuracy of the emergency compression US method done by non-radiologists or cardiologists in diagnosing lower extremities DVT.

CASE ILLUSTRATION

A 45-year-old female was admitted to the emergency department with unexplained persistent dyspnea three days before presentation and was not affected by activities or rest. The patient complained about pain in the lower extremities of both legs, with a pain visual analog scale of 7, which was partially relieved by paracetamol. There was also swelling of both legs in the past year before the presentation. The physical examination showed bilateral pitting edema positive and prolonged PT/APTT. However, initial assessment through anamnesis, physical examination, as well as laboratory and radiologic workup did not reveal any diagnosis related to the unexplained dyspnea. Echocardiography was also performed before exploring cardiovascular abnormalities, which may relate to the complaints, but no valve or functional abnormalities were found. Since the patient had swollen leg following the immobilization history, a doppler ultrasound compression emergency examination was carried out in both legs. The results showed many thrombi in both legs, which accumulated more in the left femoral artery and vein. Therefore, the patient was worked up using CT Scan to detect the lung embolization process that can cause dyspnea. The patient was diagnosed with DVT and lung emboli, which were treated with heparin and warfarin.

CLINICAL QUESTION

Is the emergency compression US performed by non-radiologists or cardiologists (general practitioners and emergency physicians) comparable to compression or doppler US performed by experts?

METHODS

A comprehensive literature search was carried out based on Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) using the predetermined PICO criteria in **Table 1** through PubMed, Cochrane, and Scopus databases. This was performed to identify studies about the potency of compression US conducted by general practitioners for diagnosing lower extremities DVT in emergency settings, until 11 February 2023 using the keywords listed in **Table 2**. A manual search was also conducted through systematic reviews or cross-referencing to include more relevant studies. However, only studies in English and Bahasa Indonesia language were also included.

The studies were screened using predetermined eligibility criteria. These included observational studies, randomized controlled trials (RCT), systematic reviews, and meta-analyses, with outpatients suspected of DVT as the population. The intervention used was emergency compression US performed by non-experts, which were defined as general practitioners or emergency physicians using limited compression US (LCUS) or point-of-care ultrasonography-based compression US. The control was compression US or doppler US performed by radiologists or non-radiologist clinical experts. The outcomes measured were sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy. Studies using the non-US method as control or based on expertise results without

Table 1. PICO Criteria.

PICO	Descriptions
Population	Patient with suspected DVT
Intervention	Emergency compression US performed by non-expert practitioners (general practitioners or emergency physicians)
Comparison	Compression US or Doppler US performed by experts (radiologists or non-radiologist clinical experts)
Outcome	Sensitivity, Specificity, and Diagnostic Accuracy

Table 2. Literature Searching Strategy.

Database	Searching Strategy (Keyword)	Hits	Articles passed inclusion criteria	Articles selected
PubMed (11 February 2023)	(Emergency OR GP OR "General Practitioner") AND ("Point-of-Care-Ultrasound" OR POCUS OR "Compression Ultrasonography" OR Ultrasonography) AND ("Deep Vein Thrombosis" OR DVT)	578	18	4
Cochrane (11 February 2023)	(("emergency"):ti,ab,kw OR (GP):ti,ab,kw OR ("general practitioner"):ti,ab,kw) AND (("point-of-care-ultrasound"):ti,ab,kw OR (POCUS):ti,ab,kw OR ("compression ultrasonography"):ti,ab,kw OR ("ultrasonography"):ti,ab,kw) AND (("deep vein thrombosis"):ti,ab,kw OR ("deep vein thromboses"):ti,ab,kw OR ("deep-vein thrombosis"):ti,ab,kw OR (DVT):ti,ab,kw))	22	3	0
Scopus (11 February 2023)	TITLE-ABS-KEY ("emergency") OR TITLE-ABS-KEY (gp) OR TITLE-ABS-KEY ("General Practitioner") AND TITLE-ABS-KEY ("Point-of-Care-Ultrasound") OR TITLE-ABS-KEY (pocus) OR TITLE-ABS-KEY ("Compression Ultrasonography") OR TITLE-ABS-KEY (ultrasonography) AND TITLE-ABS-KEY ("Deep Vein Thrombosis") OR TITLE-ABS-KEY (dvt)	479	21	2
Total		1079	42	6

experts performing the compression or doppler US, pediatric patients, and those without full-text availability were excluded. Subsequently, the included studies were appraised independently by two authors (AGIK and AP) according to the Oxford model of evidence-based medicine using the Validity-Importance-Applicability checklist for diagnostic studies, which was consulted to the third author (MSA) until consensus was reached.

RESULTS

The comprehensive literature search yielded a total of 1079 articles. The studies were screened for duplication, resulting in 870 articles being assessed further in this study. Subsequently, titles and abstracts were screened, and 42 studies that met the predetermined eligibility criteria were included. Among these 42 studies, 3 were narrative literature reviews, 7 compared methods instead of the compression US performers, 17 were non-emergency compression US studies, 6 only assessed the agreement between compression US performers, and 3 mixed the outcome of experts and non-experts, therefore, they were further excluded from this study. Finally, one prospective cohort study and 5 cross-sectional studies were included in the critical appraisal and assessed qualitatively. The detailed planned procedure of the literature searching process was illustrated in **Figure 1**.

A total of 2058 patients was involved and the included studies varied across several regions including Asia, America, and Europe. All included studies used compression US performed

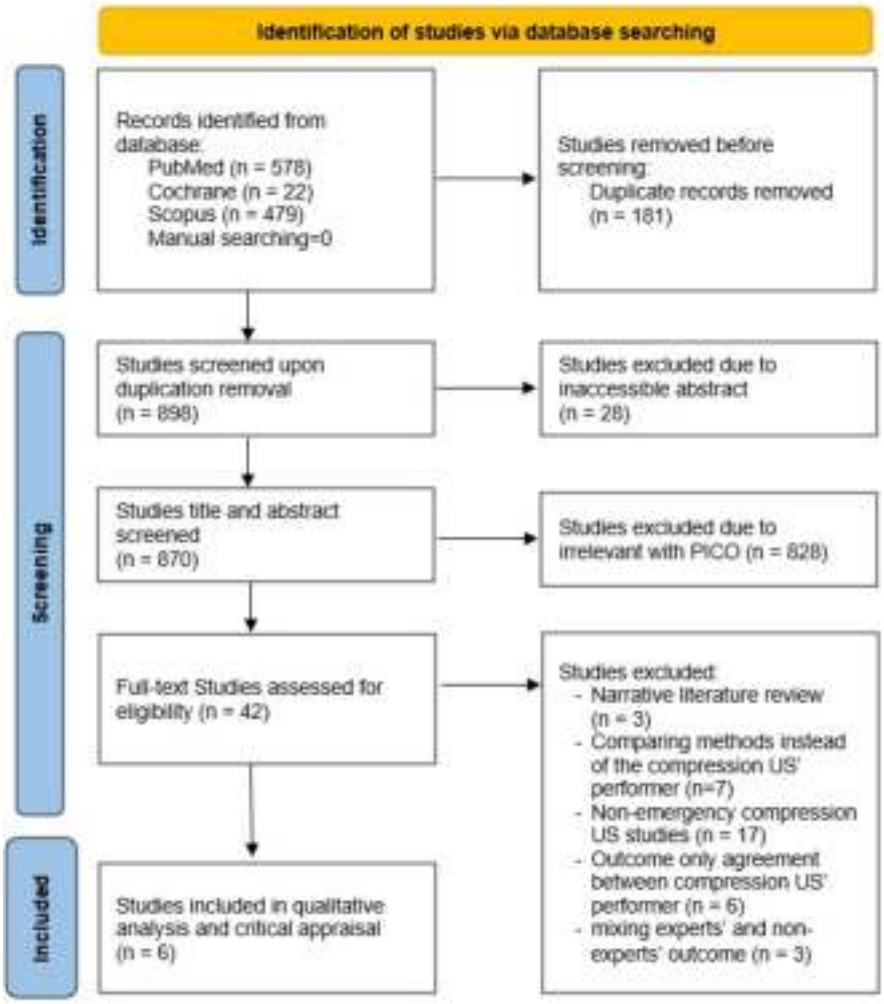

Figure 1. Detailed PRISMA flowchart of the literature search process.

by general practitioners or emergency physicians as their intervention and US by the experts, either duplex or compression in emergency settings. The detailed characteristics of the included studies and their outcomes were summarized in **Tables 3** and **4,** respectively.

After completing the eligibility screening, the included studies were appraised for their validity, importance, and applicability using the University of Oxford Center for Evidence-Based Medicine Diagnostic Critical Appraisal Tools.[19]

Table 3. Study Characteristics.

Author (Publication Year)	Study Design	Study Location	Sample Size	Median/ Range/ Mean Age	Intervention	Control
Abbasi et al (2012)[14]	Cross-sectional	Iran	81 outpatients submitted to the ED	47.2 ± 18.6 years old	Emergency Compression US by EP	Duplex US by second-year radiology residents
Canakci et al (2020)[15]	Cross-sectional	Turkey	266 outpatients submitted to the ED	63 years (IQR: 48-74)	Emergency POCUS by EP	Doppler US or Compression US by radiologists
Crisp et al (2010)[16]	Cross-sectional	America	199 outpatients submitted to the ED	18 years and above	Emergency Compression US by EP	Duplex US by radiologists
Garcia et al (2018)	Cross-sectional	Spain	109 outpatients submitted to the ED	68 ± 16 years old	Bedside Emergency POCUS by EP	Duplex US by radiologists
Kim et al (2015)[17]	Cross-sectional	Canada	296 outpatients submitted to the ED	50 years old (IQR: 37-60)	Emergency LCUS by EP	Duplex US by radiologists
Mumoli et al (2017)[13]	Prospective Cohort	Italy	1107 outpatients submitted to the ED	63.6 ± 15.2 years old (DVT); 63.8 ± 14.9 years old (without DVT)	Bilateral Proximal Compression US in lower extremities by general practitioners	Compression US by vascular US experts (Vascular Surgeons

Abbreviations: ED: Emergency Department; EP: Emergency Physician; POCUS: point-of-care ultrasonography; USG: Ultrasonography; LCUS: Limited compression ultrasonography

Table 4. Study Outcomes.

Author (Publication Year)	Sensitivity (95% CI)	Specificity	PPV	NPV	Summary of Study Outcomes
Abbasi et al (2012)[14]	85.9% (74.5-93)	41.2% (19.4-66.5)	84.6 % (73.1-92)	43.8 % (20.8-69.4)	- Lower extremities DVT diagnostic accuracy using emergency compression US by emergency physicians is 84.6% - Emergency Compression US by emergency physicians has acceptable sensitivity and accuracy, although has low specificity in diagnosing DVT. - Emergency Compression US sensitivity is higher in men compared to women
Canakci et al (2020)[15]	93% (84-98)	93% (89-96)	83% (74-89)	97% (94-99)	- Positive likelihood ratio 14 (8-24) - Negative likelihood ratio 0.08 (0.03-0.19) - POCUS has high sensitivity and specificity in examining popliteal and femoral veins performed by emergency physicians in diagnosing DVT in suspected patients

Crisp et al (2010)[16]	100% (92-100)	99% (96-100)	97.83% (88.47-99.94%)	100% (97.62-100%)	- Lower extremity emergency compression US by general practitioners and emergency physicians using portable US machines can accurately identify and exclude proximal lower extremity DVT - Emergency compression US results have been found to be equivalent directly compared to duplex US done by radiologists (Cohen Kappa 0.99 (95% CI: 0.958-1))
Garcia et al (2018)[18]	93.2% (83.8-97.3)	90% (78.6-97.3)	91.7% (81.9-96.4%)	91..8% (80.8-96.8%)	- Lower extremity DVT compression US performed by emergency physicians using emergency physician diagnostic accuracy: 91.7% (85-95.6) - Emergency physicians have an equivalent competency level compared to radiologists in diagnosing DVT with emergency compression US, although substantial training is needed to achieve and maintain their performance.
Kim et al (2015)[17]	86% (73-94)	93% (89-96)	73.44% (60.91-83.70)	96.55% (93.32-98.5)	- Positive likelihood ratio 12.11 (95% CI: 7.56-19.40) - Negative likelihood ratio 0.16 (95%CI: 0.08-0.30) - Emergency physicians who have performed LCUS training could diagnose DVT with the average accuracy - LCUS by emergency physicians has good diagnostic accuracy, although not adequately sensitive to exclude DVT as a stand-alone test.
Mumoli et al (2017)[13]	90% (88.2-91.8)	97.1% (96.2-98.1)	87.4% (85.4-89.3)	97.8% (96.9-98.6)	- DVT diagnostic accuracy using emergency compression US by general practitioners: 95.8% (94.7-97) - Agreement between general practitioners and experts were equivalent (Cohen Kappa 0.86) - Compression US could be a reliable tool to diagnose DVT in emergency patients - Although emergency compression US by general practitioners resulted in suboptimal sensitivity. However, this method could be an alternative accurate method to diagnose DVT. - Emergency compression US by general practitioners could reduce time-to-diagnosis and maximize optimal management.

Abbreviations: US= Ultrasonography; DVT = Deep Vein Thrombosis; POCUS = *Point-of-care* Ultrasonography; LCUS = Limited Compression Ultrasonography

All studies were found to have good validity and applicability. Since the included studies were not systematic reviews and meta-analyses, they were judged as level II in terms of evidence level. Although they were observational studies with good validity, importance, and applicability. A detailed summary of the critical appraisal was illustrated in **Table 5,** while a detailed critical appraisal per study was provided in the supplementary material.

DISCUSSION

DVT was found to be an emergency case that required rapid and accurate methods to diagnose. However, challenges regarding ideal radiography modality limitations can lead to late management initiation. This study highlighted that compression US in emergency settings performed by general practitioners or emergency physicians can present accurate and reliable results in detecting DVT diagnosis

Table 5. Critical Appraisal Summary of Included Studies.

Author (Publication Year)	Study Design	Validity			Importance				Applicability	LOE
		R	RS	B	Sens	Spec	PPV	NPV		
Abbasi dkk[14] (2012)	Cross-sectional	✓	✓	✓	85.9% (74.5-93)	41.2% (19.4-66.5)	77.6% (64.4-87.1)	39.1% (20.5-61.2)	✓	II
Canakci dkk[15] (2020)	Cross-sectional	✓	✓	?	93% (84-98)	93% (89-96)	83% (74-89)	97% (94-99)	✓	II
Crisp dkk[16] (2010)	Cross-sectional	✓	✓	✓	100% (92-100)	99% (96-100)	45/46 = 97,83%	153/153= 100%	✓	II
Garcia dkk[18] (2018)	Cross-sectional	✓	✓	✓	93.2 (83.8-97.3)	90.0 (78.6-97.3)	91.7% (81.9-96.4)	91.8% (80.8-96.8)	✓	II
Kim dkk[17] (2015)	Cross-sectional	✓	✓	✓	86% (73-94)	93% (89-96)	47/64 = 73.44%	224/232= 96.55%	✓	II
Mumoli dkk[13] (2017)	Prospective Cohort	✓	✓	✓	90% (88.2-91.8)	97.1% (96.2-98.1)	87.4% (85.4-89.3)	97.8% (96.9-98.6)	✓	II

Abbreviations: R = Representative, RS = Reference Standard; B = Blinding and Gold standard; Sens = sensitivity; Spec = specificity; PPV = *positive predictive value*; NPV = *negative predictive value*; App = Applicability; LOE = Level of Evidence; ✓ = Yes; ? = unclear

at the bedside settings, compared to experts-performed compression or duplex US. This was demonstrated by all included studies showing high sensitivity, specificity, and diagnostic accuracy in diagnosing lower extremities.

Crisp et al[16] (2010) revealed that lower extremity compression US performed by general practitioners and emergency physicians using portable US machines can accurately identify and exclude proximal lower extremity DVT. This was demonstrated through the high sensitivity and specificity of emergency compression US, accompanied by equivalent results compared to the radiology department-performed duplex US with the Cohen Kappa of 0.99 (95% CI: 0.958-1). The result highlighted that emergency compression US by emergency physicians was equivalent compared to the duplex US by radiologists, which needed more time. A similar previous meta-analysis in 2013[12] showed that emergency compression US performed by emergency physicians had pooled sensitivity of 96.1% (95%CI: 90.6-98.5) and pooled specificity of 96.8% (95%CI: 94.6-98.1) based on bivariate analysis. Although the meta-analysis included many types of US which were not considered in this study. The results showed the high potential of emergency compression US method used by non-experts doctors to produce accurate results when applied in clinical settings, requiring rapid diagnosis.

A study by Abbasi et al[14] (2012) showed that the overall diagnostic accuracy of the emergency compression US method in lower extremities DVT was 84.6%. This indicated that although emergency compression US had acceptable sensitivity and accuracy, low specificity was found in this method when used by emergency physicians in diagnosing DVT. This can be due to portable machine use, as well as the low experience and knowledge of the physician performing the compression US. However, Canacki et al[15], Garcia et al[18], Kim et al[17], and Mumoli et al[13] revealed that emergency compression US by general practitioners and emergency physicians had high sensitivity and specificity with the range of 86-93% and 90-97.1%, respectively. Some of these studies highlighted that the result was still suboptimal, thereby requiring substantial training.

Venography with contrasts had been the standard diagnostic criteria for patients with suspected lower extremities DVT. However, duplex US had become the first line in clinical settings and as a reference standard in clinical trials. The duplex US was commonly not available in 24-hours care settings due to the need for experts availability to interpret rapidly.[20,21] As an alternative, emergency compression US can be practically used in emergency clinical settings that required rapid and accurate diagnosis. This was related to several examinations and methods to reduce mean time to diagnosis below 15 minutes for healthcare centers where

radiologists were not available 24 hours a day. The early anticoagulation therapy initiation can also optimize rapid and accurate management, preventing mortality and long inpatient stay in the emergency department, reducing costs and other unnecessary examinations, and be beneficial for emergency patients with unstable hemodynamics who cannot be transferred to the radiology department.[11,20,21]

Emergency compression US can be used effectively and practically in emergency settings to diagnose DVT rapidly and accurately. However, the method also had several disadvantages, including operator dependent. Zitek et al[22] (2016) found that non-expert doctors who were given short training had compression US sensitivity of only 57.1% compared to those performed by radiologists. Video analysis in this study also revealed several mistakes, including suboptimal visualization of the popliteal vein and the position of the thrombus, located above the superior femoral vein that can not be visualized by two-point compression US. Therefore, standardized, and measured training was still needed to produce the best diagnostic accuracy with this rapid and accurate method. Previous studies highlighted that this method was easy to learn and perform because it only required about two hours of supervision and hands-on experience for general practitioners or emergency physicians to achieve the skills needed to produce an adequate quality radiology image.[16,23]

This study suggested that standardized training was still needed to achieve the highest sensitivity, specificity, and diagnostic accuracy. However, the emergency compression US method performed by general practitioners or emergency physicians can be the accurate and rapid method in diagnosing and excluding lower extremities DVT in emergency settings.

This study was the first evidence-based case report that analyzed the diagnostic accuracy of emergency compression US in diagnosing lower extremities DVT. The included studies had good validity and applicability, accurate sensitivity, and specificity, as well as positive and predictive values of the compression US method to be implemented in clinical settings. However, the included studies were obtained from regions with a large number of samples, excluding Southeast Asia, specifically Indonesia, and no systematic reviews or meta-analyses were involved. This indicated that the results cannot be generalized because the method used was location-specific. Therefore, further considerations were required to implement this method in Indonesia and other countries with different demographics.

CONCLUSION

Emergency compression US performed by general practitioners or emergency physicians had high diagnostic accuracy. This indicated that the method can be used for diagnosing and excluding lower extremities DVT although standardized training was still needed to produce high sensitivity, specificity, and diagnostic accuracy. Based on the results, the sensitivity and specificity ranged from 86-93% and 90-97.1%, respectively. The emergency compression US performed by general practitioners and emergency physicians reduced time-to-diagnosis, optimized management, and enhanced 24-hour healthcare settings, thereby reducing costs. This method can also be used in unstable hemodynamic patients that were not transferred. Therefore, implementing the emergency compression US method by general practitioners and emergency physicians in clinical emergency settings after routine training and expert supervision was recommended to maximize the implementation of this method. Further studies in other regions such as Southeast Asia specifically in Indonesia were still needed to analyze the potency of this method in different sociodemographic conditions.

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The authors have nothing to declare.

COMPETING INTEREST

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The Effect of Music Therapy for Improving Quality of Life in Patients with Cancer Pain: An Evidence Based Case Report

Yanuar Ardani[1], Ibrahim Achmad[2], Edward Faisal[3], Vinandia I. Poespitasari[3], Rudi Putranto[3], Hamzah Shatri[3,4]*

[1]Psychosomatic and palliative Division, Department of Internal Medicine, Faculty of Medicine Universitas Diponegoro - Dr. Kariadi Hospital, Semarang, Indonesia.
[2] Specialty Education Program, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[3]Psychosomatic and Palliative Division, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Nasional Hospital, Jakarta, Indonesia.
[3]Clinical Epidemiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia- Cipto Mangunkusumo Nasional Hospital, Jakarta, Indonesia.

***Corresponding Author**:
Hamzah Shatri, MD., PhD. Division of Psychosomatic and Palliative, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusuo Nasional Hospital. Jl. Diponegoro no.71, Jakarta 10430, Indonesia. Email: hshatri@yahoo.com; psikosomatik.paliatif@gmail.com.

ABSTRACT

***Background**: Music therapy is a frequently used complementary and creative arts treatment in psychosocial cancer care. Particularly in advanced cancer populations and palliative care, music therapy has recently received high attention in both research and clinical care. This evidence-based case report is aimed to assesed the effect of music therapy for improving quality of life in patients with cancer pain. **Methods**: the search was conducted on Pubmed, Cochrane Library, and EMBASE according to clinical question. The studies were selected based on inclusion and exclusion criteria. The selected study was critically appraised. **Results:** All selected studies significantly showed effectiveness of music therapy towards quality of life in cancer patient. **Conclusion**: Music therapy might be beneficial adjuvant for cancer patients.*

***Keywords**: Music therapy, cancer, quality of life, pain.*

INTRODUCTION

Cancer patients are often treated with combination of radiation therapy, chemotherapy, targeted therapy and surgery.[1] Several studies shown that 15%–40% of patients with cancer suffer from psychological disorders associated with anxiety and depression during therapy and more than 50% feel pain.[1–3] Therefore, patients with this condition will experience an increased response to stress even after treatment, based on almost all literature. Stress itself will cause the immune system, endocrine function and end to decrease the quality of life.[1–9] In addition to treatment for the cancer itself, additional therapies for cancer to improve quality of life can be given.[5–7,10] Psychosocial cancer care refers to the comprehensive approach that addresses the emotional, psychological, social, and spiritual needs of individuals affected by cancer. It recognizes that cancer not only affects a person's physical health but also has profound impacts on their overall well-being and quality of life. Music is one of the therapies that can be given and is an additional therapy that is often given as a part of

psychososial cancer care. Music itself does not directly affect cancer treatment, but music can be a distractor and affect endorphins which are natural painkillers of the body that can affect mood and may affect feelings and maybe raise enthusiasm for treatment.[1,2,7–9,11] Listening to music can affect a person's sensation of receiving painful stimuli.[16–19] In the human body there is a Descending Pain Modulating System (DPMS) which can inhibit and modulate pain sensations that arise from various parts of the body.[16,17,20] Music itself is a parasympathetic nerve stimulation which indirectly causes a sense of relaxation.[16,17,20,21]

CASE ILLUSTRATION

A 41-year-old female patient, hospitalized in the internal medicine ward, the patient has been diagnosed with right breast cancer since December 2021, the patient is admitted to treatment because the body feels weak and has pain in the breast that never goes away.

The first symptom was a lump in the right breast followed by bleeding from the areola of the breast. Then the patient went to a local hospital, a biopsy was done and it was probably a malignant tumor.

The patient was referred to a referral hospital, where a repeat biopsy was performed to determine treatment. After the procedure, the surgical site and surrounding tissue slowly become larger and painful. During hospitalization, patients tend to be anxious, afraid, depressed and often feel pain that is difficult to control. The patient has also been given strong opioid drugs according to the protocol, while the pain usually increases at certain times, especially if the patient feels lonely when the accompanying family leaves the patient alone in the hospital.

METHODS

A comprehensive literature searching was conducted on May 9th 2022 to answer the clinical question mentioned by exploring several online databases such as PubMed, Cochrane Library and EBSCOHost. The keywords "music therapy", "quality of life", "cancer pain", combined with the Booelean operator "AND" and "OR" were used in the search strategy. Articles obtained were screened according to predetermined selection criteria.

The articles were selected according to inclusion and exclusion criteria. Inclusion criteria included: (1) research articles including meta-analysis and systematic reviews to asses the effect of music therapy for improving quality of life in patients with cancer pain; (2) adult population with cancer pain; (3) determining the efficacy of music therapy as a therapy for cancer pain. Findings were filtered for the last 5 years. The exclusion criteria are clinical trials, case series, case reports, review articles, and other studies which were reported in language other than English and not relevant to PICO framework.

After meeting the inclusion and exclusion criteria, every article will be assessed for its validity, importance and applicability by using critical appraisal worksheet available from Centre of Evidence-Based Medicine (CEBM) University of Oxford in accordance to the type of article obtained. Level of evidence of each article would be classified according to the Oxford Central for Evidence-Based Medicine Classification. There There were 169 articles obtained after searching through online database. The queries are described in **Table 1**.

After screening through title and abstracts, we filtered three articles that suited the formulated clinical question and PICO framework. Screening and reviewing processes based on the inclusion and exclusion criteria were done. After further full-text reading there were three articles found to answer the clinical question. Flowchart of the searching strategy is presented in **Figure 1**.

RESULTS

The study that included to be appraised are a systematic review and meta-analysis study by Yanfei Li et al, Miren Perez-Eizaguirre et al, and Friederike Kohler et al. Summary of the articles presented in **Table 2**. Critical appraisals of the studies are presented respectively in **Table 3**.

DISCUSSION

Music therapy is divided into 2 categories, active and passive. active means interactively engaged and encouraged to create or describe their experiences with music, while passive the patient simply listens to either live or recorded music.[18]

Table 1. Queries used to conduct literature searching in journal database.

Journal database	Keywords	Hits	Screened
PubMed/ MEDLINE	((((music therapy[MeSH Terms]) OR (music therapy[Title/Abstract])) AND ((cancer[MeSH Terms]) OR (cancer[Title/Abstract]))) AND ((pain[MeSH Terms]) OR (pain[Title/Abstract]) AND (systematicreview[Filter]))) AND ((quality of life[Title/Abstract]) OR (quality of life[MeSH Terms]))	13	13
EMBASE	('music therapy'/exp OR 'music therapy':ab,ti) AND ('cancer'/ exp OR cancer:ab,ti) AND ('pain'/exp OR pain:ab,ti) AND ('quality of life'/exp OR 'quality of life':ab,ti)	117	40
Cochrane Lib	ID Search Hits #1 (music therapy):ti,ab,kw 3056 #2 MeSH descriptor: [Music Therapy] explode all trees 947 #3 (cancer):ti,ab,kw 177972 #4 MeSH descriptor: [Neoplasms] explode all trees 87970 #5 (pain):ti,ab,kw 202477 #6 MeSH descriptor: [Pain] explode all trees 54750 #7 (quality of life):ti,ab,kw 134852 #8 MeSH descriptor: [Quality of Life] explode all trees 28372 #9 #1 or #2 3056 #10 #3 or #4 209330 #11 #5 or #6 208936 #12 #7 or #8 134852 #13 #9 and #10 and #11 and #12 39	39	1

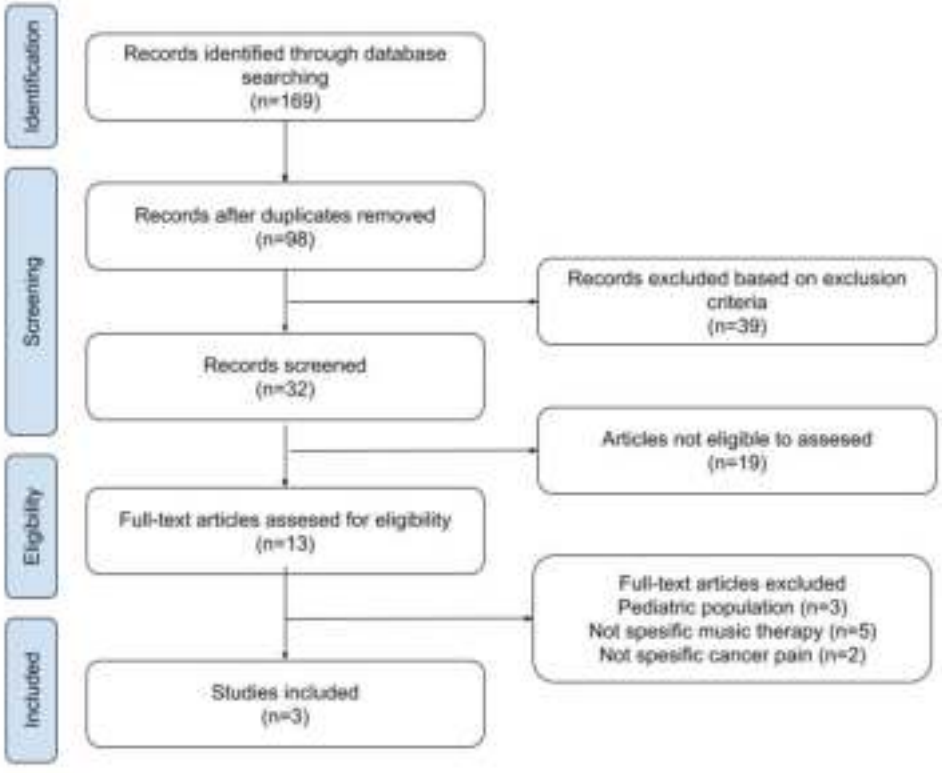

Figure 1. Flow diagram of literature searching

Listening to music has many special beneficial effects on cancer patients. Listening to music as a form of passive/receptive therapy can be easily introduced into clinical situations.[18] For example, patients receiving radiotherapy often experience anxiety, fear, stress, or feelings of loneliness. Listening to music during the treatment can help take the patient's mind off the discomfort.[18]

A systematic review and meta-analysis

Table 2. Summary of articles used in study

Reference / Study designs	Subjects	Determinants	Methods	Inclusion criteria	Exclusion Criteria	Primary Outcome	Evidence Grade
Li et al, (2019) / SR-MA	19 trials (1548 patients) with cancer)	I: Music therapy	This research was conducted in China in 2018. The subjects in the study had various types of cancer, including breast cancer, ovarian cancer, malignant lymphoma, gastrointestinal cancer, leukemia, and lung cancer	- Population with current cancer diagnosis. - Control group received standard care, such as conventional treatment, supportive care, standard treatment, and routine services, whereas the experimental group received music therapy based on standard care.	- Non-human studies. - Duplicate reports of a study. - Studies with insufficient data (e.g. protocols, conference proceeding or abstract, among others).	Effectiveness of music therapy on the quality of life, anxiety, depression and pain of patients with cancer.	1a
Perez-Eizaguirre et al (2020) / SR	19 articles included	I : Music therapy	This study is a 2020 research and was performed in Spain. The subjects in the study experienced a diverse range of cancer types.	- The term “music therapy” must appear explicitly in the title. - Any study of intervention with human beings in palliative care (including hospices and palliative care units). - Music therapy is the first choice of intervention in the articles.	- Other creative therapies. - Conferences and programme proposals.	Not spesified	1a
Kohler et al (2020) / SR-MA	21 articles included	I : Music therapy provided by a trained therapist. Active and receptive interventions.	This research was conducted in Germany in 2020. The subjects in the study had various types of cancer, including gynecological cancer, breast cancer, lymphoma, myeloma, ovarian cancer, leukemia, head and neck cancer, lung cancer, colon cancer, and uterine cancer.	Particiants : adult cancer patients at all stages Interventions : music therapy provided by a trained therapist. Active and receptive interventions. Comparators : waiting list group, treatment as usual group, active control group Study designs randomized controlled trials or controlled clinical trials	Not explicitly explained	Effectiveness of music therapy on psychogical well-being, quality of life, physical symptom distress	1a

Table 3. Critical appraisal of systematic review and meta-analysis based on worksheet by Center of Evidence-Based Medicine.

	Li et al	Perez-Eizaguirre et al	Kohler et al
Q - What question (PICO) did the systematic review address? And use it to direct the search and select articles for inclusion?	Yes	Yes	Yes
F – Did the search find all the relevant evidence?	Yes	Yes	Yes
A – Were the criteria used to select articles for inclusion appropriate and critically appraised?	Yes	No	Yes
I – Were the included studies sufficiently valid for the type of question asked?	Yes	Yes	Yes
T – Have the results been totaled up with appropriate summary tables and plots?	Yes	Yes	Yes
H – Heterogenity between studies assessed and explained?	Yes	No	Yes

conducted by Li et al[2], showed music therapy could improved overall quality of life, anxiety, depression, and pain. However, half of the trials selected in this article were conducted in China and significant statistical heterogeneity was observed in the analysis of music therapy effects on anxiety and depression.

While in a systematic review by Perez-Elzaguirre et al[8], showed significant improvements in the standardization of musical interventions. The studies show a concern for performing effective musical interventions, which are clearly reflected in the research in order to assess which are the most efficient. However, the specific musical aspects of these interventions need to be described in great depth for them to serve as a guide for preparing music therapy sessions. It is important to remember that these objectives are achieved through the music itself and through using it in the best possible way. Music serves as a channel for patient communication and its use is what distinguishes music therapy from other therapies. However, in the search for this systematization we must not forget the psychological and spiritual support on many occasions that music therapy provides in this type of population.[19]

In the systematic review and meta-analysis by Kohler et al[4] showed that music therapy overall had positive effects on a broad range of outcomes, with techniques and effects varying in different phases (during chemotherapy and radiation, surgery and transplantation, aftercare, and non-spesific treatment phases). In the meta-analysis showed significant effect on psychological well-being, physical symptom distress, and quality of life.

To effectively implement music therapy in hospitals, it is crucial to integrate it into the existing multidisciplinary cancer care framework. Collaborative efforts involving healthcare professionals, music therapists, and administrators can facilitate seamless incorporation of music therapy interventions into patient care plans. Regular interdisciplinary meetings and joint assessments can ensure coordination and enhance the effectiveness of the therapy. Providing education and training to healthcare staff is also essential for successful implementation of music therapy. Conducting workshops, seminars, and training sessions on the benefits and techniques of music therapy can raise awareness and equip healthcare professionals with the knowledge to support and promote its use.[20-21]

There are some limitations that should be considered in this study such as, several studies could not be included in meta-analysis due to insufficient data, high risk of bias in all studies, and small sample size in some studies. These factors might contribute to an underpowered analysis.

CONCLUSION

Of all three systematic reviews, results are consistently showed effectiveness of music therapy towards quality of life in cancer patient. As the diagnosis and treatment of cancer is often accompanied by challenging physical symptoms and psychological distress, even small improvements through music therapy may be relevant for patients with oncological diseases. However, long-term clinical trials with larger patient samples, are required in future studies, in order to demonstrate a more precise conclusion. Future studies should explore the differences in music therapy application, such as why patients chose a particular style of music, and what this meant to them. In addition, no fixed procedure for music therapy exists, and different people should be given different treatments. In published studies, both systematic reviews and original research lacked information on the holistic framework of music therapy, such as the optimal time for single music therapy, and most appropriate musical styles. Such theoretical results would ameliorate the scientific nature of the study and improve the possibility of clinical application. The accuracy of future meta-analyses could also be sub- stantially improved if subsequent trials can consistently report high-quality and comprehensive evidence.

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Advancing The Cardiovascular Care in Cancer Patients on Chemotherapy

Muhammad Yamin[1*], Simon Salim[1], Mohamad Syahrir Azizi[1], Lusiani Rusdi[1], Aru Wisaksono Sudoyo[2], Anneira Amanda Putri[3]

[1]Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia.
[2]Division of Hematology-Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia.
[3]Research Assistant, Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia.

Corresponding Author:
Muhammad Yamin, MD., PhD. Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - dr. Cipto Mangunkusumo National Central General Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: yamin@ui.ac.id.

ABSTRACT

Cardiotoxicity associated with chemotherapy, also known as Cancer Therapy-Related Cardiac Dysfunction (CTRCD), affects 10% of patients undergoing chemotherapy and is the most undesirable side effect of chemotherapy. Over time, it is anticipated that there would be an increase in the number of cancer patients receiving treatments that could harm their cardiovascular systems. Physicians should choose whether to continue, halt, delay, or reduce the dose of chemotherapeutic drugs to reduce the impact of cardiotoxicity.

Cardiotoxicity screening and diagnosis need a variety of methods, primarily echocardiography to evaluate Left Ventricular Ejection Fraction (LVEF) and Global Longitudinal Strain (GLS). Depending on the clinical state, these procedures may be carried out prior to, during, or following chemotherapy. It's critical to reduce cardiovascular risk factors and offer advice on leading a healthy lifestyle before giving cancer patients medicines.

There are a lot of cancer treatment facilities all around the world that don't have evidence-based perspective cardiotoxicity scores to stratify the risk of cardiovascular problems caused by cancer therapy. Additionally, comorbid conditions like diabetes and hypertension are frequently present in cancer patients, which can have a significant impact on clinical outcomes and cancer treatment. Therefore, this article aims to discuss assessment methods, clinical practice guidance, and prevention of CTRCD.

Keywords: *cardiac disease, cardiotoxicity, CTRCD, chemotherapy, cancer.*

INTRODUCTION

Cardiotoxicity associated with chemotherapy is a critical issue in cancer treatment. Cardiovascular disease and cancer are the two leading causes of morbidity and mortality, approximately contributing to 70% of death cases worldwide.[1] According to the Global Cancer Observatory data in 2020, there were 19.2 million newly diagnosed cases and 9.9 million cancer deaths. There were 396 thousand newly diagnosed cases and 234 thousand cancer death in Indonesia.[2] This situation is also observed in developed countries. Cardiovascular and cancer are diseases are also associated with high morbidity and mortality rates in the United States and Canada.[3–5] The International Agency

for Research on Cancer (IARC) estimates that globally, one in every five persons will develop cancer throughout their lifetime. Categorized by gender, the mortality proportions are one in every eight men and 1 in 11 women.[5]

The number of patients receiving cancer treatment that may impair the cardiovascular system is projected to continue increasing over time. In previous years, a study reported that post-curative treatment of breast cancer in postmenopausal women had higher risk of cardiovascular mortality than the cancer recurrence itself. Cardiotoxicity is assumed to be the cause mortality.[6] Cardiotoxicity, often identified with Cancer Therapy-Related Cardiac Dysfunction (CTRCD), is the most undesirable side effect of chemotherapy, impacting 10% of all patients. Cardiotoxicity is a process that begins with myocardial cells injury and followed by a reduction in Left Ventricular Ejection Fraction (LVEF), which leads to chronic heart failure if not treated properly.[7]

Early diagnosis of cancer and advancement of the treatment increase the survival rate.[6] To minimize the effect of cardiotoxicity, physicians should decide whether to continue, stop, postpone, or reduce the dose of chemotherapeutic agents.[6]

CHEMOTHERAPY IMPACT ON CARDIOVASCULAR

The administration of chemotherapy drugs must take into consideration both the potential adverse cardiovascular effects and the expected advantages.[1,8] Cardiotoxicity-related cancer therapy can result in severe cardiac dysfunction as a serious adverse effect. Cardiotoxicity affects the effectiveness of treatment, the quality of life, and overall survival in cancer patients.[8]

Cardiotoxicity due to chemotherapeutic agents is categorized into type 1 and type 2. The categories are made based on the effect of chemotherapeutic agents on cardiomyocytes. Type 1 cardiotoxicity is caused by irreversible cardiomyocyte cell death, either by necrosis or apoptosis. However, there is no cell death in type 2 cardiotoxicity. Type 2 toxicity is characterized by dose-dependent reversible cardiomyocyte cell damage.[9]

A common example of chemotherapy-related cardiotoxicity is the usage of anthracycline. Anthracycline-induced long-term cardiotoxicity results in the necrosis of cardiomyocyte cells and is thus classified as type 1 toxicity.[10,11] Anthracycline is one of the well-known chemotherapy agents, an anticancer drug with high effectiveness for solid tumors and hematological malignancies. However, due to the adverse cardiovascular effects, its usage is restricted. The mechanism of Left Ventricle (LV) dysfunction associated with anthracyclines has been previously investigated. The mechanism of the cardiotoxicity effect of Anthracyclines is the oxidative stress hypothesis, indicating that the reactive oxygen species and lipid peroxidation from cell membranes injure cardiomyocytes. However, this cardiotoxicity can be minimized to some extent by administration of protective agents (i.e., dexrazoxane).[12]

Anthracycline is widely known as a risk factor for induced heart failure (HF) and asymptomatic LV dysfunction.[13] Anthracycline cardiotoxicity can be divided into 3 categories based on the time of cardiotoxicity development: acute, subacute, and chronic. Arrhythmias (supraventricular tachycardias, ventricular ectopic beats), heart failure, and myopericarditis are examples of acute injuries that occur during or soon after administration of chemotherapy. Subacute cardiotoxicity develops within a few weeks. Myocarditis with edema and thickening of the left ventricular wall is a subacute clinical occurrence that contributed to diastolic dysfunction and increased mortality.[13,14]

An example of type 2 cardiotoxicity is the effect of trastuzumab, which is a HER2 inhibitors.[10] Uncertainty surrounds the mechanism underlying cardiomyopathy brought on by trastuzumab. According to some authors, it might be related to the HER2 receptor being present on the surface of cardiomyocytes. Cardiotoxicity, which manifests as heart failure and is accompanied by a decline in LVEF or an asymptomatic decline in LVEF, is one of the most frequent side effects of trastuzumab therapy.[15]

Combination chemotherapy for the treatment of solid tumors, leukemias, and lymphomas

frequently includes alkylating agents. It operates by impairing DNA transcription, which in turn causes a downregulation of protein synthesis. Although the exact mechanism of cardiotoxicity is uncertain, the most widely accepted explanation postulates that myocyte destruction and capillary microthrombi that encourage ischemia are caused by endothelium layer damage that allows potentially harmful compounds to flow into the myocardium.[9]

Antimicrotubule agents (taxanes) are frequently used to treat solid tumors. The method of action involves attaching to microtubules and preventing microtubule disintegration to prevent cell division. In comparison to other drugs, taxanes have a relatively low prevalence of LV dysfunction (0.7%). Taxanes alter the metabolism and excretion of anthracyclines, increasing the risk of cardiotoxicity.[9]

The presence of LV systolic dysfunction and congestive heart failure indicate the cardiotoxic effects of the chronic phase. Chronic cardiotoxicity can be categorized into two types: early type (symptoms arise within one year after chemotherapy) and late type (symptoms appear after one year of treatment completion). The mechanism of cardiotoxicity in the acute phase is believed to be different from that in the chronic phase, whereas the inflammatory process has a more significant role. The time-related course of cardiotoxicity differs between the pediatric group and the adult group, with the pediatric group developing cardiotoxicity during the chronic phase as chemotherapy doses accumulated. While in adults, this process occurs earlier and is affected by several comorbid cardiovascular risk factors, such as hypertension.[14] Early adverse events occur within the first year of treatment, while the late adverse events manifest after lengthy period (±7 years following treatment).[5,6] Biomarkers such as troponin I (TnI) and N-Terminal-prohormone Brain Natriuretic Peptide (NT-proBNP) can be used to monitor or identify cardiotoxicity. Aside from the chemotherapy agents mentioned above, **Table 1** shows the most prevalent cardiotoxicity adverse effects of anticancer drugs.[13]

Table 1. Cardiotoxicity side effects related to chemotherapeutic drugs.[13]

Manifested Cardiovascular toxicity	Associated chemotherapeutic drugs
Heart failure	Doxorubicin, daunorubicin, idarubicin, epirubicin, mitoxantrone, cyclophosphamide, ifosfamide, docetaxel, trastuzumab, bevacizumab, sunitinib, pazopanib, sorafenib, imatinib, dasatinib, lapatinib, nilotinib, carfilzomib, bortezomib.
Myopericarditis	Cyclophosphamide, 5-fluorouracil, cytarabine, trastuzumab, rituximab, Interleukin-2, Immune-checkpoint inhibitors.
Ischemic cardiomyopathy	5-fluorouracil, capecitabine, cisplatin, paclitaxel, docetaxel, etoposide, bevacizumab, sorafenib, sunitinib, bleomycin.
Atrial fibrillation	Cisplatin, cyclophosphamide, ifosfamide, melphalan, doxorubicin, capecitabine, 5-FU, gemcitabine, etoposide, paclitaxel, rituximab, sorafenib, sunitinib, ibrutinib, bortezomib, Interleukin-2, interferon.
Bradyarrhythmias	Cisplatin, doxorubicin, mitoxantrone, capecitabine, 5-FU, gemcitabine, paclitaxel, thalidomide, ifosfamide, bortezomib, epirubicin, Rituximab, cyclophosphamide, arsenic trioxide, interleukin-2, imatinib,
Accelerated atherosclerosis	Bevacizumab, nilotinib, ponatinib, carfilzomib, bortezomib.
Pericardial effusion	Cyclophosphamide, Immune-checkpoint inhibitors.
Venous thromboembolic disease	5-fluorouracil, cisplatin, nilotinib, ponatinib, erlotinib, bevacizumab, vorinostat, L-Asparaginase, immune-checkpoint inhibitors.
Arterial thromboembolic disease	Bleomycin, nilotinib cisplatin, carboplatin, vincristine, gemcitabine, bevacizumab, ponatinib, interferon alfa-2, immune-checkpoint inhibitors.
Arterial hypertension	Bevacizumab, sorafenib, sunitinib, axitinib, vandetanib, regorafenib.
Pulmonary hypertension	Dasatinib, cyclophosphamide.
Prolonged QT interval	Sorafenib, vorinostat, doxorubicin, axitinib, cabozantinib, dasatinib, lapatinib, crizotinib, sunitinib, nilotinib, ribociclib, arsenic trioxide, depsipeptide, vemurafenib, vandetanib.

CARDIOTOXICITY ASSESSMENT METHODS

Cardiotoxicity screening and detection require various methods.[12] Reduced LVEF has been widely used as a marker of cardiotoxicity. Among the several proposed criteria, the American Society of Echocardiography's is the most widely accepted. Cardiotoxicity is defined as a reduction in LVEF of more than 10% from the baseline to a final LVEF <53% in some clinical studies. Patients with remarkable reduced LVEF typically have poor prognosis.[9,16,17]

Advances in imaging modalities and the availability of cardiac biomarkers can contribute to early diagnosis, leading to a better quality of life in patients with cancer. Even though LVEF is the most well-known parameter to assess systolic function, LVEF has lower sensitivity to detect subclinical myocardial injuries. When a reduction in LVEF is observed in cancer patients, the myocyte cells are irreversibly damaged. As a result, other markers should be considered in order to detect subclinical cardiac toxicity earlier.[17]

The diagnostic criteria of cardiotoxicity defined by the European Society of Cardiology (ESC) are based on echocardiography, nuclear cardiac imaging, Cardiovascular Magnetic Resonance (CMR), and the use of cardiac biomarkers such as troponin, high-sensitivity troponin, BNP, and NT-proBNP.[16] Echocardiography is the method of choice for detecting myocardial dysfunction before, during, and after chemotherapy. Echocardiography can be performed in three dimensions (3D) or in two dimensions (2D) using the Simpson biplane method. The most frequently used imaging method for monitoring LVEF during and after chemotherapy is two-dimensional echocardiography. However, the intra and inter-observer variability is a major limitation. Therefore, 3D echocardiography has been recommended to be the best method for LVEF monitoring in cancer patients.[9]

The best modality to assess cardiotoxicity during or following chemotherapy is Global Longitudinal Strain (GLS). Global Longitudinal Strain can detect myocardial damage. An absolute reduction in GLS values of more than >-19% after completion of anthracycline therapy is considered a predictor for future cardiotoxicity (positive predictive value is 53% and negative predictive value is 87%).[18] The advantages of this modality are its wide availability, most negligible radiation, non-invasive method, its ability to assess hemodynamics, and its availability to be used for serial examination. However, the limitations of this modality include the poor image quality, skill requirements, as well as inter-observer and inter-vendor variability.[12,14]

Other than echocardiography, additional methods that can be used to diagnose cardiotoxicity, including Multigated Acquisition (MUGA) scanning and cardiovascular magnetic resonance (CMR). For many years, MUGA, or nuclear cardiac imaging, has been used to diagnose chemotherapy-associated cardiotoxicity with high precision and reliability. However, the usage is usually hampered by radiation exposure and its limited ability to provide information about heart anatomy and hemodynamics. The diagnostic cutoff value for MUGA cardiotoxicity has been determined as a reduction in the ejection fraction value of more than 10% to 50%.[11]

Due to its excellent sensitivity and specificity, cardiovascular magnetic resonance (CMR) can be used to assess or retest echocardiographic test results that are unclear or inconsistent between one test and another. Another advantage of using CMR is the ability to evaluate more structures such as the pericardium or the presence of severe myocardial fibrosis. However, CMR is infrequently used because it is not available at every facility and requires high standardized resources.[11,13] Nuclear cardiology imaging can be used in addition to echocardiographic methods. The criterion of cardiotoxicity in this method is a reduction of more than 10% LVEF, with a LVEF value less than 50%. The advantage of this method is its reproducibility and its minimal inter-observer variability in evaluating LVEF. Meanwhile, the disadvantages of this modality include radiation exposure and limited information on the structure and function of the heart.[12,14,17] In addition to precisely estimate LV and systolic volumes with high reproductive function, CMR is also capable for detecting myocardial edema, perfusion abnormalities,

and fibrosis.[19] In addition, CMR requires patient adaptation (claustrophobia, continuous breathing, long acquisition time). It is used when other methods are on the verge of failing and require validation from this modality.[12,19]

The cardiac biomarker measurements consist of High Sensitivity Troponin I (HS-TnI), Troponin I, NT-ProBNP, and BNP. These serum markers are helpful in detecting acute cardiotoxicity. BNP, a unique cardiac neurohormone, is released as the ventricular response to increased wall tension.[11,12] The primary role of NT-ProBNP and BNP in monitoring patients with high risk needs more investigation. The positive aspects of the method of cardiac biomarker measurements are its reliability, efficiency, accuracy, wide availability, and high sensitivity. The shortcomings include a lack of data to confirm the significance of changes, variances in the tests, and the role of periodic surveillance.[12] In general, the NT-proBNP level helps to assess initial risk stratification and predict the prognosis of LV dysfunction. Gradual changes of NT-proBNP throughout three days of chemotherapy are associated with progressive reduction in LVEF.[2,12,18,19]

Canadian Cardiovascular Society (CCS) guidelines recommend the same imaging method and modality to determine LVEF before, during, and after chemotherapy for cancer. Myocardial strain imaging can be used to detect early subclinical LV failure in cancer patients receiving potentially cardiotoxic drugs. The physician can also perform serial measurements of cardiac biomarkers.[6] A comparison of various modalities for assessing cardiotoxicity is described in the **Table 2**.

A multimodality strategy that incorporates multiple biological and imaging parameters might be useful for detecting cardiotoxicity. According to a study conducted by Sawaya et.al, the combination of an increase in hs-cTnI and a reduction in GLS >-19% has an 87% sensitivity to diagnose cardiotoxicity. However, when each parameter is used separately, the sensitivity values are 74% and 48%, respectively.[18] When paired with biomarker analysis, GLS > -17.5% and troponin T > 0.034 ng/ml have a specificity of 94%, indicating that it is a promising strategy for cardiotoxicity prediction.[20]

CLINICAL PRACTICE GUIDANCE

Before administering cancer medications, it is crucial to optimize cardiovascular risk factors and provide healthy lifestyle recommendations such as nutrition, smoking cessation, and exercise. Risk stratification and management of undesirable cardiovascular side effects in cancer treatment are two elements that physicians should consider when determining management actions. Cardiovascular risk stratification with HeartScore has been approved by the European Association of Preventive Cardiology (EAPC) to assist in the adjustment of cardiovascular risk factors.[17]

Table 2. Comparison of Diagnostic Modalities to Evaluate Cardiotoxicity[2,6,9,12–14,16]

	Echocardio-graphy 2D	Echocardio-graphy 3D	Strain Longitudinal Global	MUGA	CMR	Troponin I
Availability	++++	+++	+++	+++	++	+++
Reproducibility	++	+++	+++	+++	++++	++++
Radiation	Zero	Zero	Zero	5-10 mSv	Zero	Zero
Detection of Subclinical Toxicity	Low	Low	High	Low	Moderate	High
Additional Diagnostic Utilities	Structural information, valvular heart disease, pericardial disease, diastolic function				Characterizing tissue, pericardial disease	Has a high negative predictive value when combined with global longitudinal strains

MUGA: Multigated Acquisition. CMR: Cardiac Magnetic Resonance. mSv: millisievert.

Numerous cancer treatment centers throughout the world lack evidence-based perspective cardiotoxicity scores to stratify the risk of cancer therapy-related cardiovascular issues. However, data from clinical trials and registries allow us to identify high-risk groups. Cardiotoxicity related to anticancer therapy must be identified in future cardio-oncology research. In this regard, coordination between the cardiology and oncology departments is required. The present issue is whether the cardiotoxicity caused by cancer treatment is specific to the drugs or class of drugs.[8] Furthermore, because of a lack of long-term data on survival of the risk of cardiac dysfunction, no recommendations about the categorization-specific risks in cancer patients treated with anticancer against cardiotoxicity can be provided.[11,17]

Cardiologists have new challenges and responsibilities as part of this developing multidisciplinary cooperation. This involves optimum treatment of underlying cardiovascular risk factors as well as monitoring cardiac safety against cardiotoxicity.[11] The following are some monitoring strategies that can be implemented: Take a thorough medical history and physical examination to identify risk factors for cardiovascular disease (smoking, diabetes, hypertension, dyslipidemia, and obesity),[8] preexisting heart disease (cardiomyopathy, myocardial infarction, clinically relevant cardiac arrhythmias, moderate or severe valvular heart disease), and previous cardiotoxic treatments.[14]

Blood pressure monitoring is recommended every week during the first cycle of therapy and then every 2-3 weeks during anticancer therapy.

Electrolyte monitoring throughout treatment must be considered at the start, 7-15 days after the dose is initiated or adjusted, every month for the first three months, and then on a monthly basis for the next three months, depending on the chemotherapy agent and the patients' condition.[12]

Frequent electrocardiogram (ECG) helps detect early cardiotoxicity at baseline, every 2-4 weeks for the first three months, every three months for the next three months, and then regularly during therapy depending on the chemotherapy regimen and the patient's condition.

Cardiac biomarkers (troponin, natriuretic peptide) can be measured at the beginning of chemotherapy, followed by every 2-3 months, to help monitor cardiac tissue injury.[11]

Echocardiographic evaluations every 2-3 months will be helpful in the detection of LV dysfunction.[11] To avoid radiation in individuals with poor imaging quality, cardiac magnetic resonance is an option. Methods for monitoring must be customized to the availability of local resources during therapy. Professional expertise is required to minimize unnecessary delays in cancer therapy.[17]

All associated decisions should consider potential threats to life expectancy, quality of life, and risk of complications. Individualized management will depend on the clinical condition, characteristics, and the causative drug.[6]

COMORBIDITIES IN CANCER PATIENTS

Some anticancer therapies are known to have deleterious effects on the cardiovascular system. Although many health care providers are aware of the potential short-term cardiotoxicity associated with anticancer therapy, there is often a lack of concern for the long-term consequences of this treatment for cardiovascular health. Cancer patients often have comorbid diseases such as diabetes and hypertension, which can greatly influence cancer treatment and clinical outcomes.[1]

Myocardial ischemia and arrhythmia infarction caused by ischemia are adverse effects of several therapeutic cancers. Fluoropyrimidines, including 5-fluorouracil and capecitabine, are the most well-established cause of coronary arterial spasm leading to acute myocardial ischemia during cancer therapy.[14] The mechanism of this anticancer drug can cause myocardial ischemia and the development of premature arteriosclerosis. Prior to cancer treatment, it is critical to recognize and identify patients who have had past coronary artery disease (CAD) or other cardiovascular diseases. According to the most recent guidelines, the minimum period of dual antiplatelet medication should be pursued to a reasonable extent in patients treated by percutaneous coronary intervention who are subsequently confirmed to have malignancies to

reduce the risk of bleeding. Thrombocytopenia following chemotherapy makes multimodal treatment challenging. Medical therapy and intervention options are limited; even the use of antiplatelet and anticoagulant medications is sometimes difficult to do.[12] The key to identifying people with latent coronary artery disease is the clinical examination for myocardial ischemia. Regular ECG can be used to monitor myocardial ischemia. If cardiac ischemia occurs, chemotherapy must be put on hold and adjustments are required.[18]

The most common comorbidity in patients with malignancy is hypertension (37%). Before chemotherapy, the percentage is similar to that of the general population (29%).[11] The severity depends on the patient's age, history of hypertension, type of cancer, type of drug and dosage, the timing of use, and cancer-related therapy.[12] Evaluation of treatment compliance is needed when severe hypertension is present. Follow-up is essential to ensure improvement and antihypertensive drug tolerance. Vascular endothelial growth factor (VEGF) inhibitors are known to have an increased risk (11-45%) to induce new hypertension. To avoid interaction with VEGF inhibitors, those suffering from resistant hypertension should be sent to a cardiologist or have their blood pressure monitored.[21]

The goal of hypertension management is to diagnose hypertension (BP > 140/90 mmHg) while maintaining blood pressure stable (BP 140/90 mmHg or lower in the event of proteinuria). Before starting VEGF inhibitors, a first assessment of cardiovascular disease risk factors (including a history of hypertension and current blood pressure level) and arterial hypertension treatment must be performed. As first-line treatment, angiotensin II receptor blockers (ARBs), angiotensin-converting enzyme (ACE) inhibitors, and non-dihydropyridine calcium channel blockers (amlodipine, felodipine) can be used.[14]

Severe atherosclerotic and non-atherosclerotic Peripheral Arteries Disease (PAD) in the lower extremities can occur in up to 30% of patients who are getting cancer therapy. PAD can occur early in the first months of therapy or as an effect that is delayed several years after treatment. One recommendation is an initial PAD risk evaluation (assessment of risk factors, physical and clinical examination, measurement of the ankle-brachial index (ABI)). The Fontaine stages 1-2 (asymptomatic or with intermittent claudication) require risk control and periodic clinical, metabolic, and hemodynamic follow-up. Antiplatelet drugs should be considered primarily for symptomatic PAD. Revascularization can be considered for severe PAD at the beginning or during cancer therapy.[12]

Intra Arterial thrombotic incidents are uncommon in cancer patients, occurring at a rate of 1%. Antithrombotic treatment, thrombolysis, and/or endovascular intervention should be managed in a multidisciplinary consultation that includes a cardio-oncology team, if available. However, venous thrombosis and venous thromboembolism (VTE) are common in cancer patients, may occur in up to 20% of hospitalized patients, and are frequently underreported. They may be associated with chemotherapy, including the route of delivery (usage of permanent venous catheters), as well as the prior patient's malignancy and venous risk from thrombosis. Treatment of episodes confirmed by acute VTE in patients with stable hemodynamics consists of 3-6 months of Low Molecular Weight Heparin (LMWH).[12]

CONCLUSION

With a growing prevalence as cancer patients continue to rise, CTRCD is a serious problem in cancer therapy. Cardiotoxicity has an impact on cancer patients' overall survival, quality of life, and response to therapy. Several techniques are needed for cardiotoxicity screening and detection, even a multimodality approach including imaging and biological markers. Comorbid conditions like diabetes and hypertension are frequently present in cancer patients, which can significantly impact the clinical outcomes.

The administration of chemotherapeutic medications must evaluate both the anticipated benefits and any potential cardiovascular side effects. Clinical practice recommendations for cancer patients must be developed. Therefore,

because of this expanding multidisciplinary collaboration, cardiologists now face new challenges and responsibilities. Individualized treatment plans will be based on the clinical condition, its traits, and the substance that caused it.

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