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Raising Awareness of Acute Kidney Injury: Unfolding the Truth

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Acute kidney injury (AKI) is an extremely complex syndrome associated with severe morbidity and mortality.[1] Moreover, AKI may cause loss of kidney function in the long term.[2] Both in developed and developing countries, AKI is common among hospitalized patients, which can be up to 20% of overall patients.[3,4] There is an urgent need to increase knowledge and awareness of AKI, particularly in developing countries, including Indonesia.

AKI is rarely being recognized as it may take place without any apparent symptoms. Severe AKI is commonly found in intensive care unit (ICU) patients. A recent multinational study comprising of thousands of patients from 97 ICUs reported that 57% of patients had developed AKI within 1 week of admission. About 39% of patients had severe AKI (stage 2 or 3), in which 13.5% of them requiring kidney replacement therapy (KRT). AKI in the ICU is an independent risk factor for death, as it may cause systemic effects on other vital organs including the lung, heart, liver, brain and immune system. Some studies have reported that AKI increases susceptibility to infection, doubles the rate of respiratory failure and impairs cardiac function. Common causes of AKI in the ICU are sepsis, cardiac surgery, acute liver failure, intra-abdominal hypertension, hepatorenal syndrome, malignancy, and cardiorenal syndrome.[5]

Considering the substantial impacts of AKI in ICU patients, early implementation of preventive measures should be an essential program which consists of developing AKI risk stratification in the ICU and encouraging the use of novel AKI biomarkers (TIMP-2, IGFBP-7, Cystatin C, IL-18, KIM-1 and NGAL) as well as other risk stratification tools (clinical risk prediction scores, computer algorithms, furosemide stress test). Furthermore, after ICU patients have recovered, AKI survivors are more likely to develop chronic kidney disease (CKD) and end-stage kidney disease (ESKD), imposing significant morbidity in the future. Nephrologist intervention is expected to help patients' recovery, prevent further deterioration of renal function, and mitigate the risk of mortality as well as the development of CKD if patients survive. Recent study has shown that nephrologist intervention was associated with lower risk of starting KRT and progression of AKI.[2,6]

The coronavirus disease 2019 (COVID-19) pandemic has caused more than 800,000 deaths worldwide.[7,8] Kidney involvement in patients with COVID-19 may present as proteinuria or hematuria and may lead to acute kidney injury (AKI). Some initial reports showed that the incidence of AKI in COVID cases was negligible.[9-14] However, later reports suggested that AKI is actually prevalent in patients with COVID-19, particularly in ICU patients. The rate of AKI in COVID-19 patients was more than 20% of hospitalized patients and more than 50% of patients in the ICU.[7,15-18] AKI is now considered as a common complication of COVID-19 and it is also associated with adverse

outcomes, including development or worsening of comorbidities, yet little is known about the pathogenesis or optimal management of COVID-19-associated AKI.

Definition and Classification of AKI (KDIGO 2012)

There was a lack of definition for AKI for quite a long time. At first, the term "acute renal failure (ARF)" was used to describe an acute deterioration in renal function, which usually calls for an emergency KRT.[2] Afterwards, some experts and several specific working groups established the definition and staging of AKI. In 2007, the term ARF was officially replaced by AKI and it was first defined using the RIFLE criteria (Risk, Injury, Failure, Loss, End-Stage).[2,19] The definition has subsequently evolved and currently corresponds to the criteria published in 2012 by KDIGO (Kidney Disease: Improving Global Outcome) working group. KDIGO criteria defines staging of AKI based on serum creatinine level and urine output as follows: (1) AKI stage I with serum creatinine level of 1.5 to 2.0 baseline within 7 days or ≥ 26.4 μmol/L within 48 h and urine output of <0.5 ml/kg/h for 6-12 h; (2) AKI stage II with serum creatinine level of 2.0 to 2.9 times baseline and urine output of <0.5 ml/kg/h for ≥ 12h; and (3) AKI stage III with serum creatinine level of ≥ 3.0 times baseline or an increase in serum creatinine to ≥ 353.6 μmol/L or the initiation of KRT and urine output of < 0.3 ml/kg/h for ≥ 24 h or anuria for ≥ 12 h.[2]

Etiology and Pathophysiology of AKI

AKI is a sudden loss in renal function that may be caused by a wide variety of clinical conditions. However, the causal relationship between AKI and those clinical conditions, whether as the cause or adverse outcomes remains controversial in most studies.[20-23] Etiologies of AKI are very heterogenous and may initiate multiple pathophysiological pathways. These etiologies can be classified into three main categories: pre-renal, intrinsic and post-renal. Pre-renal AKI is caused by renal hypoperfusion that leads to a decreased GFR without any damage to the renal parenchyma, such as hypovolemia (bleeding, volume depletion, etc), impaired cardiac function, or increased vascular resistance. Intrinsic AKI is due to a variety of injury that occurs in the kidney structures (tubules, glomeruli, interstitium or renal blood vessels). Whereas, post-renal AKI etiologies include any acute obstruction of the urinary flow that increases intra-tubular pressure and thus decreases the glomerular filtration rate (GFR).

In the pathophysiological point of view, these etiologies usually cause imbalance of oxygen supply and demand that activates cascade of responses to hypoxemia and oxidative stress. Subsequently, this may lead to persistent inflammation, hyperfiltration, progressive tubular damage, glomerulosclerosis and tubulointerstitial fibrosis, eventually leading to CKD, ESKD, and other associated complications.[24-26] Currently, the pathophysiology of AKI is not completely understood and is known to be mediated by a complex interplay of multiple pathophysiological process.[2] This process will ultimately end up as irreversible renal damage. Based on such pathophysiological perspective, the long-term impact of AKI outcomes depends on the residual renal function and repair capacity after surviving renal stress.[2]

Multiple Impacts of AKI, Prevention and Early Diagnosis

AKI may have multiple clinical impacts, high risk of mortality, and risk of progressive deterioration of renal function, leading to CKD as well as ESKD. Consequently, AKI decreases the quality of life and may contribute to the increasing medical costs and becomes national financial burden covered in the universal health coverage (BPJS Kesehatan).[27,28]

Some recent studies have also identified AKI as risk factor for other adverse outcomes, including stroke, cardiovascular disease, sepsis, malignancy, bone fracture and upper gastrointestinal hemorrhage.[29,30] In a general sense, AKI-related adverse outcomes depend on the presence of preexisting comorbidities, namely cardiovascular disease, hypertension, diabetes mellitus, and most importantly, preexisting CKD.[2] It can be said that presence of comorbidities is a key player in the long-term impact of AKI. Tight control of these comorbidities should prevent the progression of AKI into CKD.[31,32] It is important to preserve

renal function as much as possible to halt further renal deterioration.

Despite great advances in the understanding of risk factors, diagnosis and management of AKI, mortality risk remains high.[33,34] Surprisingly, majority of patients had delayed consultation to nephrologists, which is known to be associated with higher mortality.[35,36] Further prevention measures may include improvement of tools used for early detection and diagnosis, identification of high-risk patients (the elderlies, patients with preexisting comorbidities and preexisting renal impairment), optimization of fluid management, proper antibiotic dosing, nutritional adjustments, withdrawal of nephrotoxic drugs, removal of hyperchloremic solutions and others.[37] Nephrologist intervention is an essential part of entire care in patients with AKI and can influence progression of AKI as well as AKI-associated mortality. Various efforts, specifically fluid adjustment, may prevent the need for KRT and decrease the progression of AKI. Considering the multifactorial nature of AKI, besides nephrologist intervention, AKI demands for multidisciplinary approach as needed, in order to provide best quality of care for patients.[6]

CONCLUSION

AKI is an important complex syndrome with multiple adverse outcomes in hospitalized patients, particularly in ICU patients. The COVID-19 pandemic has increased its complexity and thus proper treatment is vital to reduce morbidity, mortality and medical costs. Some pivotal approaches in managing AKI patients are (1) consideration of multiple risk factors and comorbidities, (2) use of early detection tools and diagnosis, and (3) implementation of preventive and therapeutic intervention as well as early nephrologist intervention as a part of multidisciplinary spectrum that lowers the risk of starting KRT and AKI progression.

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Polymorphisms of SLCO1B1 Gene in Sundanese Ethnic Population of Tuberculosis Patients in Indonesia

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ABSTRACT

Background: *The blood level of rifampicin, one of the tuberculosis (TB) drugs, depends on the organic anion transporting polypeptide 1B1 (OATP1B1) in hepatocytes. This protein is encoded by the solute carrier organic anion 1B1 (SLCO1B1) gene. Its genetic variation has been reported to have an impact on clinical outcomes and drug efficacy. However, the polymorphism in the SLCO1B1 gene has not been examined in Indonesia yet. We aimed to identify the frequency of polymorphism in SLCO1B1 gene among pulmonary TB patients in Bandung, Indonesia.* ***Methods:*** *Cross-sectional study was conducted in West Java. 145 pulmonary TB patients who were treated with first-line drugs treatment (including rifampicin 450 mg daily) were analyzed for polymorphism in SLCO1B1 gene. Patients aged between 18–64 years old and mainly came from Sundanese ethnic group (92.4%). Genetic variants were detected using Polymerase Chain Reaction (PCR) and Sanger sequencing.* ***Results:*** *Polymorphism of c.463C>A(rs11045819) was not identified, while heterozygous and homozygous polymorphism of c.85-7793C>T(rs4149032) were identified in 74 (51.0%) and 56 (38.6%) patients, respectively. The minor allele frequency (MAF) of T (mutant) allele of c.85-7793C>T(rs4149032) was 64.13% (186/209), higher than in the general population, which the MAF of rs4149032 is 53.6% based on 1000 genome database.* ***Conclusion:*** *This study highlights the presence of different allele frequencies of polymorphisms within the population, which might affect treatment outcomes.*

Keywords: *c.85-7793C>T (rs4149032), c.463C>A (rs11045819), drug transporter, gene polymorphism, West Java, Indonesia.*

INTRODUCTION

Lungs are the most commonly *M. tuberculosis (M.tb)*-infected organ. There are five standard first-line drugs for the treatment of pulmonary tuberculosis (TB), namely: rifampicin, isoniazid, ethambutol, pyrazinamide, and streptomycin.[1,2] The administration of TB drugs is divided into two categories. The first category is given to new patients, who did not have a previous history of TB treatment, while the second one is given for relapsed cases or patients that were lost to follow-up during treatments, with additional injection regimens and longer duration.[2,3] Among those five drugs, rifampicin, derived from *Amycolatopsis rifamycinica* is the backbone for TB treatment, as it is one of the most effective bactericidal agents against *M. tuberculosis (M.tb)* by inhibiting mycobacterial RNA polymerase through suppression of chain formation in RNA synthesis.[4,5]

The activity of rifampicin against *M.tb* is determined by plasma concentrations of rifampicin.[6,7] Lower plasma concentrations of rifampicin can affect the results of the treatment, including a higher rate of relapse in the continuous phase.[8] Aside from the severity of the disease, low plasma concentration of rifampicin is also related to the course and absorption of drugs inside the patients' body or pharmacokinetics. Weiner et al. mentioned that the polymorphism of c.463C>A (rs11045819) Solute carrier organic anion transporter family member 1B1 (SLCO1B1) gene significantly influenced the pharmacokinetics of rifampicin, resulting in lower plasma concentrations of rifampicin.[9] It was reported that patients with polymorphisms c.463C>A (rs11045819) in the *SLCO1B1* gene experienced lower peak concentrations of rifampicin by 42% compared to wild types.[9]

Organic anion transporting polypeptide 1B1 (OATP1B1), encoded by the *SLCO1B1* gene, plays a role in mediating drugs and their absorption in hepatocytes.[10-13] A study conducted by Kwara in 2014, reported that polymorphism c.463C>A (rs11045819) *SLCO1B1* gene resulted in lower plasma concentrations of rifampicin, especially in men.[14] Other than c.463C>A (rs11045819) polymorphism, Chigutsa et al., identified that c.85-7793C>T (rs4149032) polymorphism in *SLCO1B1* decreased rifampicin concentration in pulmonary TB patients.[8] Moreover, the study detected an allele frequency for this polymorphism of around 70%.[8] In Asia, it is found that the allele frequency for polymorphisms of c.85-7793C>T (rs4149032) *SLCO1B1* is around 56%. While the allele frequency for polymorphism of c.463C>A (rs11045819) *SLCO1B1* is approximately 3%.[8,9,15,16] Minor allele frequencies of these polymorphisms in TB patients have not been reported yet. We aimed to determine the frequency of polymorphisms of the *SLCO1B1* gene in TB patients. Knowing the frequencies of the polymorphisms is important to determine the association between these variables and the effect of TB drugs on clinical outcomes.

METHODS

This is a cross-sectional descriptive study to evaluate the frequency of c.463C>A (rs11045819) and c.85-7793C>T (rs4149032) polymorphisms in the *SLCO1B1* gene in pulmonary TB patients receiving anti-TB drug. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran, (15/UN6. C1.3.2/KEPK/PN/2016, 22). Written informed consent was obtained from all the subjects before the commencement of the study.

Population

A total of 145 TB patients receiving first-line anti-TB drugs and residing in Bandung were enrolled in this study. Patients included were patients aged between 18 and 65 years old, undergoing an intensive phase at the time of the study, and with normal liver function. Normal liver function was defined as an SGPT level below 2.5 x the upper normal limit. The ethnicity of study participants was determined by interview, which is the ethnicity of the subjects' previous two generations (parents and grandparents).

Data Collection

Two phases of data collection were done; initial screening was performed to collect and sort out pulmonary TB patients who met the inclusion and exclusion criteria. When the inclusion criteria are met, whole blood samples were taken. DNA extraction followed by PCR of

the *SLCO1B1* gene was performed. Furthermore, the PCR products were sequenced to obtain the sequence of nucleotide bases which is further analyzed for c.463C>A (rs11045819) and c.85-7793C>T (rs4049302) polymorphisms. An in-depth analysis of each with polymorphism in each patient was done.

DNA Extraction

Genomic DNA was extracted from patients' whole blood using the Salting Out method. The cleaned and washed DNA pellet was re-suspended with TE buffer to complete the process of DNA extraction.[17]

PCR Reaction

The primer mixtures were used to produce amplicons. Forward primer was GGGGAAGATAATGGTGCAAA, and reverse primer was CATCCAGTTCAGATGGACAAAA.

The amplification was done in the following condition: 10 min of initial denaturation PCR activation at 94^{O}C; 35 cycles of denaturation at 94^{O}C for 5 min; annealing at 58^{O}C for 30 seconds, elongation at 72^{O}C for 1 min; and final elongation at 72^{O}C for 7 min. The PCR products were then visualized by mixing 10 ul amplicons with SyberSafe and loading it to 2% gel agarose then electrophoresis with 1% TAE buffer was performed at 100 V for 40 min.[17]

Sanger Sequencing

The PCR product was purified by adding 2 µL of ExoSAP-IT reagent (USB Corporation, Cleveland, OH, USA) to 5 µL of PCR product and incubated for 15 minutes at 37^{O}C and 15 minutes at 80^{O}C for denaturation.[17] Furthermore, DNA concentration was measured using an ND-1000 spectrophotometer (NanoDrop Tech., Rockland, DE, USA) and dissolved up to 100 mg.[17] After adding 0.1 µM of the sequencing primer sequence, the solution was reacted with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) when the cycle sequencing is performed in a thermal cycler.[17] The result was analyzed using Bioedit software to detect SNPs.

RESULTS

Patient characteristics data related to subsequent data analysis, including age, ethnicity, sex, drugs other than anti TB drugs, body weight, body mass index, HIV status, diabetes mellitus status, and the dose of rifampicin/kg body weight were collected. The basic characteristics of the patients involved are presented in **Table 1**.

Table 1. Patient characteristics.

Characteristics	n=145
Age, years	
Median (Range)	36 (18–64)
Age categories, years (%)	
18–60	134 (92.4)
>60	11 (7.6)
Gender (%)	
Male	80 (55.2)
Female	65 (44.8)
Ethnicity (%)	
Ambonese/ South Moluccans	1 (0.7)
Bataknese	1 (0.7)
Buginese	1 (0.7)
Javanese	7 (4.8)
Minangnese	1 (0.7)
Sundanese	134 (92.4)
Body weight, kilograms	
Mean (SD)	46 (5)
Range	34–58
Body Mass Index/BMI, kg/m^2	
Mean (SD)	17.4 (1.9)
Range	13.8–23.1
BMI categories, kg/m^2 (%)	
<18.5	107 (73.8)
18.5–23.0	38 (26.2)
HIV (+) (%)	3 (2.1)
Diabetes Mellitus (%)	3 (2.1)

A total of 145 patients were enrolled in this study. Most patients were under 60 years old. The proportion of gender in this study (male vs. female) was similar. Since this study was conducted in Bandung, nine out of ten subjects' ethnicity were Sundanese. The mean weight of the patients in this study was 46 kg and about three-quarters were underweight (BMI <18.5 kg/m^2). There were three patients with HIV co-infection (2.1%) and three patients with diabetes mellitus comorbidity (2.1%).

Table 2. The number of polymorphism c.463C>A (rs11045819) and c.85-7793C>T (rs4149032) of SLCO1B1 gene

Characteristics	n=145
c.463C>A (rs11045819) *SLCO1B1* (%)	
CC	145 (100)
CA	0 (0.0)
AA	0 (0.0)
c.85-7793C>T (rs4149032) *SLCO1B1* (%)	
CC	15 (10.3)
CT	74 (51.1)
TT	56 (38.6)

There were no polymorphism c.463C>A (rs11045819) identified as presented in **Table 2**. However, 130 (89.6%) patients were identified carrying c.85-7793C>T (rs4149032) polymorphism of the *SLCO1B1* gene from the analysis of sequencing results. Half of the patients were heterozygous c.85-7793C>T (rs4149032), around 40% were homozygous (c.85-7793T>T (rs4149032) and the remaining 10% of the patients were wild type c.85-7793C>C (rs4149032). Furthermore, the minor allele frequency of c.85-7793C>T (rs4149032) in this group of patients was 64.13% (186/290). The distribution of these polymorphisms based on gender, ethnicity, body weight, and rifampicin dose are presented in **Table 3**.

DISCUSSION

Tuberculosis (TB) is one of the top global problems in the world requiring special attention. It is also the second leading cause of death from a single infectious agent, after the Human Immunodeficiency Virus (HIV).[18] The success of pulmonary TB treatment is influenced by many factors, such as the dose of the drug, the method of administration of the drug, patient compliance, and TB germs. What is important and often overlooked is the achievement of therapeutic concentrations of rifampicin in plasma.

From a previous study, it has been proved that genetic variation in the *SLCO1B1* gene affects rifampicin OATP1B1 transporter and has a negative effect on plasma rifampicin concentration, which might contribute to TB treatment outcome.[8,9,14] Several studies in Indonesia, especially in Bandung, showed low concentrations of plasma rifampicin which varied between individuals. This variation can be caused by the polymorphism of the OATP1B1 transporter encoding gene in the pharmacokinetics process of rifampicin, the *SLCO1B1* gene. Most of the patients were under 60 years and 92.4% were Sundanese.[19,20] Further study needs to be done, including other Indonesian ethnicities to generalize the result of the Indonesian population.

Rifampicin is a substrate with OATP1B1 as its transporter. This transporter is expressed on the sinusoidal membrane of hepatocytes.[13] In the liver, it has a role in metabolic processes. The OATP1B1 transporter is encoded by the *SLCO1B1* gene.[16] Solute Carrier Organic Anion Transporter family member 1B1 (SLCO1B1) is a gene that encodes the organic anion protein transporter for membrane binding (OATP1B1). This protein transporter facilitates the absorption of rifampicin in the hepatocellular level. The *SLCO1B1* gene is known to have many possibilities for polymorphisms.[10,12,14-16] SNP or single nucleotide polymorphism is the change of a single nitrogenous base in a gene that has > 1% frequency of occurrence. This genetic variation can cause molecular changes both at the level of metabolic enzymes, drug targets or receptors, and

Table 3. The distribution of polymorphism c.85-7793C>T (rs4149032) of SLCO1B1 gene based on gender, ethnicity, body weight, and rifampicin dose.

Variables	Polymorphism c.85-7793C>T (rs4149032) *SLCO1B*1			P value
	CC (reference) n=15	CT n=72	TT n=56	
Gender (%)				
- Male	8 (53.3)	32 (44.4)	39 (69.6)	0.017*
- Female	7 (46.7)	40 (55.6)	17 (30.4)	
Ethnicity (%)				
- Ambonese	0 (0.0)	1 (1.4)	0 (0.0)	0.188
- Bataknese	0 (0.0)	1 (1.4)	0 (0.0)	
- Buginese	1 (6.7)	0 (0.0)	0 (0.0)	
- Javanese	0 (0.0)	5 (6.9)	2 (3.6)	
- Minangnese	0 (0.0)	0 (0.0)	1 (1.8)	
- Sundanese	14 (93.3)	65 (90.3)	53 (94.6)	
Body weight, kg				
Mean ± SD	47.0 ± 5.3	45.5 ± 5.0	47.6 ± 5.5	0.073
Dose per kg BW				
Mean ± SD	9.7 ± 1.3	10.0 ± 1.1	9.6 ± 1.1	0.095

*: statistically significant

protein transporters.[21] Pharmacokinetic changes, caused by genetic polymorphisms, might affect drug efficacy.[22-24]

The polymorphisms of *SLCOB1* gene, c.463C>A (rs11045819) and c.85-7793C>T (rs4149032) have been reported to be associated with lower plasma rifampicin concentrations compared to wild type.[8,9,14] Genetic variations between individuals can cause differences in the concentration of rifampicin through the role of the transporter. For example, polymorphism c.85-7793C>T (rs414903232) *SLCO1B1* gene is associated with C-max, AUC, and confounding factors such as gender, ethnicity, weight, rifampicin dose per kgs of body weight.[14,16]

Pharmacogenetic polymorphism is an important factor in the high variation of plasma rifampicin concentration. Genetic polymorphisms in enzyme metabolism and drug transporters might explain the occurrence of 30% pharmacokinetics variations of the drug.[25]

In this study, two types of polymorphisms were examined, which are c.463C>A (rs11045819) and c.85-7793C>T (rs4149032) of *SLCO1B1* gene. At the end of the examination, not a single polymorphism of c.463C>A (rs11045819) *SLCO1B1* gene was identified in the group of TB patients in Bandung, Indonesia. Based on data from dbSNP150 database at NCBI (National Center for Biotechnology Information), the minor allele frequency (MAF) of rs11045819 (c.463C>A) was 0,0649/325, meaning that a minor (mutant) allele (A allele), was identified in 6,5% within the population (these data were generated from sequencing of 325 individuals/650 chromosome).[8,26,27] If the sample size is 100 (200 chromosomes), it means that the probability of allele A will be found in 6 samples with heterozygous genotype or 3 samples with homozygous genotype (minor/ mutant) or a mix between heterozygous and homozygous type. The obtained information after tracing the data on dbSNP150, MAF data of rs11045819 showed that this polymorphism only occured populations/ ethnicity of African Americans, Mexicans, and Caucasians. On the contrary, Asian ethnicities, including Japanese and Chinese (Han Chinese) do not have this polymorphism. In this study, the majority of patients came from a relatively homogeneous ethnic group which is Sundanese. The Sundanese is part of the Deutro-Melayu ethnic group which gnomically is similar to the Chinese. This might explain why this polymorphism was also not identified in this study.

In a previous study, Pasanen (2008) investigated the diversity of *SLCO1B1* gene at a global level. This study involved 941 patients within 52 populations. The study was carried out by dividing populations into 8 regions, namely: Sub-Saharan Africa, North Africa, Middle East, Europe, South/ Central Asia, East Asia, Oceania, and America. The allele frequency of polymorphism c.463C>A was found to be 0.4% in the East Asian region. This number was considered very small compared to Europe (17%) and the United States (7.2%).[27,28] The presence of *SLCO1B1* polymorphisms correlated with geography.[29] This is consistent with a study conducted by Niemi et al (2011) which stated that polymorphism in c.463C>A associated with decreased rifampicin concentration in plasma, only be around 0–3% of the population in East Asia.[16]

Polymorphism heterozygous and homozygous mutant of c.85-7793C>T (rs4149032) of the *SLCO1B1* gene was found in 74 and 56 patients, respectively (51.0% and 38.6%). Hence the MAF of this polymorphism in this group of TB patients is 64.13% (186/290). A previous study by Chigutsa et al. in Africa found at least 70% of allele frequency of polymorphism c.85-7793C>T (rs4149032) of *SLCO1B1* gene.[8] In other reports, allele frequency of polymorphism c.85-7793C>T (rs4149032) was 75% in Nigerians, 29% in Caucasians, and 56% in Asian.[8,26,27]

This study found a higher minor allele frequency of polymorphism c.85-7793C>T (rs4149032) *SLCO1B1* gene than that in Caucasians, and other Asian but lower than that in the African population. Preliminary studies conducted by OD Sampurno in Jakarta, included 30 healthy people, 60% of men aged between 25–58 years received the results of the *SLCO1B1* gene with the SNP in c.463C>A, with the proportion of genotype CC 46.7%, CA 46.7%, and AA 6.6% whereas in rs4149032 there was no polymorphism detected.[30] This is

likely due to differences in study location setting, patients, and ethnicity. The relative difference in the contribution of transporter polymorphisms that affect the pharmacokinetics depends on the patient's ethnic background.[31]

Presence of polymorphism found in this study highlights the possibility of suboptimal plasma rifampicin concentration in TB patients treated with first-line drugs in Bandung. It is necessary to determine the optimal dose in different populations to ensure that each patient is given ideal treatment. Since examination of SLCO1B1 polymorphisms before starting therapy is rather difficult to implement, increasing the dose of rifampicin may be considered. Previous study showed that increasing the dose of rifampicin by 150 mg daily in subjects with homozygous polymorphism c.85-7793TT (rs4149032) and heterozygous polymorphism c.85-7793CT (rs4149032) will give the same plasma rifampicin concentration value as the wild type c.85-7793CC (rs4149032).[8] Higher dose of rifampicin will increase the peak concentration (C_{max}) in the polymorphism group.[8] The possibility of suboptimal treatment also accentuates the need of drug monitoring during TB treatments.

This study's limitation is that it focused on the Sundanese population, therefore it is essential to characterize the frequency of polymorphisms and their functional consequences in other ethnic groups, as different genetic variations may be observed in different ethnic groups. There is also a need for further study regarding rifampicin pharmacokinetics and *SLCO1B1* polymorphisms. The relationship between the polymorphisms and treatment outcomes also still needs to be identified. It is recommended to discover all the polymorphisms that might occur in pulmonary TB patients by examining samples with whole-genome sequencing.

CONCLUSION

This study reported the high presence of polymorphism c.85-7793C>T (rs4149032) of the *SLCO1B1* gene in TB patients in Bandung, especially in the Sundanese population. There were no polymorphisms c.463C>A. (rs11045819) of the *SLCO1B1* gene identified in this study. This study highlights the presence of polymorphism allele frequency in TB patients, which may affect treatment outcomes.

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CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

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Lower Number and Percentage of Activated Natural Killer Cells in Colorectal Cancer Patients

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ABSTRACT

Background: *Colorectal cancer is a type of cancer that begins in the colon and/or rectum tissue. Natural killer (NK) cells play a critical role in the first line of defense against infection and tumors, as well as in autoimmunity and hypersensitivity reactions. NK cells also play a role in regulating tumor cell growth and metastasis. The number and percentage of activated natural killer cells have been determined in patients with colorectal cancer and benign lesion.* ***Methods:*** *This was a cross-sectional observational analytic study. The number and percentage of activated NK cells in peripheral blood were determined using the flow cytometry method in 50 samples from patients who underwent colonoscopy and obtained a mass as evidenced by histopathological examination.* ***Results:*** *Among the 50 samples, 24 samples included in the colorectal cancer group and 26 samples from benign lesion group. The mean number of NK cells in colorectal cancer was 161.71 ± 62.666 cells/µL, benign lesion was 553.92 ± 269.173 cells/µL. The mean percentage of activated NK cells in colorectal cancer was 2.82 ± 1.19%, benign lesion was 5.10 ± 2.48%. There was a significant difference in the number of NK cells and the percentage of activated NK cells between colorectal cancer and benign lesion patients (p = 0.000).* ***Conclusion:*** *The number and activity of NK cells decreases in patients with colorectal cancer.*

Keywords: *The number of NK cells, percentage of activated NK cells, colorectal cancer, cancer.*

INTRODUCTION

Colorectal cancer is a type of cancer that begins in the colon and/or rectum tissue. Recently the incidence of colorectal cancer has been increasing in both Western and developing countries. Colorectal cancer is the third most common type of cancer worldwide, the second leading cause of death from cancer, and the leading cause of death from gastrointestinal cancer.[1] Colorectal cancer is the third most common type of cancer and the third leading cause of death in men and women in the United States, according to the American Cancer Society. In 2018, 19,113 men (11.9% of new cancer cases) and 10904 women (5.8% of new cancer cases) were diagnosed with colorectal cancer in the world.[2] Data in Dr. Soetomo Surabaya Hospital, Indonesia, recorded as many as 852 patients diagnosed with colorectal cancer from 1 January 2012 to 31 December 2017. Other data obtained were 201 patients were recorded as having colorectal tumors at the Gastroentero-

hepatology Center Dr. Soetomo Hospital from June 2013 to May 2015. The risk of developing colorectal cancer can be associated with aging, poor dietary habits, lack of exercise, smoking, and obesity.[3] In general, it is stated that the development of colorectal cancer is an interaction of various factors, namely environment and genetics. Natural killer (NK) cells are a type of large granular lymphocyte with a distinctive morphology that participates in innate immunity. NK cells play a role in the early stages of infection and tumor defense and may also play a role in autoimmunity and hypersensitivity reactions. NK cells protect the body by killing specific cells and secreting chemokines and cytokines (innate immune system), as well as assisting other immune cells in eliminating the targeted cells (adaptive immune system). NK cell functions are regulated by two types of receptors, namely receptor activation, and inhibition.[4]

NK cells are the primary cells in cancer immune surveillance. The role of NK cells in colorectal cancer can predict the occurrence of postoperative recurrence and metastases. A previous study shows that the number of NK cells is related to the life expectancy of people with colorectal cancer.[5] The decrease in NK cell activity was not associated with the cancer staging, is associated with a lower life expectancy.[4] Colorectal cancer was tenfold more likely to develop in patients with low NK cell activity.[6]

The incidence of colorectal cancer is increasing in developing countries such as Indonesia. A recent study compared patients at high risk of colorectal cancer, specifically those over the age of 40 who had colonoscopy.[6] This study compares the number and percentage of activated natural killer cells in colorectal cancer patients and benign lesion patients based on this description.

METHODS

The method used was observational analytic with a cross-sectional design. The population of this study was all patients who underwent colonoscopy at Dr. Soetomo Hospital, Surabaya, East Java, Indonesia. The study sample was patients who met the inclusion and exclusion criteria, and had a mass taken in colonoscopy which was proven by histopathological examination. The inclusion criteria were consenting to participate in the study with informed consent, aged 20-60 years and not undergoing anticancer treatment, including surgery and chemotherapy. While the exclusion criteria were patients who are not pregnant and not currently using contraception, currently has no active bacterial or viral infection, had hepatitis B or C infection, had a history of using corticosteroids and immunosuppressants for the past six months, had autoimmune diseases (rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, kidney disease), intestinal inflammation including Crohn's disease, type 1 diabetes, Guillain Barre syndrome), currently has no active TB or a history of HIV infection. The number of subjects was obtained after calculating the minimum sample size using the formula unpaired numerical analytic. The number of samples was obtained with 24 colorectal cancer samples and 26 benign lesion samples.

Colorectal cancer patients are patients whose masses are suspected of malignancy originating from the colon and/or rectum on colonoscopy examination, as well as evidenced by histopathological examination, and benign lesion patients are patients who do not show a mass suspected of malignancy (polyps, internal and external hemorrhoids, normal) as evidenced by histopathological examination. This study has approved by the Ethics Committee of dr. Soetomo Hospital (Ref. No. 2028/KEPK/VII/2020).

The research location was the Gastroentero-Hepatology Center, Internal Medicine Department/SMF at Dr. Soetomo Hospital, Surabaya, East Java, Indonesia. Samples were taken in the period July 2020 to February 2021. Sampling was done by consecutive sampling until the sample size was met. This research was conducted by taking the peripheral blood of the research subject to check the number and percentage of activated NK cells using the flow cytometry method. After conducting research and obtaining the desired data, the data were collected and analyzed using SPSS version 25 software.

RESULTS

Based on the 50 subjects obtained, 26 subjects had colorectal cancer and 24 subjects had benign lesion. Based on the age profile, the mean age of the subjects for colorectal cancer was 44 years while for benign lesion it was 49 years as shown in **Table 1**. Most of the subjects (57.69%) were female in the benign lesion group (15 people), and most 75% were male in the colorectal cancer group (18 people).

Characteristics of the Research Subject's Laboratory Examination

From a total of 50 research subjects, 24 subjects had colorectal cancer, and 26 subjects had benign lesion. As can be seen in **Table 2** shows that the average Hb value in the benign lesion was higher than the colorectal cancer group. The average value is not more significant. Leukocyte levels in benign lesion subjects were higher (10353.46 cells/µL) than in the colorectal cancer group. At the same time, the platelet level was higher (371037.50 cells/µL) in the colorectal cancer group. Lymphocyte levels were greater (2110.77 cells/µL) in the benign lesion group. The mean *Serum Glutamic Oxaloacetic Transaminase* (SGOT) was 30.09 mg/dL for the colorectal cancer group. Mean *Serum Glutamic Pyruvic Transaminase* (SGPT) level 34.50 mg/dL for benign lesion.

The average Blood Urea Nitrogen (BUN) level was 7.07 mg/dL for the colorectal cancer group. The average albumin level in the colorectal cancer group was 3.55 g/dL. Examination of the Serum Creatinine (SK) test showed the mean SK level for the benign lesion group was 3.32 mg/dL. The average Na level for the colorectal cancer group was 135.79, while the average K level in the benign lesion group was 4.05.

Distribution of Colorectal Cancer and Benign Lesion Based on Histopathological Type

The results of this study found that the most frequent histopathological types found in the colorectal cancer group was adenocarcinoma (36%), followed by carcinoma well differentiated (12%). While for the benign lesion group, non-specific chronic colitis was most frequent (28%),

Table 1. Demographic characteristics.

Characteristics	Colorectal cancer n = 24	Benign lesion n = 26	p-value
Age (years)			
Mean ± SD	44.83 ± 12.11	49.35 ± 10.94	0.540
Median	46.0	54.0	
Gender			
Women (%)	25	57.69	0.019
Men (%)	75	42.30	
Body Massa Index (kg/m^2)			
Mean ± SD	25.754 ± 0.84	22.096 ± 3.07	0.034
Median	25.60	21.75	
Ethnicity			
Javanese (%)	79.17	76.92	0.957
Madura (%)	12.5	15.38	
Tionghoa (%)	8.33	7.7	
Symptoms			
Hematochezia (%)	29.17	38.46	
Diarrhea (%)	29.17	23.07	
Constipation (%)	8.33	3.85	0.821
Abdominal pain (%)	33.33	34.62	

Table 2. Characteristics of the research subject's laboratory examination.

Laboratory parameters	Colorectal cancer n=24	Benign lesion n=26	p-value
Hemoglobin (g/dL)			
Mean ± SD	12.27 ± 2.10	12.85 ± 2.21	0.565
Median	12.65	12.85	
Leukocytes (cell/µL)			
Mean ± SD	9618.67 ± 5994.57	10353.46 ±12677.35	0.432
Median	8385.00	6755.00	
Platelets (cell/µL)			
Mean ± SD	371037.50 ± 193426.06	326938.46 ± 135021.05	0.352
Median	4.20	4.20	
Lymphocytes (cell/µL)			
Mean ± SD	1147.50 ± 449.44	2110.77 ± 635.13	0.069
Median	1090.00	2300.00	
SGOT			
Mean ± SD	30.09 ± 27.26	27.46 ± 17.75	0.191
Median	20.500	24.500	
SGP-T			
Mean ± SD	31.15 ± 17.71	34.50 ± 21.11	0.651
Median	28.00	28.00	
BUN			
Mean ± SD	7.07 ± 3.41	6.19 ± 2.35	0.652
Median	6.00	6.00	
Albumin			
Mean ± SD	3.55 ± 0.69	2.90 ± 0.94	0.232
Median	3.70	2.80	
SC			
Mean ± SD	2.47 ± 2.12	3.32 ± 2.67	0.321
Median	1.3200	1.3200	
Na			
Mean ± SD	135.79 ± 6.70	131.08 ± 24.60	0.363
Median	135.00	134.50	
K			
Mean ± SD	3.99 ± 0.49	4.05 ± 0.35	0.390

*SGOT : Serum Glutamic Oxaloacetic Transaminase
SGP-T : Serum Glutamic Pyruvic Transaminase
SC : Serum Creatinine
Na : Natrium
K : Kalium

followed by non-specific chronic colitis with mild dysplasia (12%), and active chronic colitis (12%).

Number of NK Cells in Colorectal Cancer and Benign Lesion Group

The mean number of NK cells in the colorectal cancer group was 161.71 ± 62.66 cells/µL, which was greater than the average number of NK cells in the Benign lesion group, which was 553.92 ± 269.17 cells/µL. The lowest number of NK cells in the colorectal cancer group was 25, while the highest number was

Table 3. Histopathological classification.

Variables	n (%)
Colorectal cancer	
Adenocarcinoma	18 (36)
Carcinoma well differentiated	6 (12)
Benign lesion	
Non-specific chronic colitis	14 (28)
Non-specific chronic colitis with mild	6 (12)
dysplasia	6 (12)
Active chronic colitis	
Total	(100)

244. The lowest number of NK cells in the benign lesion group was 272, while the highest number was 1095. The range of minimum and maximum values in both groups was also quite large, as indicated by the very high standard deviation of 62.66 in the colorectal cancer group and 269.17 in the benign lesion group. Comparative analysis of the number of NK cells in patients with colorectal cancer and benign lesion patients were performed using the Mann Whitney's test. The analysis results show a significant difference between the number of NK cells in patients with colorectal cancer and benign lesion (p-value 0.000).

Percentage of Activated NK Cells in Colorectal Cancer and Benign Lesion Group

The average percentage of activated NK cells in the colorectal cancer group is 2.82 ± 1.19%, which is smaller than the benign lesion group with 5.10 ± 2.48%. The lowest percentage of activated NK cells was 0.92, and the highest was 4.67 in the colorectal cancer group, while the lowest percentage of NK cells in the benign lesion group was 1.23, and the highest was 11.44. The range of minimum and maximum values in both groups was also quite large, as indicated by the high standard deviation values, namely 1.19 in patients with colorectal cancer and 2.48 in patients without colorectal cancer. The analysis results show a significant difference between the percentage of activated NK cells in patients with colorectal cancer and benign lesion with a p-value of 0.000.

DISCUSSION

Colorectal cancer group is a patient whose mass is suspected of malignancy originating from the colon and/or rectum on colonoscopy examination, as well as evidenced by histopathological examination.[1] The number and activity of natural killer cells (NK cells) decreased in patients with colorectal cancer in this study. NK cells are effector lymphocytes of the innate immune system that regulate the growth and spread of several types of tumors. NK cells are a promising cell type for adoptive immunotherapy. Transplantation of tumor-infiltrating lymphocytes has demonstrated some remarkable responses in patients with metastatic melanoma.[7] Although NK cells have a limited ability to infiltrate the colorectal cancer microenvironment, a subpopulation of colorectal cancer patients had lesions that are sufficiently infiltrated with NK cells for statistical analysis. NK cell infiltration was previously detected in approximately 30% of colorectal tumor specimens.

Notably, NK cell infiltration was not found to be associated with disease progression.[8] Following a 5-year follow-up, the beneficial effect of NK cell-T cell cooperation on the clinical course of colorectal cancer is diminished. The mechanism underlying this phenomenon is obscure. One could hypothesize that tumor-infiltrating NK cells lose their helper function over time as a result of altered activities induced by cancer cells via a variety of mechanisms. These include indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2) production by malignant cells, metalloproteinases (MMPs), transforming growth factor b1 (TGFb1), and integrin b2 production by malignant cells (ITGB2, also known as LFA1).[9]

Another study found that the mean age of patients with colorectal cancer was 42 years.[10] Most colorectal cancer sufferers are at the age of 40-49 years. Other studies mostly found colorectal cancer at the age of over 40 years. The age of 40 years is when the diagnosis of colorectal cancer begins to increase sharply.[11] In this study the colorectal cancer group was also found to be at an average age of 44 years.

This situation may occur due to the slow metabolic process, inactivity, and more frequent food consumption. As many as 70% of colorectal cancer cases are sporadically caused by poor lifestyle, such as a diet low in fiber and fruits, excessive red meat and saturated fat consumption, lack of physical activity, alcohol consumption, and smoking.[12] Based on gender, the proportion of female subjects in the benign lesion group significantlt larger than that the colorectal cancer group. As for the colorectal cancer group, there was a significantly higher proportion of males. In most studies, colorectal cancer were mostly found in male patients. The incidence of colorectal cancer in men is related

to the level of the hormone estradiol, which in normal amounts, functions in spermatogenesis and fertility. However, excessive amounts of estradiol will inhibit the secretion of gonadotropin proteins such as luteinizing hormone (LH), further reducing testosterone secretion. A high amount of testosterone is associated with a reduced risk of colorectal cancer.[13]

Another study discovered that the majority of colorectal cancer patients had an overweight BMI. Obesity causes hormone accumulation, increased insulin levels, and insulin-like growth factor-1 (IGF-1), triggering tumor growth regulators, impaired immune response, and oxidative stress, thus triggering colorectal carcinoma.[14] Based on ethnicity, the proportion of subjects with Javanese ethnicity in the colorectal cancer group was more significant than the benign lesion group. Another study found colorectal cancer patients of Javanese ethnicity. The ethnic groups chosen for this study are critical because they will influence cancer treatment strategies, cancer prevention strategies, early detection, and appropriate treatment.[15] Ethnic variations in the risk of colorectal cancer should affect results if adjusted for known or suspected risk factors and environmental exposures.[16] Although there was no statistically significant difference in the number of NK cells in the peripheral blood of healthy donors and colorectal cancer patients, the colorectal cancer group had 10.10 cells/L in the CD45+ CD56+ cell population. Whereas in healthy donors, the results were 12.3 cells/μL.[17] Increased loss of CD16 expression via release could explain the increase in the frequency of the CD56-dimmed CD16 population. CD16 release can also be induced by activating NK cells with cytokines such as IL-2, IL-15, and IL-18, TNF, or target cells (such as tumor cells). Many of these cytokines are increased in the blood of patients with colorectal cancer. When NK cells are activated via CD16 or NKG2D signaling, the ADAM17 metalloprotease, which also cleaves CD16 in NK cells, is increased.[18] The increase in CD56+CD16 + NK cell counts in colorectal cancer patients is statistically significant. CD16 is a low-affinity FcRIII that recognizes antibody-coated targets and signals antibody-dependent cytotoxicity (ADCC). CD16 binds specifically to the Fc moiety of IgG antibodies on the surface of coated cells and induces degranulation of intracellular granules, resulting in the death of infected or tumor cells.[19] NKG2D expression was significantly downregulated in NK cells isolated from patients with colorectal cancer. The decreased expression of NKG2D may be associated with the suppression of NK cell activity in colorectal cancer.[20] NKG2D is required for NK cell activation, and decreased NKG2D expression may result in decreased NK cell activity in patients with colorectal cancer. In colorectal cancer patients, the NKG2 pathway can be used to inhibit NK cell-mediated antitumor immune responses. The imbalanced expression of NKG2A and NKG2D may contribute to the suppression of NK cell activity in colorectal cancer patients, thereby allowing tumor cells to escape NK-mediated lysis. Numerous cytokines, including IL-2, IL-12, IL-15, and IFN-, can increase NKG2 expression and NK cell-mediated cytotoxicity. Due to the decreased level of NKG2D expression in colorectal cancer patients, which may be related to NK cell suppression, tumor cells can evade NK cell control via the NKG2 pathway.[21]

CONCLUSION

A significant difference was found between the number and percentage of activated NK cells in colorectal and benign lesion patients.

CONFLICT OF INTEREST

The authors declare that they have no conflicting interests.

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Diagnosis of Chronic Lymphocytic Leukemia Using iwCLL 2018 Compared with NCI-WG96 Criteria in Cipto Mangunkusumo Hospital: A Practical Consideration in Resource Limited Setting

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ABSTRACT

Background*: The diagnosis of chronic lymphocytic leukemia (CLL) is mainly based on blood count, morphology, and immunophenotyping. In Indonesia, the diagnosis is more challenging as the availability of immunophenotyping tests is limited. The European Society of Medical Oncology (ESMO) stated flowcytometry as a prerequisite to establishing diagnosis of CLL, meanwhile in the original International Workshop on Chronic Lymphocytic Leukemia (iwCLL) 2018 criteria, which has been widely accepted by physicians caring for patients with CLL, the diagnosis of CLL can be made in patients with cytopenia using bone marrow biopsy where flowcytometry test is not available. The aim of the study was to compare the utility of iwCLL 2018 compared with National Cancer Institute Working Group 96 (NCI-WG96) criteria in the diagnosis of CLL in Indonesia, especially in limited resource settings.* ***Methods****: The data of newly diagnosed CLL patients, including baseline demographic, clinical, and laboratory characteristics was retrieved retrospectively from medical records in Cipto Mangunkusumo General Hospital from 2015 until 2021. Diagnosis of CLL using iwCLL 2018 diagnostic criteria were then compared with NCI-WG96 criteria.* ***Results****: Thirty-eight patients were enrolled to this study. The median age was 59.5 years and dominated by males. Most of them were classified in the late-stage disease (63.4% in Binet C and about 70% in Rai III-IV). Four cases were CD5-negative CLL. Based on NCI-WG96 guideline, only 24 patients (63.2%) fulfilled all four criteria for CLL. Similarly, using the iwCLL 2018 flowcytometric criteria without biopsy data, 26 patients (68%) were diagnosed as CLL. However, if bone marrow biopsy in patient with cytopenia was taken into account, all patients (100%) can be confirmed as CLL.* ***Conclusion****: The iwCLL 2018 criteria which included bone marrow biopsy in the presence of cytopenia was more applicable to establish the diagnosis of CLL in Indonesia where flowcytometry is not available.*

Keywords: *chronic lymphocytic leukemia, diagnosis, NCI-WG96 criteria, IWCLL 2018 criteria.*

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is defined as a clonal expansion of small, mature, CD5-positive neoplastic B-cell lymphocytes having a characteristic immunophenotype in the peripheral blood, spleen, bone marrow, and other lymphoid tissues.[1] This disease is the most prevalent leukemia among adults in Western countries, accounting for 37% of newly diagnosed leukemia in the United States, followed by acute

myeloid leukemia (AML).[2] However, CLL in Asia differs from Western countries due to its lower prevalence.[3,4] This might be explained by variation in genetic background between different regions.[5] Another reason for this lower incidence is due to the asymptomatic nature of most CLL cases, many are left undiagnosed, particularly in resource limited countries. Until now, there is no population-based study regarding epidemiology of CLL in Indonesia, especially regarding prevalence and diagnostic aspects of CLL. This lack of data can hinder the efforts to evaluate the incidence and trends of the disease among different populations.

Over the last few decades, the criteria to diagnose CLL has undergone substantial revisions. The first consensus criteria to standardize the diagnosis of CLL was available in 1988. At that time, automated blood counter and immunophenotyping test were not routinely available, thus absolute lymphocyte counts (ALC) above $5x10^9$/L was used as the threshold for diagnosis.[6,7] In the 2008 revisions to the CLL diagnostic criteria, B-cell count rather than ALC above $5x10^9$/L was adopted as the basis for diagnosis.[8] Nowadays, the diagnosis of CLL is mainly based on laboratory features, including blood count, morphology, and immunophenotyping, as described in the iwCLL 2018.[9] Society guidelines for CLL stressed on the importance of CLL diagnosis from peripheral blood using B-cell count and flowcytometry alone. In the National Comprehensive Cancer Network (NCCN) guidelines, bone marrow biopsy is recommended if the initial workup is non-diagnostic.[10] Moreover, the European Society of Medical oncology (ESMO) guidelines suggest doing bone marrow biopsy if flowcytometry result remain inconclusive.[11] This is considered convenient for patients since these recommendations prevent the procedural risk and unnecessary pain from bone marrow biopsy. However, flowcytometry is not widely available in developing countries. After thorough review of the iwCLL 2018 diagnostic criteria, it was stated that "the presence of a cytopenia caused by a typical marrow infiltrate establishes the diagnosis of CLL regardless of the number of peripheral blood B lymphocytes or of the lymph node involvement."[7] Therefore, in the absence of immunophenotyping facility, bone marrow biopsy can be used to establish CLL diagnosis.

The present study aimed to evaluate the diagnosis of CLL using iwCLL 2018, which allowed CLL diagnosis from bone marrow biopsy where flowcytometry test is not available, compared with NCI-WG96 and to determine demographic and immunophenotypic profiles of CLL in Indonesia , . This is the first study to compare the diagnosis of CLL according to the NCI-WG96 and the iwCLL 2018 guidelines in such settings.

METHODS

Based on the NCI-WG96 guideline, the diagnosis criteria of CLL include: (1) Peripheral absolute lymphocyte count $>5x10^9$/L; (2) Lymphocytes were positive for CD5 and CD19/CD20/CD23; (3) Atypical cells (prolymphocytes) <55%; (4) Bone marrow lymphocytes ≥ 30%.[12] Meanwhile, in iwCLL 2018 criteria, CLL diagnosis can be made if there are: (1) Peripheral B-cell count $\geq 5x10^9$/L; AND (2) Lymphocytes were positive for CD5 and CD19/CD20/CD23. Alternatively, CLL can also be diagnosed by "the presence of cytopenia caused by a typical bone marrow infiltrate, regardless of the number of peripheral B-cell count or lymph node involvement".[9]

This was a cross-sectional study done at Department of Hematology and Medical Oncology, Cipto Mangunkusumo General Hospital, Jakarta from 2015 until 2021. Patients with newly-diagnosed CLL were recruited from the database of patients who visited the Hematology and Medical Oncology outpatient clinic. Sampling was done by total sampling obtained from the list of CLL patients in the registry. Inclusion criteria was patients diagnosed with CLL. Exclusion criteria was incomplete laboratory data.

Information regarding baseline demographic, clinical (including age, sex, Rai and Binet stage, lymphadenopathy, hepatosplenomegaly), and laboratory (including complete blood count, absolute lymphocyte count, erythrocyte sedimentation rate, LDH, serum protein electrophoresis, and immunophenotyping)

characteristics were obtained retrospectively from medical records. The sample of bone marrow aspirate and biopsy were collected to confirm the diagnosis by flow cytometry immunophenotyping and morphologic analysis were performed to examine the percent of bone marrow lymphocytes.

The study protocol was approved by the Local Ethics Committee of Faculty of Medicine Universitas Indonesia (No. KET-452/UN.2.F1/ETIK/PPM.00.02/2021). The data was expressed in frequency (percentage, %) for categorical variables and median (range) for continuous variables. All analysis was performed using SPSS version 24 for Mac.

RESULTS

There were thirty-eight CLL cases in Cipto Mangunkusumo General Hospital, Jakarta between 2015 and 2021, which were recorded in the registry. All subjects fulfilled the inclusion criteria and were recruited into this study. The characteristics of the subjects are summarized in **Table 1**. Their age at diagnosis ranged from 41-82 years (median 59.5 years) and was dominated by individuals aged ≤65 years (89.5%) and male (60.5%). Two of those patients (5.3%) was diagnosed as prolymphocytic leukemia (PLL), the aggressive form of CLL. Hypertension (28.9%), dyspepsia/GERD/gastritis (15.8%), and diabetes mellitus (13.2%) were the most common comorbidities in the patients. Two of the patients had history of malignancy: thyroid cancer (2.6%) and breast cancer (2.6%). Most of the patients with CLL at time at diagnosis were found in the late stage of the disease, 63.4% of the patients were in Binet stage C and around 70% of the patients were in Rai stage III-IV. Additionally, palpable splenomegaly was presented in 63.2% of the patients.

The median hemoglobin, leucocyte count and absolute lymphocyte count at diagnosis were 8.9 g/dL, 59.86 x 10^9/L, and 47.83 x 10^9/L, respectively. Serum LDH levels were available in 24 of 38 patients, and only 9 of 38 patients (23.7%) had an elevated LDH levels.

Table 1. Clinical and laboratory characteristics of the subjects

		CLL/PLL (n=38)
Age at diagnosis (years), median (range)		59.5 (41-82)
Age group (years), n (%)	≤65	34 (89.5)
	>65	4 (10.5)
Sex, n (%)	Male	23 (60.5)
	Female	15 (39.5)
Diagnosis, n (%)	CLL	36 (94.7)
	PLL	2 (5.3)
Comorbidities, n (%)	Hypertension	11 (28.9)
	Dyspepsia/ GERD/ gastritis	6 (15.8)
	Diabetes mellitus	5 (13.2)
	Hyperuricemia	4 (10.5)
	Chronic kidney disease	2 (5.3)
	Congestive heart failure	2 (5.3)
	Stroke	1 (2.6)
	AIHA	1 (2.6)
	Beta thalassemia trait	1 (2.6)
	Thyroid cancer	1 (2.6)
	Breast cancer	1 (2.6)
	Hepatitis C	1 (2.6)
	Asthma/ allergy	2 (5.2)

Binet, n (%)	A	11 (28.9)
	B	3 (7.9)
	C	24 (63.2)
RAI, n (%)	0	1 (2.6)
	I	2 (5.3)
	II	7 (18.4)
	III	20 (52.6)
	IV	8 (21.1)
Lymphadenopathy >1 cm, n (%)	Yes	16 (42.1)
	No	22 (57.9)
Palpable hepatomegaly, n (%)	Yes	9 (23.7)
	No	29 (76.3)
Palpable splenomegaly, n (%)	Yes	24 (63.2)
	No	14 (36.8)
Hemoglobin (g/dL), median (range)		8.9 (2.8-16.1)
Hematocrit (%), median (range)		28.25 (12.6-47.1)
Leukocyte count (x10^9/L), median (range)		59.86 (4.04-558.35)
Absolute lymphocyte count (x10^9/L), median (range)		47.83 (2.00-530.43)
Platelet count (x10^9/L), median (range)		163.5 (41-468)
ESR (mm/h), median (range)		57.5 (2-153)
LDH, n (%)	Above the limit (>350 IU/L)	9 (23.7)
	Normal levels	15 (39.5)
	N/A	14 (36.8)
Serum protein electrophoresis, n (%)	Monoclonal	5 (84.2)
	Polyclonal	1 (2.6)
	N/A	32 (84.2)

ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; N/A, not available

Immunophenotyping results from thirty-eight subjects were shown in **Table 2**. Cells from all cases were positive with CD19, and the majority were CD5, CD20, CD23, and kappa positive. Lambda were positive in a minority of cases. Four cases were CD5-negative.

Table 2. Membrane markers of CLL/PLL cases

Marker	Number of cases		% Positive
	Tested	Positive	
CD5	31	27	87.1%
CD19	33	33	100%
CD20	36	34	94.4%
CD23	21	17	81%
Kappa	15	11	73.3%
Lambda	9	3	3.3%

For NCI-WG 1996, 37 patients fulfilled criteria of absolute lymphocytosis > 5x10^9/L in the peripheral blood, 27 patients fulfilled immunophenotyping criteria (lymphocytes were positive for CD5 and > 1 B-cell marker), 36 patients fulfilled atypical cells (prolymphocytes) < 55%, and 35 patients fulfilled bone marrow lymphocytes > 30% (**Figure 1**). Using the immunophenotyping criteria, a total of eleven patients that did not fulfill the criteria. However, it should be noted that 7 out of 11 patients did not have complete immunophenotyping examinations.

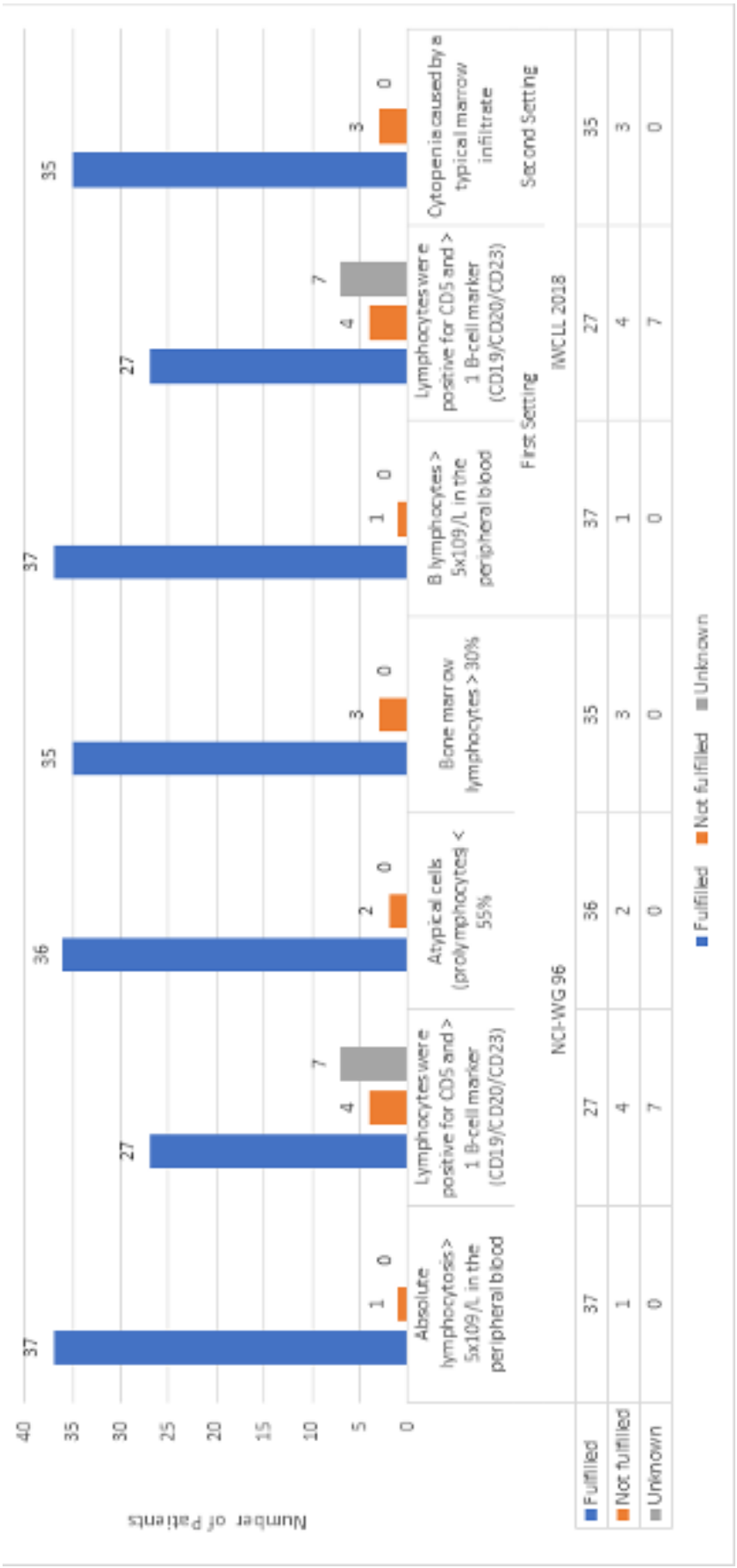

Figure 1. Number of Patients that Fulfilled NCI-WG 96 Guideline and iwCLL 2018 Diagnostic Criteria.

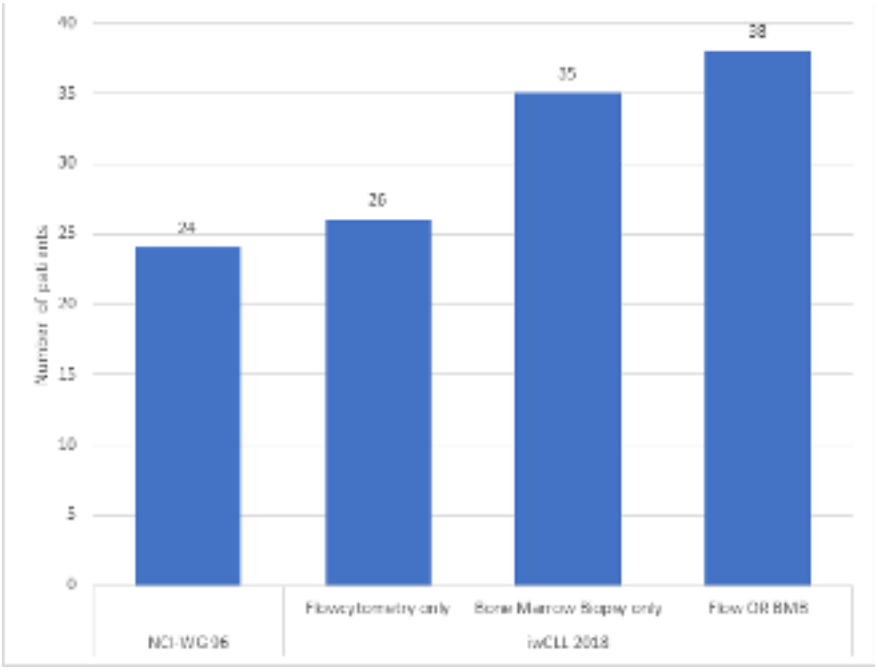

Figure 2. Number of patients categorized as CLL based on NCI-WG 96 guideline, iWCLL 2018 guideline flowcytometric criteria, bone marrow biopsy criteria, and flowcytometry OR bone marrow biopsy. Flow: flowcytometry; BMB: bone marrow biopsy.

Based on NCI-WG96 guideline, only twenty-four patients (63.2%) fulfilled all four criteria and thus were diagnosed as CLL (**Figure 2**). Similarly, using the iwCLL 2018 criteria without bone marrow biopsy, twenty-six patients (68%) were diagnosed as CLL. Using the iwCLL 2018 bone marrow biopsy part of the criteria only, there were thirty-five cases (92%) of CLL (**Figure 2**). Overall, using the bone marrow biopsy criteria of iwCLL 2018 yielded more CLL diagnosis than using all four NCI-WG96 criteria for CLL diagnosis or iwCLL flowcytometric criteria. If bone marrow biopsy in patient with cytopenia was taken into account in the patients who did not fulfill the flowcytometric criteria of iwCLL 2018, all patients (100%) can be confirmed as CLL.

DISCUSSION

In the present study, the median age of study population at diagnosis was 59.5 years and CLL cases were predominantly among males. This results is in accordance to prior studies.[13,14] Chronic illnesses, such as hypertension, gastrointestinal problem (dyspepsia or gastritis), and diabetes mellitus were the top three comorbid conditions in our patients. Although CLL incidence may be secondary to aging, chronic illnesses may be a contributing factors despite the mechanisms remaining unclear.[15–17]

According to CLL international data, CLL incidence rate (IRs) is highest in Western countries (e.g. North America, Europe), and the lowest in Asian countries.[18–20] Indeed, CLL incidence was varied by race as noted in a multicenter study by Dores et al that the IRs of CLL among whites, blacks, and Asian/pacific islander were 4.18, 3.01, and 0.84 cases per 100,000 people, respectively.[20] The variability of CLL incidence cases between different regions might be influenced by the variations in genetic background.[5] The sustained low IRs of CLL in Asians who have migrated to the United States support that genetic influence is greater than environmental factors.[21,22] This low IRs in Asians might explain why we had limited sample size. In addition, underdiagnosis and shortened life expectancy may contribute to the low incident cases. Since patients with early stage CLL are mostly asymptomatic, the diagnosis is often overlooked. Moreover, in Indonesia there is lack of infrastructure to diagnose CLL properly. Flowcytometry, which is essential to determine B-cell clonality, is only available in few large cities.

Call et al demonstrated that the majority of CLL incident cases diagnosed using the NCI-WG96 criteria were observed in the early stages: 60.9%, 33.9%, and 5.2% for Rai stage 0, I/II, and III/IV, respectively as it was mostly diagnosed in primary care settings.[23] Molica et al, Villavicencio et al, and Strati et al also provided similar results.[16,24,25] This is truly contrast to our data where most of CLL cases was diagnosed in the advanced-stage disease, either Binet C or Rai III-IV, because patients in Indonesia usually sought medical help when they developed symptoms (B-symptoms, lymphadenopathy, marrow failure); and only a minority of the patients were diagnosed through incidental finding of an ALC above the defined threshold. Apparently, most of the cases were diagnosed in tertiary care setting because of the limited availability of flow cytometry immunophenotyping.

The typical immunophenotypic feature of B-cell clone in CLL is the co-expression of CD5, CD19, CD20, and CD23. However, some cases of CLL have CD5-negative B-cell clone. Its incidence varies from 7% to 20%.[26,27] In our study, the incidence of CD5-negative CLL was 10.5% (four in 38 cases) and all of them had splenomegaly. Similarly, several studies reported a higher incidence of splenomegaly in CD5-negative CLL compared to CD5-positive CLL cases. Furthermore, some studies pointed out that CD5-negative CLL patients had a more advanced stage of disease and shorter survival.[26,28,29] In the present study, all those four patients was in Binet C and Rai III.

One patient in the present study had the ALC of $2x10^9$/L and no organomegaly, but presented with B-symptoms, anemia, typical bone marrow infiltrate, and immunophenotyping of CLL. In other cases, the diagnosis of CLL in CD5-negative cases was made according to clinical and morphological features. Accordingly, these patients met the iwCLL 2018 bone marrow criteria for CLL which only requires the presence of cytopenia caused by typical bone marrow infiltrate, regardless of B-cell count or nodal involvement.[9] The disadvantage of the immunophenotyping test is the cost and it is not widely available in Indonesia. In addition, very few pathologists have completed the flowcytometry interpretation training. The expensive testing cost pose another problem since it is not covered by the National Health Insurance. Hence, the use of immunophenotyping as stated in the flowcytometric criteria of iwCLL 2018 is not applicable in Indonesia. Based on our data, more than 90% of the patients can be diagnosed as CLL by bone marrow biopsy alone. Meanwhile, only 63.2%% of the patients fulfilled all the NCI-WG96 criteria and 68% of patients met the iwCLL flowcytometry without bone marrow biopsy) criteria. These findings showed that in our CLL patients, bone marrow biopsy alone is more sensitive to diagnose CLL, even without flowcytometry data.

This study depicted another insight into the iwCLL 2018 CLL diagnostic criteria. In the absence of flowcytometric immunophenotyping, bone marrow trephine biopsy showing marrow infiltrate can be utilized to diagnose CLL. In contrast to flowcytometric immunophenotyping, trephine biopsy is reimbursable by the national health insurance. The procedure can be performed in remote areas with limited resources, with the pathologic review done in large centers using the preserved samples sent in 10% formalin preservatives. In our study, only three patients (8%) did not fulfill the iwCLL 2018 bone marrow criteria for CLL. However, all three patients were confirmed for CLL by flowcytometry. It can be proposed that bone marrow biopsy is mandatory in diagnosis of CLL, especially in limited settings where flowcytometry is not available.

Our study is subject to several limitations. This study was a single center study which only had small number of subjects. Race or ethnicity was not gathered in our study as it might influence the incidence of CLL. Additionally, immunophenotypic analysis was not available for all patients. Immunophenotyping test is still limited and only available in several urban areas across Indonesia. Moreover, as CLL is usually diagnosed incidentally and patients present with minimal signs and symptoms, thus financial barriers to accessing primary health care providers or specialists or laboratory tests may hamper the detection rate of CLL. Further studies are needed to confirm the findings of this study,

especially with bigger sample size and emphasis on diagnostic agreement.

Another drawback of the bone marrow trephine biopsy is that this procedure is not routinely performed in diagnosis of CLL in all centers in Indonesia. This study showed that bone marrow biopsy in addition to bone marrow aspirate is the best available modality to diagnose CLL in Indonesia when flowcytometry is not available.

CONCLUSION

The iwCLL 2018 criteria involving bone marrow biopsy in the presence of cytopenia were more applicable to establishing CLL diagnosis in Indonesia. Also, it may be applied to other countries with limited access to immunophenotyping tests.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY DATA

The 1996 National Cancer Institute-sponsored Working Group guidelines (NCI-WG 96) Guideline

No	Criteria
1	Absolute lymphocytosis > $5x10^9$/L in the peripheral blood
2	Lymphocytes were positive for CD5 and ≥ 1 B-cell marker (CD19/CD20/CD23)
3	Atypical cells (prolymphocytes) < 55%
4	Bone marrow lymphocytes ≥ 30%.

The 2018 International Workshop on chronic lymphocytic leukemia guidelines (IWCLL 2018) Guideline

B lymphocytes > $5x10^9$/L in the peripheral blood	AND	CLL cells were positive for CD5 and B-cell antigens (CD19/CD20/CD23)
OR		
Presence of cytopenia caused by a typical marrow infiltrate regardless of the number of peripheral blood B lymphocytes or of the lymph node involvement		

Antiviral Treatment in COVID-19 Outpatients: A Systematic Review of Randomized Controlled Trials

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ABSTRACTS

Background: *Most COVID-19 patients have mild or moderate illnesses that can progress to severe illness, leading to hospitalization and/or mortality. The use of antivirals to prevent the progression of COVID-19 in non-hospitalized patients shows conflicting result and efficacy remain unclear. This study evaluates the efficacy and safety of antivirals therapy in COVID-19 outpatients.* ***Methods:*** *Search were conducted in Pubmed, ScienceDirect, Cochrane Library, Springer, medRxiv, Journal Storage [JSTOR], and Directory of Open Access Journals [DOAJ] for articles investigating antivirals in COVID-19 outpatients. In addition, clinical and virological outcomes, COVID-19 hospitalization, all caused mortality, and adverse events were assessed.* ***Results:*** *Thirteen studies were included in this review. The consecutive data from these studies suggested that favipiravir is more optimally used in early disease, but improvement in symptoms shows inconsistent results. Meanwhile, molnupiravir shows consistent results, which can reduce hospitalization and mortality risk. In addition, remdesivir and nirmatrelvir-ritonavir have the potential to prevent the progression of COVID-19 in outpatients, but the data provided in this study are very limited. Finally, there is no significant difference in serious and non-serious adverse events, highlighting that antivirals have a good safety profile.* ***Conclusion:*** *This study provides an overview of the role of various antivirals therapy in COVID-19 outpatients. Molnupiravir, remdesivir, and nirmatrelvir-ritonavir have shown potential to prevent the progression of COVID-19 in early disease. However, this review was based on very limited data. Therefore, further clinical trials are needed to confirm this finding.*

Keywords*: COVID-19, SARS-COV-2, antiviral, outpatients, systematic review.*

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization on March 11th, 2020.[1] As of March 2nd, 2022, there were 437 million confirmed cases and 5.9 million deaths were caused by COVID-19 worldwide.[2] The clinical manifestation of COVID-19 can range from asymptomatic status, acute respiratory disease, and pneumonia, to acute respiratory distress syndrome. One of the factors of disease progression of COVID-19 are comorbidities such as chronic hypertension, organ damage, and coagulation dysfunction.[3] Currently, therapy is used based on the severity of COVID-19. In hospitalized COVID-19, corticosteroids and antivirals are recommended

for severe COVID-19. In addition, the majority of the patients were classified as mild or moderate illnesses with some of them progressing into severe illness and needing hospitalization.[4] Because of that, prevention of illness progression in an outpatient setting is important to decrease the risk of death and healthcare workload.

The choice of treatment for outpatient COVID-19 patients is still a matter of debate. Neutralizing antibody exhibits a significant antiviral effect when administered early in COVID-19 outpatients. However, the presence of the SARS-CoV-2 variant may escape the neutralizing antibody response.[5] Even so, antivirals are one of the treatment options in COVID-19 outpatients since they are not affected by spike-protein variants. Several antivirals have been used in clinical trials by COVID-19 outpatients, including remdesivir, favipiravir, tenofovir disoproxil fumarate, and molnupiravir which are antivirals groups that inhibit RNA synthesis.[6,7] The active form of these drugs will act on the RdRp enzyme and can interfere with the transcription process. RdRp is an enzyme that works on the viral genome (+gRNA) and will form a complementary strand (-gRNA) through the transcription process, so it will be able to kill the virus via chain termination and mutagenesis.[8] In addition, protease inhibitors such as nirmatrelvir, lopinavir, and ritonavir can inhibit the translation of polypeptides into protein components by inhibiting 3-chymotrypsin-like protease (3CLpro). This enzyme plays a role in the viral life cycle by breaking down polyproteins (PP1A and PP1AB) into functional viral proteins.[6,9,10] Then there is umifenovir, a drug with a mechanism of action targeting spike protein, angiotensin-converting enzyme 2 (ACE2), and inhibiting viral envelope membrane fusion[11]. Moreover, there are sofosbuvir and daclatasvir which are NS5B polymerase inhibitors and NS5A inhibitors that can inhibit the viral replication process.[12]

Studies on the use of antivirals in COVID-19 outpatients are still scarce. According to its capability, antivirals can potentially prevent worsening of clinical manifestation especially when given earlier in the disease manifestation. Several randomized controlled trials (RCTs) on antiviral therapy in COVID-19 outpatients have recently been published and produced conflicting results. Therefore, in this systematic review, we aim to comprehensively evaluate the efficacy and safety of antivirals therapy in COVID-19 outpatients. The parameters of efficacy were assessed based on clinical outcomes such as WHO average score, time to alleviation of symptoms, and COVID-19 related symptoms. Meanwhile, safety is assessed from non-serious adverse event and a serious adverse event. Non-serious adverse events is defined as any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of an investigational product serious adverse events are defined as events that, at any dose, result in the following: death, life-threatening, in-patient hospitalization or prolongation of existing hospitalization, and persistent or significant disability.

METHODS

This systematic review and meta-analysis are written based on the 2020 guideline for Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA),[13] and registered in the database for PROSPERO (CRD42022313970).

Eligibility Criteria

This study used randomized controlled trials (RCTs) as the required type of study. Two authors (DSB and PO) scanned through the titles and abstracts for each journal based on the eligibility criteria as follows: (1) COVID-19 outpatients; (2) studies involving antiviral therapy; (3) reported at least one of the outcomes of interest (4) English language literature. The primary outcomes included clinical recovery, the need for hospitalization, and adverse events with the secondary outcomes being laboratory outcomes. Reviewed articles, non-human studies, irrelevant articles, and duplicates are excluded.

Search Strategy and Selection of Studies

Two authors (PO and FA) have been conducting keyword searches on September 10th, 2021 for related materials published in databases (Pubmed, ScienceDirect, Cochrane Library, Springer, Journal Storage [JSTOR], and Directory of Open Access Journals

[DOAJ]). The following keywords were used: "((Covid) OR (SARS-COV-2)) AND ((Antiviral) OR (Remdesivir) OR (Molnupiravir) OR (Favipiravir) OR (Nirmatrelvir)) AND ((Outpatient) OR (Non-hospitalized))". We also performed manual searches, extended from September 11th, 2021 to March 10th, 2022. Additional details about the search strategy can be found in ***Supplementary Materials***. Titles and abstracts were screened individually from every article gathered until this point to identify potentially eligible studies, to then having full text screening. Any disagreements between these two authors were resolved by discussion with all authors until consensus was reached.

Data Extraction

Relevant data were independently extracted using a structured and standardized format from each study selected by two authors (DSB and PO). The following information was extracted: first author name and year of publication, study design, country of origin, sample size, patient age, disease severity, antivirals dose and duration, combination therapy and outcomes (clinical outcome, laboratory outcome, and adverse events).

Quality Assessment

The methodological quality of each study was assessed independently by two authors (DSB and PO) using the Cochrane Risk of Bias Tool for Randomized Trials (RoB ver.2).[14] Studies were classified as "low risk of bias", "some concerns" or "high risk of bias".

Statistical Analysis

Considering the important differences in the comparison of each study and various outcome measures, we could not generate meta-analyses of the included studies; instead, we narratively synthesized the evidence.

RESULTS

Study Selection

From the database and manual research, we acquired 5946 and 125 records, respectively. After a screening process of titles and abstracts, 36 potentially eligible articles were selected for review. After a full-text assessment, 13 studies were included for a systematic review. The study selection process is summarized in the PRISMA flow chart **(Figure 1).**

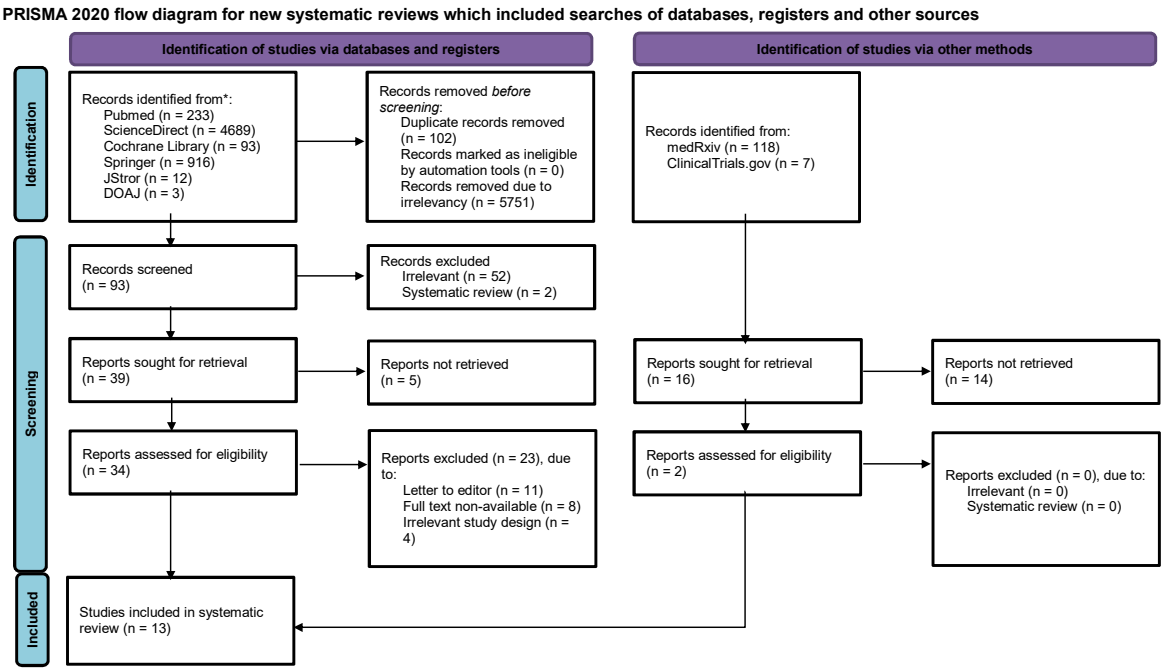

Figure 1. PRISMA flow chart.

Quality Assessment

Ten RCTs[15–24] were considered to be low-risk of bias studies and three RCTs[25–27] have some concerns according to Cochrane's Risk of Bias 2 (RoB2) assessment. In addition, details of the quality of assessment are summarized in ***Supplementary Materials*. (Table S2)**

Study Characteristics

Thirteen studies were found with a total of 3078 COVID-19 outpatients belonging to the antivirals therapy group and 2839 patients belonging to the placebo or standard therapy as a control group. In this review, all studies are RCTs conducted in the United States, France, Iran, and multiple countries, including several centers in various countries. In this review, there are several antivirals used including favipiravir,[15–17,25] molnupiravir,[18,19,26] remdesivir,[20] tenofovir disoproxil fumarate,[27] nirmatrelvir-ritonavir,[21] lopinavir-ritonavir,[22] umifenovir,[23] sofosbuvir-daclatasvir.[24] Meanwhile, standard therapy consisted of hydroxychloroquine, corticosteroid, antibiotics (such as azithromycin), and vitamin supplements.[23–26] In clinical outcomes, several criteria are used, such as WHO average score, time to alleviation of symptoms, and COVID-19 related symptoms. The eight-category ordinal scale defined by WHO consists of the following categories: no clinical or virological evidence of infection (score = 0), no limitation of activities (score = 1), limitation of activities (score = 2), hospitalized, no oxygen therapy (score = 3), oxygen by mask or nasal prongs (score = 4), non-invasive ventilation or high flow oxygen (score = 5), intubation and mechanical ventilation (score = 6), ventilation support, PRC, ECMO (score = 7), and death (score = 8).[23] The characteristics and outcomes summary for each study is presented in **Table 1** and **Table 2.**

Patients Characteristics

The mean patient age was 45 ± 10 years. Regarding disease severity, 61.6% of the outpatients were mild, and 38.4% were moderate.[15–27] Meanwhile, 6 studies consisted of a high-risk population that had comorbidities such as age >60 years old; active cancer; chronic kidney disease; chronic obstructive pulmonary disease; pulmonary hypertension; obesity; severe heart conditions; diabetes mellitus; history of transplantation; immunocompromised status due to disease or medication.[16,18–22] While 7 studies consisted of low-risk populations in which comorbid factors were excluded.[15,17,23–27] In addition, 6 studies are reporting on the vaccination status of which 4 studies used the unvaccinated population,[16,18,19,21] while 1 study used the vaccinated population where at least 1 dose of vaccine was used,[20] and 1 study used both vaccinated and unvaccinated populations.[17]

Clinical Outcomes

Seven studies report clinical outcomes with different parameters, such as time to alleviation of symptoms, WHO average score, and COVID-19 related symptoms.[15,16,20,23–25,27] The use of favipiravir reported no significant difference in median time to alleviation of symptoms between favipiravir versus placebo in the study conducted by Bosaeed et al., 2022 (7 days [IQR: 4-11] vs 7 days [IQR: 5-10]], p=0.51),[15] and Holubar et al., 2021 (15 days [IQR: 12-26] vs. 14 days [IQR: 11-18], p=0.43).[16] Meanwhile, Ruzhentsova et al., 2021 reported significant results regarding the median time to alleviation of symptoms between favipiravir compared with standard therapy (6.0 days [IQR: 4.0-12] vs 14 days [IQR: 5.0-12], p=0.019).[25] The remdesivir as an intervention of antivirals therapy reported an alleviation of symptoms on day 14 between the remdesivir group versus the placebo group of 23/66 patients (34.8%) vs 15/60 (25.0%), rate ratio of 1.41; 95% CI 0.73 to 2.69.[20] Meanwhile, the combined use of tenofovir disproxil fumarate plus emtricitabine did not show a greater improvement in COVID-19 symptoms compared to standard therapy (6/30 (20%) vs 3/30 (10%), p=0.29).[27] Meanwhile, the use of sofosbuvir plus daclatasvir also did not show significant results in terms of reducing the symptoms of COVID-19 on day 5 compared to standard therapy (12/27 patients (44%) vs. 12/28 (43%), p= 1.00).[24] In addition, umifenovir showed a difference in the mean WHO score compared to placebo in the Mild-asymptomatic group on day 5 (0.45 ± 0.11 vs. 0.88 ± 0.13, p= 0.019). These results contrast the moderate population where umifenovir compared with

Table 1. Characteristics of the included studies.

References	Study design	Country	Sample size		Age, years Mean ± SD/Median (IQR)		Disease severity	Dosage and administration	
			Intervention (n)	Control (n)	Intervention	Control		Intervention	Control
Bosaeed et al., 2022[15]	RCT	Saudi Arabia	112	119	37 [31.5-45]	36 [32-44]	Low risk, non-hospitalised, mild illness	Oral favipiravir 1800mg twice daily on day 1 followed by 800mg twice daily for a total duration of 5 to 7 days therapy	Placebo
Holubar et al., 2021[16]	RCT	United States	75	74	42.5 ± 12	42.8 ± 12	Unvaccinated, low risk non-hospitalised, mild illness	Oral favipiravir 1800mg twice daily on day 1 followed by 800mg twice daily for a total duration of 10 days therapy	Placebo
Lowe et al., 2022[17]	RCT	United Kingdom	180 (favipiravir+lopinavir-ritonavir= 61 Favipiravir =59 Lopinavir-ritonavir= 60)	60	Favipiravir++lopinavir-ritonavir= 40.3 ±13.1 favipiravir= 40.3 ±12.1 lopinavir-ritonavir= 38.6 ±11.5	40.6 ±12.2	Both vaccinated and unvaccinated, low risk, non-hospitalised, mild illness	Oral favipiravir 800 mg twice daily on Day 1, followed by 400 mg four times daily from day 2 to day 7 Oral lopinavir-ritonavir 400mg/100 mg twice daily on day 1, followed by 200mg/50mg four times daily from day 2 to day 7 Oral favipiravir (1800mg twice daily on day 1 followed by 400mg four times daily on days 2-7) PLUS lopinavir-ritonavir (400mg/100mg twice daily on day 1, followed by 200mg/50mg four times daily on days 2-7)	Placebo
Bernal et al., 2021[18]	RCT	Multiple countries	716	717	44 ± 15	45 ± 15	Unvaccinated adult, High risk non-hospitalised, mild to moderate illness	Oral molnupiravir 800 mg for 5 days twice daily	Placebo

Fischer et al., 2022[19]	RCT	United States	140 : 23=200mg 62=400mg 55=800mg	62	200 mg: 34.76 ± 11.92 400 mg: 43.73 ± 13.55 800 mg: 42.17 ± 10.97	Median age (range): 39 (19-71) 39.92 ± 11,18	Unvaccinated adult, high risk non-hospitalised, mild to moderate illness	Oral molnupiravir 200 mg, 400 mg, and 800 mg for 5 days twice daily	Placebo
Gottlieb et al, 2022[20]	RCT	United States, Spain, Denmark, United Kingdom	279	283	50 ± 15	51 ± 15	Vaccinated, High-risk non-hospitalized, mild to moderate illness	IV remdesivir (200 mg on day 1, 100 mg on days 2 and 3)	Placebo
Hammond et al., 2022[21]	RCT	Multiple countries	1120	1126	45 ± 10	46 ± 10	Unvaccinated adult, High risk, non-hospitalised, mild to moderate illness	Oral nirmatrelvir (300 mg) + Ritonavir (100 mg) for 5 days twice daily	Placebo
Reis et al., 2021[22]	RCT	Brazil	244	227	54 [18-94]	53 [18-80]	high-risk, non-hospitalized, mild to moderate illness	Oral lopinavir-ritonavir loading dose of 800 mg and 200 mg, respectively, every 12 hours in day 1, followed by 400 mg and 100 mg, respectively, every 12 hours for the next 9 days	Placebo
Ramachandran et al., 2022[23]	RCT	India	60	63	46.08 ± 1.93	47.35 ± 1.96	Low risk, non-hospitalised, mild to moderate illness	Oral umifenovir 800 mg for 14 days twice daily + Standard of care	Standard of care
Roozbeh et al., 2020[24]	RCT	Iran	27	28	43.3 ± 3.7	46.8 ± 3.9	low risk non hospitalized, mild to moderate illness	Oral sofosbuvir (400 mg) + daclatasvir (60 mg) + hydroxychloroquine (200 mg) for 7 days twice daily	Standard therapy
Ruzhentsova et al.2021[25]	RCT	Russia	83	44	41,7 ± 10,6	42,0 ± 10,4	low risk non hospitalized, mild to moderate illness	Oral favipiravir loading dose 1800 mg BID on day 1, followed by 800 mg BID on days 2-10	Standard of care

Khoo et al., 2021[26]	RCT	United Kingdom	12 Molnupiravir (300 mg, N=4; 600mg, N=4; 800 mg, N=4)	6	300 mg: 56.0[51.0-80] 600 mg: 43 [22-60] 800 mg: 39[25-63]	Median age of Standard of care (range): 59.0 (22.0-63.0)	Non hospitalized, asymptomatic, mild illness	Oral molnupiravir 300 mg, 600 mg, and 800 mg twice daily	Standard of care
Parienti et al.,2021[27]	RCT	France	30	30	39.9 ± 14.8	42.6 ± 16.7	low risk non hospitalized, mild to moderate illness	Oral single tablet of 245 mg tenofovir disoproxil fumarate + 200 mg emtricitabine (2 tablets on day 1, 1 tablet on days 2-7)	Standard of care

SD, standard deviation; IV, intravenous; NA, not available

Table 2. Data extraction for each individual studies.

Reference	Clinical outcomes		Covid-19 related hospitalization N (%)		Mortality N (%)		Laboratory parameters (Mean ± SD / Median)		Adverse events N (%)	
	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control
Bosaeed et al., 2022[15]	Time to Alleviation of Symptoms (Median) : 7 days (IQR: 4-11)	Time to Alleviation of Symptoms (Median) : 7 days (IQR: 5-10)	6/112 (5.3%)	2/119 (1.6%)	0/112 (0%)	0/119 (0%)	Viral clearance within 15 days: 42/112 (37.5%)	Viral clearance within 15 days: 49/119 (41.1%)	Any AE: 8/112 (7.1%)	Any AE: 7/119 (5.8%)
Holubar et al., 2021[16]	Time to Alleviation of Symptoms (Median) : 15 [12-26]	Time to Alleviation of Symptoms (Median) : 14 [11-18]	0/75 (0%)	4/74 (5%)	0/75	0/74	Viral clearance at day 7: 10/42 (24%)	Viral clearance at day 7: 10/47 (21%)	Any AE: 19/75 (25.3) Serious AE: 0/75	Any AE: 10/74 (13.5) Serious AE: 1/74
Lowe et al., 2022[17]	NA	NA	NA	NA	0	0	Viral clearance at day 5 favipiravir: 25 (46.3%)* lopinavir-ritonavir: 17 (30.4%) favipiravir + lopinavir-ritonavir: 20 (35.7%)	Viral clearance at day 5 : 14 (26.9%)	Any AE: favipiravir: 38 (64.4) lopinavir-ritonavir: 59 (98.3)* favipiravir + lopinavir-ritonavir: 55 (90.1)	Any AE: 39 (65.0)
Bernal et al., 2021[18]	NA	NA	28/385 (7.3%)	53/377 (14.1%)	1 (0.1%)*	9 (1.3%)	Change of viral load: at days 3= -1.08±1.287* at days 5= -2.09 ±1.49*	Change of viral load: at days 3= -0.84±1.258 at days 5= -1.79±1.513	Any AE= 216 (30.4%) Serious AE=49 (6.9%)	Any AE= 231 (33.0%) Serious AE= 67 (9.6%)

Fischer et al., 2022[19]	NA	NA	NA	NA	NA	NA	Change of viral load: day 5 200 mg: -1.471 (0.212) 400 mg: -1.754 (0.128)* 800 mg: -1.867 (0.126)* Positive Covid-19 at day 5= 200mg:1/22(4.5) 400mg: 0/42* 800mg: 0/53*	Change of viral load: Day 5: -1.320 (0.150) Positive Covid-19 at day 5= 6/54(11.1)	Any AE: Pooled: Any AE of 200 mg = 11 (47.8) Any AE of 400 mg = 20 (32.3) Any AE of 800 mg = 11 (20.0) Any Serious AE of 200 mg = 0 Any Serious AE of 400 mg = 2 (3.2) Any Serious AE of 800 mg = 1 (1.8)	Any AE = 18 (29.0) Any Serious AE = 1 (1.6)
Gottlieb et al, 2022[20]	FLU-PRO Plus questionnaire: 61/169 (36.1%) reported improvement in symptoms on day 14	FLU-PRO Plus questionnaire: 33/165 (20.0%) reported improvement in symptoms on day 14	2/279 (0.7%)*	15/283 (5.3%)	0/279 (0%)	0/283 (0%)	Change of viral load at day 7: 6.31±1.75 to 4.11±1.36 = −1.24 log10 copies per milliliter	Change of viral load at day 7: 6.28±1.79 to 4.06±1.19 = −1.14 log10 copies per milliliter	Any AE: 118 (42.3%) Serious AE: 5 (1.8)	Any AE: 131 (46.3%) Serious AE: 19 (6.7)
Hammond et al., 2022[21]	NA	NA	8/1039 (0.77%)*	65/1046 (6.31%)	0/1039 (0%)*	12/1046 (1.15%)	NA	NA	Any AE = 251 (22.6%) Serious AE = 18 (1.6%)	Any AE= 266(23.9%) Serious AE= 74(6.6%)
Reis et al., 2021[22]	NA	NA	14/244 (5.7%)	11/227 (4.8%)	2/244 (0.8%)	1/227 (0.4%)	viral clearance: 125/201 (62.2%)	viral clearance: 112/195 (57.4%)	Serious AE: 20/232 (8.6%)	Serious AE: 12/220 (5.5%)
Ramachandran et al., 2022[23]	Average WHO scores for Mild-asymptomatic group at day 5: 0.45 ± 0.11* for Moderate group at day 5: 1.60 ± 0.32	Average WHO scores for Mild-asymptomatic group at day 5: 0.88 ± 0.13 for Moderate group at day 5: 1.95 ± 0.32	NA	NA	0 (0)	0 (0)	Viral clearance of mild-asymptomatic patients at day 5: 29/40(73%)*	Viral clearance of mild-asymptomatic patients at day 5: 17/42 (40%)	Any AE = 7 (11.7%)	Any AE = 7 (11.1%)

Roozbeh et al., 2020[24]	COVID-19 related symptoms: day 5 = 12/27 (44%) day 7= 7/27 (26%)	COVID-19 related symptoms: day 5 = 12/28 (43%) day 7= 7/28 (28%)	1 (4%)	4 (14%)	NA	NA	NA	NA	NA	NA
Ruzhentsova et al., 2021[25]	Time to Alleviation of Symptoms (Median) : 6.0 [4.0-12]*	Time to Alleviation of Symptoms (Mean ± SD / Median) : 14 [5.0-12]	3/112 (3.6%)	2/56 (4.5%)	NA	NA	Rate of viral clearance: day 3=80/112 (71.4%)* day5=91/112 (81.2%)*	Rate of viral clearance: day 3=32/56 (57.1%) day5=38/56 (67.9%)	Any AE: 80 (74.1%) Serious AE=2 (1.9%)	Any AE: 33 (60.0%) Serious AE: 0 (0%)
Khoo et al., 2021[26]	NA	NA	NA	NA	NA	NA	NA	NA	Any AE: 300 mg = 4 (100.0) 600 mg = 4 (100.0) 800 mg = 1 (25.0)	Any AE: 5 (83.3)
Parienti et al.,2021[27]	symptoms related to COVID-19 at day 7: 6/30 (20%)	symptoms related to COVID-19 at day 7: 3/30 (10%)	NA	NA	0 (0%)	0 (0%)	NA	NA	Serious AE: 2 (6%)	Serious AE: 1 (3%)

NR, not reported; SD, standard deviation; NA, not available; AE, adverse events* $p<0.05$ ** $p<0.001$

placebo did not show significant results (1.60 ± 0.32 vs. 1.95 ± 0.32, p = 0.281).[23]

COVID-19 Related Hospitalization

Eight studies reported hospitalization that was correlated with COVID-19.[15,16,18,20–22,24,25] Three RCTs using favipiravir conducted by Bosaeed et al., 2022, Holubar et al., 2021 and Ruzhentsova et al., 2021 have consistently shown that the favipiravir group did not reduce the risk of COVID-19 related hospitalization when compared to the control group (6/ 112 (5.3%) vs 2/119 (1.6%), p= 0.16), (0/75 (0%) vs 4/74 (5%), p= 0.06), (3/112 (3.6%) vs 2/56 (4.5%), p=0.494), respectively.[15,16,25] Meanwhile, lopinavir-ritonavir also did not show any difference in terms of hospitalization compared to placebo (14/244 (5.7%) vs 11/227 (4.8%, p>0.05).[22] Sofusbufir plus daclatasvir therapy reported that 1/27 (4%) patients needed hospitalization, which was not significantly different from the standard therapy group 4/28 (14%) (p=0.352).[24] Remdesivir showed a lower risk of COVID-19 related hospitalization by 87% in the remdesivir group compared to placebo group (hazard ratio, 0.13; 95% confidence interval [CI], 0.03 to 0.59; P = 0.008).[20] Meanwhile, the combination of nirmatrelvir plus ritonavir showed lower hospitalizations rate compared to placebo (8/1039 (0.77%) vs. 65/1046 (6.31%), p<0.001).[21] In addition, molnupiravir showed a lower mean hospitalized or death rate than placebo at day 29 (7.3% [28 of 385 participants vs 14.1% [53 of 377 participants, a treatment difference of 6.8 percentage points (95% confidence interval [CI], 11.3 to 2.4; P = 0.001).[18]

Mortality

Three studies are reporting all-cause mortality outcomes.[18,21,22] Bernal et al., 2022 reported one death in the molnupiravir group and nine deaths in the placebo group on day 29. The risk of all caused mortality was lower by 89% (95% CI, 14 to 99) with molnupiravir than with placebo.[18] Meanwhile, nirmatrelvir plus ritonavir showed lower mortality in that there were 0 out of 1039 participant deaths in the intervention group compared to 12 out of 1046 participant deaths in placebo (p < 0.001).[21] Meanwhile, Lopinavir-ritonavir did not show any significant difference when compared with placebo (2/244 (0.8%) vs. 1/227 (0.4%), p>0.05).[22]

Laboratory Parameters

Nine studies reported laboratory outcomes including rate and time of viral clearance, and change of viral load.[15–20,22–25] Giving favipiravir can increase the rate of viral clearance significantly compared to the standard therapy group on day three and day five (80/112 (71.4%) vs. 32/56 (57.1%), p=0.03) and (91/112 (81.2%) vs 38/56 (67.9%), p=0.022), respectively.[25] Meanwhile, on day 7, the rate of viral clearance did not show any difference between the favipiravir group compared to standard therapy (95 (84.8%) vs 46 (82.1%), p=0.296).[25] In addition, Bosaeed et al., 2022 reported that the rate of viral clearance at day 15 also showed no significant difference between recipient favipiravir versus placebo (42/112 (37.5%) vs. 49/119 (41.1%), p>0.05).[15] Meanwhile, giving favipiravir showed a significant viral clearance at day 5 compared to control group (25 (46.3%) vs. 14 (26.9%), p=0.03).[17] Next, Holubar et al., 2021 reported no significant viral clearance between the favipiravir group versus control group on day 7 (10/42 (24%) vs 10/47 (21%), p=0.80).[16] Administration of molnupiravir was associated with greater reductions from baseline in mean viral load than the control group on day 3 (-1.08±1.287 vs -0.84±1.258) and day 5 (-2.09 ±1.490 vs -1.79±1.513).[18] Furthermore, Fischer et al., 2022 reported that at 400 mg and 800 mg doses of molnupiravir, the least-squares mean viral load change from baseline was significantly greater at day 5 than in the placebo group, with differences of -0.434log10 copies/ml (p =0.030) and 0.547log10 copies/ml (p=0.006), respectively.[19] In addition, administration of 400 mg and 800 mg of molnupiravir significantly increased viral clearance at day five compared to placebo (0/42 (0.0) vs 6/54 (11.1), p= 0.034) and (0/53 (0.0)) vs. 6/54 (11.1), p=0.003).[19] Meanwhile, the administration of remdesivir showed no difference in the least-squares mean viral load change from baseline on day 7 compared to placebo administration, with differences (-1.24 log10 copies per milliliter vs -1.14 log10 copies per milliliter, p=0.07).[20]

Administrations of lopinavir plus ritonavir (OR, 1.04; 95% CI, 0.94-1.16) showed no difference in viral clearance compared to placebo.[22] In mild-asymptomatic patients receiving umifenovir showed greater viral clearance than standard therapy on day 5 (29/40(73%) vs 17/42 (40%), p=0.002).[23]

Adverse Events

Non-serious Adverse Events

Ten studies reported minor adverse events after receiving antiviral therapy.[15–21,23,25,26] In four studies using Favipiravir it was found that there was no significant difference between the favipiravir group compared to the control group (8/112 (7.1%) vs. 7/119 (5.8%), p>0.05);[15] (19/75 (25.3) vs 10/74 (13.5), p=0.11);[16] (38 (64.4) vs 39 (65.0), p>0.05);[17](80 (74.1%) vs 33 (60.0%), p>0.05).[25] The most common adverse events reported were dizziness and nausea.[16] Meanwhile, the three studies using molnupiravir also consistently reported no significant difference in the occurrence of minor adverse events (216 (30.4%) vs. 231 (33.0%), p>0.05).[18] The most common minor adverse events related to molnupiravir therapy include nausea, diarrhea, and dizziness.[18,19] Gottlieb et al., 2022 reported several minor adverse events occurring in 118/279 participants (42.3%) in the remdesivir group and 131/283 participants (46.3%) in the placebo. The most common minor adverse events were nausea, headache, and cough but the difference were not statistically significant (p>0.05).[20] The incidence of minor adverse events in the nirmatrelvir plus ritonavir group compared with placebo was not significant (= 251 (22.6%) vs 266 (23.9%), p>0.05), in detail the minor adverse events that occurred included dysgeusia, diarrhea, fibrin D-dimer increase, mild transaminitis, and headache.[21] In a study conducted by Ramachandran et al., 2022, it was found that umifenovir showed few minor adverse events such as nasal discharge, headache, and stomach ache which were distributed almost similar to the placebo group (p>0.05).[23]

Serious Adverse Events

Eight studies are reporting serious adverse events after receiving antiviral therapy.[16,18–22,25,27] Two favipiravir-related studies showed consistently insignificant results between the favipiravir group compared to controls in which the study conducted by Holubar et al., 2021 reported serious adverse events in the placebo group. In contrast, serious adverse events did not occur in the favipiravir group (p> 0.05).[16] In addition, a study conducted by Ruzhentsova et al., 2021 reported that 2 participants (1.9%) experienced serious adverse events, while in the controls group there were no serious adverse events (p>0.05).[25] Serious adverse events include bone fracture and a decrease in saturation[25]. Meanwhile, serious adverse events were also found in molnupiravir, Bernal et al., 2022 reported that there were at least 49 (6.9%) participants experiencing serious adverse events when compared to the control group with 67 (9.6%) participants experiencing serious adverse events, this number is less numerically, but in an insignificant manner (p>0.05).[18] In addition, Fischer et al., 2022 reported four serious adverse events requiring hospitalization. Two participants in the 400 mg molnupiravir experienced a cerebrovascular accident and the other experienced a decrease in oxygen saturation, while those in 800 mg molnupiravir experienced acute respiratory failure. Therefore, despite the treatment with molnupiravir, the worsening condition of COVID-19 was suspected to be the cause, considering that in the placebo group one participant experienced acute respiratory failure cause hypoxia that led to death 31 days after the onset of serious adverse events.[19]

Administration of remdesivir in COVID-19 outpatients reported some serious adverse events than placebo 5 of 279 participants (1.8%) vs. 19 of 283 participants (6.7%).[20] More serious adverse events were reported in the lopinavir-ritonavir group compared with placebo (20/232 (8.6%) vs 12/220 (5.5%).) In the tenofovir disoproxil fumarate plus emtricitabine, two (6%) participants experienced serious adverse events, while one (3%) participant experienced serious adverse events in the standard therapy group.[27] In detail, two serious adverse events in the tenofovir disoproxil fumarate plus emtricitabine experienced dyspnea (22 breaths/min), very high RT-PCT viral load (14 Ct), and inflammatory syndrome (CRP = 21 mg/L) and one other participant need hospitalization for severe

COVID-related pneumonia requiring high flow oxygen, which recovered without mechanical ventilation. One participant in the standard of care group experienced severe COVID-related pneumonia requiring oxygen (6 L/min) and recovered.[27]

DISCUSSION

Prevention of COVID-19 illness progression is an important topic to minimize mortality risk, and antivirals have the potential because apart from the therapeutic effect they are not affected by the SARS-CoV-2 spike protein mutation.[28] In this study, several antivirals as monotherapy or combination have gone through clinical trials in early disease COVID-19 outpatients, including favipiravir, molnupiravir, remdesivir, umifenovir, tenofovir disoproxil fumarate, nirmatrelvir plus ritonavir, lopinavir plus ritonavir, and sofosbuvir plus daclatasvir.

The use of favipiravir showed conflicting results in time to alleviation of symptoms in which two studies had insignificant results,[15,16] while one study was significant.[25] This could be influenced by the different baseline characteristics among the three RCTs, where insignificant results were found in patients with mild disease, while an acceleration of time to alleviate symptoms occurred in patients with moderate disease. In addition, different initiations of favipiravir may influence the outcome which in Bosaeed et al., 2022 was initiated in the first 5 days of the onset.[15] Meanwhile, Ruzhentsova et al., 2021 initiated favipiravir administration within 3-6 days.[25] In addition, the consistent administration of favipiravir increased the rate of viral clearance significantly compared to the standard therapy group on the third and fifth days. However, above the 7th day, there was no difference. This maybe correlated with negative RT-PCR results where the number of negative RT-PCRs on day 5 is significant compared to controls,[25] while on day 7 the results are insignificant.[16] However, favipiravir consistently does not reduce the risk of hospitalization in COVID-19 outpatients.[15,16,25] Meanwhile, an RCT conducted by Ruzhentsova et al., 2021 reported two serious adverse events on favipiravir administration, including bone fractures and decreased saturations, but these were not correlated with investigational drugs. The most common non-serious adverse events were dizziness and nausea.[25] Nevertheless, favipiravir has been used in various countries such as China, Hungary, India, Korea, Poland, Portugal, Russia, Serbia, Thailand, and Turkey.[29] In the previous study, favipiravir did not reduce mortality and mechanical ventilation in moderate-severe patients.[30] Meanwhile, when used in mild to moderate, favipiravir could promote viral clearance, which is in line with the results of this study.[31]

In contrast to favipiravir, administration of molnupiravir in COVID-19 outpatients has been shown to reduce the risk of being hospitalized or dead compared to placebo. The mortality risk was lower by 89% with molnupiravir therapy.[18] In addition, molnupiravir was associated with greater reductions from baseline in mean viral load than placebo on days 3 and 5,[18,19] which is accompanied by a decrease in COVID-19 patients.[19] The serious adverse event of molnupiravir was not significant compared to placebo.[18,19] Molnupiravir was well tolerated with no increase in treatment-related or serious adverse events. In addition, there is no evidence of hematological, renal, or hepatic toxicity related to molnupiravir.[19] These results are in line with the previous systematic review which stated that molnupiravir could reduce disease progression and reduce the risk of hospitalization and/or death.[8] At the same time, in the safety profile, we found that there were serious adverse events that occurred although they were not statistically significant. Currently, there is no evidence that reports a mechanical relationship related to the duration of use and dosage of molnupiravir on serious adverse events such as acute respiratory failure. This opens the topic of the importance of a longer-term investigation of the safety profile of molnupiravir after receiving prophylaxis, which is currently still in the process of recruiting participants (ClinicalTrials.gov identifier: NCT04939428).

Like molnupiravir, remdesivir, and tenofovir disoproxil fumarate target the RNA-dependent RNA-Polymerase (RdRp) enzyme used by the coronavirus for transcription and replication of its viral RNA genome.[32] Administration of remdesivir

in COVID-19 outpatients showed a lower risk of hospitalization than in the placebo group. However, there was no difference in least-squares mean viral load change from baseline between remdesivir and placebo. In terms of safety profile, remdesivir caused nausea, headache, and cough the most but was insignificant when compared to placebo and the remdesivir group had few serious adverse events compared to placebo.[20] Administration of 3 days of remdesivir has qualitatively similar efficacy compared to single-dose neutralizing monoclonal antibodies.[33–35] However, intravenous administration of remdesivir is the same as neutralizing antibodies, which is less efficient than other oral antivirals. In this study, tenofovir disoproxil fumarate plus emtricitabine did not significantly improve COVID-19 symptoms compared to standard therapy.[27] In a study conducted by Parienti et al., 2021, gastrointestinal symptoms caused by COVID-19 may resemble tenofovir disoproxil fumarate plus emtricitabine adverse events, so the assessment of clinical tolerance and clinical resolution of symptoms may be biased.

Several antiviral protease inhibitors were analyzed in this study, including nirmatrelvir, lopinavir, and ritonavir.[21,22] The combined use of lopinavir-ritonavir did not reduce the risk of hospitalization compared to placebo in COVID-19 outpatients.[22] In contrast, the nirmatrelvir-ritonavir combination showed a lower hospitalization rate than the placebo in COVID-19 outpatients.[21] In addition, the risk of mortality was also decreased with nirmatrelvir-ritonavir compared with placebo, whereas with lopinavir-ritonavir there was no difference in mortality risk.[21,22] Next, for the virological outcomes was not associated with viral clearance. The safety profile of nirmatrelvir-ritonavir showed fewer serious adverse events than the placebo group.[21] In this study, the combination of nirmatrelvir plus ritonavir had better efficacy and safety than lopinavir plus ritonavir. Important, nirmatrelvir-ritonavir uses the unvaccinated and high-risk population, which is the most important population to receive interventions to prevent the progression of COVID-19. Unlike protease inhibitors and RNA synthesis inhibitors, umifenovir-related RCTs and the combination of sofosbuvir plus daclatasvir are still very limited. However, prior RCTs using umifenovir in COVID-19 outpatients have shown improvement in WHO clinical score analysis and greater viral clearance at day 5 if given earlier in mild disease.[23] Meanwhile, the combination of sofosbuvir plus daclatasvir did not show any reduction in COVID-19 symptoms when compared to standard therapy.[24] However, due to the lack of studies related to umifenovir and the combination of sofosbuvir plus daclatasvir, other RCTs are needed to confirm these results.

Real-world populations tend to have confounders that are difficult to control. For instance, patients may receive different standard therapies which may influence the outcomes. Additionally, population of these studies are COVID-19 outpatient in which the severity criteria of the disease varies between each centers.[36] This could lead to differences in clinical outcome. Thus, the administration of standard therapy such us corticosteroids and hydroxychloroquine on top of the antiviral therapy could potentially obscure the effects of antivirals in COVID-19 outpatients, especially in viral clearance and COVID-19 related hospitalization endpoint. It is also important to note that the small sample size could affect the findings of this study.

To the best of our knowledge, this is the first systematic review investigating the efficacy and safety of various antivirals in COVID-19 outpatient. However, this systematic review has some limitations. First, this study mainly discusses Favipiravir and molnupiravir because most published RCTs are both favipiravir and molnupiravir associated studies, and existing studies on antivirals are scarce. Second, several RCTs have small samples which can undermine the result and cause failure in detecting slight differences. Third, some studies did not have comparable RCTs so results still need to be confirmed. Therefore, further studies are required to address the limitation of our systematic review.

CONCLUSION

Various antivirals show different therapeutic effects in COVID-19 outpatients. Favipiravir

has shown inconsistent results concerning the time of improvement in COVID-19 symptoms and is more optimal when used in early disease. Meanwhile, molnupiravir has shown consistent results, which can reduce the risk of hospitalization and mortality, this is supported by a decreased change of viral load compared to baseline. Remdesivir and the combination of antivirals nirmatrelvir-ritonavir may have potential because they can prevent the progression of COVID-19 in early disease. However, the conclusion remains inconclusive due to limited data and the number of studies related to remdesivir and nirmatrelvir-ritonavir combinations. The safety profile of antivirals is relatively safe where there are no greater serious adverse events than controls. Therefore, further studies are needed to confirm this finding.

AUTHORS' CONTRIBUTIONS STATEMENT

DSB developed conceptualization, data curation, methodology, visualization, writing - original draft, writing - review & editing. PO developed conceptualization, data curation, writing - original draft, writing - review & editing. TPA developed writing - review & editing, manuscript validation, and supervision, PL developed conceptualization, writing - review & editing, data analysis, manuscript validation, and supervision. FA and RI contributed to the writing review and editing, and visualization. TT, DF and NL developed data curation and methodology. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

We declare no competing interests.

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Usefulness of Combining NT-proBNP Level and Right Atrial Diameter for Simple and Early Noninvasive Detection of Pulmonary Hypertension Among Adult Patients with Atrial Septal Defect

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ABSTRACT

Background: *Atrial septal defect developed pulmonary hypertension (ASD-PH) at first diagnosis due to late presentation are common in Indonesia. Transthoracic echocardiogram (TTE) is a common tool to detect ASD-PH, before proceeding to invasive procedure. The NT-proBNP measurement to screen ASD-PH is not yet considered the standard approach, especially in limited resource conditions. The objective of this study is to assess the value of NT-proBNP, along with simple TTE parameter, to screen PH among adults with ASD.* ***Methods:*** *This was a cross-sectional study. The subjects were adult ASD-PH patients from the COHARD-PH registry (n=357). Right heart catheterization (RHC) was performed to diagnose PH. Blood sample was withdrawn during RHC for NT-proBNP measurement. The TTE was performed as standard procedure and its regular parameters were assessed, along with NT-proBNP, to detect PH.* ***Results:*** *Two parameters significantly predicted PH, namely NT-proBNP and right atrial (RA) diameter. The cut-off of NT-proBNP to detect PH was ≥140 pg/mL. The cut-off of RA diameter to detect PH was ≥46.0 mm. The combined values of NT-proBNP level ≥140 pg/mL and RA diameter ≥46.0 mm yielded 46.6% sensitivity, 91.8% specificity, 54.3% accuracy, 96.5% positive predictive value and 26.2% negative predictive value to detect PH, which were better than single value.* ***Conclusion:*** *NT-proBNP level ≥140 pg/mL represented PH in adult ASD patients. The NT-proBNP level ≥140 pg/mL and RA diameter ≥46.0 mm had a pre-test probability measures to triage patients needing more invasive procedure and also to determine when and if to start the PH-specific treatment.*

Keywords**:** *atrial septal defect, N-terminal pro-BNP, pulmonary arterial hypertension, diagnostic test.*

INTRODUCTION

In developed countries, registries of congenital heart disease (CHD) in adults associated with pulmonary hypertension (PH) are dominated by heart congenital systemic-to-pulmonary shunt defects.[1,2] In this context, its prevalence is much higher in developing countries due to PH complication as a late diagnosis and uncorrected systemic-to-pulmonary shunting.[3-5] Adult patients with undetected and delayed diagnosis of CHD seek medical advice mostly due to complaints related to PH such as dyspnea, easily fatigued, dizziness, presyncope and chest discomfort.[5,6] The most common CHD with overlooked diagnosis is atrial septal defect (ASD), which accounts for the most common uncorrected CHD in adults.[3,5,6]

In ASD patients, PH is a result of protracted hypercirculation and overloaded blood volume in the pulmonary circulation. Its consequences are represented by: endothelial dysfunction, pulmonary vascular remodeling, increased pulmonary artery pressure and augmented pulmonary vascular resistance.[7] The current guideline compels the utilization of right heart catheterization (RHC) to diagnose the hemodynamics of PH and guidance to confirm pulmonary artery hypertension (PAH), by directly measuring mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR) and pulmonary capillary wedge pressure (PCWP).[8] The precise values of mPAP ≥20 mmHg, PVR >3 Wood Units and PCWP ≤15 mmHg are determined for a confirmation of PAH diagnosis.[8,9] In ASD, the values of RHC are not only used for PAH diagnosis, but also for selecting patients suitable for defect closure as a definitive therapeutic choice which should have been done as early as possible in childhood.[6]

In Indonesia, due to their late presentation at cardiologist visit, the majority of adult patients with ASD have already developed PH at initial diagnosis.[5] Moreover, the number of hospitals which can provide RHC and heart defect correction are limited. The chest-X ray, electrocardiogram and transthoracic echocardiogram (TTE) are the most common tools to detect suspicion of PH due to ASD (ASD-PH), before proceeding to RHC. The current guideline on diagnosis and management of pulmonary hypertension by Indonesian Heart Association includes the TTE as a first diagnostic tool to detect PH and the use of biomarker, NT-proBNP, as a prognostic component of PAH. Although TTE measurements are widespread, the use of NT-proBNP as a diagnostic tool to help screen for ASD-PH is not yet considered a standard approach. Currently, in PAH patients the use of NT-proBNP is recommended only as prognostic value.[8,9] Therefore, the diagnostic role of NT-proBNP for ASD-PAH needs to be further investigated, especially in the settings with limited resources and scarce facilities for more advanced procedures, such as RHC and surgical closure. Consequently, we conducted a study to assess the sensitivity, specificity, accuracy and predictive value of circulating level of NT-proBNP in early and non invasive detection of the PH presence among adult patients with ASD.

METHODS

This was a cross-sectional design study. The variable tested for this study was the cut-off level of NT-proBNP for diagnostic power of the presence of PH in adults with ASD. We enrolled patients with diagnosis of PH already confirmed by RHC as a gold standard. The studied population was represented by adult patients in hospital with uncorrected ASD, who were mostly symptomatics.[5]

The subjects of this study were patients registered into the COngenital HeARt Diseases in adult and Pulmonary Hypertension (COHARD-PH) registry. The COHARD-PH registry is a single-center, observational, and prospective registry which enrolls adult patients with CHD and CHD-associated PH in Dr. Sardjito Hospital, Jogjakarta, Indonesia.[5] The subjects selected for this study fulfilled the inclusion and exclusion criteria as follows. Inclusion criteria were: (1) patients diagnosed with ASD, (2) patients evaluated by RHC, and (3) patients in whom NT-proBNP circulating levels was obtained. Exclusion criteria were: (1) patients who underwent a previous ASD closure procedure, (2) patients with other congenital defects or multiple septal defects, (3) patients with significant valve diseases other than tricuspid or pulmonal valves

regurgitation, (4) patients with component of post-capillary PH by RHC (i.e. mean left atrial pressure (mLAP) or PCWP >15 mmHg) and (5) the incomplete haemodynamic result of RHC.

The sample size estimation was determined from formula to calculate the accuracy index by receiver operating characteristics (ROC) curve.[10] The minimal sample size requirement was 114 for each case and control.[10]

All subjects signed an informed consent form as part of the inclusion in the COHARD-PH registry and its subsequent studies. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by Medical and Health Research Ethical Committee of Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada, Indonesia (Ref. no. KE/FK/0738/EC/2020 and KE/FK/1189/EC/2021).

Data Collection

The demographics, clinical characteristics, laboratory data and echocardiograms were retrieved from the COHARD-PH registry database. The data were collected from the index of ASD diagnosis, i.e. during TTE and transoesophageal echocardiography (TOE) examinations (G.E Vivid 7 (G.E Healthcare, U.S.A), G.E Vivid S6 (G.E Healthcare, U.S.A) or Phillips HD 15 (Philips N.V, The Netherlands)). The measurement of defect diameter, right atrial (RA) diameter (minor dimension), right ventricle (RV) diameter (maximal minor dimension), tricuspid annular plane systolic excursion (TAPSE) and left ventricle ejection fraction (LVEF) were performed based on standard procedures.[11] The image acquisitions were conducted by experienced sonographers. The validation and confirmation of TTE and TOE results were performed by cardiologist consultants as described previously.[5,12]

The results of RHC data were retrieved from the COHARD-PH registry database. During RHC, the hemodynamic measurements and calculations were determined by indirect Fick methods, as previously described.[5] The PH diagnosis in this study was determined as mPAP ≥20 mmHg and PCWP or mLAP ≤15 mmHg by RHC, at any calculated PVR index.

The blood sample was collected from each patient during RHC by venipuncture from peripheral veins (for hemoglobin and hematocrit measurement by hemocytometer) and from inferior vena cava (for NT-proBNP measurement by electrochemiluminescence immunoassay (ElecsysProBNP II) and a Cobas e immunoassay analyzer (Roche Diagnostics, Germany). These measurements were performed in our hospital's central laboratory.[5]

Statistical Analysis

The presentation of numerical data was in mean and standard deviation (SD) (for normal distribution of numerical data) or median and interquartile range (IQR) (for non-normal distribution of numerical data). The Kolmogorov-Smirnov test was applied as determination of normal and non-normal numerical data distribution. The presentation of categorical data was in percentage. The Student T test or Mann-Whitney test was used to compare numerical data. The chi-squared test was used to compare categorical data. Univariate and multivariable analyses were performed with logistic regression tests to analyze the independent predictors for PH. A correlation test was performed with either Pearson (for normal distribution of numerical data) or Spearman test (for non-normal distribution of numerical data). An ROC curve was constructed to analyze the area under the curve (AUC) and to determine the best cut-off point for accuracy of the diagnostic test for PH. The diagnostic tests were performed using the determined cut-off value. A p value <0.05 was considered statistically significant.

RESULTS

From July 2012 until December 2020, 910 consecutive adult patients with ASD included in the COHARD-PH registry were enrolled into the study. Among them, 620 had undergone RHC procedure. As many as 436 patients had NT-proBNP measurement during the RHC procedure. After being selected based on inclusion and exclusion criteria, 357 subjects were considered eligible to be included in this study. Seventy-nine subjects were excluded due to incomplete RHC results. **Figure 1** showed the flow-chart of subjects' enrollment and selection

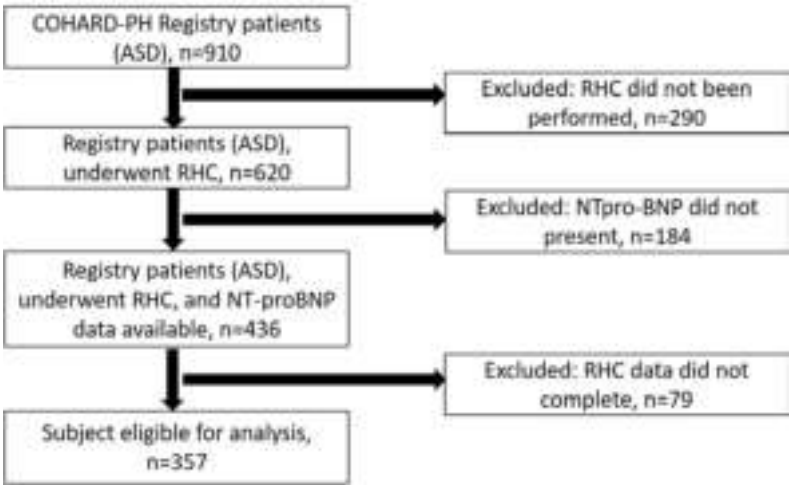

Figure 1. The flow-chart of enrollment and selection of subjects from the COHARD-PH registry.

from the COHARD-PH registry.

The majority of subjects were females (82.7%) with ages in their mid-thirties. The mean ASD diameter was 2.4±0.8 cm. Most subjects had already developed PH (82.9%), based on the criteria. Subjects with ASD-PH had significantly older ages, less bodyweight, less oxygen saturation, with higher hemoglobin and hematocrit levels. From TTE result, they had larger septal defect diameter, greater RA and RV diameters, lower TAPSE and higher LVEF. The NT-proBNP level was significantly higher in subjects with ASD-PH compared to ASD patients with no PH. **Table 1** shows the demographic, clinical, laboratory, echocardiogram and hemodynamic characteristics of all subjects and their comparisons based on the presence of PH.

Among variables that were associated with PH in the univariate analysis, only NT-proBNP level (adjusted OR 1.004, 95% CI: 1.001-1.006,

Table 1. The demographic, clinical, laboratory, structural (TTE) and hemodynamic (RHC) characteristics of all subjects and their comparison based on the presence of PH.

Characteristics	Total (n=357)	ASD, no PH (n=61)	ASD-PH (n=296)	*P* value
Age (years) [mean±SD]	34.7±12.1	30.7±10.7	35.5±12.2	0.005
Female sex, n (%)	292 (82.7)	53 (86.9)	239 (81.8)	0.344
Body weight (kg) [mean±SD]	47.9±10.4	50.9±9.3	47.4±10.5	0.014
Body mass index [mean±SD]	19.9±7.1	20.5±3.0	19.8±7.7	0.445
Oxygen saturation (%)[mean±SD]	95.4±5.0	98.3±0.9	94.9±5.3	<0.001
Hemoglobin (g/dL) [mean±SD]	14.1±2.1	13.2±1.9	14.3±2.1	<0.001
Hematocrit (%) [mean±SD]	42.3±6.2	39.7±5.2	42.8±6.3	<0.001
NT-proBNP (pg/mL) [median(IQR)]	383.8 (147.1-1309.0)	109.1(55.2-197.3)	606.1 (177.5-1706.3)	<0.001
Defect diameter (cm) [mean±SD]	2.4±0.8	2.1±0.9	2.5±0.8	<0.001
RA diameter (mm) [mean±SD]	45.8±6.7	42.0±5.6	46.6±6.6	<0.001
RV diameter (mm) [mean±SD]	43.2±6.8	38.7±5.5	44.1±6.8	<0.001
TAPSE (mm) [mean±SD]	25.1±5.3	27.3±4.5	24.6±5.4	<0.001
Left ventricle EF (%)[mean±SD]	69.8±8.7	67.5±7.8	70.2±8.8	0.026
mPAP (mmHg) [median(IQR)]	36.0 (22.0-56.0)	16.0(14.5-18.0)	43.0 (27.0-59.8)	<0.001
PVRi (Wood Unit.m^2)[median(IQR)]	3.5(1.5-13.4)	1.3(1.0-2.0)	5.8 (2.2-17.3)	<0.001
mRAP(mmHg) [median(IQR)]	8.0(5.0-11.0)	5.0(3.0-8.0)	9.0(6.0-11.0)	<0.001
mLAP (mmHg) [median(IQR)]	9.0 (6.0-11.0)	6.0(5.0-9.0)	9.5 (7.0-12.0)	<0.001
Aorta saturation (%)[mean±SD]	91.8±7.6	96.2±4.2	90.9±7.9	<0.001

TTE: transthoracal echocardiogram; RHC: right heart catheterization; ASD: atrial septal defect, PH: pulmonary hypertension, SD: standard deviation, RA: right atrial, RV: right ventricle, TAPSE: tricuspid annular plane systolic excursion, EF: ejection fraction, mPAP: mean pulmonary artery pressure, PVRi: pulmonary vascular resistance index, mRAP: mean right atrial pressure, mLAP: mean left atrial pressure

p=0.008) and RA diameter (adjusted OR 1.13, 95% CI: 1.01-1.28, *p*=0.028) were independently associated with PH diagnostic criteria. **Table 2** shows the results of the univariate analysis of covariables and multivariable logistic regression analysis which indicated that only NT-proBNP level and RA diameter were significantly and independently associated with PH.

The ROC curve to determine the accuracy and cut-off point of NT-proBNP level to detect PH among patients with ASD is shown in **Figure 2**. The AUC of NT-proBNP was 84.4% (95%CI: 80.1%-88.8%, *p*<0.001) to predict PH. The best cut-off of NT-proBNP level to detect PH was ≥140 pg/mL. The NT-proBNP level ≥140 pg/mL had a sensitivity of 85.1%, a specificity of 62.3% and accuracy of 81.2%. Its positive predictive value was 91.6%, positive likelihood ratio was 2.26, negative predictive value of 46.3% and negative likelihood ratio was 0.24 to detect PH. **Table 3** indicates the results of the diagnostic tests with NT-proBNP level ≥140 pg/mL to detect PH.

The ROC curve to determine the accuracy and cut-off point of RA diameter to detect PH among patients with ASD was demonstrated in **Figure 3**. The AUC of RA diameter was 69.9% (95%CI: 63.2%-76.1%, *p*<0.001) to predict PH. The cut-off value of RA diameter to detect PH was determined at ≥46.0 mm. This value had a sensitivity of 51.0%, a specificity of 78.7% and accuracy of 55.7%. Its positive predictive value was 92.1%, positive likelihood ratio was 2.40, negative predictive value of 52.2% and negative likelihood ratio was 0.62 to detect PH. **Table 4** indicates the results of the diagnostic tests with RA diameter of ≥46.0 mm to detect PH.

Table 2. The results of the univariate and multivariable analyses of covariables associated with ASD-PH.

Covariables associated with ASD-PH	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age (years)	1.04 (1.01-1.07)	0.005	1.04 (0.99-1.08)	0.100
Bodyweight (kg)	0.97 (0.94-0.99)	0.016	1.00 (0.96-1.05)	0.863
Oxygen saturation (%)	0.54 (0.39-0.75)	<0.001	0.72(0.49-1.04)	0.076
Hemoglobin (g/dL)	1.33 (1.14-1.55)	0.003	1.66 (0.77-3.59)	0.200
Hematocrit (%)	1.10 (1.04-1.16)	0.001	0.86(0.66-1.11)	0.271
NT-proBNP (pg/mL)	1.005 (1.003-1.007)	0.019	1.004 (1.001-1.006)	0.008
Defect diameter (cm)	1.92 (1.32-2.77)	0.001	1.81 (0.98-3.37)	0.060
RA diameter (mm)	1.13 (1.07-1.19)	<0.001	1.13 (1.01-1.28)	0.038
RV diameter (mm)	1.15 (1.09-1.22)	<0.001	1.08 (0.97-1.19)	0.160
TAPSE (mm)	0.91 (0.86-0.96)	<0.001	0.95 (0.86-1.05)	0.344
Left ventricle EF	1.04 (1.01-1.06)	0.028	1.03 (0.97-1.08)	0.321

ASD: atrial septal defect, PH: pulmonary hypertension, RA: right atrial, RV: right ventricle, TAPSE: tricuspid annular plane systolic excursion, EF: ejection fraction

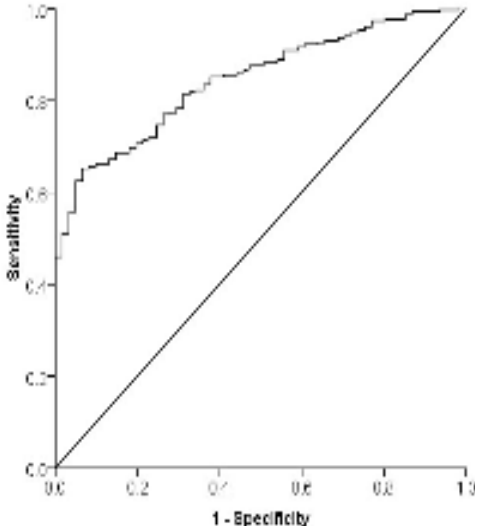

Figure 2. The ROC curve and AUC of NT-proBNP (84.4%, 95%CI: 80.1%-88.8%, p<0.001) performance to detect the presence of PH among ASD patients.

Table 3. The results of the diagnostic tests with NT-proBNP cut-off value of ≥140.0 pg/mL to detect ASD- PH.

	ASD-PH (n=296)	ASD-No PH (n=61)
NTproBNP ≥ 140.0 pg/mL (n=275)	252 (85.1)	23 (37.7)
NTproBNP < 140.0 pg/mL (n=82)	44 (14.9)	38 (62.3)
Sensitivity: 252/296=85.1%	Accuracy: 290/357 = 81.2%	
Specificity: 38/61=62.3%	Prevalence: 296/357 = 82.9%	
Positive predictive value: 252/275=91.6%		
Negative predictive value: 38/82=46.3%		
Positive likelihood ratio: 85.1/ (23/61) =2.26		
Negative likelihood ratio: 14.9/ (38/61) =0.24		

The combined values of NT-proBNP level ≥140 pg/mL and RA diameter ≥46.0 mm yielded a sensitivity of 46.6%, a specificity of 91.8% and accuracy of 54.3%. Its positive predictive value was 96.5%, positive likelihood ratio was 5.7, negative predictive value of 26.2% and negative likelihood ratio was 0.58 to detect PH. **Table 5** indicates the results of the diagnostic tests with the combined values of NT-proBNP level ≥140 pg/mL and RA diameter ≥46.0 mm to detect PH.

NT-proBNP level showed a significant positive correlation with both mPAP and PVRi in all ASD subjects (r value=0.639, p<0.001 and r value=0.587, p<0.001, respectively) and in ASD-PH subjects (r value=0.517, p<0.001 and r value=0.515, p<0.001, respectively). The NT-proBNP level had significant negative correlations with aorta saturation in ASD subjects (r value=-0.543, p<0.001) and in ASD-PH subjects (r value=-0.451, p<0.001). **Table 6** shows the correlation test results between NT-proBNP levels and hemodynamic parameters by RHC.

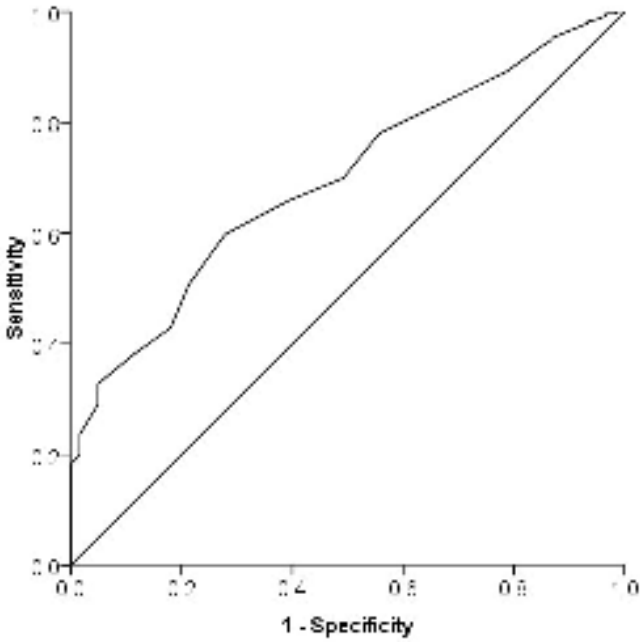

Figure 3. The ROC curve and AUC of RA diameter (69.6%, 95%CI: 63.2%-76.1%, p<0.001) performance to detect the presence of PH among ASD patients

Table 4. The results of the diagnostic tests with RA diameter cut-off value of ≥46.0 mm to detect ASD-PH

	ASD-PH (n=296)	ASD-No PH (n=61)
RA diameter ≥ 46.0 mm (n=164)	151 (51.0)	13 (21.3)
RA diameter < 46.0 mm (n=193)	65 (49.0)	48 (78.7)
Sensitivity: 151/296 = 51.0%	Accuracy: 199/357 = 55.7%	
Specificity: 48/61 = 78.7%	Prevalence: 296/357 = 82.9%	
Positive predictive value: 151/164 = 92.1%		
Negative predictive value: 48/92 = 52.2 %		
Positive likelihood ratio: 51.0/21.3 = 2.4		
Negative likelihood ratio: 49.0/78.7 = 0.62		

Table 5. The results of the diagnostic tests of NT-proBNP ≥140 pg/mL and RA diameter ≥46.0 mm todetect ASD-PH.

	ASD-PH (n=296)	ASD-No PH (n=61)
NT-proBNP ≥ 140 pg/mL and RA diameter ≥ 46.0 mm (n=143)	138 (46.6)	5 (8.2)
No both values (n=214)	158 (53.4)	56 (91.8)
Sensitivity: 138/296 = 46.6%	Accuracy: 194/357 = 54.3%	
Specificity: 56/61 = 91.8%	Prevalence: 296/357 = 82.9%	
Positive predictive value: 138/143 = 96.5%		
Negative predictive value: 56/214 = 26.2%		
Positive likelihood ratio: 46.6/8.2 = 5.7		
Negative likelihood ratio: 53.4/91.8 = 0.58		

Table 6. The correlation between NT-proBNP level with hemodynamic parameters measured by RHC in all subjects and in ASD-PH subjects.

Variables*	All subjects		ASD-PH	
	r value	*P*value	r value	*P*value
mPAP	0.636	<0.001	0.517	<0.001
PVRi	0.587	<0.001	0.515	<0.001
mRAP	0.082	0.128	-0.056	0.342
mLAP	0.045	0.399	-0.095	0.105
Aorta saturation	-0.543	<0.001	-0.451	<0.001

* Spearman correlation test
RHC: right heart catheterization; mPAP: mean pulmonary artery pressure, PVRi: pulmonary vascular resistance index, mRAP: mean right atrial pressure, mLAP: mean left atrial pressure

DISCUSSION

Our study results revealed that NT-proBNP level ≥140 pg/mL demonstrated a diagnostic value to detect the presence of PH within adult patients with ASD. The NT-proBNP for this prediction effect showed a sensitivity of 85.1% and a specificity of 62.3%. These values represented the ability of NT-proBNP ≥140 pg/mL to screen for early detection of PH within adult patients with ASD and ruling-out PH among those with NT-proBNP level <140 pg/mL, and selecting those with NT-proBNP level ≥140 pg/mL for PH and further PAH confirmation with invasive procedure, i.e. RHC. The NT-proBNP level ≥140 pg/mL showed 91.6% positive predictive value, which indicated its excellent ability to detect PH in adult patients with ASD coming to hospital, in which the prevalence of PH is more than 80%. This ability was also supported by the 2.26 positive likelihood ratio of this cut-off value to detect PH among adult patients with ASD.

By combining the values of NT-proBNP level ≥140 pg/mL and RA diameter ≥46.0 mm, obtained at TTE evaluation, the specificity was increasing (91.8%), which denoted it had more power with the combined values to identify adult patients with ASD as having PH. These combined values also had increasing positive predictive value and positive likelihood ratio, further supporting its role in detecting PH among adult patients with ASD coming to hospital where there is a high prevalence of PH. The use of the RA diameter parameter measured by TTE as a complement to the NT-proBNP level has an added benefit since it is an already standardized measurement and easily non-invasively be

obtained by any echocardiograph-dedicated machine.[11] Furthermore, these measured parameters are already approved by Indonesian guideline. As a screening tool, this measurement meets the requirements regarding expediency and availability. The TTE findings can be used to examine the hemodynamic consequences of ASD-related shunting, such as RA dilation, RV dilation, RV function, tricuspid annular dilation and tricuspid regurgitation, as an estimation of the presence of PAH or severity of PH.

In developing countries with limited resources or scarce facilities then we propose the triage of adult patients with ASD combining the NT-proBNP level and RA diameter non invasive evaluation to decide the urgent need to perform more advanced and invasive procedures, such as RHC (**Figure 4**). Adult ASD with NT-proBNP value <140 pg/mL can be ruled-out for the presence of PAH. In these patients, there is less urgency to perform RHC, however the RHC would be eventually still required to correct the defect and measure hemodynamic parameters, especially in secundum ASD suitable for non-surgery closure. Patients with NT-proBNP ≥140 pg/mL represent those with high probability of PAH, and by adding the measurement of RA diameter, those with RA diameter ≥46.0 mm indicate the most urgent cohort of patients needing to undergo RHC to measure hemodynamic parameters and determine correctability criteria. The semi-urgent status for RHC is indicated in adult ASD patients with both NT-proBNP ≥140 pg/mL and RA diameter <46.0 mm. By producing this triage, the scheduling of RHC for adult patients with ASD visiting the hospitals can be more efficiently performed. Those with NT-proBNP ≥140 pg/mL may also benefit from PAH-specific medication before undergoing the RHC procedure, if any World Health Organization (WHO) functional class was indicated.

Large ASD is associated with increased morbidity and mortality overtime if left uncorrected, from 0.6% and 0.7%/year in the first two decades of life until 4.5%/ year in the fourth decade of life.[13] The most common morbidities and mortality are related with PH and right heart failure.[13] Adult patients with ASD usually have a prolonged asymptomatic presentation or insidious obscured symptom courses which go undetected.[5] The delayed symptoms most often arise in the third or fourth decades of life.[14] At early phase of the disease, most symptoms occur during physical exertion which relate to the decrease in cardiac output because of the interatrial shunting.[13] Overtime, the severity of symptoms increases and patients seek medical help. Our registry indicated that the prevalence of PH and PAH among symptomatic adults with ASD was 82.9% by RHC examination.[5]

The RHC is important to evaluate the pulmonary pressures and direction of the shunt flow in ASD with PH and to guide the decision of appropriateness of defect closure, particularly in adult patients with ASD. The combination of clinical signs, WHO functional capacity, TTE/TOE parameters, and RHC hemodynamic results determines the recommendation of defect closure.[14] Therefore, through PH screening among adult patients with ASD with easy, comfortable and non-invasive tools, the selection of who will get the most urgent working up by more invasive procedures can be timelier and more efficiently executed. For patients with ASD-PH, the urgency to undergo RHC for diagnostic, therapeutic and prognostic purposes are applicable in the current guidelines.[8,16] For adult ASD patients without PH, the RHC is needed for therapeutic purposes to close the defect. In this scenario, adult patients with ASD

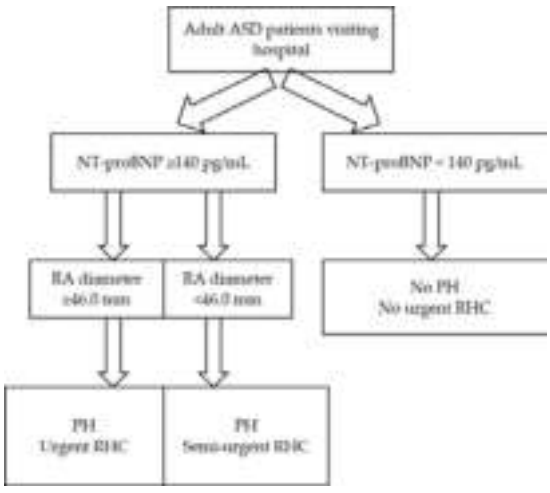

Figure 4. The triage scheme for urgency of invasive procedure by RHC among adult ASD by using NT-proBNP level and proceed by RA diameter

without PH can undergo RHC with less urgency.

The utility of NT-proBNP to triage, diagnose and risk stratify acute and chronic heart failure has been established.[17] Besides LV, NT-proBNP is an established biological indicator of RV strain and overload.[17,18] Its circulating level correlates with cardiac and pulmonary hemodynamics in PH patients.[19] In ASD patients, NT-proBNP was associated with RV dysfunction which improves after ASD closure.[20] The level of NT-proBNP has been validated as a prognostic and therapeutic response biological marker in patients with PAH.[21, 22] The European Society of Cardiology/ European Respiratory Society guidelines use the NT-proBNP threshold levels of 300 pg/ mL and 1400 pg/mL and categorize the risks as low (<5%), intermediate (5%–10%), or high (>10%) of 1-year mortality in PAH, of which also being adopted by Indonesian guideline.[8] While the adaptation of these prognostic and therapeutic markers has been widely accepted in patients with PAH, including ASD, the broader utilization of NT-proBNP as a diagnostic marker of PAH in ASD patients is not yet confirmed. This is because ASD has the hemodynamic consequences of pulmonary chronic vascular overflow and right atrial/ventricle chronic volume overload before resulting in PH and PAH. These long-term routes make it demanding to determine the timeliest point of PH diagnosis. The measurement of NT-proBNP level in adult patients with ASD can determine which patients experience PH and which had not. Our study, which included subjects coming to hospitals due to the symptoms they felt, showed that the NT-proBNP level identified and distinguished those who had already developed PH. Prompt decisions for invasive procedures and initiation of therapy in adult ASD patients with levels above the upper cut-off of NT-proBNP may avoid further RV failure, and thereby reducing early morbidity and mortality.[23]

The result of this study needs to be externally validated in the cohorts of adult ASD patients who are still frequently found in Indonesia due to the lack of screening processes in childhood. These combination of NT-proBNP level and RA diameter by TTE was a simple parameter to discriminate patients who needed more invasive procedure which will need external validation especially from district hospitals, which become the first-line treating hospitals, and also referral hospitals, which had more advanced facility to treat ASD patients with/without PH. In the future, the external validation process is important to test the usability and generability of this study finding.

This study had several notable limitations. Firstly, the sample size for control group did not meet the minimum requirement based on sample size calculation formula. Secondly, it did not exclude subjects with Eisenmenger syndrome since its current clinical and hemodynamic definitions are not clearly defined. Third, patients with corrected ASD who still developed PAH were excluded. Fourth, the time interval between index of ASD diagnosis by TTE and NT-proBNP level measurement during RHC varied. And lastly, this study was conducted in a single PH center in Indonesia, which needs further corroboration and externally validated by a large multicenter study.

CONCLUSION

NT-proBNP circulating level ≥140 pg/mL seems to represent PH in adult patients with ASD. The NT-proBNP level ≥140 pg/mL can be used together with RA diameter ≥46.0 mm at TTE as pre-test probability measures to triage patients needing more invasive procedures and also to determine when and if to start the PAH-specific treatment, especially in developing countries in which the adult ASD delayed presentation is high and the facility for PH and PAH diagnosis is limited and scarce.

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CONFLICT OF INTEREST

All authors had no conflict of interests regarding the manuscript.

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Diagnostic Performance of *Mac-2-Binding Protein Glycosylation Isomer* (M2BPGi), compared to Transient Elastography to Assess Liver Stiffness in Treatment Naïve Chronic Hepatitis C Patients

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ABSTRACT

Background: *Liver fibrosis is an essential factor in the management of Hepatitis C virus infection. Its assessment is crucial in decision-making regarding the therapeutic decisions, and the patients' follow up. However, the established liver measurement methods have several limitations. Therefore, this study aims to assess the role of Mac-2-Binding Protein Glycosylation Isomer (M2BPGi) as a novel biomarker to measure liver stiffness in treatment naïve Chronic Hepatitis C Indonesian patients.* ***Methods:*** *This study used a cross-sectional design to determine the correlation between serum M2BPGi and the degree of liver stiffness, Transient Elastrography, and differences in serum M2BPGi levels in chronic hepatitis C patients. Serum M2BPGi level and Transient Elastography results were evaluated in 56 Chronic Hepatitis C patients and 48 healthy controls. Pearson correlation analysis was conducted to find the correlation between the level of M2BPGi and Transient Elastography result. ROC analysis was conducted to find the optimum cut-off to assess fibrosis's degree among Chronic Hepatitis C Patients.* ***Results:*** *The level of serum M2BPGi and Transient Elastography result was strongly correlated with the median level of serum M2BPGi. It was also significantly higher among Chronic Hepatitis C Patients than among healthy controls (r: 0.708, p<0.001; 0.590 COI vs. 4.130 COI, p<0.001). Among the Chronic Hepatitis C patients, the median serum of M2BPGi increased according to the degree of liver fibrosis: 1.500 COI (F0-F1), 2.985 COI (F2-F3) and 8.785 COI (≥F4). The optimum cut-off value for diagnosing significant fibrosis (F2-F3) was 1.820 COI (AUC: 90.8%) and for diagnosing cirrhosis (≥F4) was 3.770 COI (AUC: 89.3%).* ***Conclusion:*** *Serum M2BPGi was a reliable diagnostic tool for identifying liver fibrosis in Indonesian patients with Chronic Hepatitis C.*

Keywords: *Chronic Hepatitis C, Mac-2-Binding Protein Glycosylation Isomer, Transient elastography.*

INTRODUCTION

Chronic Hepatitis C remains a global burden of disease due to its high prevalence. It is estimated that around 177.5 million adults (2.5% of the total global population) live with HCV infection.[1] In Indonesia, the prevalence of anti-HCV is 2.5% of the total population. This data shows an increase by 0.4% from the

previous survey conducted in 2007.[2] Moreover, nearly 400 thousand people die annually from the complication of HCV infection, such as liver cirrhosis, hepatic decompensation, or hepatocellular carcinoma.[3]

In clinical practice, the quantification of liver fibrosis in patients with Chronic Hepatitis C is crucial in decision making regarding the start of therapeutic regimens and the adequate follow up of the patients. According to the current clinical practice guidelines, Chronic Hepatitis C treatment is recommended when significant fibrosis is present.[4] For those detected to have liver cirrhosis, the treatment duration will be prolonged up to 6 months, and regular screening for complications and hepatocellular carcinoma surveillance must be initiated.[4,5] Moreover, the fibrosis stage is also found to be the main predictive factors of complication once the virus is eradicated. Therefore, fibrosis regression has become a new surrogate goal of Hepatitis C Virus infection therapy.[6]

The gold standard for assessing the degree of liver fibrosis is a liver biopsy. However, due to its invasive method, it could lead to some potential complications. A previous study found that around 6% of complications were observed from 1806 biopsy procedures in which 75% of these patients reported moderate and severe pain, while 33% of the others underwent prolonged hospital observation or surgical intervention due to excessive bleeding.[7] Furthermore, errors in sampling and interpretation are often found in biopsy procedures. Consequently, many clinicians prefer to use non-invasive approaches.[8]

Several non-invasive approaches have been used in assessing liver fibrosis in Chronic Hepatitis C patients. One of the commonly used non-invasive modalities that have been highly validated is Transient Elastography (TE). It works to quantify the mechanical responses of liver tissues through a shear-wave velocity produced by a piston. However, the generated mechanical waves diffuse in nonviscous liquids. Hence, TE could not be used in patients with ascites condition.[9]

In addition, the assessment of liver fibrosis through biomarkers has also been widely used. Some methods are considered as a "direct" method since they are directly involved in the cellular matrix accumulation process. On the other hand, several biomarkers are considered an "indirect" method because they could only portray the epiphenomena associated with the fibrogenesis process.[10]

One commonly used indirect biomarker for assessing liver fibrosis is AST to platelet ratio index (APRI). Its diagnostic performance has been extensively evaluated and consistently depicted more robust positive diagnostic performance when assessing advanced fibrosis and cirrhosis. However, since it is an indirect biomarker to evaluate liver fibrosis, its application will also need adjustment according to the specific condition of the patients.[10]

Mac-2-binding protein glycosylation isomer (M2BPGi) was recently found to be an alternative serum for detecting liver fibrosis. Acting as a juxtracrine messenger sent by Hepatic Stellate Cells (HSCs) to Kupffer cells during liver fibrosis enables it to depict the liver fibrosis progression directly.[11] Previous studies have confirmed that M2BPGi was useful to measure liver fibrosis in several conditions, such as Hepatitis B or C infection, autoimmune hepatitis, biliary atresia, primary biliary cirrhosis, and Non-Alcoholic Fatty Liver (NAFLD).[12–16] Furthermore, a meta-analysis study also found that M2BPGi could be a reliable predictor for determining the stage of liver fibrosis.[12]

However, another study also found that the value of M2BPGi might be different according to the etiology of the liver fibrosis, even when the stage of liver fibrosis is similar. For instance, the level of M2BPGi among Non-alcoholic steatohepatitis (NASH) tends to be lower than those in Chronic Hepatitis C or B infection with the same degree of liver fibrosis.[13] Given this uniqueness and the lack of research on the role of serum M2BPGi in assessing liver fibrosis in treatment naïve Chronic Hepatitis C patients in the Indonesian population, this study aims to find the correlation of M2BPGi level with the fibrosis measurement by using Transient Elastography, measure the cut-off point to assess significant fibrosis and cirrhosis, and evaluate its diagnostic performance on Hepatitis C Chronic patients in these population.

METHODS

This study used a *cross-sectional* design to determine the correlation between serum M2BPGi and the degree of liver stiffness, transient Elastrography, and differences in serum M2BPGi levels in chronic hepatitis C patients**.** And, based on the minimum sample calculation for differences of two means.

Patients

56 treatment naïve Chronic Hepatitis C patients in one of the National General Hospital in Jakarta were recruited for the study. Inclusion criteria were: age >18 years old with Hepatitis C. Exclusion criteria were: (i) diagnosed with Hepatitis B, HIV, or HCC, (ii) had a history of Hepatitis C treatment. Furthermore, 48 healthy controls were also recruited as a comparison group. The Ethics Committee at the Faculty of Medicine Universitas Indonesia approved the study with the number of approval letter: 85/UN2.F1/ETIK/2019. Informed consent was collected from each participant before the data collection process.

Liver Stiffness Measurement

Liver Stiffness Measurement was assessed using Fibro Scan®, and the result was used as the reference standard. A skilled full physician performed the assessment of LSM after the measurement of serum M2BPGi on the same day. The median of ten LSM values was used as the final score. Significant fibrosis was defined as of LSM ≥8 kPA, while cirrhosis was defined as the level of LSM ≥12 kPA. Moreover, probe XL was used to assess liver fibrosis among obese patients.

Serum M2BPGi Measurement

M2BPGi level was assessed through a lectin-antibody sandwich immunoassay using the HISCL-5000 immune analyzer (Sysmex Corporation, Hyogo, Japan). The blood sample was taken before the measurement of LSM measurement. The final value of M2BPGi was obtained by using the following equation:

Cut Off Index (COI) = ([M2BPGi]sample – [M2BPGi]NC)/([M2BPGi]PC-[M2BPGi]NC) in which [M2BPGi]sample is the M2BPGi found in the serum sample, PC is a positive control, and NC is the negative control. The positive control was used as the calibration solution preliminarily standardized, which provided a cut off value of 1.0. The result of M2BPGi level is automatically calculated by the instrument.[17]

Statistical Analysis

Correlation between Liver Stiffness Measurement and serum M2BPGi level was analyzed using Pearson correlation. Differences between numeric variables were assessed by using independent T-test analysis for the parametric data and Mann-Whitney U test for the nonparametric data. Optimal serum M2BPGi cut off value was evaluated by performing ROC and AUC analysis based on the optimal sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

RESULTS

Baseline Clinical Characteristic of Patients

The baseline of the study participants is shown in **Table 1**. The enrolled patients in the

Table 1. Baseline characteristic.

Characteristic	Cases (n = 56)	Control (n=48)	P value
Sex (M/F)	33/23	22/26	>0.05
LSM, kPA, median (range)	14.35 (3.0 – 75.0)	5.1 (3.5 – 8.7)	<0.001
Fibrosis stage (F0-F1/ F2-F3 /≥F4)	14/8/34	47/1/0	<0.001
ALT			
F0-F1	51.6 ± 30.2	-	-
F2-F3	67.5 ± 45.3	-	-
≥F4	63.1 ± 53.9	-	-
AST			
F0-F1, mean ± SD	42.5 ± 21.9	-	-
F2-F3 mean ± SD	52.0 ± 15.5	-	-
≥F4, mean ± SD	65.3 ± 39.7	-	-
M2BPGi, COI, median (range)	4.13 (0.72 – 26.00)	0.59 (0.23 – 1.27)	<0.001

case group were dominated by male participants (59%), while the control group was dominated by female participants (54%). In the Hepatitis C infected group, the median of liver stiffness measurement (LSM) was significantly higher than the healthy control group (14.3 kPA vs. 5.1 kPA, p<0.001). Most of the Hepatitis C group were in the cirrhosis stage (≥F4), accounting for 60% of the total participant in the case group. Moreover, the M2BPGi level also differed significantly between the case and control groups (4.13 COI vs. 0.59 COI, p<0.001).

Correlation between M2BPGi and Liver Stiffness Measurement in Treatment Naïve Chronic Hepatitis C Patients

Based on the Pearson correlation analysis, the level of serum M2BPGi was found to have a strong positive correlation with the liver stiffness measurement by Transient Elastography (r=0.708, p<0.001) (**Figure 1a**). Moreover, the median level of serum M2BPGi increased significantly according to the severity of liver fibrosis stages as 1.50 COI for F0-F1, 2.985 COI for significant fibrosis (F2-F3) and 8.785 COI for cirrhosis (≥F4). The analysis also showed that the level of M2BPGi between healthy control and F0-F1 Hepatitis C group differed significantly (0.585 COI vs 1.500 COI, p<0.001) (**Figure 1b**).

Cut-off Values of Serum M2BPGi To Detect Significant Fibrosis and Cirrhosis

ROC analysis was carried out to evaluate the diagnostic performance of serum M2BPGi to evaluate significant liver fibrosis and cirrhosis. The AUCs were 0.908 (95%CI: 0.807 – 1.000) for evaluating significant fibrosis and 0.893 (95%CI: 0.806 – 0.980) for evaluating cirrhosis (**Figure 2**).

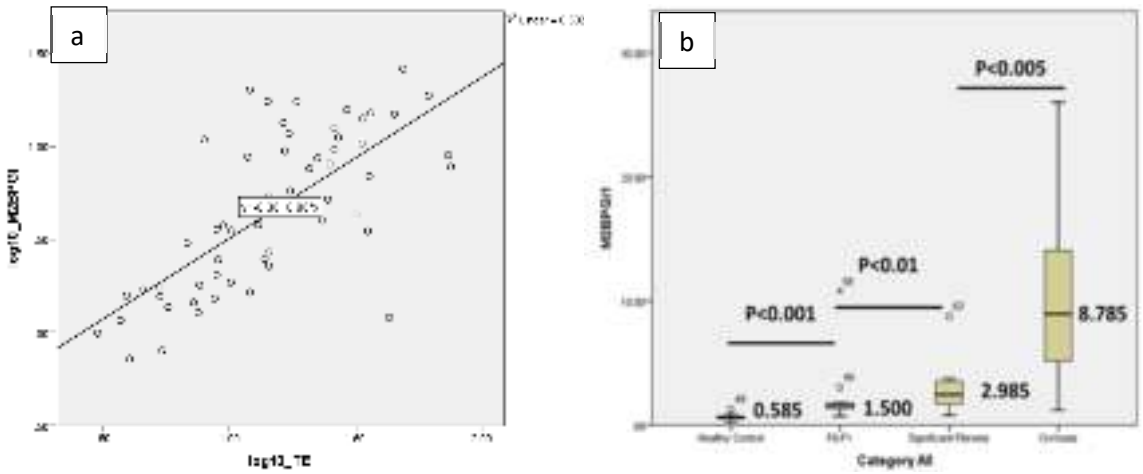

Figure 1. a) Correlation of log10 Serum M2BPGi with log10 Liver Stiffness Measurement by Transient Elastography. **b**) The Difference of Median Level of Serum M2BPGi Across Fibrosis Stages.

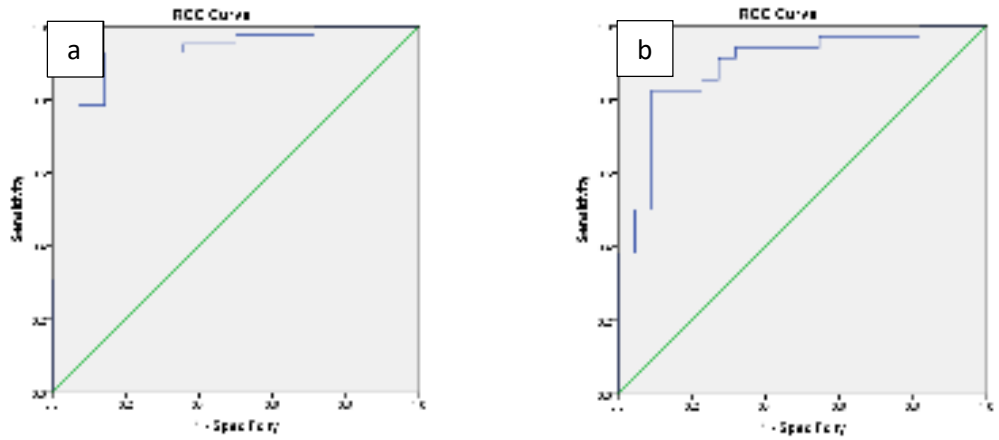

Figure 2. ROC Graphs of Serum M2BPGi for Evaluating **a**. Significant Fibrosis and **b**. Cirrhosis

The optimal cut-off value for diagnosing significant fibrosis and cirrhosis were 1.820 COI and 3.770 COI, respectively. The sensitivity, specificity, negative predictive value, and positive predictive value for each cut-off were presented in **Table 2**.

DISCUSSION

Hepatitis C Virus infection could progress to numerous extra-hepatic manifestations and affect the achievement of SVR. However, due to its high cost, not all patients infected with the virus would undergo therapy. In this case, liver fibrosis measurement is essential in the decision-making to start treatment and determine the patients' adequate follow-up.[6] Furthermore, the fibrosis stage has also been a primary predictive factor of complications after eradicating the virus.[18]

The gold standard for assessing liver fibrosis is a liver biopsy. However, it has several limitations, such as the risk of tremendous side effects due to its invasive nature and the possibility of sampling bias also inter-observer variability[7,19] Several non-invasive modalities have been developed to overcome this barrier, such as Transient Elastography, which is accessible, accurate, and safe for patients. It has also been widely investigated and validated; therefore, it is also recognized as the liver biopsy's surrogate modality.[20]

In this study, it was found that the level of serum *Mac-2-binding Protein Glycosylation Isomer* (M2BPGi) in treatment naïve Chronic Hepatitis C patients had a strong positive correlation with liver stiffness measurement by using Transient Elastography ($r=0.708$, $p<0.001$). Moreover, the level of serum M2BPGi tended to increase according to the severity of liver stiffness stages (figures 1a-b). These findings were in line with the previous study, which also found that serum M2BPGi was positively correlated with Transient Elastography ($rho=0.504$, $p<0.001$).[17]

Moreover, a significant difference of serum M2BPGi level was found between the treatment naïve Chronic Hepatitis C groups and healthy control (4.13 COI vs. 0.59, $p<0.001$). A previous study has suggested that M2BPGi is sent by the Hepatic Stellate Cells (HSCs) as a juxtracrine-acting messenger to Kuppfer Cells during the occurrence of liver fibrosis. Hence, the alteration of serum M2BPGi between healthy control and Chronic Hepatitis C patients could demonstrate the progression of liver fibrosis which occurs among the Chronic Hepatitis C patients.[11]

ROC analysis also showed an excellent diagnostic accuracy for detecting significant fibrosis (AUC: 0.908) and cirrhosis (AUC: 0.893) using the cut-off levels of 1.820 COI and 3.770 COI, respectively, with high sensitivity and specificity, especially for significant fibrosis (Table 2). Hence, this marker could be reliable for screening, distinguishing patients with significant liver fibrosis from those who do not. Although the ROC, sensitivity, and specificity of serum M2BPGi for diagnosing cirrhosis was found lower than for diagnosing significant fibrosis, the result was still considered to be satisfying.

There are several limitations to this study. Firstly, there was no liver biopsy assessment, which could lead to bias, especially when the liver stiffness measurement falls into the grey area. Secondly, based on the previous studies, it was stated that the specificity of M2BPGi could be an issue due to the influence of other medical conditions, such as acute liver injury and adenocarcinoma (21,22). However, this study also excluded participants who had been diagnosed with Hepatitis B Virus infection and hepatocellular carcinoma. Therefore, the bias could be minimized. Moreover, best to our knowledge, this is the first study about the role of M2BPGi in detecting liver fibrosis in Indonesian patients who have treatment naïve Chronic Hepatitis C.

Table 2. Diagnostic Performance of Serum M2BPGi for Evaluating Significant Fibrosis and Cirrhosis.

Fibrosis Stages	Cutoff	AUC	Sen	Spe	NPV	PPV
Significant Fibrosis (≥F2)	1.820	0.908 (0.807 – 1.000)	0.93	0.86	0.80	0.95
Cirrhosis (≥F4)	3.770	0.893 (0.806 – 0.980)	0.82	0.91	0.77	0.93

CONCLUSION

Serum M2BPGi was strongly positively correlated with liver stiffness measurement and high diagnostic performance to assess significant fibrosis and cirrhosis. Thus, the level of serum M2BPGi could be a simple and reliable diagnostic modality for evaluating liver fibrosis in treatment naïve Indonesian patients with Chronic Hepatitis C conditions.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

ASS proposed, designed, and conducted the study. IH, CRAL, JK, COMJ, SN, and KFK performed the research. RSH, DR collected and analyzed the data. ASS wrote the draft of the manuscript. RAG reviewed the manuscript. ASS was the research coordinator.

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The Effect of Vitamin D Supplementation on Symptoms of Depression in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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ABSTRACT

Background: *The effect of vitamin D supplementation on depressive symptoms in people with type 2 diabetes is still up for debate. The aim of this paper was to investigate the effect of vitamin D supplementation on symptoms of depression in type 2 diabetic patients.* ***Methods:*** *The protocol for this review has been registered in PROSPERO:CRD42021231713. Searching for literature was conducted using Pubmed, EBSCOhost, and EMBASE. Randomised controlled trials (RCTs) regarding vitamin D supplementation in type 2 diabetic patients with depression were retrieved through a systematic search. The outcome measured was a change in depressive symptoms evaluated with any validated rating scale. Independent data extraction was conducted, and the study quality was assessed. A meta-analysis was carried out to calculate the improvement in depressive symptoms in the group receiving vitamin D and the control group. The available evidence in RCTs was analysed using the PRISMA approach, and clinical significance was determined using the GRADE system. Risk of bias was assessed using the Cochrane Risk of Bias Tool.* ***Results:*** *Four RCTs were reviewed and three RCTs were meta-analysed. In two studies, vitamin D was statistically effective in improving depressive symptoms in type 2 diabetic patients. Three randomised controlled trials were included in the meta-analysis with 161 subjects using depression score as an outcome assessment. Vitamin D is significantly more effective than placebo (95% CI: -0.70 to - 0.08, p = 0.01).* ***Conclusion:*** *Vitamin D supplementation may improve the depressive symptoms in type 2 diabetic patients. Future research with different geographical areas and larger samples should be done to further assess the benefits.*

Keywords*: depression, type 2 diabetes mellitus, vitamin D.*

INTRODUCTION

Diabetes mellitus and depression are both significant chronic diseases that diminish life expectancy, reduce quality of life, and increase functional disability.[1] Diabetes and depression occur together approximately twice as frequently

as they would be predicted by chance alone. Comorbid diabetes and depression pose a significant clinical problem because the outcomes of both disorders are exacerbated by the presence of the other.[2] Despite the existence of biological, psychological, and environmental explanations, the underlying pathophysiology of depression is unknown, and several processes may be involved.[3]

A study by Holt et al. showed that diabetic patients have an increased risk of developing depression. This phenomenon can be caused by several factors, either by patients' perception of a disease or by biological changes that occur within the body. The diagnosis of diabetes mellitus often frightens patients. Some of the reasons are that they are afraid that the disease cannot be cured and that it requires high discipline and compliance to take medications regularly to prevent further complications. Moreover, diabetic patients should also change their lifestyle, which is not suitable and comfortable for some of them. On the other hand, diabetes can also cause decreased neurogenesis, which can further increase the risk of depression.[2,3] The symptoms of depression in diabetic patients are correlated with a decreased quality of life, a higher risk of developing further complications, and increasing mortality. Several solutions can be given to patients, and one of the solutions is giving anti-depressants. However, several anti-depressants have side effects of decreasing glycaemic control and causing weight gain, which are not suitable for diabetic patients. As a result, other solutions to this problem are being sought.[4,5]

In the past few years, other treatment options besides anti-depressants have been investigated to treat depressive symptoms. One of these treatment options is vitamin D. Vitamin D receptors are found on neurons and glia in various parts of the brain, including the cingulate cortex and hippocampus, which have been linked to depression pathophysiology.[6] Furthermore, vitamin D is a supplement that is easily accessible and inexpensive. These reasons serve as a foundation for utilizing vitamin D supplementation to treat depressive symptoms in patients with type 2 diabetes. Even though vitamin D is theoretically useful to treat depressive symptoms in type 2 diabetic patients, there are not many studies that are able to give conclusive conclusions regarding this matter.[7-9] This review aimed to compile all the available evidence on the effectiveness of vitamin D in type 2 diabetes patients with depression when compared to placebo or other vitamin D doses in relieving depressive symptoms.

METHODS

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (PRISMA).[10] The protocol for this review is registered with PROSPERO (CRD42021231713).[11]

Inclusion Criteria

We considered trials to be eligible if they included: (1) studies with the intervention of vitamin D supplementation and placebo as comparison – intervention can be single or combined with other drugs, such as anti-depressants, psychotherapy treatment, or other adjuvant therapies (micronutrients, probiotics, etc.); (2) the patients in the studies were older than 18 years old; (3) type 2 diabetes mellitus patients diagnosed based on ADA/WHO criteria with any depression questionnaire; (4) experimental studies; (5) studies in the form of randomized controlled trials (RCTs); (6) studies written in English; and (7) full-text articles; original articles; and (9) studies that were published between January 1, 2009 and December 31, 2021.

Exclusion Criteria

The exclusion criteria in this study include (1) children and adolescents as subjects; (2) pregnant and breast-feeding mothers; (3) subjects with progressive illness (such as: chronic kidney disease, hepatic cirrhosis, known history of seizure and other neurological disorders, and previous history of depression); (4) correspondence, reviews, editorials, and conference abstracts.

Outcomes

Our primary outcome for all studies was a change in depressive symptoms evaluated with any validated rating scale.

Table 1. Literature Search Strategy.

Database	Keywords	Results
PubMed	((((Depression[Title/Abstract] OR Depressive[Title/Abstract] OR Mood[Title/Abstract] OR Mental[Title/Abstract])))) AND ((Vitamin D[Title/Abstract] OR Vitamin- D2[Title/Abstract)) AND ((Diabetes Mellitus[Title/Abstract] OR Diabetes[Title/Abstract]))	135
EBSCOhost	("Depression" OR "Depressive" OR "Mood" or "Mental") AND "Vitamin D" AND ("Diabetes Mellitus" OR "Diabetes")	95
EMBASE	(Depression OR Mood OR Mental OR Affective Disorder*) AND (Vitamin D OR Cholecalciferol OR Vitamin D3 OR Ergocalciferol OR Vitamin D2 OR Alfacalcidol) AND (Type 2 Diabetes Mellitus OR Type II Diabetes Mellitus OR Diabetes Mellitus OR Diabetes)	69

Search Strategy

The literature search was conducted in six databases: PubMed, EBSCOhost, and EMBASE (up to 31 March 2022). The keywords used in the literature search were "depression" in conjunction with "diabetes mellitus" and "vitamin D". Synonyms used in the literature search keywords are obtained from the MeSH Terms (**Table 1**). Supplemental 2 provides the detailed search process. The search was limited to studies in English and was bound to articles that were published between January 1, 2009 and December 31, 2021. Studies that have no available full-text reports were not looked into further. Available full-text articles from the three databases were then screened based on their titles and abstracts. Eligibility criteria were applied to the articles for further screening. Suitable texts that fulfil all the criteria were taken into deeper analysis. In addition, all included publications' reference lists were thoroughly checked to ensure that no relevant studies were missed.

Study Selection

Following the removal of duplicates, all titles and abstracts were evaluated by three independent researchers (RP, KH, and SS). When studies were found eligible, the researchers collected full texts and conducted additional screening. Consensus was used to settle disagreements.

Data Extraction

To address the differences, the three researchers (RP, KH, and SS) did data extraction and reviewed the results. Some papers were eliminated from the data extraction process because they did not meet the study's objectives, and the remaining articles were extracted by two different researchers independently (EDS and SRFS).

Study characteristics (first author's name, year of publication, study location, publishing year, and study design), diagnosis, participant characteristics (mean age and gender of intervention and control group subjects, health condition of subjects, and the number of subjects in each group), types of intervention (type, dose, and duration of supplementation), mean and SD or percentage of clinical variables were collected from each study.

Quality Assessment

We assessed the risk of bias of RCTs using the Cochrane Risk of Bias Tool.[12] The following categories were examined: (1) method of randomisation, (2) allocation concealment, (3) blinding of subjects and personnel, (4) blinding of outcome assessment, (5) incomplete outcome data, (6) selective outcome reporting, and (8) other biases. Each domain was labelled as low risk of bias (+), high risk of bias (-) or unclear risk of bias (?). Two independent reviewers performed the quality assessment and resolved disagreements via discussion. Review Manager (RevMan) 5.4 software was used to assess the risk of bias.[13]We intended to assess publication bias using funnel plot techniques[14], Begg's rank test[15], and Egger's regression test[15], as appropriate, given the known limitations of these methods.

Statistical Analysis

On the basis of pre-to-post intervention changes, the effects of vitamin D supplementation were investigated. For all continuous outcomes, we utilized the standardized mean difference (SMD) and 95 percent confidence intervals (CIs). A fixed-effects model was applied to pool SMDs across studies by RevMan 5.4 software.[13] The chi-squared test and I-squared values were used to measure statistical heterogeneity. Moderate to substantial heterogeneity was indicated by I-squared >75 percent, mild heterogeneity by I-squared 50-75 percent, and low or no heterogeneity by I-squared ≤ 50 percent.

Sensitivity Analysis

We utilized a "leave-one-out" evaluation procedure to assess the stability of the estimated measures in the sensitivity analysis. This evaluation is an iterative procedure in which one trial was excluded from each iteration, and a meta-analysis was conducted on the remaining sample of studies. This analysis demonstrates how each study influences the overall estimate of the other studies.

We used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach to evaluate the certainty of depression score as the outcomes, which values certainty at one of four levels, to objectively analyse the power of the included research (high, moderate, low, and very low).[16]

RESULTS

After a thorough search and selection, our searches yielded 19,375 references. After duplicates were removed, 232 references remained for title and abstract screening. Of these, 6 were identified and retrieved for full-text screening; all were in English. After a full text review, four RCTs were included for the systematic review and three for the meta-analysis. The phases of the literature search are illustrated in **Figure 1**.

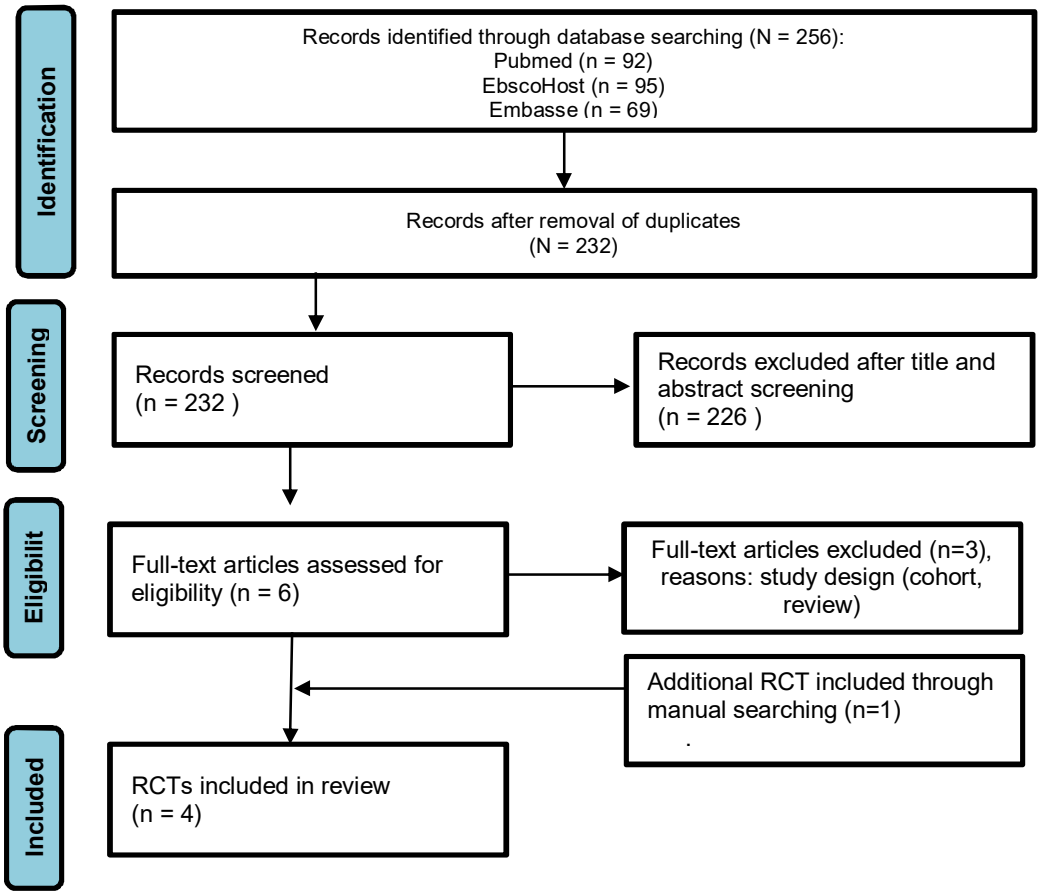

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Diagram of Study Selection

Table 2. Study Characteristics of Selected Articles.

Study	Country	Year	Subjects	N,Gender	Design	Intervention	Comparison	Duration	Validated scale used	Outcomes
Raygan et al.[17]	Iran	2018	Type 2 diabetic people with CHD, 45-85 years old	60 (30 Male, 30 Female)	RCT	50,000 IU vitamin D + probiotic	Placebo	12 weeks	Beck Depression Inventory (BDI II) scale	Improvements in BDI total score (-2.8 vs -0.9, p=0.01)
Omidian et al.[18]	Iran	2019	People with type 2 DM and mild- moderate depressive symptoms, 30-60 years old	68 (40 Male, 28 Female)	RCT	4000 IU	Placebo	3 months	Beck Depression Inventory (BDI II) scale	BDI-II scores decreased from 15.2 to 9.8 (p value <0.001)
Fazelian et al.[19]	Iran	2019	Women with type 2 diabetes and vitamin D deficiency, 20 – 60 years old	51 women	RCT	50,000 IU vitamin D3	placebo	16 weeks	Depression, Anxiety, Stress Scales (DASS-21)	Depressive changes were not significantly different between groups (p>0.05). Within group-analysis, it showed significant decrement in depression score in vitamin D group (p=0.03)
Mirzavandi et al.[20]	Iran	2020	Patients with type 2 diabetes mellitus and vitamin D deficiency, 30 – 60 years old	50 (Male 15, Female 35)	RCT	200,000 IU vitamin D injection at week 0 and week 4	none	8 weeks	Beck Depression Inventory (BDI II) scale	No significant difference in depression score between groups

In **Table 2**, we summarized the characteristics of the four RCTs. The samples ranged from 50 to 68 subjects and the mean sample size was 57.25. A total of 229 patients within the studies were evaluated. Among all subjects, 85 were men and 144 were women. The age of subjects included in these studies ranged from 30 to 85 years old, with a mean age of 52.7 in the experimental group and 54 in the placebo group. All of them were diagnosed with type 2 diabetes mellitus based on the American Diabetes Association or World Health Organization criteria. Only subjects in the study by Raygan et al. [15] had a comorbidity of coronary heart disease. The dose of vitamin D used ranged from 4000 IU to 200000 IU. The extracted scales used to measure depression in the selected studies included the BDI [17,16,20] and DASS -21.[19] For the post-intervention score, means and SD values were calculated from medians and ranges. Two of the studies reported no adverse events, while the other two did not report anything regarding side effects.

Assessment of Risk of Bias

The risk of bias of included RCTs was assessed using the Cochrane risk- of-bias tool for randomized trial (RoB 2).[21] Two reviewers independently assessed the risk of bias of the included RCTs using the technique developed by Higgins and Green in the Cochrane Handbook for Systematic Reviews of Interventions.[12] Selection bias (random sequence generation and concealment of allocation), performance bias (blinding of subjects and personnel), detection bias (blinding of researchers conducting outcome assessments), attrition bias (incomplete outcome data), reporting bias (selective reporting), and other sources of bias were all assessed. A judgement of 'low risk' of bias, 'high risk' of bias, or 'unclear risk' of bias was made for each domain. Any disagreements were resolved by discussion or by involving a third reviewer until consensus was reached (**Figure 2** and **3**). There were insufficient numbers of included studies to appropriately assess a funnel plot or more advanced regression-based assessments; hence, publication bias was not assessed.[22]

Outcome Evaluation and Meta-Analysis

There was a statistically significant improvement in depressive symptoms in the vitamin D supplementation group as compared to the control group (95% confidence interval: -0.70 to - 0.08, p = 0.01). Only three studies were included because one study did not report the mean and SD, so it was not estimable.[18] Statistical heterogeneity was assessed using the chi-squared test and I-squared values. Our meta-analysis showed 0% ≤ I-squared ≤50 % low or no heterogeneity.

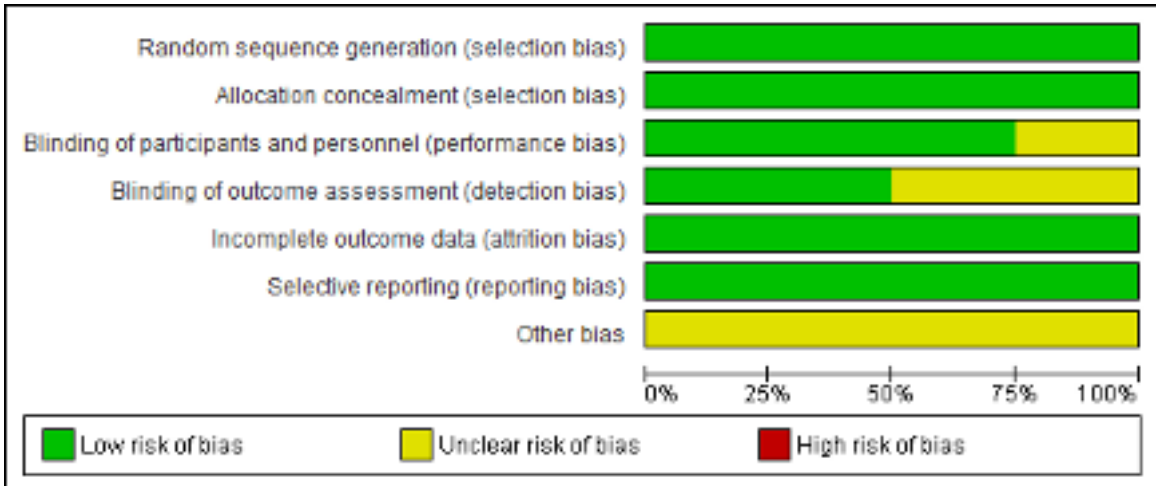

Figure 2. Risk of bias graph: review author's judgements about each risk of bias item presented as percentages across all included studies

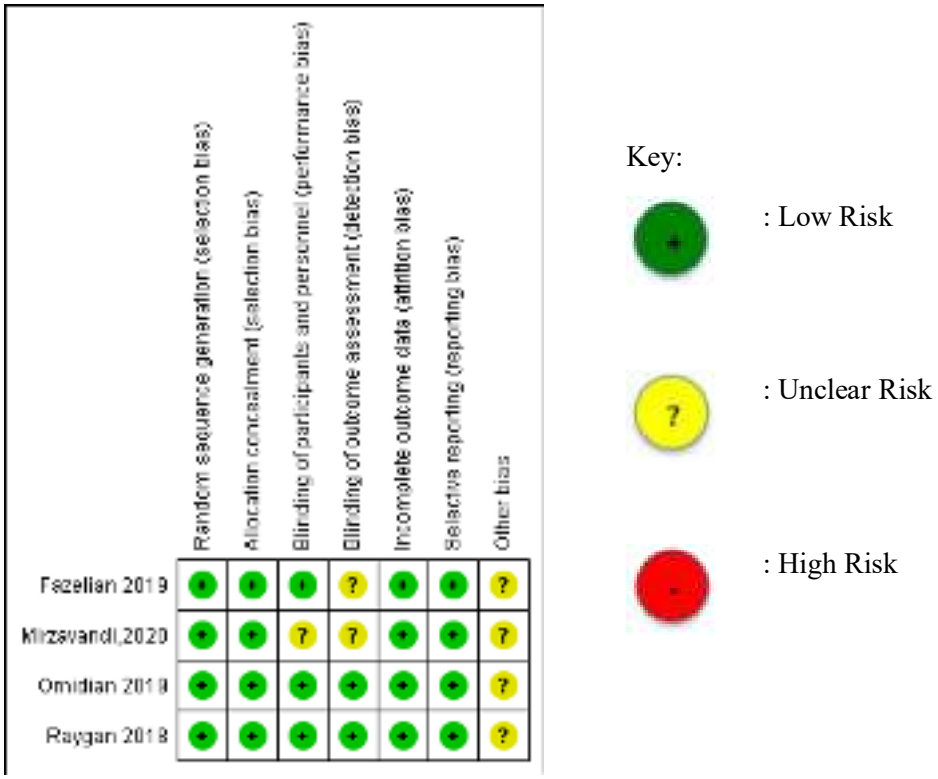

Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study

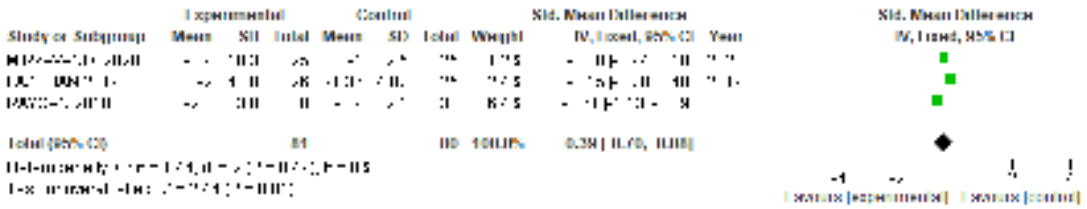

Figure 4. Meta-analysis of Randomised Controlled Trials of the Effect of Vitamin D Supplementation in Improving Depressive Symptoms in Patients with Type 2 Diabetes Mellitus.

Confidence in Cumulative Evidence

For the effectiveness of treatment, the confidence in the cumulative evidence was considered moderate (Table 3). The possibility that the actual effect may be significantly different from the estimated effect reduced our confidence in the efficacy of the treatment effect estimate.

Potential limitations, such as even rates and a small sample size, failure to assess compliance, and a non- representative sample are likely to reduce confidence in the effect estimate. In addition, we found disparities in treatment effect estimates, unexplained heterogeneity in subgroup analyses, and minimal overlap of confidence ranges (CI). Some of the findings were consistent with substantial benefit and substantial harm, implying that imprecision should be rated lower.

DISCUSSION

In this meta-analysis of 3 randomized controlled trials with a total of 161 subjects, vitamin D supplementation was significantly associated with improving depressive symptoms ($p = 0.01$). All four studies analysed have strong points and were conducted with a high level of evidence, adequate duration of therapy, multiple disguises, proper randomization using computer-generated randomization, similarities between groups during baseline, and similarities in the

Table 3. The three studies included in the meta-analysis were graded using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) evidence profile for the role of vitamin D supplementation for depression symptoms.

Certainty Assessment							Effect		
Number of studies	Study Design	Risk of Bias	Inconsistency	Indirectness	Imprecision	Other Considerations	Relative (95%CI)	Certainty	Importance
Outcome: Depressive symptoms score									
3	RCT	Not serious	Not serious	Not serious	Not serious	None	- 0,70 - 0,08	Moderate	Important

given therapy (high dose of vitamin D). All studies had similar sample size of around 50–68 patients. There were a number of patients who were not available for a follow-up, but none were attributable to the side effects or complications of the regimen given. The number of samples was low due to restrictive inclusion and exclusion criteria applied to these four studies.

Lower serum vitamin D levels have been linked to an increasing risk of depression. Depressive symptoms in those with very low vitamin D levels could be alleviated with vitamin D supplementation.[23-25] Vitamin D supplementation increased the well-being in three pilot studies, and symptoms of depression were reduced when high doses of vitamin D3 (100 mcg per day) were given for 1 to 3 months.[23]

The studies by Raygan et al.[17] and Omidian et al.[18] showed that vitamin D supplementation led to significant improvement in depressive symptoms compared to placebo. In contrast, the study by Fazelian et al.[19] and Mirzavandi et al.[20] resulted in no statistically significant decrement of depression scores between groups. Nevertheless, the study by Mirzavandi et al. used non-experimental groups rather than the placebo group – control groups were not given intervention. This methodology makes the blinding process impossible to carry out, which may lead to an increasing risk of bias.[20] Moreover, the study by Fazelian et al. stated that according to the within-group analysis, patients who had a low serum vitamin D level at baseline had a significant decrement in their depressive symptoms score.[19] Therefore, further research to assess the effect of vitamin D supplementation on the improvement of depressive symptoms in patients with low levels of serum vitamin D is still needed.

The studies results by Omidian et al.[18] and Mirzavandi et al.[20] is highly applicable in type 2 diabetes mellitus patients with depression. Raygan et al.[17] did a study in diabetic patients with the comorbidity of coronary heart disease, while Fazelian et al.[19] did a study on depressed diabetic patients with low, moderate, or severe anxiety disorder. Nevertheless, the generalizability of these studies to a larger population is still questionable since all of the studies were performed in Iran.

The studies by Omidian et al.[18] and Mirzavandi et al.[20] reported that vitamin D supplementation did not show any significant side effects, while Raygan et al.[17] and Fazelian et al.[19] did not report anything about adverse effects. These findings show that the benefits of vitamin D are much greater than the potential losses.

Consistent with the results of our study, other clinical trials that have different population samples also showed that vitamin D supplementation was associated with improvement of depressive symptoms.[26,27] A study by Mozzafari et al. recruited depressed patients with vitamin D deficiency as their subjects and found that after 3 months of injected vitamin D, there was a significant improvement in depressive symptoms.[26] Penckofer et al. reported a significant effect of vitamin D supplementation on depressed diabetic women.[7] A further study by Penckofer et al. in the Sunshine 2 study showed that there was a significant improvement in depressive symptoms in diabetic women over time, regardless of the vitamin D3 dose.[28] Furthermore, Khoraminya et al. reported that

vitamin D supplementation as an adjunctive therapy to an anti- depressant drug was effective.[29] Moreover, two cross-sectional studies have also proved that there is a correlation between low serum vitamin D and depressive symptoms.[30,31] However, there are some observational studies that have found no association between these two variables.[32,33]

In this systematic review of RCTs, the effect of vitamin D supplementation was significant for improvement in depressive symptoms in patients with type 2 diabetes mellitus. Even though one of the RCTs showed no significant effect of vitamin D supplementation, the study did show a possible trend of depressive symptom improvement by giving a vitamin D injection. The decrement of the BDI score was higher in the experimental group (-3.9 10.3) than in the non-experimental group (-1.0 2.5) [20]

Limitations

To the best of our knowledge, this study is the first study to review the effect of vitamin D supplementation on the improvement of depressive symptoms in type 2 diabetic patients. Moreover, this systematic review only included RCTs with a high level of evidence, which ensured less study bias and was more reliable in assessing the effectiveness of medical treatment. In addition, the author also did some grey literature searching, which minimized the plausibility of missing evidence.

The limitations of this study include the questionable generalizability of the findings because of concentrated patient samples, i.e., all studies were conducted in Iran. Furthermore, even though the included articles were focused on the effect of vitamin D supplementation on the improvement of depressive symptoms in type 2 diabetic patients, most of the studies lacked detailed information on the mechanisms of how vitamin D may affect depressive symptoms.

Due to the limitations of the study, the authors provide recommendations as follows: (1) larger patient samples and more varied patient demographics since all available studies were conducted in Iran; (2) more studies that include within-group analysis, especially based on the level of serum vitamin D, are highly recommended to further explore the effect of vitamin D; (3) the addition of more different populations in the next research topic because the existing studies only examine specific populations, namely patients with type 2 diabetes mellitus who do not have complications from diabetes mellitus; and, (4) the dosing and form of vitamin D used should be more standardized for further studies.

CONCLUSION

The results of the systematic review and meta-analysis demonstrated that vitamin D supplementation may improve the depressive symptoms in type 2 diabetic patients.

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CONFLICT OF INTEREST

The authors report no declarations of interest.

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Long-Term Use of Omeprazole: Effect on Haematological and Biochemical Parameters

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ABSTRACT

Background: *Long-term use of proton pump inhibitors (PPIs) is believed to have various potential adverse events. Omeprazole is a part of PPIs most commonly prescribed worldwide; it irreversibly binds to H+-K+ ATPase enzyme system in the gastric parietal cells to reduce secretion of H+ ions into the lumen of stomach. The main objective of the current work is to assess the adverse effects of omeprazole medication on certain haematological and biochemical parameters in patients who were on treatment for one year and more.* ***Methods:*** *We conducted a comparative cross-sectional study between October 2021 and March 2022. A total of 90 participants of both sexes were enrolled in this study, aged between 25-58 years. The participants were categorized into two groups: 40 patients on long-term omeprazole medication (40 mg) as a patients group and 50 healthy subjects as a healthy group who did not use omeprazole. Complete blood count and biochemical parameters were measured for both groups.* ***Results:*** *Patients of a group 1 had remarkable significant reductions in the number of red blood cells (RBCs) ($p<0.001$) and the indices. Omeprazole elevated the cholesterol level ($p<0.001$) and triglyceride ($p<0.001$) as well as low-density lipoprotein ($p<0.01$). However, no impact was found with high-density lipoprotein (HDL) ($p>0.05$). Alkaline phosphatase (ALKP) ($p<0.001$) and aspartate aminotransferase (ASAT) ($p<0.01$) levels were elevated in long-term patients treated with omeprazole. In contrast, no significant change was found in the level of alanine aminotransferase (ALAT) ($p>0.05$). Creatinine level ($p<0.001$) and nitrogen blood urea ($p<0.0001$) were significantly increased in patients group treated with omeprazole medication. The results also showed that group 1 had a high significant decline in serum ferritin ($p<0.0001$), vitamin D3 ($p<0.01$) and calcium levels ($p<0.001$) than that of healthy group.* ***Conclusion:*** *Prolonged use of omeprazole might result in adverse effect on hematological profile, particularly RBCs and their indices leading to develop anemia in patients on this medication. Furthermore, it might result in disturbances in biochemical profile, levels of minerals and vitamins as consequences of affected absorption.*

Keywords: *Omeprazole, blood count, hypocalcemia, vitamin D, kidney function, cholesterol, triglyceride.*

INTRODUCTION

Omeprazole is a member of substituted benzimidazoles class, that inhibits protons pump of the gastric parietal cells.[1] It inhibits gastric secretion by inhibiting the enzyme H+-K+ ATPase that is responsible for gastric acid production.[2] Omeprazole is used to manage and treat several conditions where the gastric acid inhibition can be very beneficial, including gastric ulcers, peptic ulcer, gastroesophageal reflux disease, erosive esophagitis, Zollinger-Ellison syndrome. It is superior to conventional therapies as well as it is used as over the counter drug in uncomplicated heartburn.[3] Side effects are rare when the drug is taken short-term. The probable common side effects include headaches, vomiting or diarrhea stomach upset, and constipation. While the serious side effects are very rare, they include liver problems, joints pain due to subacute cutaneous lupus erythematosus due to long term use and allergic reaction. Other signs of long-term use may include a decrease in the levels of magnesium in the blood after taking omeprazole for more than 3 months. Taking omeprazole for more than a year may increase the chances to develop other side effects such as bone fractures, gastrointestinal infections and vitamin B12 deficiency.[4] Furthermore, it has been found that long-term proton pump inhibitors (PPIs) treatment may affect haematological indices.[5] The change in gastric acidity may also affect the intestinal absorptive ability of microelement nutrients in a way that could result in iron deficiency and a decrease in the concentration of zinc, selenium and copper. Impact of proton pump inhibitors on these important trace elements may reduce their antioxidant activity in the body. However, a previous study has indicated the necessity for further studies about the role of PPI in reducing certain body parameters.

The common therapeutic uses of omeprazole for a wide spectrum of gastric-related health problems, being an over the counter treatment and the development of certain negative health indicators in the patients who were on long-term treatment, is the rationale for the objective of the current work, which is to elucidate the adverse influences of omeprazole medication on certain haematological and biochemical findings in patients who were on treatment for one year and more.

METHODS

Study Population and Plan of Work

A comparative cross-sectional study was conducted between October 2021 and March 2022 in Basrah city, Iraq. A total of 90 participants of both sexes aged between 25-58 years were involved in the study. They were categorized into two groups: 40 patients on long-term omeprazole medication (40 mg), as patients group 1 and 50 individuals as healthy group who were healthy and did not take any medications, including omeprazole.

Patients were received and interviewed in an outpatient clinic and selected according to certain criteria. The omeprazole duration medication was one year and more. The study design was reviewed and approved by the Ethics Committee of the Al-Zahraa College of Medicine, University of Basrah, Iraq. The aim of the research was explained for all participants before enrolling in the study and written consents were obtained. The work complied with the Declaration of Helsinki ethical principles. Initially required information of all participants were obtained using a questionnaire form.

Collection of Samples

5 ml of venous blood were drawn from each participant enrolled in this study, 2 ml of blood was collected in anticoagulated test tube with ethylenediaminetetraacetic acid (EDTA). The rest of blood sample was collected immediately in a gel plain tube in order to prepare serum for performing further tests. Haematological and biochemical profiles were performed in a private laboratory.

Clinical Biochemical Analyzes

Clinical biochemical tests were done including lipid profile, alkaline phosphatase (ALKP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), total bilirubin (TBIL), direct (DBIL) and indirect bilirubin (IDBIL) as well as urea and creatinine. Serum samples were analysed by fully automated chemistry analyser smart 150 (Gento TEK, USA).

Determination of Vitamin D3 Levels

Sera from total patients and healthy control subjects were investigated to measure levels of serum circulating 25-hydroxycholecalciferol(25[OH]D) using mini VIDAS (Biomerieux, France).

Quantitative Measurement of Ferritin

Ferritin level was estimated for all participants by an enzyme-linked immunosorbent serologic assay (ELISA) in accordance with instructions provided by the manufacturer (Pointe Scientific Inc, USA).

Serum Calcium Assay

The serum levels of total calcium were measured for both groups using high resolution inductively coupled plasma mass spectrometry ICP-MS (Element 2, Thermo Scientific, Germany). Normal range of serum calcium levels in adult is 8.5-10.5 mg/dL.

Complete Blood Count (CBC) Test

Haematological indices were measured using 2 ml anti-coagulated blood, including red blood cells (RBCs) count, haemoglobin (HGB) concentration (g/dL), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), total count and differential count (neutrophils, monocytes and lymphocytes) of white blood cells, platelets (PLTs) count and mean platelet volume (MPV). Blood sample was immediately analysed after collection by Emerald Haematology System (Abbott, USA).

Statistical Analyzes

Collected data were inputted in an Excel spreadsheet for all participants. Then, differences between the two groups were assessed using an unpaired t-test. This comparison was carried out through GraphPad Software (Version 8, Software Inc, United States). P-values (<0.05) were used to indicate statistical significance. All values in the tables are presented as mean±SD.

RESULTS

Analysis of data to compare between the two groups of the study patients on long-term omeprazole and healthy group revealed several variations in haematological indices, despite no significant variation in mean age, gender percentage, body mass index (BMI) and healthy status as clarified in **Table 1**.

Statistical analyses have found no variations ($p>0.05$) in WBC count (7.86±2.67 vs. 8.96±2.79 $\times 10^9$/L), neutrophils % (57.04±12.84 vs. 52.53±13.61), monocytes % (9.11±1.76 vs. 8.83±2.34) and lymphocytes % (33.39±11.57 vs. 37.23±12.08) between patients group and healthy group, as illustrated in **Table 2**. On the other hand, significant reductions were seen in red blood cell count (4.15±0.76 vs. 4.55±0.92 ×1012/L; $p<0.001$); HGB concentration (10.13±1.95 vs. 12.38±1.72 g/dL, $p<0.001$); MCV (78.71±10.29 vs. 85.83±12.75 fl, $p<0.01$); MCH (24.39±2.80 vs. 27.32±2.48 pg, $p<0.001$); and MCHC (30.79±2.90 vs. 31.98±1.70 g/dL, $p<0.05$) in patients group compared to healthy

Table 1. General characteristics of the studied groups.

Parameters	Patients group (N = 40)	Healthy group (N = 50)	P-value
Females%	17 (42.5%)	22 (44%)	NS
Males%	23 (57.5%)	28 (56%)	
Age (years)	(26-58)	(25-55)	
(Mean ± SD)	42.05±9.57	40.75±8.03	NS
Weight (Kg)	88.14±2.07	85.71±2.03	NS
BMI (Kg/m^2)	31.25±5.91	29.62±6.55	NS
Comorbidities			
- Diabetes mellitus		N/A	
- Hypertension		N/A	
- Hyperlipidemia		N/A	
- Other diseases		N/A	

*Significance at level <0.05. NS: non-significant differences.

group. The t-test also did not show any significant changes (P>0.05) in platelets count and MPV values between patients group and healthy group (304.40±70.74 vs. 272.90±83.67 $\times 10^9$/L; 9.27±1.30 vs. 8.98±1.27 fl, respectively).

Regarding biochemical parameters, it has been found that the long-term use could exert a variation in certain parameters. In serum cholesterol levels, we found a significant elevation (p<0.001) in the levels of total cholesterol in patients group (225.45±23.48 mg/dL) in comparison to healthy group (182.69±39.15 mg/dL), alongside significant elevation (p<0.001) of triglyceride levels in patients group (215.46±35.98 mg/dL) compared to healthy group (158.81±48.16 mg/dL). Analysis of lipoprotein parameters such as direct low-density lipoprotein (DLDL) and very low-density lipoprotein (vLDL) showed significant increases (p<0.01) in patients group (152.60±34.82; 47.96±14.73 mg/dL), compared to healthy group (120.92±24.46; 33.20±8.62 mg/dL). However, there were no significant differences (p>0.05) between the two groups in high-density lipoprotein (HDL), as seen in **Table 3**.

Table 2. Comparison of haematological parameters between patients group and healthy group.

Parameters	Patients group (N = 40)	Healthy group (N = 50)	P-value
WBCs ($\times 10^9$/L)	7.86±2.67	8.96±2.79	NS
Neutrophils (%)	57.04±12.84	52.53±13.61	NS
Monocytes (%)	9.11±1.76	8.83±2.34	NS
Lymphocytes (%)	33.39±11.57	37.23±12.08	NS
RBCs ($\times 10^{12}$/L)	4.15±0.76	4.55±0.92	<0.001*
HGB (g/dL)	10.13±1.95	12.38±1.72	<0.001*
MCV (fl)	78.71±10.29	85.83±12.75	<0.01*
MCH (pg)	24.39±2.80	27.32±2.48	<0.001*
MCHC (g/dL)	30.79±2.90	31.98±1.70	<0.05*
PLTs ($\times 10^9$/L)	304.40±70.74	272.90±83.67	NS
MPV (fl)	9.27±1.30	8.98±1.27	NS

*Significance at level <0.05. Data are presented as mean±SD. NS: non-significant differences.

Table 3. Comparison of biochemical parameters between patients group and healthy group.

Parameters	Patients group (N = 40)	Healthy group (N = 50)	P-value
Cholesterol (mg/dL)	225.45±23.48	182.69±39.15	<0.001*
Triglyceride (mg/dL)	215.46±35.98	158.81±48.16	<0.001*
HDL (mg/dL)	46.10±14.73	50.57±19.05	NS
DLDL (mg/dL)	152.60±34.82	120.92±24.46	<0.01*
vLDL (mg/dL)	47.96±14.73	33.20±8.62	<0.01*
ALKP (U/L)	87.23±8.37	72.47±15.89	<0.001*
ALAT (U/L)	27.62±14.76	23.54±26.87	NS
ASAT (U/L)	23.32±4.74	14.73±6.29	<0.01*
Ferritin (mg/dL)	18.19±16.19	69.85±53.70	<0.0001*
Creatinine (mg/dL)	1.39±0.45	0.78±0.25	<0.001*
Urea (mg/dL)	45.75±14.22	26.27±14.77	<0.0001*
Vitamin D3 (ng/ml)	17.30±11.14	25.02±13.47	<0.01*
S. Calcium (mg/dL)	7.81±0.86	9.30±1.78	<0.001*
TBIL (mg/dL)	1.23±1.17	0.89±0.32	NS
DBIL (mg/dL)	0.35±1.00	0.26±0.83	NS
IDBIL (mg/dL)	0.47±0.23	0.45±1.62	NS

*Significance at level <0.05. Data are presented as mean±SD. HDL: high-density lipoprotein; DLDL: direct low-density lipoprotein; vLDL, very low-density lipoprotein; S. calcium: serum calcium; NS: non-significant differences.

Indicators of liver functions were also compared between the groups (**Table 3**). Significant increases in ALKP ($p<0.001$) and ASAT ($p<0.01$) levels were detected in patients group (87.23±8.37; 23.32±4.74 U/L) compared to healthy group (72.47±15.89, 14.73±6.29 U/L). Whereas, no significant change ($p>0.05$) in ALAT level (U/L) was found.

Renal function parameters were compared between patient and healthy groups. As shown in **Table 3**, creatinine level was significantly increased ($p<0.001$) in patients group (1.39±0.45 mg/dL) in comparison to healthy group (0.78±0.25 mg/dL). Likewise, significant differences ($p<0.0001$) were observed in levels of blood urea between patients group (45.75±14.22 mg/dL) and healthy group (26.27±14.77 mg/dL).

Moreover, patients group had significantly lower ($p<0.0001$) serum levels of ferritin (18.19±16.19 mg/dL) than healthy group (69.85±53.70 mg/dL). Serum calcium concentration in long-term patients group (7.81±0.86 mg/dL) was lower ($p<0.001$) than healthy group (9.30±1.78 mg/dL). Similarly, a significant decline in the levels of vitamin D3 ($p<0.01$) was found in patients group (17.30±11.14 ng/ml) compared to healthy group (25.02±13.47 ng/ml) as illustrated in **Table 3**.

No significant changes were noticed between the groups in the levels of TBIL, DBIL and IDBIL (1.23±1.17 vs. 0.89±0.32 mg/dL, $p>0.05$; 0.35±1.00 vs. 0.26±0.83 mg/dL, $p>0.05$; 0.47±0.23 vs. 0.45±1.62 mg/dL, $p>0.05$, respectively) (**Table 3**).

DISCUSSION

Over recent years, the focusing on the adverse effects of using PPI medications for long-term therapy has gained increasing concerns. Omeprazole is commonly used for treating multiple acid-dependent gastrointestinal disorders. The present study was planned to detect the adverse effects of prolonged use of omeprazole on haematological and biochemical parameters. The result of this study demonstrated that omeprazole might interfere with the blood profile in patients with long-term treatment.

In order to reveal if long term omeprazole use may exert an adverse effect on hematological indices or not, blood test was performed for 40 outpatients who were on omeprazole medication and visited a private clinic. We found that the means of RBCs and HGB in these patients as well as other RBC indices were significantly lower than those in healthy group. These findings are similar to what were reported by previous studies.[6,7]

A retrospective cohort study has examined the impact of PPIs use on haematological indices among individual patients who received PPI medications for over 1 year. The study revealed a significant reduction in values of haemoglobin, haematocrit and mean corpuscular volume and suggested that the chronic use of PPIs may cause iron mineral deficiency, long-term therapy may reduce absorption of non-heme iron.[8] Another study conducted in a group of patients using PPI medications for long-term period, Kaczmarczyk et al. showed that using of PPIs might cause a reduction in the number of RBCs and levels of HGB and some serum micronutrients. This suggested that prolonged use of PPIs might give rise to iron deficiency anemia.[7] Iron absorption usually occurs in the proximal small intestine, and this process is facilitated by gastric acid secretion which is necessary to convert the iron mineral from ferric state to ferrous state.[9] Two biological mechanisms have been put forward that chronic use of PPIs causes anemia. One of these mechanisms is the suppression of absorption of iron in the small intestine is due to inhibition of H+-K+ ATPase and increase the pH of stomach.[10] The another mechanism contributes in the development of anemia is the suppression of absorption of vitamin B12, food-bound vitamin B12 is liberated in the acidic medium and is bound to the glycoprotein haptocorrin for readily absorption in the ileum.[11] Proton pump inhibiters are powerful agents that inhibit production of gastric acid, a reduction in gastric acid production as a result of PPIs use may influence the absorption of minerals and vitamins in the gastrointestinal tract. A consequence of iron and vitamin B12 deficiency is anemia.[6] Means of MCV, MCH and MCHC were low in patients group in compare to healthy group. It is likely that these patients developed iron deficiency anemia because omeprazole may suppress secretion of gastric acid and thence

inhibit absorption of iron minerals.

Numbers of white blood cells in patients group were not significantly affected by chronic use of omeprazole medication in comparison to healthy group. Omeprazole medication demonstrated a non-significant reduction in the number of WBC. Although, this result differed from some published studies,[7,12] it was consistent with those of other studies.[13,14] We also found no statistical variation between the groups in the numbers of platelets. Our results did not show any reduction in the numbers of the platelets due to use of omeprazole. Literature data regarding the influence of PPIs on platelet numbers are conflicting. The present findings seem to be consistent with other researches which found no differences in the numbers of platelets between PPIs user and control group as well as having platelet counts which were within normal range in PPIs user.[7,13] On the other hand, only one case report has described the role of omeprazole medication in inducing thrombocytopenia.[15] A few number of studies over the past two decades have demonstrated thrombocytopenia with various types of PPIs therapy.[6,16,17]

Suppression of gastric acid secretion is linked with alterations in the digestion process of dietary lipids. It has been demonstrated that using omeprazole result in increased lipid absorption. Process of lipid absorption is associated with the underlying mechanism gastric acidity suppression resulting from using omeprazole, thereby increasing the lipolytic activities of gastric juices that lead to increased absorption of lipid in the small intestine.[18] Proton pump inhibitors might be involved in metabolism of cholesterol.[19,20] This fact may explain the findings of the current study, cholesterol level is significantly increased in patients with long-term use of omeprazole medication compared to healthy group, alongside significant elevation in triglyceride plasma level and LDL in patients group. These results are consistent with what were reported by other researches.[21-24]

Plasma concentration of minerals must be maintained within stable range, so that cellular metabolism processes can work properly. Our results showed that plasma concentration of calcium decreased in patients with long-term treatment of omeprazole. This finding is consistent with reduction in intestinal calcium absorption.[25-27] Acidic environment is necessary for absorption of intestinal calcium mineral, as this process is inhibited by omeprazole intake via blocking the gastric H+-K+ ATPase enzyme system that is located in the apical membrane of stomach parietal cells, which cause achlorhydia. Maintenance low gastric acid reduces lipolysis which is essential for calcium absorption in the gastrointestinal region and hence reduced absorption of calcium mineral in the gut causing hypocalcemia. Additionally, dietary protein increases the intestinal calcium solubility and absorption efficiency.[28,29] Hypocalcemia possibly mediates cardiovascular adverse events of omeprazole. It has been shown that hypocalcemia was observed in patients with long-term treatment of PPI.[30,31] It may cause life-threating arrhythmias and heart failure. Hypocalcemia is usually accompanied with hypomagnesemia and both these mineral abnormalities can give rise to cardiovascular instability.[32,33]

Measurement of liver function biomarkers revealed a marked raised in serum ASAT and ALKP levels in patients group compared to healthy group, with no significant changes in ALAT and bilirubin levels, visible in **Table 3**. Aminotransferases and alkaline phosphatase are enzymes that exist primarily in the hepatic parenchymal cell. Increased levels of these enzymes in the bloodstream are indicators of tissue damage of the liver.[34] A case study illustrated that liver clinical markers including serum level of ASAT, ALAT and γ-glutamyl transferase of old age patient suffering from gastroesophageal reflux disease receiving omeprazole (20 mg) and ranitidine were increased. However, levels of these enzymes were decreased and returned to normal values after cessation of omeprazole and replaced by herbal products as well as regulation of diet.[35] Recently, effect of PPIs treatment on possible complication and prognosis in liver cirrhosis patient without acute-on chronic liver failure has been investigated. Each of ASAT and ALAT significantly decreased in patients with liver cirrhosis in comparison to the group who did not receive PPIs. Also, there was no significant

change in bilirubin level between these two groups.[14] Although, there were significant changes in the levels of ASAT and ALKP between patients group and healthy group, these changes in the concentrations of these enzymes were within normal limits.

In the current study, significant elevations in serum creatinine and blood urea concentrations in patients group were observed in comparison to the healthy group. Similar findings were observed in previous researches as well. Deterioration of kidney function tests was demonstrated in users of PPIs compared to nonusers with marked elevation of serum creatinine and blood urea levels.[36,37] Decreased serum creatinine clearance is not associated with H2-receptor blockers and other PPI nonusers.[37] Despite of the results, these clinical markers are not optimal for detecting kidney diseases, as they are often used to find out whether patients have developed kidney diseases or not. Reduction of glomerular filtration rate leads to accumulation of nitrogen waste products in circulation, evidenced by abnormal increase in serum creatinine and blood urea levels.[38] The precise mechanisms between PPIs and adverse kidney outcomes are unclear.[37] Our results contradict those of Mélo and colleagues which found that level of serum creatinine did not change, while blood urea level was decreased in group treated with omeprazole compared with control group.[21] Omeprazole may be associated with development of kidney diseases by increasing levels of serum creatinine and blood urea.

We found a significant decline in vitamin D3 in patients group compared to the healthy group. Interestingly, this result is the first finding demonstrating the role of long term omeprazole use in causing vitamin D deficiency. It is well known that vitamin D plays a significant role in homeostasis of calcium through regulating calcium absorption from the gastrointestinal tract, therefore, it maintains serum level of calcium within normal range.[39] It has been found that vitamin D insufficiency could contribute to the development of secondary hyperparathyroidism, osteoporosis, and in elderly, reduced muscle strength. This category of people is more likely to be at risk of bone fracture due to reduced mineral density.[40] Although, the main mechanism underlying the relation between long-term use of PPIs and increased of bone fracture risk is still unclear, several studies have focused on two possible mechanisms. One of these mechanisms focuses on serum calcium homeostasis. Calcium ion is insoluble in an alkaline environment. Acidic pH is mandatory for dissolution of calcium salts to be absorbable in the small intestine.[41] Blockage of H+-K+ ATPase by PPIs renders the stomach parietal cells incapable to secrete gastric acid thereby increasing risk of bone fractures.[42] Moreover, hypergasterinemia induced by PPIs can give rise to hyperparathyroidism, and consequently, increased rate of bone resorption.[43,44] The other possible mechanism of clinical fracture induced by using PPIs focuses on the cells of the bone, particularly the osteoclasts.[45] PPIs directly affect metabolism of bone via inhibition of vacuolar H+-ATPase, specific proton pumps that are located on the cell membrane of osteoclasts.[46] These pumps are responsible to create acidic environment for bone resorption. Bone matrix resorption occurs at the convoluted ruffled border membrane of osteoclasts by lowering the pH.[47] It has been indicated by a previous clinical article about the necessity of calcium and vitamin D intake by elderly who were treated with long-term PPIs, especially with high-doses.[48]

We also found a significant reduction in the levels of serum ferritin in patients group treated with omeprazole medication in comparison to healthy group. This finding is matched with those observed in earlier studies. In an open label prospective study on 250 adult participants, administration of PPIs (omeprazole, esomeprazole, lansoprazole or pantoprazole) for one year resulted in significant reduction in iron body stores (levels of ferritin).[49] On the other hand, in an early study, it was reported that serum ferritin levels were decreased in 3 of 34 patients with esophageal reflux due to use of omeprazole medication continuously over a long-term period. The study suggests that ferritin shortage seldom occurs, even when using of PPIs for long-term period.[50] The results may indicate that decreased absorption of iron in the gastrointestinal tract is due to use omeprazole medication, therefore, it

may reduce levels of serum ferritin and storage of iron as ferritin in the body tissues. Low levels of serum ferritin may be clinically indicative of iron deficiency anemia.

Although the study has successfully demonstrated the impact of long-term use of omeprazole on blood parameters, it has certain limitations in terms of lack of information particularly the relation between PPIs use and vitamin D. Another limitation is the relatively small number of the enrolled patients. Additionally, levels of serum iron were not investigated, a biomarker of iron deficiency which might have also confirm the results of the current study beside measurement of serum ferritin levels.

CONCLUSION

In this study, the objective was to assess the effect of long term omeprazole medication on blood parameters. Omeprazole is very effective in controling gastric acid secretion. Accumulating data suggest that long-term use of omeprazole may lead to a reduction in the numbers of circulating RBCs and their indices, ultimately leading to anemia. We have demonstrated for the first time that long-term use of omeprazole causes vitamin D deficiency. Low level of vitamin D is due to inhibited production of gastric acid which is necessary for calcium mineral absorption, consequently resulting in hypocalcemia. Both hypocalcemia and hypomagnesemia may affect the cardiovascular system, therefore, levels of serum magnesium should also be measured to evaluate any abnormality in the serum mineral levels and their relation with cardiovascular health. Omeprazole may be associated with development of kidney functional disorders, therefore, physicians should be cautious when prescribing PPIs because of their adverse effects. A further study with higher number of enrolled patients could assess the various long-term adverse effects of omeprazole medication on the organ systems by performing comprehensive blood and biochemical tests.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this work.

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The Profile of COVID-19 in Patients with Autoimmune Disease: A Case Series

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ABSTRACT

Autoimmune diseases are known to be a risk factor for severe COVID-19 infection. This is the first case series of patients with autoimmune disease suffering from COVID-19 infection in Jakarta, Indonesia. There were 12 confirmed cases of COVID-19 infection in autoimmune patients from March 2020 until February 2021. We select 5 patients in this case series. Three of them had systemic lupus erythematous (SLE), one of them had rheumatoid arthritis, and one of them had ankylosing spondylitis. Three of them had high BSR Risk Stratification. Most of them had used daily steroid therapy. Fatigue, abdominal pain, diarrhea, and cough were the common symptoms found. None of the patients were admitted to ICU, used mechanical ventilators, and all of them survived. Most of the patients were prescribed anti-coagulant therapy. This first comprehensive case series can provide valuable information regarding the clinical characteristics of COVID-19 infection in the Indonesian autoimmune disorder patient population.

Keywords*: autoimmune diseases, COVID-19, case series, Indonesia.*

INTRODUCTION

Coronavirus Disease-19 (COVID-19) is a new global pandemic that started in Wuhan, China, at the end of 2019. It is caused by a new virus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The virus is transmitted via droplets, and its spike protein (protein S) can infect the respiratory cells as it has Angiotensin-converting enzyme-2 (ACE-2) receptors. In addition to causing respiratory symptoms, it can also manifest in other organ systems, for example kidney injury, heart damage, and coagulation in the blood vessels. Based on the latest number in Indonesia, there are more than 1 million cases of COVID-19 infection with mortality cases over

25,000 (2.9% mortality rate).[2] The spectrum of severity in COVID-19 is variable, ranging from asymptomatic, mild symptoms, moderate symptoms, and life-threatening conditions such as acute respiratory distress syndrome (ARDS) and multi-system organ failure. Patients with chronic comorbid conditions have a higher risk of morbidity and mortality of COVID-19.[3] The severity of COVID-19 is related to a combination between uncontrolled inflammation and dysfunctional lymphocyte response.[4]

Autoimmune disease is a known risk factor for severe COVID-19 infection as patients usually receive immunosuppressive therapy to control the disease.[5] Autoimmune disease occurs when the immune system attacks the body's cells due to immunologic tolerance breakdown towards autoreactive immunity. It is a multifactorial disease that is usually related to genetics, environments, and infections. Common examples of autoimmune disease include systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, etc.[6] Steroids are currently used in treating COVID-19 infection, and it may have a dual effect on the patient. Steroids can either exacerbate the infection or protect the host from cytokine storms resulting from the viral clearance in the early stages.[7] Current prevalence of the autoimmune disease in Indonesia is expected to be more than 2000 patients, and the number is increasing annually, yet the exact number is still unknown.[8]

To our knowledge, this is the first comprehensive case series in Indonesia reporting the characteristics and clinical course of COVID-19 in patients with autoimmune diseases.

CASE ILLUSTRATION

Cases were collected retrospectively from medical records between March 2020 until February 2021. Cases were confirmed by RT-PCR (qRT-PCR) detection of SARS-CoV-2 RNA through analysis of nasopharyngeal swabs. The severity of COVID-19 infection was classified based on the Indonesia National Guideline of COVID-19.[9] The specific diagnosis of autoimmune diseases was made based on the revised classification criteria of the American College of Rheumatology. British Society of Rheumatology (BSR)- Risk Stratification was used to know whether the patient had a low-moderate risk of COVID-19 infection (score less than three) or clinically extremely vulnerable (score three or more).[10] Acute Respiratory Distress Syndrome (ARDS) was diagnosed using Berlin's criteria.[11] Data was analyzed using SPSS software. The results were presented in percentage, mean and standard deviation if data distribution was normal or median and interquartile range if data were not normally distributed.

Ethical approval was obtained from Director of Kramat 128 Hospital. Informed consent was obtained from all patients for being included in the study. Proxy consent occurs when an individual was provided with the legal right to make decision on behalf of another, for example in patient with altered mental status. The patients also give written inform consent for publishing their clinical records.

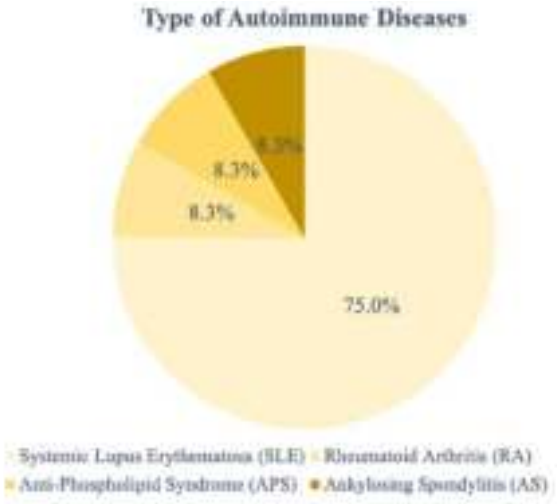

Figure 1. Type of autoimmune diseases

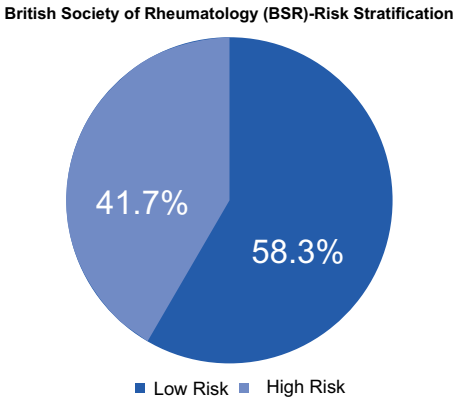

Figure 2. British Society of Rheumatology (BSR) – risk stratification.

There were 60 autoimmune patients who were hospitalized between March 2020 until February 2021, with 12 confirmed cases of COVID-19 infection. Most of the patients (75.0%) had systemic lupus erythematosus (SLE) (**Figure 1**).

Based on the British Society of Rheumatology (BSR)- Risk Stratification, more than 50% of the patients had low-moderate risk (**Figure 2**).

8 out of 12 patients were categorized as moderate COVID-19 infection, three as mild COVID-19 infection, and only one patient without any symptoms (**Figure 3**).

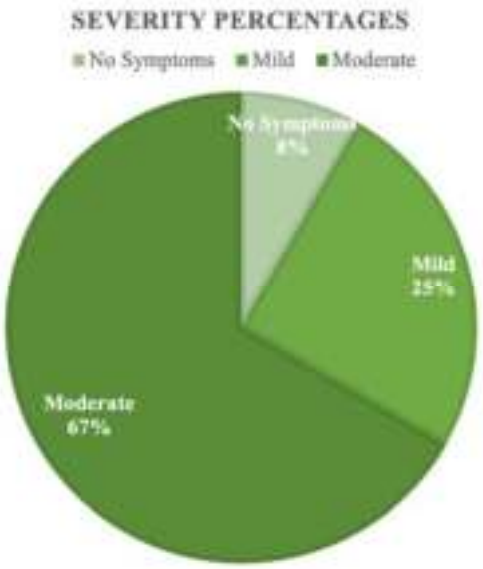

Figure 3. Severity percentages.

We select five COVID-19 patients with autoimmune diseases included in this case series. Three of them had systemic lupus erythematous as the base autoimmune diseases, one with rheumatoid arthritis, and one with ankylosing spondylosis. **(Table 1)**

Case 1

Female, 46 years old, had Systemic Lupus Erythematous (SLE) with hypertension and hyperthyroidism came into the ER with moderate COVID-19 severity. She used Methylprednisolone 2x4 mg daily with BSR Risk Stratification categories of high. She had symptoms such as fever, chills, muscle pain, joint pain, abdominal pain, diarrhea, nausea, cough, fatigue, and dyspneu. Upon hospital admission, she was hypertensive (177/96 mmHg), tachycardic (107x/minute), and feverish (37.6^{o}C). She was given oxygen supplementation of 5 liter/minute using nasal cannule and her oxygen saturation was 98%. She had body mass index category of overweight (22.1 kg/m2). She had absolute lymphocyte count (ALC) of 1317/uL with Neutrophil-Lymphocyte Ratio (NLR) of 1.93. She had normal D-dimer result with ground glass opacity as found in the thorax CT-Scan. She was treated with vitamins, intravenous Remdesivir, and Heparin prophylaxis dose (2x5000U).

Case 2

Female, 46 years old, had Rheumatoid Arthritis (RA) with hypertension, heart disease, hyperthyroidism, and chronic kidney disease came into the ER with moderate COVID-19 severity. She used Methylprednisolone 1x4 mg daily with Sulfasalazine three times a day. She had low BSR Risk Stratification. She

Table 1. Patient's characteristics.

Patient No.	Sex	Age	Auto-immune Disease	Comorbidities	Autoimmune Medication	Prednisolone Equal Dose (mg/ day)	BSR Risk Stratification	Charlson Comorbidity Index	Length of Stay	COVID Severity
1	F	46	SLE	HT, Thyroid	Glucocorticoid	10	High	0	7	Moderate
2	F	46	RA	HT. Heart Disease, Thyroid, CKD	Glucocorticoid, Sulfasalazine	5	Low	5	10	Moderate
3	F	36	SLE	Heart disease, Tuberculosis	Glucocorticoid	10	High	2	15	Moderate
4	M	33	AS	HT, Obesity	None	N/A	Low	0	0	Mild
5	F	27	SLE	Lymphadenitis TB	Glucocorticoid	20	High	0	8	Mild

had symptoms such as sore throat, joint pain, abdominal pain, diarrhea, cough, fatigue, and dyspneu. Upon hospital admission, she was normotensive (122/75 mmHg), bradycardic (58x/minute), and her oxygen saturation was 98% in room air. She had body mass index category of overweight (22 kg/m^2). She had absolute lymphocyte count (ALC) of 1644/uL with Neutrophil-Lymphocyte Ratio (NLR) of 1.15. She had normal D-dimer result with normal thorax x-ray. She was treated with vitamins and Heparin prophylaxis dose (2x5000 U).

Case 3

Female, 36 years old had Systemic Lupus Erythematous (SLE) with congestive heart failure with (Ejection Fraction of 55%), and hypercoagulable state came into the ER with moderate COVID-19 severity. She used Methylprednisolone 2x4 mg, Aspirin 1x80 mg, Nitroglycerine 1x2.5 mg, Furosemide 1x40 mg, Spironolactone 1x100 mg, and Diltiazem 1x200 mg. She had high BSR Risk Stratification. She had symptoms such as fever, headache, fatigue, ageusia, abdominal pain, nausea, vomiting, and diarrhea. Upon hospital admission, she was normotensive (120/80 mmHg), heart rate was 100x/minute, and her oxygen saturation was 97% on room air. She had body mass index category of obese (28 kg/m^2). She had absolute lymphocyte count (ALC) of 2034/uL with Neutrophil-Lymphocyte Ratio (NLR) of 2.03. She had increased D-Dimer of 1400 with bilateral infiltrate on the thorax x-ray. She was treated with vitamins and Heparin prophylaxis dose (2x5000 U).

Case 4

Male, 33 years old had ankylosing spondylitis (AS) with hypertension came into the ER with mild COVID-19 severity. He did not use any routine medications. He had low BSR Risk Stratification. He had symptoms of cough, runny nose, fatigue, muscle pain, joint pain, and diarrhea. Upon hospital admission, he was hypertensive (149/94 mmHg), heart rate was 72x/minute, and her oxygen saturation was 99% on room air. He had body mass index category of obese (28 kg/m^2). He had absolute lymphocyte count (ALC) of 3400.3/uL with Neutrophil-Lymphocyte Ratio (NLR) of 1.43. He had normal D-dimer result with normal thorax x-ray. He was treated with vitamins and sent to do self-isolation.

Case 5

Female, 27 years old had Systemic Lupus Erythematous (SLE) with tuberculosis of the glands came into the ER with mild COVID-19 severity. She used Methylprednisolone 16 mg daily. She had high BSR Risk Stratification. She had symptoms of fever, headache, runny nose, anosmia, ageusia, fatigue, and joint pain. Upon hospital admission, she was normotensive (107/68 mmHg), heart rate was 76x/minute, and her oxygen saturation was 99% on room air. She had body mass index category of overweight (22 kg/m^2). She had absolute lymphocyte count (ALC) of 2125.2/uL with Neutrophil-Lymphocyte Ratio (NLR) of 7.64. She had normal D-dimer result with normal thorax x-ray. She was treated with vitamins and Heparin prophylaxis dose (2x5000 U). She had increased D-Dimer (800) with normal thorax x-ray. She was treated with Methylprednisolone high dose (2x125 mg) for 2 days due to increased disease activity and Heparin continuous drip 10.000 unit/24 hour.

DISCUSSION

There have been several studies investigating the impact of the presence of autoimmune diseases in COVID-19. Below we highlighted several studies and correlating them with the results from our case series.

Risk of Developing COVID-19

Autoimmune diseases have been postulated as risk factors for developing infection through the generation of autoantibody to cytokines. For example, autoantibody to IFN-γ is associated with an increased risk of tuberculosis infection.[12] Autoimmune disease, primarily systemic connective tissue disease such as systemic lupus erythematosus (SLE), was associated with delayed type 1 interferon response. This dysfunctional interferon response could predispose to a higher risk of SARS-CoV-2 infection and worse outcome.[13]

Several studies reported that patients with autoimmune diseases were at higher risk of developing COVID-19 than general population. Pablos JL et al. conducted a retrospective observational study from seven hospitals in Spain, investigating the difference between the prevalence of PCR + COVID-19 in rheumatology patients compared with matched reference populations from the same hospitals.[14] The results showed that patients with chronic inflammatory diseases had higher odds of developing COVID-19 (OR 1.3, 95% CI 1.15-1.52) compared with the reference population. This higher prevalence was observed in systemic autoimmune or immune-mediated diseases, except for inflammatory arthritis or SLE. Besides, patients receiving biologic or targetted synthetic disease-modifying antirheumatic drugs (bDMARD or tsDMARD) but not conventional-synthetic DMARD (csDMARD) had a higher prevalence than the reference population.[14] Similarly, a meta-analysis of 62 observational studies showed that patients with autoimmune diseases were at higher risk of developing COVID-19 than the control patients (OR 2.19; 95% CI 1.05 - 4.58). Unlike the previous study, the SLE/ Sjögren's syndrome/ Systemic sclerosis subgroup had a higher prevalence of COVID-19 than other disease subgroups. The meta-regression analysis results also showed that studies with a higher proportion of steroid use had a higher prevalence of COVID-19. Meanwhile, the use of DMARDs was not associated with increased risk.[15]

However, not all studies supported those results. An online survey involving 1,381 respondents with rheumatic diseases in Ireland reported that the COVID-19 PCR positivity rate was 0.46%, similar to general Irish population positivity rate.[16] Similarly, a large cross-sectional study performed in Italy reported no difference in the incidence of COVID-19 between patients with autoimmune disease and those with no autoimmune disease.[17] Recommendation from European League Against Rheumatism (EULAR) also stated that so far, there had been not enough evidence that autoimmune diseases increased the risk of COVID-19.[18]

Our case series was not designed to estimate the risk of SARS-CoV-2 infection in autoimmune disease patients. Thus, we were unable to draw any conclusion regarding the risk of infection in our patient population. Our study identified 12 patients with autoimmune diseases and confirmed COVID-19. Although men have a higher risk of SARS-CoV-2 infection in the general population, most of our subjects were female because they had higher risk of developing autoimmunity. Based on the BSR risk score, about half of the patients in this series were classified as low risk and the other half as high risk. Two of the patients were healthcare workers. Although one of them (patient no. 9) was classified as BSR low risk, they were at higher risk of SARS-CoV-2 exposure than the general population due to their clinical duty.

Clinical Characteristics

The most common symptom was fatigue, a non-specific symptom, followed by anorexia, fever, and cough. This finding was pretty similar to a case series in New York, where the most common symptom in the patient population of autoimmune diseases and COVID-19 was fever, followed by cough and dyspnea.[19] Similarly, data from the German national registry for patients with rheumatic diseases and COVID-19 showed that the patients' common symptoms included cough, fever, and fatigue.[19]About 40% of patients in our case series experienced arthralgia, which might reflect the underlying rheumatic disease activity or disease flares triggered by SARS-CoV-2 infection. In our case series, the most common autoimmune disease was SLE, which was similar to an online survey performed by the Indonesian Rheumatology Association where 63.6% of subjects with confirmed COVID-19 in that study had SLE.[20] One patient in our series had asymptomatic COVID-19 and was hospitalized due to other reasons.

The most prevalent comorbidity in our patient population was hypertension, similar to findings from other studies.[19] However, surprisingly, the prevalence of tuberculosis (TB) was relatively high in our case series (4/12 patients). Three of them had pulmonary tuberculosis, while one patient had lymphadenitis TB. Two of them had active pulmonary TB, and one required streptomycin injection as part of

the TB treatment regimen.

Tuberculosis in COVID-19 has not been extensively reviewed. Past studies about tuberculosis in SARS infection showed that the SARS-CoV infection suppressed cellular immunity, thus causing the reactivation or new infection of *M. tuberculosis*.[21] Besides, tuberculosis patients who suffered from influenza infection had a higher risk of mortality with symptoms lasting for more than seven days. Moreover, tuberculosis is an independent risk factor for hospitalization caused by influenza-associated illness.[22] A systematic review and meta-analysis investigating the relationship between tuberculosis and COVID-19 by Gao et al. stated that the prevalence of tuberculosis was higher in severe COVID-19 patients than non-severe COVID-19 patients (OR=2.10; 95% CI: 0.61-7.18).[22] However, this result was not statistically significant. There was still conflicting data regarding whether tuberculosis affects the mortality rate in patients with COVID-19 or not. One possible mechanism is that active tuberculosis infection can increase the proinflammatory cytokines, such as IFN type I and III, which are upregulated in both COVID-19 and tuberculosis infection.[23] More high-quality studies providing a clear relationship between tuberculosis and COVID-19 are still needed.

Risk of Hospitalization in Patients with Autoimmune Diseases and COVID-19

The COVID-19 Global Rheumatology Alliance (C-19 GRA) is a global physician-reported registry gathering data on patients with rheumatic diseases diagnosed with COVID-19. There have been several publications based on the data, which helped clarify the relationship between rheumatic diseases and the risk or clinical course of COVID-19. One of the earlier publications from the C-19 GRA registry investigated factors associated with increased risk of hospitalizations in autoimmune patients with COVID-19. Around 600 patients from 40 countries were included in the study. The study results showed that older age, comorbidities, and steroid use with a prednisolone-equivalent dose of ≥ 10 mg/day were associated with higher odds of hospitalization. Neither antimalarial use (such as hydroxychloroquine) nor NSAID uses positively or negatively impacted the odds of hospitalization. In contrast, the use of bDMARD or tsDMARD monotherapy was associated with lower odds of hospitalization (OR 0.46; 95% CI 0.22-0.93). This finding was especially true for anti-TNF, as the number of patients who used other classes of drugs was too small to draw conclusions.[24]

The number of patients whose age was ≥ 60 years old in our study was just one patient, and she had a mild course of COVID-19. The age range of patients who had moderate COVID-19 in our study was 28-59 years old. None had a severe course of COVID-19. Half of our patients had taken steroids with a prednisolone-equivalent dose of ≥ 10 mg/day, which was associated with a higher hospitalization rate and worse clinical outcomes.

Risk of Mortality in Patients with Autoimmune Diseases and COVID-19

Strangfeld et al. analyzed 3729 patients with rheumatic diseases from the registry to investigate factors associated with mortality in confirmed or presumptive COVID-19 cases. Rheumatoid arthritis was the most common rheumatic disease in the study, followed by connective tissue diseases such as SLE. Most patients were in remission or had low disease activity. Deaths occurred in 10.5% of patients in the study, and half were hospitalized. More than half of the patients had comorbid disease, the most common being hypertension. Other prevalent comorbidities include chronic lung disease, obesity, other cardiovascular diseases, and chronic kidney disease. Regarding therapy for autoimmune diseases, around 40% of patients only received either csDMARDs, immunosuppressants, or a combination of both. In contrast, approximately 20% of patients did not receive any DMARD or immunosuppressant except for steroids. Almost 40% of patients were treated with steroids, whereby 10% of them received a dose exceeding >10 mg/day.[25]

The results of the multivariate analysis from the study revealed the following factors to be associated with increased risk of deaths: 1) age >65 years old, 2) male sex, 3) chronic lung disease, 4) presence of both CVD and hypertension, 5) patients who did not receive any DMARD, 6)

treatment with rituximab, 7) treatment with sulfasalazine, 8) immunosuppressants, 9) steroid treatment with a prednisolone-equivalent dose of >10 mg/ day, 10) high/ severe/ moderate disease activity. The association between increased age, male sex, and comorbidities had been established as factors that could increase the risk of COVID-related mortality in other populations. High disease activity and absence of DMARD being risk factors highlighted the importance of autoimmune disease control even in patients with COVID. Rituximab worked by depleting B-cells, which could impair immunity to COVID. Likewise, a high dose of steroid and immunosuppressants could cause dysfunction of the host's immune system, leading to a more severe presentation of COVID. The association with sulfasalazine was surprising, given that it was a weak immunosuppressant, but the result was consistent with studies from IBD-COVID patient population. However, as the authors noted, an association was not the same as causation, and further prospective studies were needed.[25]

In our case series, only two patients had taken csDMARD (methotrexate and sulfasalazine) while none had taken bDMARD or tsDMARD. Most of our patients (75%) did have comorbidities that predisposed them to a higher risk of mortality, such as hypertension, cardiovascular disease, and chronic lung disease (tuberculosis). Another factor that might increase mortality in our study was steroid use with a dose of ≥ 10 mg/ day. Despite that, no mortality occurred in our patient population. There were several possible reasons: no patient had severe COVID-19, the autoimmune disease was pretty well controlled without bDMARD or tsDMARD, most patients were <65 years old and were female.

Limitations of our study stemmed from the fact that this was a descriptive case series. Thus we could not perform any statistical analysis. None of our patients in this series had severe COVID-19. Besides, most of our patients had SLE, and patients with other types of autoimmune disorders were underrepresented. No patients in our study received bDMARD or tsDMARD. Therefore, we could not describe the clinical characteristics in those populations. We also did not use a formal scoring system to assess the disease severity. However, although our study was only a descriptive study, our case series provide valuable information regarding the clinical characteristics of COVID-19 in the Indonesian autoimmune disorder patient population. To our knowledge, only one similar study focusing on Indonesian autoimmune disorder patients had been published previously, and most of their subjects did not undergo PCR confirmation of COVID-19.[20] Conversely, all of the COVID-19 diagnoses in our patients were confirmed by PCR testing.

CONCLUSION

This first comprehensive case series provides characteristics of autoimmune patients having positive COVID-19 infection, of which study is still limited in Indonesia. Protecting high-risk group such as autoimmune patients are important, especially during this pandemic.

DATA AVAILABILITY

The data supporting the result of this article will be made available by the authors, without undue reservation.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Additional Chromosomal Abnormalities in Chronic Myeloid Leukemia Patient Treated with First-Line Tyrosine Kinase Inhibitor Therapy: Good or Poor Prognosis?

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ABSTRACT

A 33-year-old male came to Policlinic of Hematology-Medical Oncology Dr. Cipto Mangunkusumo General Hospital for routine control of chronic myeloid leukemia (CML) treatment. He was treated with Imatinib Mesylate (IM) for two years. At the beginning of therapy, he showed good treatment response. However, after two years of treatment, he lost complete hematological response (CHR) occured and major molecular response (MMR) was not achieved. This demonstrated drug resistance even with good compliance. Evaluation of therapy through cytogenetic karyotype testing showed complex additional chromosomal abnormalities (ACA) in addition to the Philadelphia chromosome (Ph). Tyrosine kinase inhibitor (TKI) therapy in this type of patients should be replaced with other alternative TKIs. A mutation profiling test is needed to determine alternative TKI. Monitoring in the treatment of CML patients is very important. The presence of ACA indicates disease progression and poor prognosis. Time to change therapy in CML patients must be done appropriately based on the results of hematological, molecular, and cytogenetic testing.

Keywords: *chronic myeloid leukemia (CML), additional chromosomal abnormalities, drug resistance*

INTRODUCTION

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder caused by translocation t(9;22)(q34;q11) that results in a Philadelphia chromosome (Ph).[1] When first diagnosed, most CML patients (90-95%) are in chronic phase.[1,2] The onset age of CML patients in Asia is lower than in western countries.[3] Proportion of Ph(+)/BCR-ABL(+) chronic phase (CP) CML patients in Dr. Cipto Mangunkusumo General Hospital is 90%.[4]

CML treatment has changed dramatically in the last decade. Imatinib and nilotinib are tyrosine kinase inhibitors (TKIs) which are commercially used for treatment of CML patients in Indonesia. Treatment with TKI results in 85-95% overall survival after five years.[5] Imatinib mesylate (IM) is the first TKI approved to treat CML-CP patients. IM competitively inhibits adenosine triphosphate (ATP) attachment sites on BCR-ABL oncoprotein, thus inhibiting phosphorylation of proteins involved in cell signal transduction. This efficiently inhibits BCR-ABL kinase, however, it also blocks platelet-derived growth factor (PDGF) receptors and KIT tyrosine kinase.[5]

We expected that CML patients who were treated with TKI to have prolonged survival which is similar to normal people. However, patients with CML responded differently to

TKI. We are reporting a case of a CML patient who showed disease progression after being treated with first-line IM therapy and showed additional chromosomal abnormalities (ACA) from cytogenetic testing.

CASE ILLUSTRATION

A 33-year-old male visited Polyclinic of Hematology-Medical Oncology Dr. Cipto Mangunkusumo General Hospital for his routine check-up of CML treatment. At the time of the visit, he had no complaints. He had been treated with oral IM 1 x 400 mg/day for two years. He had good compliance in taking his medication and did not take any other drugs. He had no history of any other diseases. He regularly came to Policlinic of Hematology-Medical Oncology for a check-up and to get IM. Physical examination did not show splenomegaly. After two years of IM therapy, laboratory testing revealed an increase in white blood cell (WBC) count to 91,140/uL (normal range: 4000-11,000/uL), platelet (Plt) count to 1,761,000/uL (normal range: 150,000-400,000/uL), basophil to 9% (normal range: 0-2%), myeloblast 1% (normal: no immature cells). Quantitative BCR-ABL was 63% IS. Bone marrow aspiration revealed a hypercellular morphology with M:E ratio 6.5:1 and expansion of granulopoiesis with 5.5% of blast cells.

On his first visit two years ago, he complained of feeling nauseous and bloated. Vital signs were normal. Physical examination indicated anemia in both eyes conjunctiva and massive splenomegaly (Schuffner 8). There was neither hepatomegaly nor any other abnormal findings. The result of blood test revealed anemia with hemoglobin (Hb) count of 5.9 g/dL (normal range: 13.2-17.3 g/dL), leukocytosis with WBC count of 251,030/uL, normal Plt value of 186,000/uL, basophil 1%, promyelocytes 1%, myeloblast 3%, and myelocytes 1%. He underwent bone marrow aspiration testing. Qualitative BCR-ABL testing was positive. Based on history, splenomegaly, peripheral blood, bone marrow aspiration, and BCR-ABL testing, we established the diagnosis of CML-CP. Sokal score was 1.3 points and Eutos score was 87 points.

DISCUSSION

Our patient was a 33-year old male who came to Policlinic of Hematology-Medical Oncology Dr. Cipto Mangunkusumo General Hospital with CML diagnosis who was treated with IM since two years ago. He routinely came for control of his IM treatment and had no complaints. We evaluated the treatment response of CML patients regularly. Response to TKI therapy is determined by the measurement of hematologic (normalization of peripheral blood counts), cytogenetic (decrease in the number of Ph-positive metaphases using bone marrow cytogenetics), and molecular responses (decrease in the amount of BCR-ABL chimeric mRNA using qPCR). The goal of TKI therapy is to achieve a complete hematologic response (CHR) within three months, a complete cytogenetic response (CCyR) and major molecular response (MMR) within 12 to 18 months after first-line TKI therapy and to prevent disease progression to accelerated or blastic phase or CML.[5,6] Since patients with CML on TKI are expected to live just like normal people, surrogate markers of outcome are important. Achieving a deeper response faster is associated with better outcome.[7]

This patient achieved CHR within three months after he started taking IM and continued the treatment until two years with good compliance. After two years of treatment, his peripheral blood counts revealed leukocytosis, thrombocytosis, basophilia, and the presence of immature cells. Quantitative BCR-ABL was 63% IS. Thus, the patient loss of CHR and did not achieve MMR after 24 months of imatinib therapy while he had no symptoms. We should evaluate patient compliance and drug interaction. In this patient, he had good compliance and did not take any other medications. Patients with disease that is resistant to primary treatment with imatinib should be treated with bosutinib, dasatinib, or nilotinib in the second-line setting.[6]

We performed bone marrow aspiration and, despite the two-year treatment with IM, the bone marrow still showed hypercellular morphology and expansion of granulopoiesis with 5.5% of blast cells (**Figure 1**). Cytogenetic analysis showed complex additional chromosomal abnormalities (ACA) such as 41,Y,-X,-11,-16,-

17,-18[1]/44,XY,-17,-20[2]/45,XY,18[1]/45, XY,ob(13;22)(q10;q10),22[1]/46,XY,t(9;22) (934;q11)[2]/46,XY[6], while Ph was still there (**Figure 2**). Ideally, we also need to perform BCR-ABL mutation profiling to guide the selection of alternative TKI.[5-7] However, we have a limitation in performing mutation profiling due to lack of facilities.

Cytogenetic monitoring should be performed by analysis of marrow cell metaphases, reporting the proportion of Ph+ metaphases with 20 metaphases analyzed minimally. The cytogenetic response is defined as complete (CCyR) with 0% Ph+ metaphases, partial (PCyR) with 1%-35% Ph+ metaphases, minor with 36%-65% Ph+ metaphases, minimal with 66%-95% Ph+ metaphases, and none if >95% Ph+ metaphases.[5] Our patient did not achieve cytogenetic response at all at 2 years of treatment with IM, even the cytogenetic also showed additional chromosome abnormalities which indicate a warning to treatment response.

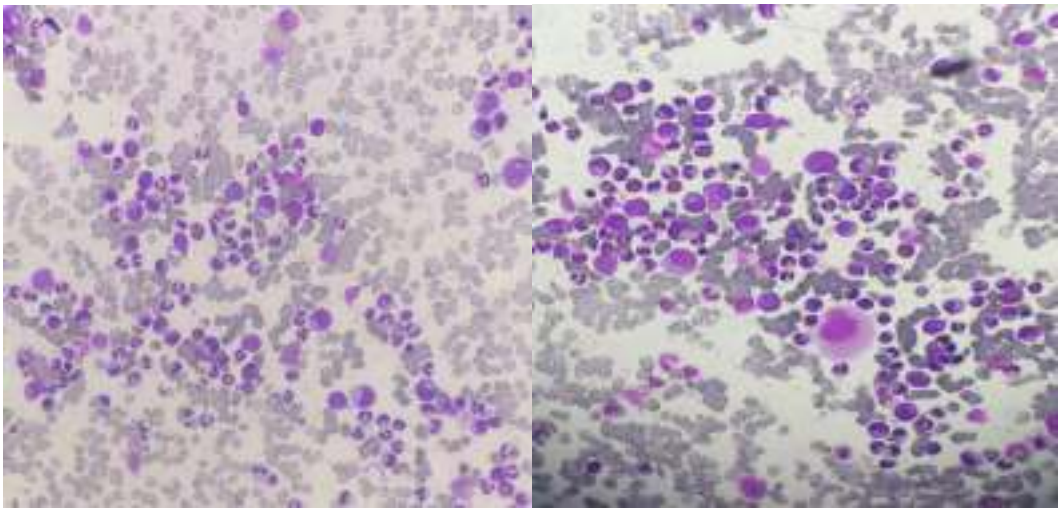

Figure 1. Morphology of bone marrow after 2 years on imatinib mesylate treatment.

Figure 2. Cytogenetic testing after 2 years on imatinib treatment

The emergence of additional chromosomal abnormalities (ACAs) in Ph (+)/ BCR-ABL (+) CML, known as clonal evolution, is an indicator of multistep disease progression. It is a reflection of genetic instability that characterizes disease evolution in CML.[8,9] Beside for diagnosis, cytogenetics is an important tool for prognosis after treatment with TKI. Frequently, ACAs are found in Ph+ cells and interfere with the progression of CML. ACAs increase in the advanced stage, from 30% in accelerated phase to 80% in blastic crisis. ACAs are related to poor prognosis, with a lower rate of treatment response with Imatinib.[10,11] We believe that ACAs in our patient is a hallmark of poor prognosis to treatment with Imatinib, even ACAs also a sign of progression into an accelerated phase of CML so that we should change Imatinib to the second generation TKIs, such as nilotinib, dasatinib, or bosutinib, or even ponatinib. There is a general consensus that patients who fail after imatinib should change without hesitation to either nilotinib or dasatinib. The choice should be guided by the mutation profile, if relevant, the comorbidities of the patient, the side effects of the drugs, and the availability of the drugs. The presence of BCR-ABL mutations is a way to guide to which one of TKIs should the clinician choose as a second-line treatment after Imatinib failure. Direct sequencing of DNA after qRT-PCR is most often used by clinicians to identify specific mutations in the BCR-ABL kinase domain. In a survey of BCR-ABL mutations in 386 CML subjects, Branford and colleagues identified specific mutations, which conferred significant resistance to nilotinib (E255K/V, Y253H, and F359C/V) and dasatinib (V299L and F317L). Ponatinib is the only approved TKI that binds to the T315I BCR-ABL mutant protein.[11-15]

CONCLUSION

Treatment monitoring in CML management is very important. The presence of ACAs is a sign of disease progression and poor prognosis. We need to properly decide when to change to alternative therapy based on hematology, molecular and cytogenetic testing.

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Persistent ST Segment Elevation After Repeated Percutaneous Coronary Intervention: A Dressler Syndrome?

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ABSTRACT

In the era of percutaneous coronary intervention (PCI), Dressler syndrome has become an extremely rare phenomenon. Originally known as post-myocardial infarction syndrome, it is characterized by fever, pleuritic chest pain, and pericardial or pleural effusion after myocardial infarction. It is one of the sub-entities of post-myocardial infarction pericarditis (PMIP).

A 62-year-old man presented with persistent chest pain and diffuse ST segment elevation even after repeated PCIs. This condition was accompanied by fever and bilateral pleural effusion upon chest X-ray. The patient showed improvement in ST segment elevation and clinical condition after 2 weeks of steroid administration. The findings in this case suggest the possibility of PMIP. Although uncommon, physicians should be aware of the potentials of this condition in the differential diagnosis of chest pain after myocardial infarction and PCI so that immediate effective treatment can be given.

Keywords: *Case report, percutaneous coronary intervention, myocardial infarction, Dressler syndrome, post myocardial infarction pericarditis*

INTRODUCTION

Patients with acute myocardial infarction are recommended to undergo myocardial reperfusion, with primary percutaneous coronary intervention (PCI) as the preferred reperfusion strategy.[1] However, in some cases, patients who have undergone PCI can experience persistent chest pain and ST segment elevation in electrocardiograms (ECG). The possible mechanisms are early stent thrombosis, which in turn leads to reinfarction,[2] and injuries to the pericardium, such as acute pericarditis and Dressler syndrome.

Dressler syndrome was originally known as post-myocardial infarction syndrome, which can be followed with or without pericardial effusion.[3] It was first described in 1956 by William Dressler after he observed the late period development of an acute myocardial infarction.[4] Dressler syndrome usually develops 2–10 weeks after myocardial infarction and is believed to be caused by immune complex formation, which results in a systemic immune-inflammatory response.[5] Nowadays, it has become quite rare in developed countries due to advanced developments in myocardial infarction management, with less than 5% incidence.[6]

CASE ILLUSTRATION

A 62-year-old man presented to the emergency department with 11 hours of worsening back pain. The pain was described as a burning sensation that radiated to the chest and was not influenced by level of activity. The patient had a history of smoking and hypertension and denied any family history of cardiovascular or metabolic diseases.

Upon physical examination, the patient's blood pressure was 170/92 mmHg, heart rate 72 beats/min, respiration rate 20 breaths/min, temperature 36.5°C, and pulse oximetry 99% on room air. There was no sign of pulmonary edema or other problems. The results of the laboratory tests were leukocytosis (leucocyte 12.86 x 10^9/L), hemoglobin 15.8 g/dL, platelet count 280000/µL, potassium 3.7 mEq/L, urea 35.7 mmol/L, creatinine 1.1 mg/dL, and estimated glomerular filtration rate (eGFR) 71 mL/min/1.73m^2. Cardiac enzymes were within normal limits. A chest radiograph showed no abnormalities. The initial ECG from admission showed an inverted T wave on lead V2–V6. The patient was then diagnosed with unstable angina pectoris and admitted to the Intensive Coronary Care Unit (ICCU). Echocardiography showed segmental hypokinetic with ejection fraction (EF) at 46% and no pericardial effusion. A serial ECG was done 10 hours after the first ECG, and the results showed worsening conditions (**Figure 1**), so the patient was sent to have urgent percutaneous coronary intervention (PCI).

Coronary angiography revealed 70–80% diffuse stenosis in the proximal to distal left anterior descending artery (LAD); thus, two drug eluting stents were deployed in the proximal and distal LAD. Post-stenting angiography with LAD injection showed adequate flow in the proximal to distal LAD (**Figure 2**). However, we failed to notice that the first LAD diagonal branch (D1) showed no continuity of dye flow.

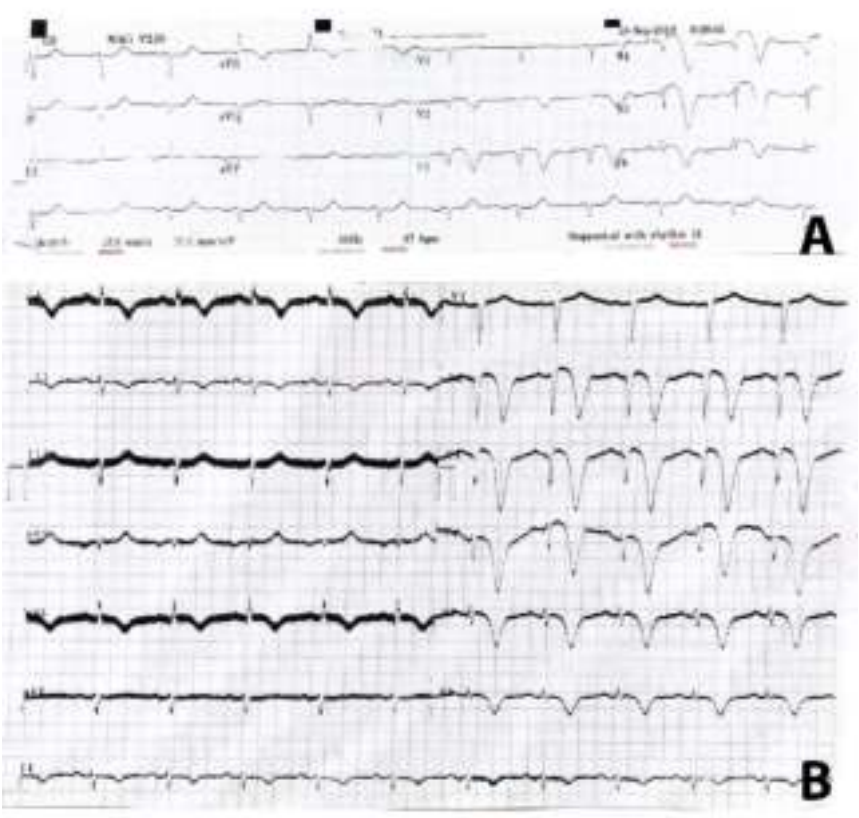

Figure 1. Twelve lead electrocardiogram reading of the patient before PCI. (A) Admission ECG demonstrating widespread inverted T wave on lead V2-V6. (B) serial ECG in ICCU 10 hours after admission showed deeper T inversion than before.

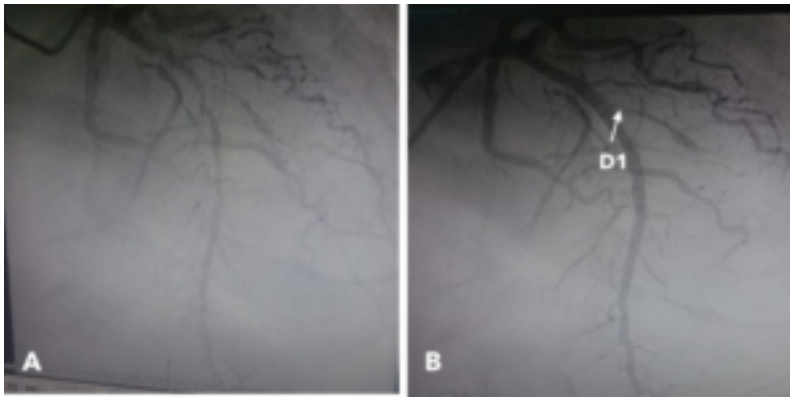

Figure 2. Coronary angiogram showing percutaneous coronary intervention of the LAD (A) pre-stent LAD showing 70-80% diffuse LAD disease (B) post-stent LAD showing adequate flow in most vessels with near occluded first diagonal branch of LAD. Notice that the first LAD diagonal branch (D1) still showed no continuity of dye flow.

Over the next 24 hours after the first PCI, the patient suddenly experienced chest pain that persisted even after nitroglycerin drips. The patient also began to develop a fever (38°C). Leukocyte count was increased to 25.4 x 10^9/L. A repeat echocardiography revealed a segmental akinetic and hypokinetic low systolic left ventricle, EF further reduced to 29%, and moderate pericardial effusion. Another ECG showed ST segment elevation in lead I, aVL, and V2–V6 (**Figure 3**), and a chest X-ray showed bilateral pleural effusion. As a result, the patient had an urgent PCI for the second time due to suspected acute stent thrombosis.

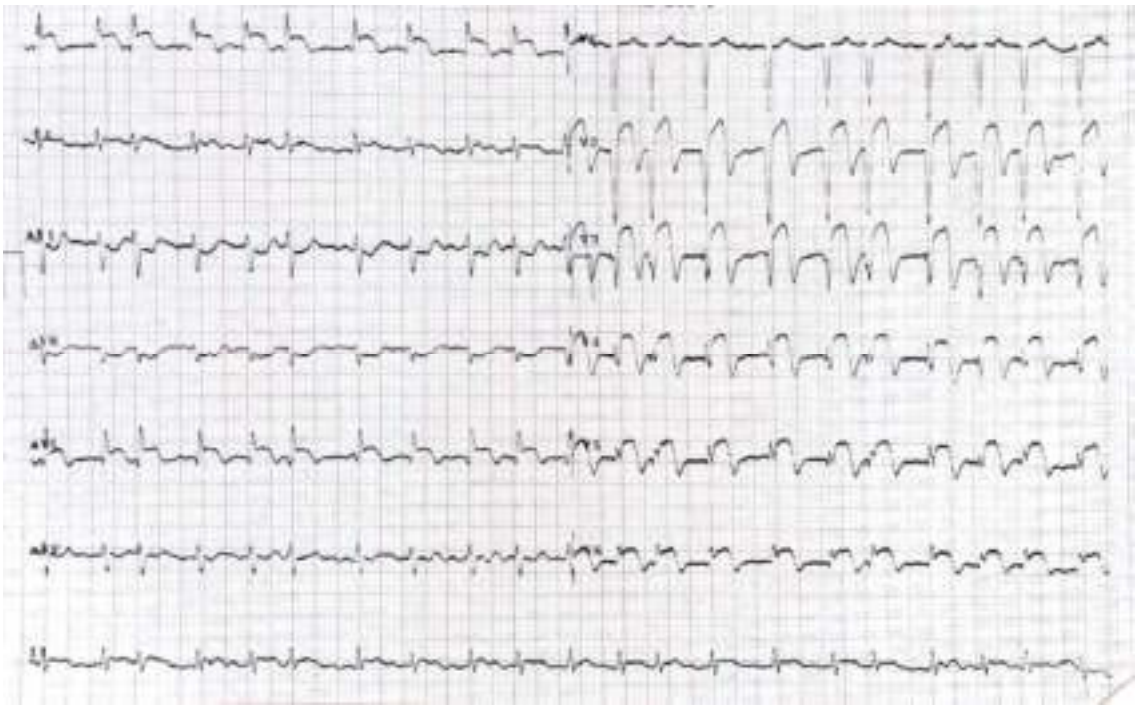

Figure 3. ECG taken one hour after first PCI showed diffuse ST segment elevation with T inversion in all leads.

The angiography results showed patency of both stents in the LAD, and we decided to open the occlusion of the D1 of the LAD with a drug-eluting stent in the osteo-proximal D1. The final angiogram post-stenting showed optimal results without any complications (**Figure 4**). But even after the second PCI was done, there was no significant improvement in the patient's clinical symptoms or ECG changes. The patient still complained of chest discomfort, epigastric pain, and breathing difficulty. The fever also persisted, and an ECG showed a worsened ST segment elevation in I, aVL, and V2–V6 **(Figure 5**).

After evaluating the patient's overall symptoms, Dressler syndrome was considered, and the patient was given ibuprofen 3 x 600 mg and a morphine drip 0.5 mg/hour. The next day, the patient's creatinine increased from 1.1 mg/dl to 4 mg/dl and urine production decreased to 0.4 ml/kg/hour, so ibuprofen was stopped and replaced with methylprednisolone 1 x 62.5 mg, and hemodialysis was performed on the patient. Furthermore, the patient felt subjective improvement, leukocytes returned to normal at 10.030 x 10^9/L, and the patient was finally discharged from the hospital 12 days later. An ECG showed improvement but a remaining, slight persistent ST segment elevation (**Figure 6**).

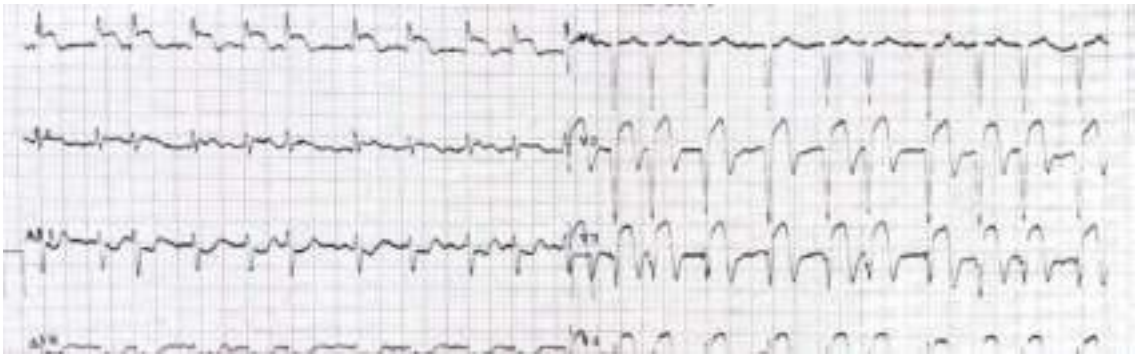

Figure 4. Coronary angiogram in second PCI. (A) Corangiography before stenting showed nearly occluded first diagonal branch (D1) of LAD. (B) View of the LAD after deployment of drug eluting stents in osteo-proximal first diagonal branch.

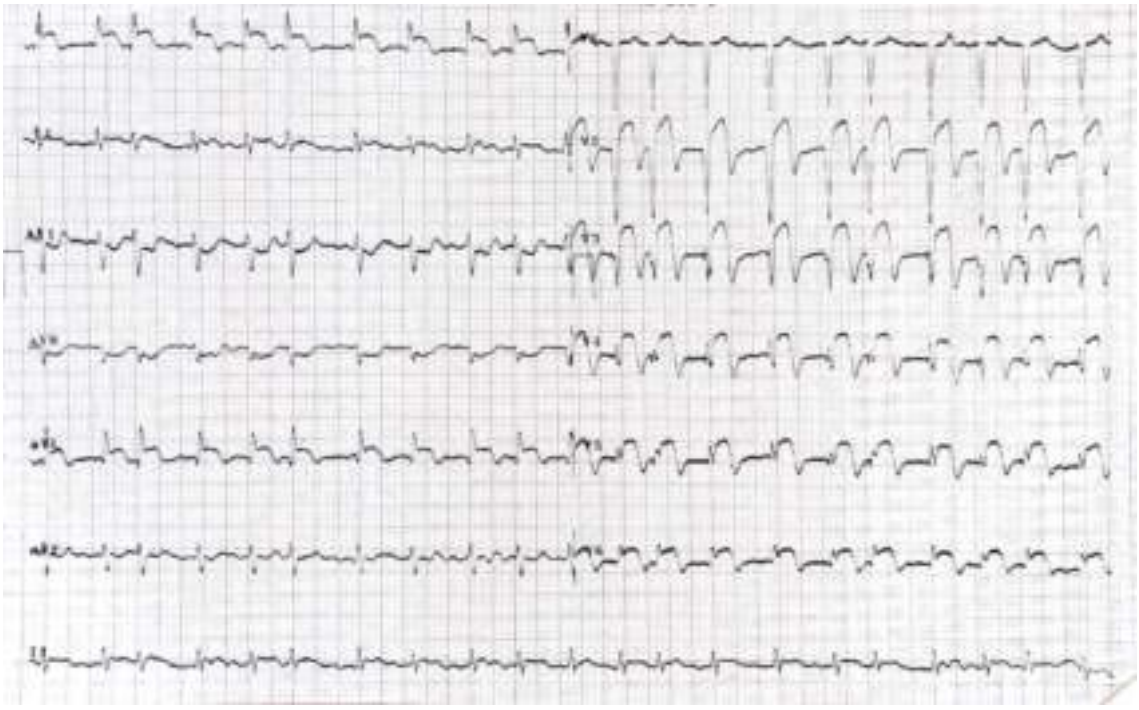

Figure 5. ECG taken after second PCI showed no improvement and persistent diffuse ST segment elevation in all leads.

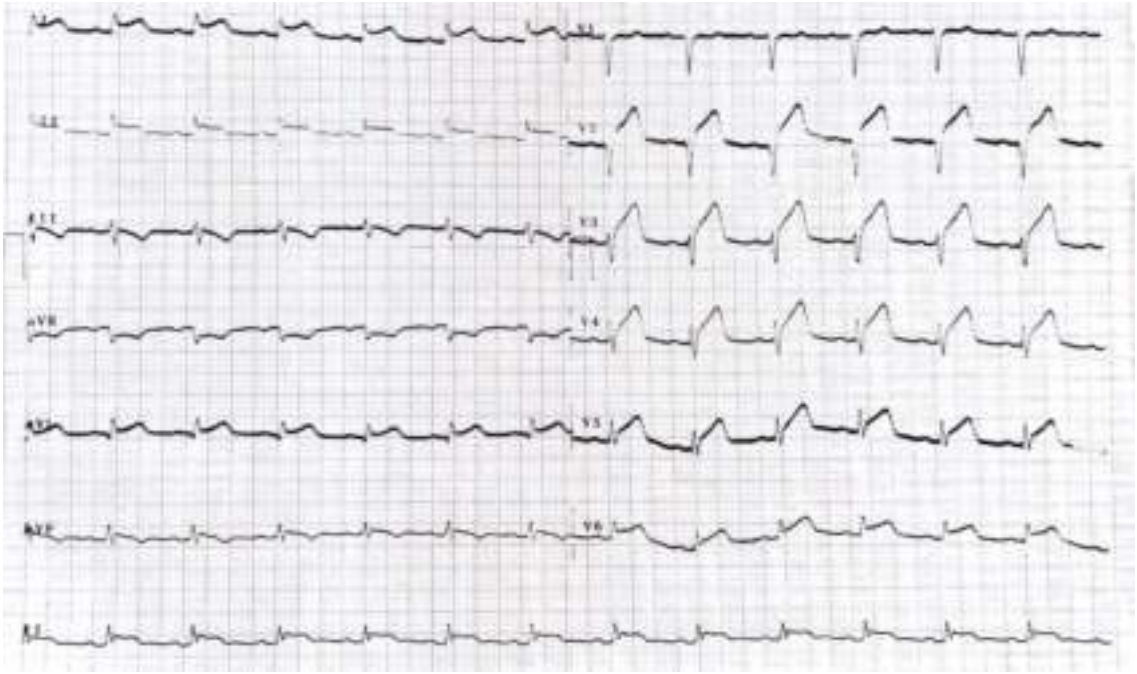

Figure 6. ECG taken 2 weeks after of steroid therapy showed marked improvement of ST segment elevation and diminished T inversion

DISCUSSION

Dressler syndrome is a form of secondary pericarditis that occurs as a result of heart or pericardium damage. It was first reported in 1956 as a benign triad of fever, pericarditis, and pericardial effusion post-myocardial infarct. Dressler syndrome should be suspected if the patient complains of prolonged weakness, especially after cardiac surgery or myocardial infarction.[7] The exact cause of Dressler syndrome is still unknown, but it is thought to be an immune-related event triggered by the initial damage to pericardial and/or pleural tissues as caused by myocardial necrosis, which in turn causes a systemic inflammatory response in the patient's body.[8] This condition is a sub-classification of post-cardiac injury syndrome (PCIS), which refers to a heterogeneous group of autoimmune-mediated conditions resulting from various injuries to the pericardial, epicardial, and myocardial.[9]

A PCIS diagnosis may be established if a patient presents with at least two of these clinical criteria after cardiac injury: (i) fever without alternative causes, (ii) pericarditis or pleuritic chest pain, (iii) pericardial or pleural rubs, (iv) evidence of pericardial effusion, and/or (v) pleural effusion with elevated C-Reactive Protein (CRP).[10] Therefore, patients with Dressler syndrome may experience complaints of fever, pleuritic chest pain, malaise, shortness of breath, palpitations, arthralgia, or irritability. Upon physical examination, tachycardia can be obtained with a friction rub upon auscultation, which can occur due to accumulated pericardial fluid. Pulsus paradoxus can also be found in the patient. In the thorax, pleural effusion can be found, though not always.[11]

On further investigations, the most sensitive diagnostic procedure for evaluating Dressler syndrome is echocardiography, which can reveal pericardial effusion, evaluated cardiac output, and contractility. If it is difficult to see posterior pericardial effusion or loculated-pericardial effusion, cardiac magnetic resonance imaging (MRI) can be used. Chest radiography can be done if echocardiography is not available. Flattened costophrenic angles can be found along with enlargement of the heart, which can indicate the presence of pleural and pericardial effusion. ECGs can have global ST segment elevations and T wave inversions, as with pericarditis. Low voltage QRS can also be used if pericardial effusion is high. Blood cultures in

Dressler syndrome show negative results. Other blood tests can reveal increased white blood cells with shift to the left and increased acute phase reactants (CRP).[4,5]

The management of Dressler syndrome is by administering anti-inflammatory drugs (NSAIDs) (e.g., ibuprofen, aspirin), tapered down in 4–6 weeks, while evaluating pericardial effusion. If the patient does not respond to NSAIDs or is contraindicated, corticosteroids (e.g., prednisone) can be given, which are reduced in 4 weeks.[12] Recurrence is possible, and colchicine can be given as prophylaxis.

In this current case, our patient was initially diagnosed with late onset angina pectoris (11 hours) that worsened over time, as proven by the deepening of the T inversion in the serial ECG, thus indicating an ongoing ischemic process of the myocardium. Coronary angiography showed diffuse stenosis of the LAD. After undergoing the first and second PCI successfully, the patient still experienced chest pain, accompanied by fever and evidence of pericardial and pleural effusion. His ECG showed persistent global ST segment elevations and T wave inversions. Laboratory examination also revealed leukocytosis. These findings are consistent with the clinical criteria of Dressler syndrome. After being given steroids, the patient showed clinical improvement. Leukocytes returned to normal and chest radiography showed remission of pleural effusion. The dramatic clinical improvement after the administration of corticosteroids strongly suggests an immune-mediated etiology in this case.

The clinical criteria and response to standard corticosteroid treatment strongly suggest a diagnosis of Dressler syndrome. However, according to 2015 European Society of Cardiology (ESC) guidelines, Dressler syndrome is defined as late post-myocardial infarction pericarditis (PMIP) with intervals of 2–8 weeks after infarction, while our patient's condition arose within the same week of the myocardial infarction event. Per the newest guidelines, PMIP can be grouped based on timing. Late infarct–associated pericarditis, known as Dressler syndrome, and early infarct–associated pericarditis typically occur less than 7 days post-myocardial infarct. The diagnostic criteria does not differ from acute pericarditis, which is the presence of two of four clinical criteria: (i) pericardial chest pain, (ii) pericardial rubs, (iii) ECG changes, and (iv) pericardial effusion. In early PMIP, also termed peri-infarction pericarditis, ECG changes may be overshadowed by changes due to myocardial infarction, but elevated ST segments may still occur.[10] Patients are usually asymptomatic, and the treatment is generally supportive, as most cases are self-limited. Still, for patients who have persistent symptoms that require more than supportive care, the choice of therapy remains the same as with other PCIS.[10,13]

While the timing of the pericarditis better matched early PMIP or acute pericarditis criteria, the clinical findings in this case are more suggestive of Dressler syndrome since PMIP or acute pericarditis do not usually present with severe symptoms, such as those in our patient. Both diagnoses have the same choice of therapy.

PMIP has been reported to be declining in incidence in recent years, which is why physicians nowadays sometimes overlook this disease when faced with patients with similar conditions and thus focus more on the possibility of myocardial infarction evolution. Thorough examination and understanding of the course of disease is required to diagnose this condition accurately. Differential diagnosis may include acute myocarditis, acute stent thrombosis, or reinfarction.

CONCLUSION

Our case supports the fact that any persistent ST segment elevation after a percutaneous coronary intervention should be thoroughly evaluated, as well as that the possibility of PMIP should be considered even in the early phase. Physicians must be aware of this entity and suspect it whenever they encounter patients with pericarditis-like symptoms after myocardial infarction, whether PCI was done or not. An immediate diagnosis is necessary to initiate proper treatment.

CONFLICT OF INTEREST

The author(s) declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

TH and EG participated in manuscript ideation. TH wrote the draft. EG reviewed the draft, and finally approved it.

ETHICAL APPROVAL AND INFORMED CONSENT

Ethical approval is not required for case reports at our institution. Written informed consent was obtained from the patient for clinical and education purposes as per standard practice at our institution.

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Ulcerative Colitis as the Rarer Phenotype of Inflammatory Bowel Disease to Coexist with Psoriatic Arthritis: A Case Report

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ABSTRACT

Psoriatic arthritis (PsA) has been linked to various diseases associated with immune dysregulation, such as Inflammatory Bowel Disease (IBD). Numerous studies have shown strong correlation between PsA and one of the phenotypes of IBD, Crohn's disease. On the other hand, the studies regarding the association of PsA with ulcerative colitis (UC) are less robust and have conflicting findings. We herein report a case of 56-year-old woman with a history of psoriatic arthritis, who developed chronic diarrhea and significant weight loss. The colonoscopy and histopathologic findings were suggestive of pancolitis with backwash ileitis, from which the working diagnosis of ulcerative colitis was carried out. The patient fit the typical epidemiological profile of a PsA patient with concomitant UC, but some aspects of the clinical features observed in this case, such as the development of anterior uveitis was rarely documented in similar studies. A conducted bidirectional meta-analysis also showed that there were more cases where UC preceded the diagnosis of psoriasis, which makes the late development of UC in this case atypical. Due to the uncommon nature of the concurrent development of these two disease entities in this case, this study could provide additional insights to the association of PsA and UC.

Keywords: *psoriatic arthritis, psoriasis, ulcerative colitis, inflammatory bowel disease.*

INTRODUCTION

Psoriasis is a chronic inflammatory disease of the skin that is often accompanied with concurrent systemic manifestations.[1] The prevalence of psoriasis varies greatly across countries, ranging between 0.09% and 11.4%.[2] Furthermore, the pathogenesis of psoriasis is multifactorial, involving a complex interplay between immune system dysregulation and genetic association.[3]

One of the conditions that commonly affects 30% of psoriasis patients is psoriatic arthritis (PsA).[4,5] PsA is part of the spondyloarthropathy (SpA) spectrum, which affects both peripheral and axial joints. The prevalence of PsA is roughly equal in men and women.[6,7] PsA has a potential to cause irreversible damage to the joints involved, which is linked to the deterioration of functional

capacity and marked impairment of psychosocial status in patients with psoriasis.[8] Moreover, PsA is also associated with increased mortality from cardiovascular disease by many studies.[9] The diagnosis of psoriatic arthritis may pose challenges, but an instrument such as CASPAR (Classification Criteria for Psoriatic Arthritis) criteria can help in diagnosing PsA with 98.7% specificity and 91.4% sensitivity.[10] Biologic agents, combined with disease-modifying antirheumatic drugs (DMARD) have been the mainstay of treatment for PsA.[11]

Inflammatory bowel disease is an inflammatory disease that is characterized by chronic relapsing inflammation of the digestive tract. This condition encompasses two phenotypes, Crohn's disease (CD) and ulcerative colitis (UC).[12] Ulcerative colitis is characterized by inflammation within the mucosa and submucosa of the colon.[13] The exact cause of IBD remains largely unknown, but recent research has shown that an individual's genetic susceptibility, the influence of the external environment, intestinal flora dysbiosis, and abnormal immune responses can cumulatively orchestrate the pathogenesis of IBD.[14]

Individuals with psoriasis are at greater risks of developing various diseases, making psoriasis a disease entity associated with many multisystem comorbidities.[15] IBD was one of the commonly identified comorbidities associated with psoriasis and the incidence of IBD in psoriasis is higher in CD compared to UC in multiple studies.[15,16] Furthermore, CD also shows strong positive correlation with psoriasis, while on the contrary, some studies including a large scale study failed to identify the association of UC to psoriasis.[15] In addition, a study conducted by Li et al. showed that when psoriasis was complicated with arthritis, the risk for developing CD increased significantly with a relative risk of 6.43, while the risk for developing UC was comparable to general population.[16]

Many studies have shown that the coexistence of psoriasis, especially when complicated with PsA and UC are less common than CD. The unusual coexistence of these two disease entities in this case drives the authors to explore this case further and connect the clinical characteristics of these two diseases in a more detailed manner. To the best of our knowledge, this is the first case report of PsA and UC in Indonesia.

CASE ILLUSTRATION

A 58-year-old woman was first diagnosed with psoriatic arthritis in 2002 at the age of 40, with complaints of debilitating pain and swelling in several joints, especially the finger joints and area of both heels, which worsened over weeks. Concurrent with the pain in the joints, patient also noticed the appearance of red, scaly patches on her skin all over her body. The patient stated that she had never experienced these symptoms before and that no family members had these symptoms or that they had been diagnosed with psoriasis. The patient denied a history of smoking and excessive alcohol consumption. The patient also had a history of cataract on the right eye as a complication of chronic anterior uveitis and she also underwent intraocular lens replacement surgery for her right eye.

The physical examination showed multiple erythematous plaques covered with hard scales on scalp, lower arms, chest, back, and knees with a total of more than 10% of her body surface area on the first presentation. There were edema and swelling of the fingers, yellow discoloration and onycholysis of the nails were later documented. The patient also stated that these symptoms negatively affect her daily activities and mental health. There were no remarkable results of the patient's blood panel and she tested negative for rheumatoid factors. The radiological examination of the hands later on was suggestive of arthritis, but specific signs for PsA such as bone proliferation and pencil-in-cup deformity were not identified. Furthermore, the patient fulfilled 5 of the CASPAR criteria with arthritis, negative rheumatoid factor, skin lesions, psoriasis nail, onycholysis and oil spot. Therefore, the confirmation of psoriatic arthritis as the working diagnosis was carried out. The patient was treated with methotrexate and marked decrease in Psoriasis Area and Activity Index (PASI) scores were documented. The patient achieved remission with her symptoms of psoriasis and PsA until today with methotrexate

12.5 mg per week.

In 2017, the patient had complaints of unresolving bloody diarrhea and significant weight loss. She reported passing ten to twelve liquid stools a day, with occasional appearance of blood and mucus around the stools. She also felt feverish at times, although she did not measure her body temperature with a thermometer. She was referred to gastroenterology clinic and was scheduled for a colonoscopy. The colonoscopy findings showed evidence of colitis with grossly hyperemic and edematous mucosa of sigmoid colon extending to the caecum and terminal ileum, which was suggestive of pancolitis with backwash ileitis. Some aphthous ulcers with whitish base were also identified. The histopathological findings showed abundant deposition of chronic inflammatory cells in the lamina propria of the ileum mucosa. The colonic crypts were distorted and the lamina propria was also fully occupied with chronic inflammatory cells. From the colonoscopy findings and the histopathological findings, the diagnosis of ulcerative colitis was carried out.

The patient was given mesalazine for the treatment of UC and her symptoms resolved. She achieved remission for both UC and PsA with mesalazine 1,000 mg twice a day and methotrexate 12.5 mg per week. The patient is also prescribed with folic acid 5 mg per week for additional supplementation and topical steroid for her skin lesions. The prognosis of this patient was good, as shown in disease remissions without biologic agents nor invasive interventions needed. The patient was also counselled to build adequate awareness with the disease course. The patient also routinely visited the outpatient department of Gastroenterology Cipto Mangunkusumo National General Hospital, as shown in the medical record data. The patient admitted that she adhered to medications given as per advised on every consultation and reported no adverse events of medications administered during the disease course.

Figure 1. Discreetly distributed erythematous nummular plaques on back region with fine scales after treatment (left); Psoriatic nail with onycholysis and oil spot (middle); Deformity of the distal interphalangeal joints which are the predilection sites for psoriatic arthritis (right)

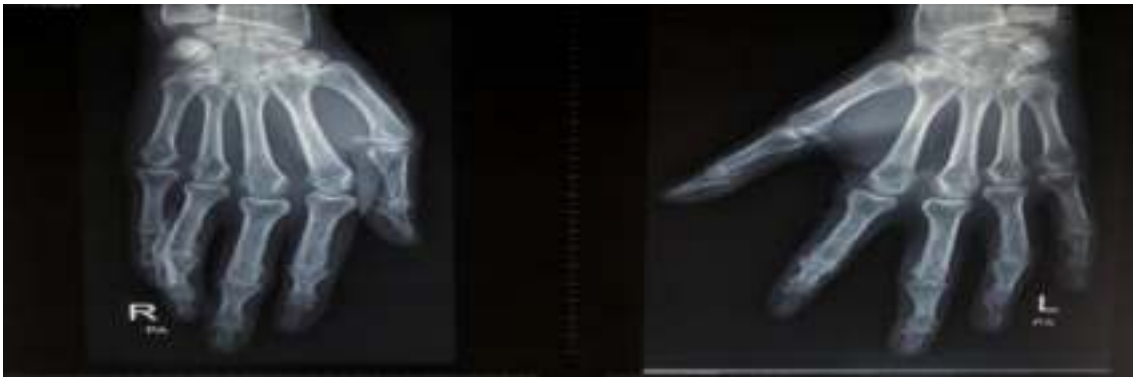

Figure 2. Radiography of the hands showed interphalangeal space narrowing which indicated arthritis, classic findings of pencil in cup deformity and bony proliferation were not found in this case

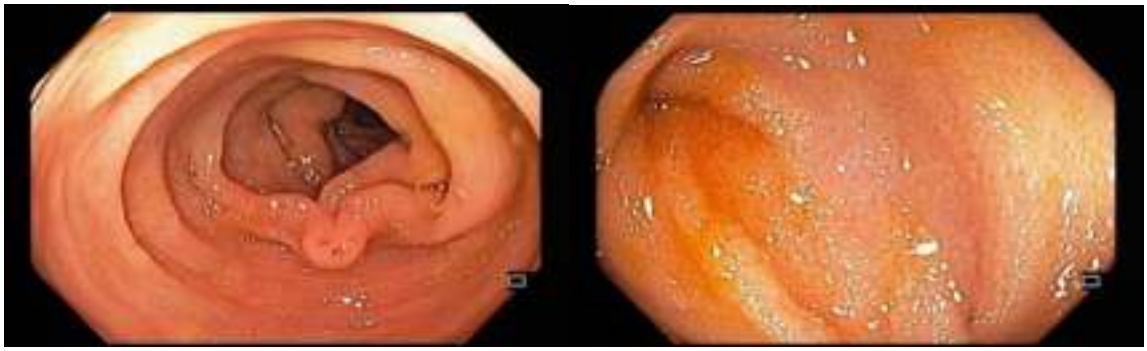

Figure 3. Hyperemic and edematous colonic mucosa with some aphthous ulcers (left), extending up to the terminal ileum (right), suggestive of pancolitis with backwash ileitis

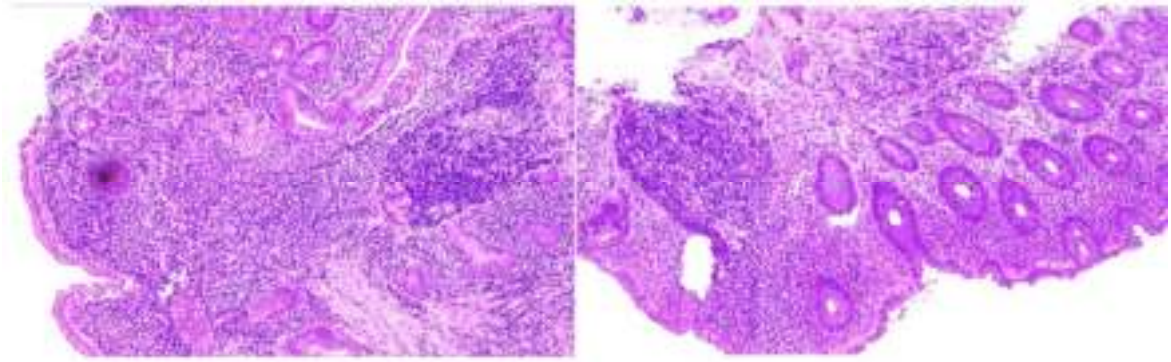

Figure 4. Histopathology (hematoxylin and eosin, original magnification of 100x). Deposition of chronic inflammatory cells in the lamina propria of the ileum mucosa (left); Distorted colonic crypts with deposition of chronic inflammatory cells (right)

DISCUSSION

Psoriasis and IBD share some common pathways in their pathogenesis, involving immune cells of Th17 and T-reg cells and various chromosomal loci which map for genes associated with innate and adaptive immunity.[17] The link between PsA (as part of the spondyloarthropathies spectrum) and IBD is presumed to lie in the proposed model of "the gut-synovium axis". Some studies have also hypothesized the upregulation of adhesion molecules such as E-cadherin and α-Eβ7 integrin, as an integral part of SpA development in IBD.[18]

According to the study conducted by Eppinga et al., it was reported that patients with psoriasis concurrent with UC were mostly female, with the median age of psoriasis diagnosis at 46 years old.[19] Compared to the coexistence of psoriasis and UC, patients with psoriasis concurrent with CD have younger onset of psoriasis with the median age of 28 years and were associated with greater disease severity. They also revealed that PsA patients were also more likely to have IBD than psoriasis-only patients.[19] A case control study conducted by Lolli et al. showed different results where IBD was associated with milder severity of psoriasis and lower incidence of PsA.[20] The contradictive findings from different studies suggested that the characteristic profiles of patients with psoriasis, especially PsA, concurrent with IBD need to be explored further. In this case, the patient fits the typical epidemiological profile from the sex and age of psoriasis diagnosis.

The patient also had a history of anterior uveitis. A study regarding this matter has shown that there were indeed substantial risks for PsA patients to develop uveitis and/or CD, but not for PsA and UC, which makes the development of uveitis in this case uncommon.[21] In cases of psoriasis concomitant with UC, nail manifestation was also rarely documented, even though nail manifestation is perceived to be a strong predictor of arthritis development in psoriasis.[19,22] Plaque-type psoriasis was reported as the most common cutaneous phenotype of

psoriasis to be documented in UC, followed by phenotypes of capitis, inverse, psoriatic palmar pustulosis, and lastly guttate.[19,20] Cutaneous manifestation usually precedes the diagnosis of PsA by an average of 10 years, while in this patient, cutaneous lesions and arthritis manifested simultaneously at diagnosis, which is rare.[7]

This patient had been diagnosed with psoriasis complicated with PsA for 15 years before the diagnosis of UC was carried out. The findings from a bidirectional meta-analysis of psoriasis and IBD conducted by Fragoulis et al. have shown that that there were more cases where UC preceded the diagnosis of psoriasis than the other way around, as the prevalence of UC in psoriasis (0.7%) was lower than the prevalence of psoriasis in UC (1.8%).[23] In support of that notion, CD was also more commonly observed with other spectrum of SpA (either axial and peripheral) other than PsA as well, rather than UC.[18]

There has not been any documented endoscopic findings of patients with PsA concomitant with UC, but it has been suggested that isolated proctitis in UC has a scarce association with rheumatic manifestations. On the other hand, CD patients with evidence of colitis show more association with joint involvement compared to CD patients with findings of ileitis.[18] Aside from IBD, there are many common gastrointestinal diseases (celiac disease, esophagitis, irritable bowel disease) that are associated with psoriasis in other studies, and these findings highly suggest that psoriasis might have a concrete link to gut inflammation in general.[24] The results from a study conducted by Scarpa, et. al supported this notion by showing that the bowel mucosa of patients with PsA exhibit microscopic changes, even when appearing normal macroscopicallly and in absence of gastrointestinal symptoms.[25]

To the best of our knowledge, there has not been any study documenting the radiologic findings of PsA concurrent with IBD, but a large scale study conducted by Geijer et al. concluded that male PsA patients showed more pronounced radiological abnormalities than female patients.[26] In this case, the radiologic findings were suggestive of arthritis because of the narrowing of the interphalangeal joint spaces. But, classic radiological findings were not visualized.

To the best of our knowledge, there has been no published consensus on the most appropriate treatment options for PsA complicated with IBD, although these two entities share similar medications with overlapping effects on both diseases.[27] A study conducted by Eppinga et al. showed that most patients with psoriasis concomitant with UC were treated with sulfasalazine (64.3%), steroids (64.3%), and methotrexate (57.1%); while patients with psoriasis concomitant with CD were mostly treated with steroids (58.3%), azathioprine (50%), and Anti-TNFα (50%).[19] This patient was treated with methotrexate for PsA and sulfasalazine for UC, and administration of these medications exhibited favorable response and maintained remission in this patient. Furthermore, methotrexate was associated with improvement of PsA and maintenance of remission in CD, but studies have shown that methotrexate has no effects on maintaining remission in UC.[28] Mesalamine has been regarded as the first-line treatment for mild-to-moderate UC which has been documented by many studies to induce clinical response and maintain clinical remission in UC.[29] Although there are not enough data regarding the association of 5-ASA compound in PsA, sulfasalazine has been shown to improve symptoms and exhibited beneficial outcomes in patients with PsA as well. However, there was not any supporting evidence that sulfasalazine can halt the progression of joint damage in PsA.[30]

Emerging biologic agents, such as infliximab and adalimumab have been used to treat PsA concomitant with IBD as they share common predisposing genes and inflammatory pathways. However, there are less data on their efficacy on UC compared to robust evidences on CD. Certain biologic agents that were shown to exhibit favorable response in both PsA and UC are infliximab and adalimumab.[28] Besides the two favorable biologic agents that are beneficial to both PsA and UC, secukinumab, a biologic agent which is primarily used for PsA may aggravate IBD manifestations in some patients.[31]

CONCLUSION

PsA is commonly observed with IBD, especially with CD, while cases of UC concomitant with PsA are less documented. There was robust evidence regarding the association and correlation between PsA and CD from the clinical findings to treatment, while some studies showed conflicting findings with PsA and UC.

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The Application of Coronary Contrast Emptying Time in Diagnosing Coronary Slow Flow Phenomenon: A Serial Case Report

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ABSTRACT

The Coronary Slow Flow Phenomenon doesn't achieve as much attention as its counterpart Coronary Arterial Disease because it is considered a rather benign entity. But now it is proven that coronary slow flow phenomenon can also manifest as an acute coronary syndrome, myocardial ischemia, malignant arrhythmia, and even sudden cardiac death.

This entity is usually diagnosed from coronary angiography study when a delayed coronary contrast filling time is found without the presence of significant epicardial narrowing of the related arteries. But, in our center's years of experience, we frequently found cases in which myocardial ischemia or infarction was suggested or proven clinically, on the other hand, angiography study showed no significant epicardial coronary artery narrowing neither delayed coronary contrast filling time. Furthermore, we observed that this group of patients exhibited a rather prolonged coronary contrast emptying time instead.

In this serial case report, we presented some of our cases where microvascular disorders were suspected. We demonstrated that not all coronary contrast filling times in ischemic or infarction-related arteries were prolonged, on the other hand, prolongation of coronary contrast emptying time showed a more consistent result.

Keywords: *Coronary Slow Flow Phenomenon, Microvascular disorder, myocardial ischemia, myocardial infarction.*

INTRODUCTION

Traditionally, the coronary Slow Flow phenomenon is an angiographic entity characterized by delayed contrast filling of distal coronary arteries without significant stenotic lesions. Non-significant stenotic lesion defined as a stenotic lesion with less than 40% lumen diameter reduction.[1] Delayed contrast filling was commonly diagnosed either with Gibson's or TIMI method. Based on Gibson criteria, diagnosis of a delayed contrast filling is when the total frame count from the moment contrast entered the proximal of the related coronary artery until it reached the distal end exceeds 27 frames, with 30 frames/second angiographic frame speed. Meanwhile, LAD needs a correction factor, where the total frame count should be divided by 1.5. Whereas with TIMI criteria,

a delayed contrast filling is diagnosed when the time from the moment contrast entered the proximal of the related coronary artery until it reached the distal end takes more than three heartbeats. Other methods for measuring coronary blood flow velocity, such as the one that Gibson proposed using guidewire and Kelly clamps, were not commonly used.[2,3,4]

Years of experience in our center showed frequently found cases that do not fit the delayed filling time criteria by Gibson's or TIMI method but with clear myocardial ischemic or infarction evidence. The current method for diagnosing coronary slow flow phenomenon based on measuring contrast filling time can only detect this abnormality in its intermediate and late form. Thus, we propose coronary contrast emptying time measurement as a marker to diagnose coronary slow flow phenomenon in the earlier stage. We define coronary contrast-emptying time as the period that starts when it entered the related artery until its complete emptying with prolonged emptying time is more than 3 seconds (45 frames with 15 frames/second angiographic frame speed or 90 frames with 30 frames/second angiographic frame speed).

In this serial case report, we presented coronary slow flow phenomenon cases in our center. We demonstrate the measurement of coronary contrast emptying time and its comparison to Gibson's method for diagnosing coronary slow flow phenomenon. Here, we presented four patients whom we suspected to have myocardial ischemia or infarction episodes.

CASE ILLUSTRATION

The first case was a 35-year-old woman. She has no previous history of chest pain, neither history of diabetes or hypertension. She was complaining of squeezing chest pain started 2 hours before admission. On anamnesis, she informed that her father died after collapsing suddenly. The patient hemodynamic was stable. The ECG recording showed an ST elevation in septal and lateral leads. Further echocardiography examination showed a hypokinetic movement of basal and mid anteroseptal with a 45% ejection fraction.

There was no significant coronary lesion found during a coronary angiography study. The study showed filling time of LAD was 26,6 total frame count (with a picture-taking speed of 30 frames per second), which is within range. However, the coronary contrast emptying time was prolonged, with a total of 136 frame counts (with the picture-taking speed of 30 frames per second). Meanwhile, the filling time of LCx was within the recommended filling time limit (26 total frame counts). However, coronary contrast emptying time was prolonged, which was 110 total frame count. Filling time in RCA was 38 total frame count, which is also longer. Coronary contrast emptying time of RCA was 132 total frame count, showing another prolonged duration.

The second case was a 34 years old male who came to our clinic with chest discomfort on performing moderate activities. He also complained of having some palpitation episodes and get fatigued while performing daily activities. Fixed splitting of second heart sound was present. Otherwise, the physical examination findings were unremarkable. The ECG recordings showed a first-degree AV block with interchanging morphology between complete and incomplete RBBB. Cardiac MRI showed dilatation of theright atrium and right ventricle with mild tricuspid regurgitation, whereas left ventricle structure and function showed no abnormalities.

There was no significant coronary lesion found during a coronary angiography study. The study showed a total of 20 frame counts of LAD, which is normal. Yet coronary contrast emptying time was prolonged, with a total of 92 frame counts. The filling time of LCx was prolonged as well, with a total of 32 frame counts. Coronary contrast emptying time was 92 total frame count, which also showed a prolonged duration. Furthermore, the filling time and coronary contrast emptying time of RCA show an increment, with 46 frame counts and 178 total frame counts, respectively.

The third case was a 41 years old male. He was referred to our hospital with retrosternal chest pain starting 7 hours before admission. The patient was a smoker and had a history of dyslipidemia before, with other physical examinations showing normal findings. The

electrocardiography examination showed a marked ST elevation on inferior leads. The patient then underwent a fibrinolytic procedure successfully. Echocardiography showed concentric left ventricular hypertrophy with preserved systolic function.

There was no significant coronary lesion found during a coronary angiography study. The study showed filling time of LAD was 32 total frame counts, which showed a prolonged filling time. Coronary contrast emptying time was longer, with 104 frame counts. The filling time of LCx was good, with 26 total frame counts. However, the coronary contrast emptying time was 104 frame counts, which showed a prolonged duration. Filling time in RCA was also good, with 24 frame counts. Coronary contrast emptying time of RCA was 150 total frame count, which showed a marked prolonged duration.

The fourth case was a 45 years old female who came to our hospital with the typical chest pain symptoms, induced by moderate activities such as walking 100 meters or climbing stairs, but further relieved with short rest. The patient has a history of hypertension for four years, which she consumed amlodipine 5 mg daily. Physical examinations on the patients show no remarkable findings. The electrocardiography displayed a complete LBBB with ST- elevation on avR. Meanwhile, the echocardiography showed left ventricular hypertrophy with a 63% ejection fraction, with no segmental wall motion abnormalities.

There was no significant coronary lesion found in the coronary angiography study. Its filling time was 24 total frame counts. Nevertheless, its coronary contrast emptying time was 108 total frame count, which fulfilled our criteria as prolonged contrast emptying duration. Although the coronary contrast emptying time increased to 96 frame counts, the filling time of LCx was good, with a total of 26 frame counts. Filling time in RCA was 32 total frame counts, which showed a prolonged duration. Coronary contrast emptying time of RCA was 110 total frame count, which is long.

DISCUSSION

We proposed several postulates to explain why measuring the coronary emptying time as a whole is critical in assessing coronary microvascular disorders and not just coronary filling time. Classically, there are two compartments of coronary vasculature, which are epicardial coronary arteries and microvascular. There are three subcategories for microvascular, which are arteriole, periarteriole, and capillary beds. In normal conditions, arteriole contributes to 25% of total blood flow resistance in the coronary vasculature, arteriole contributes to 55% of total resistance, while the rest comes from capillary beds. Several different factors play roles in regulating microvascular tones.[5]

Endothelial and neural factors, mainly induced by the shear stress of the vascular wall, regulate periarteriole and large-arteriole tones. Meanwhile, the myogenic factors and physical factors control the medium-sized arteriole tones. Take, for example, the presence of extravascular compression and increased intraventricular end-diastolic pressure. Metabolic factors, on the other hand, regulate the small-sized arteriole tones. While we have a predominant mechanism for controlling resistance on each part of the microvascular compartment, this is more like a continuum than a clear-cut separation.[5]

Table 1. Summary of myocardial ischemia/infarction in each patient, with coronary filling time dan emptying time measurements

Patient	Evidence suggesting myocardial ischemia/infarction	Coronary Filling time			Coronary Emptying Time		
		LAD	LCx	RCA	LAD	LCx	RCA
35 years old woman	Anterolateral ST elevation Hypokinetic wall motion of anteroseptal LV wall on echo	26.6	26	38	136	110	132
34 years old male	Palpitation and fatigueness 1st degree AV block with RBBB	20	32	46	92	92	178
41 years old male	Inferior ST elevation	32	26	24	104	104	150
45 years old female	Typical chest pain Complete LBBB	24	26	32	108	96	110

In coronary angiography study, the first compartment blood and contrast entered after occupying epicardial arteries is arterioles-large arterioles compartment. When the blood can not enter the arterioles-large arterioles compartment, the coronary angiography contrast will also face difficulties in occupying epicardial space, known as the delayed filling time in the slow coronary flow phenomenon. Endothelial dysfunction and subclinical atherosclerosis are widely accepted. They are also the most studied etiologies for the slow coronary flow phenomenon. Cin and colleagues on their study with intravascular ultrasound found a diffuse intimal thickening and widespread calcification with no luminal irregularities observed from coronary angiography in patients with coronary slow flow phenomenon. In other study, Pekdemir and colleagues also found increased endothelin-1 concentration in coronary slow flow patient during rapid atrial pacing compared to patient without coronary slow flow phenomenon. These entities will affect primary, large arterioles, and also a proportion of medium-sized arterioles. So our first suggestion is that a milder but more diffuse form will cause difficulties of blood entering the 'medium-sized arterioles' compartment on coronary angiography study, which in turn will cause difficulty for contrast entering the 'prearteriole- large arteriole' compartment. Rather than delayed contrast filling, this phenomenon will manifest more as delayed contrast emptying.[6,7,8]

Several factors theoretically might contribute to microvascular disturbance but are not as extensively studied. Those factors are the physical factors, myogenic and metabolic factors. Since they mainly affect medium to small-sized arterioles resistance, these factors will cause delayed contrast emptying rather than filling in coronary angiography. It further lays the foundation for the second suggestion where physical factors like left ventricular hypertrophy and myocardial fibrosis could contribute to coronary microvascular disorders incidence. Furthermore, it manifests as delayed contrast emptying time rather than filling time on coronary angiography.[8]

Although we emphasize the contrast emptying time aspect of the microvascular disorder, its measurement should not be separated from contrast filling time measurement. The reason is that we accept that endothelial dysfunction and subclinical atherosclerosis are the main risk factors for the slow coronary flow phenomenon until now. Moreover, these compartments previously described are a continuum rather than separate compartments. Thus, we propose new criteria for diagnosing slow coronary flow phenomenon with coronary contrast emptying time, which counts from the first time the contrast entering the related epicardial arteries until it fully empties from that artery. This process will take no more than three seconds.

Epicardial coronary stenosis can have a direct impact on coronary filling time. Its presence consequently excludes the diagnosis of coronary slow flow phenomenon by Gibson's criteria. Ramakrishnan and his colleagues from their observational study concluded that dyslipidemia, hypertension, and smoking are strongly associated to coronary slow flow phenomenon incidence. Since they have relatively the same risk factors, these two entities can be present in one patient at once. Patel and colleagues demonstrated the presence of these two entities at once on her study by measuring myocardial perfusion at rest and stress with quantitative positron emission tomography. That is why we also propose that in the significantly narrowed epicardial coronary artery, prolongation of coronary emptying time with a normal-filling time could indicate a presence of microvascular disorder in conjunction with epicardial stenosis.[9,10]

Different operators lead to the variability of contrast injection duration, and it will cause bias without using an automatic contrast injector device. Thus we proposed the contrast injection duration to be 1 to 1,5 seconds for three cc contrast each shot.

CONCLUSION

We propose that measurement of epicardial contrast duration might serve as a better marker in terms of sensitivity than measurement of contrast filling time.

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Preventing Thrombosis in Cancer Patients

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ABSTRACT

Thromboembolism events, either venous (VTE) or arterial thromboembolism (ATE) remain a highly prevalent complication in cancer patients. Thrombosis is a leading cause of death, contributor to significant morbidity, the reason of delayed cancer treatment, leading to increased cancer financing and expenses. Both cancer and its treatment are recently found to be related to vascular inflammation through the induction of tissue factor (TF) expression and promoting a procoagulant state which triggers the activation of coagulation system. Several risk factors may also coexist such as dehydration, immobilization, smoking, obesity, previous DVT, etc. Even in patients with asymptomatic deep vein thrombosis (DVT), they have a three-fold increase in mortality. The high morbidity and mortality of VTE raises the need for thromboprophylaxis to reduce the incidence of overt thrombosis, albeit against its possible side effects related to anticoagulant prescription. This article highlighted the clinical perspectives for thromboprophylaxis while counting on the risk stratification in a particular cancer patient.

Keywords*: thrombosis, cancer, inflammation, thromboprophylaxis.*

INTRODUCTION

Cancer is a chronic disease with a high morbidity and mortality rate despite treatment advances. However, today many patients can survive longer due to progress in early diagnosis and progress in its treatment.[1] Cancer has long been known to be related to thrombosis and patients are reported to have a 7-fold increased probability compared to the general population.[2] Studies reported that the incidence of thrombosis in patients with cancer has been increasing overtime, partly due to its increasing incidence in recent years.[3] Therefore, management of complications especially thrombosis during the disease course are becoming more clinically relevant. Optimal strategies to manage cancer-associated thrombosis remains a major concern that challenges clinicians in daily clinical practice; due to the fact that thrombosis is a preventable complication.[3,4]

Venous and arterial thrombosis are the already known two spectrums of thrombosis.[4] Venous thromboembolism (VTE) represents a clinical condition whether the thrombus is developed in the venous vasculature of the lower extremities and pelvic veins, as well as visceral or splanchnic vein thrombosis. Thrombus migration proximally can travel along the bloodstream.

Pulmonary embolism (PE) can unexpectedly develop when the thrombus embolization occurs in the pulmonary artery or its branches which is the major cause of morbidity and mortality in patients with DVT.[5] In addition to VTE, arterial thromboembolism (ATE), including the myocardial infarction (MCI), cerebrovascular accident (CVA), and peripheral arterial disease (PAD) are also prevalent in patients with active cancer compared to non-cancer population.[4] Thrombosis events, either VTE or ATE, are the second-leading cause of mortality after cancer progression itself.[6,7] This make cancer-associated thrombosis a clinical conditions in which relevance should be increasingly recognized both for physician and medical oncologists.

This article aims to described the need for thromboprophylaxis treatment in cancer patients and how to identify those who would benefit, irrespective of the risks. The dogma that "prevention is better than cure" is not an exaggeration in terms of reducing the burden of thrombosis. The decision to prescribe anticoagulants as a prophylactic measure should be based on the risks of morbidity and mortality related to VTE/ATE, thrombosis recurrence, anticoagulant-related bleeding, as well as on social values and patient preferences, particularly in Indonesia.

THE BURDEN OF VTE (AND ATE) IN ONCOLOGICAL PRACTICE

Cancer patients will experience complications during the course of their disease, which includes disease progression, infections, side effects of chemotherapy, as well as thrombosis, which is a frequently occurring complication among others.[6,8] To weigh the benefits against the risks of thromboprophylaxis, clinicians need to be familiar with the burden of thrombosis in cancer patients (**Table 1**). The decision to provide thromboprophylaxis should be based on careful assessment of the benefits, such as reduction in VTE and possible arterial thromboembolism, against its harms including the side effect of bleeding from anticoagulant.[7] The risk of thrombosis in cancer patients, the purpose of anticoagulation, and the consequences in this population underlines the need for clinicians to carefully assess all factors before deciding to recommend any thromboprophylactic strategies.[9,10]

Thrombosis in cancer patients can ultimately interfere with cancer treatment, reduce the quality of life, lead to additional diagnostic tests, increase treatment cost, and prolong length of stay. Patients with a history of VTE have a higher risk of recurring thrombosis and an increased mortality rate.[7,11] Approximately 95% of blood clots originate from the proximal portion of the lower extremities. However, pulmonary embolism may also occur without prior DVT. Thrombosis can occur without the presence of any symptoms, referred to as incidental thrombosis. A study conducted in Dr. Kariadi Hospital reported the incidence of asymptomatic DVT to be 25.6% among cancer patients. Without prophylaxis, PE or even fatal PE can be the initial manifestation of VTE. Despite that, thrombotic events in cancer patients has not gained enough attention as seen by the lack of practice of thromboprophylactic use in clinical practice, although the international[4,5] and Indonesian national guidelines have been published since 2018.[12]

Table 1. Thromboprophylaxis and dire consequences of thrombosis in cancer patients.

Ultimate goals for thromboprophylaxis	
Prevent thrombosis	
Reduced risk of thrombosis recurrences	
Short-term (immediate) consequences	**Long-term consequences**
Morbidity caused by DVT and/or PE	Post-thrombotic syndrome
Interruption of cancer treatment	Chronic thromboembolic pulmonary hypertension
Reduced quality of life	Long-term bleeding risk
Financial consequences	
Increased mortality	

Abbreviations: DVT, deep vein thrombosis; PE, pulmonary embolism
Source: Mulder FI, *et al*. Cancers 2020, with modifications.

EPIDEMIOLOGY

Horsted et al.[13] reported that the incidence rates of venous thrombosis in cancer patients could be stratified by the background risk of VTE. The incidence among cohorts with average-risk patients was estimated to be 13 per 1,000 person-years (95% CI: 7-23). Among cohorts with high-risk characteristics, the overall incidence rate was incredibly high with 68 per 1,000 person-years (95% CI: 48-96). In terms of the type of cancer, certain cancer can interestingly be more hypercoagulable than others, such as those in the gastrointestinal tract, including gastric, esophageal, and pancreatic cancers (**Table 2**). Patients with brain and lung cancers also showed an increased risk of VTE by more than ten-fold compared to the general population.[14]

Table 2. Incidence of VTE among various types of cancer.

Type of cancer	First VTE per 100 person-years (95% CI)
Bladder	2.7 (2.4-3.0)
Breast	3.2 (2.9-3.4)
Prostate	4.4 (4.0-4.7)
Hematologic	4.5 (4.1-4.8)
Colon	6.7 (6.3-7.2)
Lung	10.1 (9.5-10.8)
Stomach	10.8 (9.5-12.3)
Ovary	11.9 (10.6-13.2)
Brain	12.1 (10.3-14.0)
Pancreas	14.6 (12.9-16.5)

Source: Cohen AT, *et al*. *Thromb Haemost* 2017.

Cancer patients have a 4- to 7-fold risk of developing VTE compared to non-cancer patients. According to Iorga et al.,[15] the prevalence of VTE in patients with cancer was 15% and correlated with poor treatment outcomes. Moreover, 20-30% of all VTE cases occurrs in cancer patients.[15,16] Data from a cohort study of 21,002 inpatients in California showed that 20% (4,368 patients) of cancer patients were found to have thrombosis.[16] A study in Korea reported that the cumulative incidence of VTE in 2 years has increased to 24.4% in patients with metastatic gastric cancer.[17] A retrospective cohort study conducted in Dharmais Cancer Hospital Jakarta showed that chemotherapy is a risk factor of DVT in patients with cancer (OR 5.0, p= 0.012).[18]

The risk of thrombosis can vary depending on the disease status. It generally increases during periods of active disease, hospitalization, tumor-directed therapy, and decreases during remission.[19,20] Chew *et al*. also reported an increased risk of VTE in all types of cancer with advanced metastasis.[21] The early phases following initial diagnosis is the period with the highest risk of developing VTE (**Figure 1**), where some prothrombotic mechanisms are involved in the CAT mechanism. The incidence of VTE is also high in patients undergoing chemotherapy. Cases of VTE in cancer patients is not limited to DVT and PE, but also thrombosis in unusual sites, such as the upper extremities, cerebral veins, and splanchnic veins.[22]

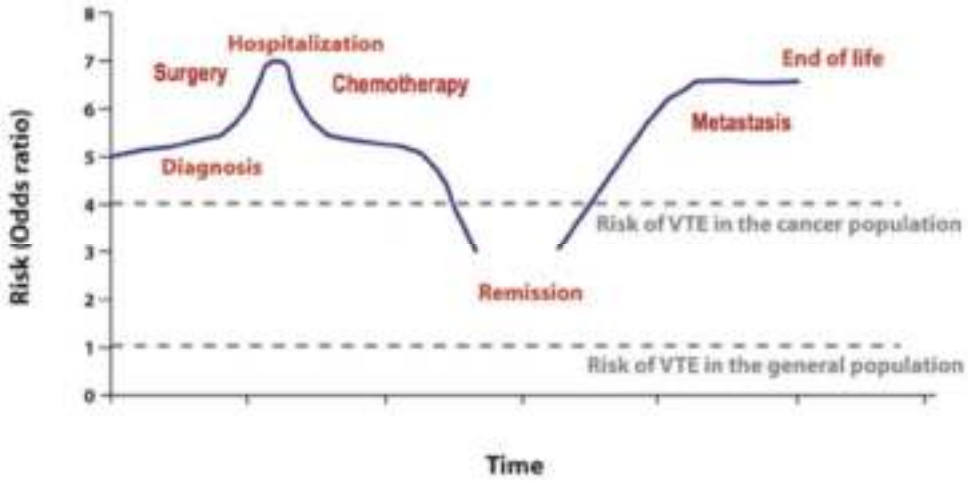

Figure 1. Dynamic changes in the risk of VTE along the course of cancer.
Reproduced with permission from Streiff MB. *Clin Adv Hematol Oncol* 2013.

RISK FACTORS FOR THROMBOSIS

The risk factors for thromboembolism are divided into patient characteristic risks, tumor-related risks, and therapy-related risks.[22] Thrombosis events in cancer patients are generally based on the interactions of each risk factor (**Table 3**). A person with more risk factors had a greater chance of developing thrombosis. Certain types of cancer have a higher incidence of thrombosis. This risk is also higher in the later stage of cancer and metastatic disease. As a concrete example, 60-70% of patients with pancreatic cancer has VTE as found in autopsy.[23]

PATHOPHYSIOLOGY OF THROMBOSIS IN CANCER PATIENTS

On the basis of thrombosis, there is the so-called Virchow's triad of endothelial injury, hypercoagulability, and venous stasis. Cancer cells can activate coagulation pathways by direct and indirect mechanisms. The direct mechanism involves production of procoagulant factors, such as tissue factors, which is constitutively expressed by cancer cells that bind to circulating FVIII and activate coagulation pathways. The indirect mechanism involves an exposure of proinflammatory cytokine stimulation in the tumor microenvironment,[24] and the administration of chemotherapy also causes damage to endothelial cells, therefore triggering an inflammatory response.[25,26] Inflammatory stimuli from cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL) -1a, IL-6, IL-17, and IL-18, as well as epidermal growth factors (EGF) that mediate inflammatory responses activated through interactions with Toll-like receptors (TLRs), IL-1 receptors (IL-1R), IL-6 receptors (IL-6R), and TNF receptors (TNR).[26,27]

In **Figure 2**, multiple mechanism of cancer-associated thrombosis is illustrated. Oncogenic MET, RAS, p53, or PTEN activation, besides promoting cancer, can also induced gene transcription involved in the hemostasis regulation such as PAI-1, COX-2, and TF. Tumor hypoxia also causes HIF-1α overexpression that directly controls the expression of hemostasis factors through the activation of PAI-1 and COX-2, or through MET.[25] The figure also shows that tumor-derived cytokines (IL-2, TNF and VEGF) can activate monocytes, platelets and endothelial cells. Tumor cells adhesion molecules (P-selectin, L-selectin) can bind the inflammatory cells which activate coagulation and stimulate fibrin production. Some predisposing factors can add to the overall prothrombotic phenotype in an individual cancer patients, such as obesity, diabetes, smoking habit, older age, hospitalization, surgery, central venous catheter (CVC) insertion, tumor compression stasis, ascites, and chemotherapy.[8,20,22]

Table 3. The risk factors for cancer-associated thrombosis.

Patient Characteristics	Cancer-Related Factors	Treatment-Related Factors	Biomarkers
Female sex	Site or origin of cancer	Hospitalization	High tissue factors expression
Older age	Tumor histology	Cancer therapy	Pre-chemotherapy platelet count > 350,000/uL
Race (African ethnicity)	Advanced stage and metastatic cancer	Erythropoiesis-stimulating agents	Pre-chemotherapy WBCs > 11,000/uL
Common comorbidities: diabetes, obesity, previous VTE, atherosclerosis, inflammation, others	Being in initial period after cancer diagnosis	Venous catheter	Elevated D-dimer
Inherited thrombophilia			High levels of: Plasma tissue factor Soluble P-selectin C-reactive protein von Willebrand factor Low expression of: ADAMTS13 gene

Abbreviations: VTE, venous thromboembolism; WBC, white blood cells; ADAMTS13,
Source: Eichinger S. *Thromb Res* 2016

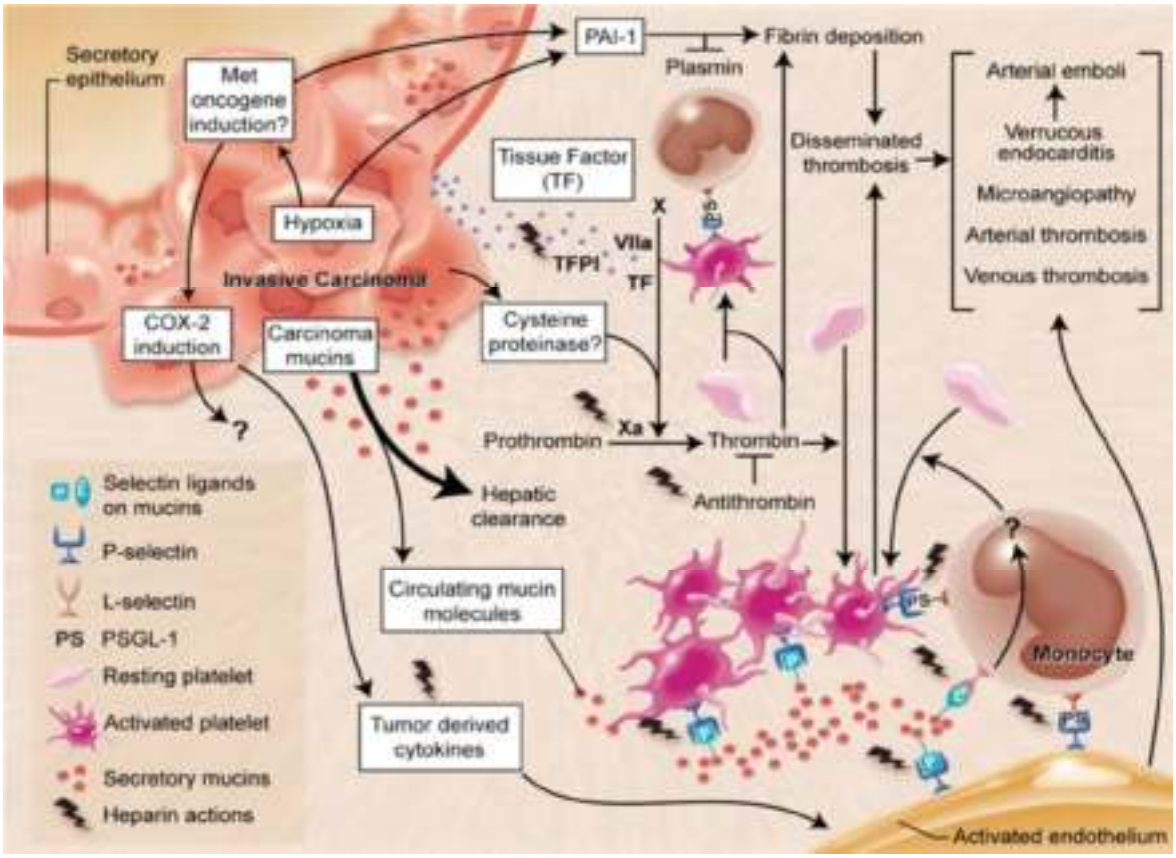

Figure 2. Multiple mechanisms in the pathophysiology of cancer-associated thrombosis. There are overlapping and interacting mechanisms that can explain the increased incidence of thrombosis (both arterial and venous thrombosis) in cancer patients. Hypercoagulability is ultimately the result of intrinsic and extrinsic risk factors. Reproduced with permission from Varki A, *Blood* 2007.

The administration of chemotherapy can lead to an inflammatory condition,[25] which triggers NF-κB and MAPK signaling pathways to produce proinflammatory cytokines, including IL-6, TNF-α, IL-1, IL-8, and CRP.[26] Proinflammatory cytokines play a role in thrombus formation in cancer patients and those undergoing chemotherapy. Inflammatory markers such as hs-CRP is correlated with Wells score and D-dimer, which can be used to predict the incidence of DVT in cancer.[28] Chemotherapy-induced vascular endothelial cell activation (VECA) is demonstrated by increased binding of circulating endothelial cells and von Willebrand factors (vWF) in the plasma.[29] vWF triggers platelets adhesion, factor VIII binding and transport, as well as thrombus formation.[30] Our study revealed that pre-chemotherapy levels of vWF:Ag and ADAMTS-13 are independent risk factors for DVT incidence among cancer patients.[31]

VTE RISK STRATIFICATION

In order to assess VTE risk in cancer patients, various factors need to be considered. Some risk models have been developed and validated. The most known is the Khorana risk score which is stratified into low (score 0), intermediate (score 1-2) and high risk (score 3) based on several variables such as cancer site, platelet count, WBCs count, hemoglobin levels or use of ESA, and BMI, as shown in **Table 4**. This model had a negative predictive value of 98.5%, positive predictive value of 7.1%, sensitivity of 40%, and specificity of 88%, as reported by a cohort study of 2,701 patients which was then validated into a prospective independent cohort study of 1,365 patients.[32] Some variations have been published such as PROTECH,[33] CONKO,[34] and Vienna CATS score,[35] which elaborate other biomarkers like D-dimer and soluble P-selectin.[36] The COMPASS-CAT[37] and ONKOTEV56[38] models were subsequently developed, which included

variables such as cardiovascular risk factors, history of VTE, presence of CVC, chemotherapy or hormonal therapy, tumor stage, and platelet count.

The risk of major bleeding must be considered when choosing pharmacological VTE prophylaxis in cancer patients for an optimal outcome. Regardless of the selection of anticoagulation, the primary contraindication to prophylactic anticoagulant are bleeding episodes.[39,40] The evidence-derived IMPROVE Bleeding Score used 13 clinical and laboratory factors and designated a score of seven or more to identify a patient cohort (10% of the population) at a high risk of bleeding (major bleed risk), 4.1% vs. 0.4%. Patients with a score of less than seven were considered at a lower risk of bleeding (**Table 5**).[41] Sex and age are the fixed risk factors, while the remaining are modifiable risk factors. When deciding whether anticoagulant can be safely initiated in a prophylaxis setting, clinicians should always optimize the patient's current clinical status.

Table 4. Predictive models for chemotherapy-related VTE in ambulatory cancer patients (Khorana risk score).

Patient Characteristics	VTE Risk Score
Cancer origin	
Very high risk	2
Primary brain, gastric, or pancreatic tumors	
High risk	1
Lung, lymphoma, gynecologic, or genitourinary tumors, excluding the prostate, and myeloma	
Low risk	0
Breast, colorectal, or head and neck tumors	
Other characteristics	
Platelet count ≥ 350x10^6/uL	1
WBCs count > 11 x 10^3/uL	1
Hemoglobin <10 g/dL or use of red blood cell growth factors	1
BMI ≥ 35 kg/m^2	1
NOTES: Low risk: 0 score; intermediate risk 1 or 2 score; high risk: 3 or higher score	

Abbreviations: BMI: body mass index; WBC, white blood cells
Source: Khorana AA, et al. Blood 2008.

Table 5. IMPROVE bleeding risk score.

Variables			Bleeding Risk Score
Fixed (non-modifiable) risk factors	Age	≥ 85 years	3.5
		40 to 84 years	1.5
		< 40 years	0
	Gender	Male	1
		Female	0
Modifiable risk factors	Kidney function	Severe kidney impairment (GFR ≤ 30 mL/min/m^2)	2.5
		Moderate kidney impairment (GFR 30 to 59 mL/min/m^2)	1
		Normal kidney function (GFR ≥ 60 mL/min/m^2)	0
	Liver function	Liver failure (INR ≥ 1.5)	2.5
		Normal liver function (INR <1.5)	0
	Platelets	<50 x 10^6/uL	4
		≥50 x 10^6/uL	0
	Other factors	Active gastric or duodenal ulcers	4.5
		Prior bleeding within last 3 months	4
		Admission to ICU or CCU	2.5
		Central venous catheter	2
		Active malignancy	2
		Rheumatic disease	2
NOTES: Low risk: score <7; increased risk: score ≥7			

Abbreviations: INR: international normalized ratio, GFR: glomerular filtration rate, ICU: intensive care unit, CCU: coronary care unit
Source: Skeik N, Westegard E. *Ann Vasc Dis* 2020.

CURRENT EVIDENCE

The VTE prophylaxis guideline in cancer patients with anticoagulants such as unfractionated heparin (UFH), low molecular weight heparin (LMWH), direct oral anticoagulant (DOAC) including rivaroxaban or apixaban has been recommended by the American Society of Clinical Oncology (ASCO),[42] International Initiative on Thrombosis and Cancer (ITAC),[43] National Comprehensive Cancer Network (NCCN)[44] and also the national guideline from *Perhimpunan Trombosis Hemostasis Indonesia* (PTHI) or the Indonesian Society on Thrombosis Hemostasis (InaSTH).[12]

The results of recent clinical trials support the benefits and safety of VTE prophylaxis in medical patients. These clinical trials have compared enoxaparin, dalteparin, and fondaparinux to placebo in patients with acute medical illnesses. The use of enoxaparin in the Medical Patients with Enoxaparin (MEDENOX) trial,[45] dalteparin in the Prevention of VTE in Immobilized Patients (PREVENT) trial,[46] fondaparinux in the Arixtra for Thromboembolism Prevention in a Medical Indications Study (ARTeMIS) trial,[47] and rivaroxaban in the CASSINI trial[48] were each compared to placebo. All studies show a significant decrease in the incidence of VTE. These results support the evidence-based recommendations for the use of thromboprophylaxis in clinical practice.

PRIMARY THROMBOPROPHYLAXIS: CHOICE, DOSE AND DURATION

Routine thromboprophylaxis is not recommended in all patients with cancer, particularly ambulatory patients. Thrombo-prophylaxis should be offered to patients with a high risk of thrombosis, including patients with myeloma receiving thalidomide or lenalidomide, and specific strategies for patients with myeloproliferative diseases should be determined.

We proposed a Khorana score-based decision algorithm for thromboprophylaxis administration to cancer patients. An aggregate score of zero indicates low risk (0.8% risk of VTE over the course of 4 chemotherapy cycles), score 1-2 indicates intermediate risk (1.8%) and score 3 or greater indicates high risk (7.1%). Cumulative VTE risk have been estimated at 17.7% in the high risk group.[39] More recent publications have suggested that high risk may be reflected by a score of 2 or greater when accommodating both inpatient and outpatient cancer populations.[49,50] The second mentioned was based on Khorana risk score ≥2 associated with the presence of metastasis, vascular compression, and previous VTE.

Thromboprophylaxis may be recommended in patients with a Khorana score of <2 whether there were addition of other risk factors such as prior VTE, known thrombophilia, or BMI >40 kg/m^2. Caution should be in mind for patients with high bleeding risk, unresected tumors, impaired or fluctuating renal function, highly emetogenic chemotherapy agents limiting reliable oral intake, and drug-to-drug interactions. The proposed thromboprophylaxis chart is illustrated in **Figure 4**.

Prophylaxis for medical patients:[42]

1. Pharmacologic thromboprophylaxis is recommended for hospitalized patients with acute medical illness and reduced mobility, in the absence of bleeding and other contraindications.
2. Routine pharmacologic thromboprophylaxis is not recommended in patients admitted for minor procedures or chemotherapy infusion, or in patients undergoing bone marrow transplantation.

Prophylaxis for cancer patients undergoing systemic chemotherapy:[42,43]

1. Routine pharmacologic thromboprophylaxis is not recommended for all cancer outpatients.
2. High-risk cancer outpatients (Khorana score of 2 or higher), can be recommended to receive thromboprophylaxis with apixaban, rivaroxaban, or low-molecular-weight heparin (LMWH) prior to starting a new chemotherapy regimen, provided that there are no significant risk factors for bleeding and in the absence of drug interactions. Considerations for such therapy should be accompanied by a discussion with the patient about the relative benefits and harms, drug costs, and duration of prophylaxis.
3. Patients with multiple myeloma receiving thalidomide or lenalidomide-based regimens

with chemotherapy and/or dexamethasone are recommended to receive pharmacologic thromboprophylaxis with either aspirin or LMWH for low-risk patients and LMWH for high-risk patients.

Prophylaxis for cancer patients undergoing surgery:[42]

1. All cancer patients undergoing major surgery is recommended to receive pharmacologic thromboprophylaxis with either unfractionated heparin (UFH) or LMWH unless contraindicated due to active bleeding, high bleeding risk, or other contraindications.
2. Mechanical prophylaxis may be added to pharmacologic thromboprophylaxis but should not be used as monotherapy for VTE prevention unless pharmacologic methods are contraindicated due to active bleeding or high bleeding risk.
3. A combined regimen of pharmacologic and mechanical prophylaxis may improve efficacy, especially in high-risk patients.
4. Pharmacologic thromboprophylaxis for cancer patients undergoing major surgery should be continued for at least 7 to 10 days. Extended prophylaxis with LMWH for up to 4 weeks post-operatively is recommended for patients undergoing major open or laparoscopic abdominal or pelvic surgery for high-risk patients, such as those with restricted mobility, obesity, history of VTE, or with other risk factors.

The algorithm for thromboprophylaxis need to be individualized and the expected benefits should always outweigh the risk of bleeding. As depicted in **Figure 3,** major surgery and hospitalization are important risk factors for VTE in cancer patients. If the bleeding risk is fair or low, then primary thromboprophylaxis can be recommended.

Information about anticoagulant dosing for thromboprophylaxis in cancer patients are provided based information on **Table 7**.

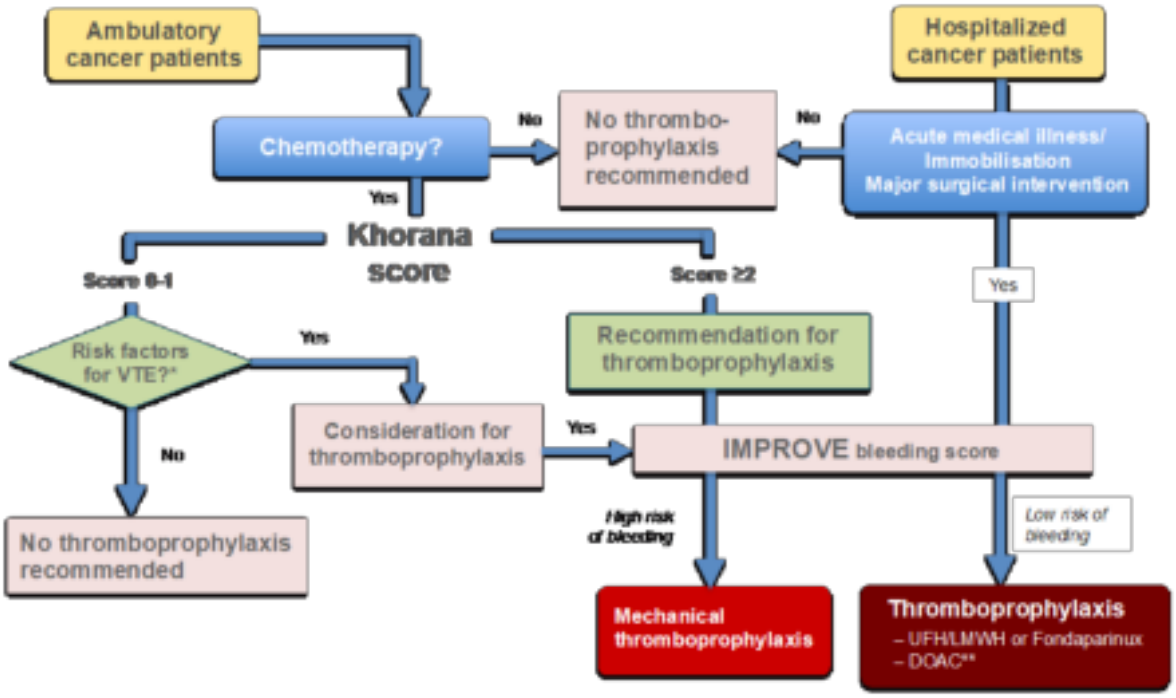

Figure 3. Daily practice algorithm for individual decisions for thromboprophylaxis in cancer patients.
Abbrebiations: LMWH, low moeluclar weight heparin; UFH, unfractionated heparin; VTE, venous thromboembolism
*Additional risk factors for thrombosis include: prior VTE, known thrombophilia, or BMI >40 kg/m^2 (see text for detail).
** DOAC can be considered only in non-gastrointestinal cancers

Table 7. Anticoagulant dosing regimens for prophylaxis in cancer patients.

Clinical background	Agent	Dose
Hospitalized cancer patients for medical reason(s)	UFH	5000 U every 8 hours sq
	Dalteparin	5000 U every 24 hours sq
	Enoxaparin	40 mg every 24 hours sq
	Fondaparinux	2.5 mg every 24 hours sq
Cancer patients undergoing surgery	UFH	5000 U 2-4 hours sq preoperatively and then every 8 hours thereafter
	Dalteparin	2500 U 2-4 hours sq preoperatively and 5000 U every 24 hours thereafter or 5000 U 2-4 sq hours preoperatively or 10-12 hours preoperatively and 5000 U every 24 hours thereafter
	Enoxaparin	40 mg 2-4 hours sq preoperatively or 10-12 hours preoperatively and 40 mg/24 hours thereafter
	Fondaparinux	2.5 mg every 24 hours sq beginning 6-8 hours postoperatively
Outpatient setting	Dalteparin	5000 U every 24 hours sq
	Enoxaparin	40 mg every 24 hours sq
	Fondaparinux	2.5 mg every 24 hours sq
	Apixaban	2.5 mg orally every 12 hours po
	Rivaroxaban	10 mg orally every 24 hours po

Abbreviations: p.o., per oral; sq, subcutaneously; UFH, unfractionated heparin
Source: Key NS et al. J Clin Oncol 2019.

SECONDARY PROPHYLAXIS

The concept of anticoagulant therapy (with proven existing VTE) can involve prolonged therapy for more than 3-6 months by noting that active cancer is a risk factor for VTE, with an annual recurrence rate of 10 to 29%. Considerations include: cancer type and activity, burden of disease, therapy, patient preference, immobilization, and life expectancy.[51] The NCCN guidelines recommends LMWH as the preferred treatment for the first 6 months, or DOAC (rivaroxaban) if the patient refuses to be injected or is not a candidate for subcutanoues medication for several reasons.[52]

THE NEW PARADIGM OF CANCER-RELATED THROMBOPROPHYLAXIS

Despite the existence of published guidelines and studies regarding the benefits and safety of VTE prophylaxis, we continue to see low adoption of such recommendations, and VTE prophylaxis remains underused.[53,56] The reason behind the low provision of prophylaxis in patients with high risk of VTE is most often due to cost considerations,[53,55,57] concerns of bleeding complications,[54,56] lack of knowledge and confidence,[54] lack of awareness,[55,58] and reluctance to give daily injections of anticoagulants as prophylaxis.[54]

Recent advances in understanding the mechanism of VTE demonstrate the pivotal role of the immune system and inflammation in its pathogenesis, and show that it is an immunity and inflammation-related process rather than merely coagulation-dependent thrombosis. The above paradigm opens new ideas for further research on new therapeutic options for VTE prophylaxis by inhibiting immune and inflammatory processes, instead of inhibiting the coagulation factors on the coagulation cascade directly, thereby reducing the risk of bleeding that can occur with the administration of anticoagulants as VTE prophylaxis.[59] Currently, there is no specific guideline for arterial thrombosis in cancer patients due to the lack of specific data available. However, usual care is recommended.

CONCLUSION

Thromboembolism events remain highly prevalent in cancer patients. Venous

thromboembolism is a leading cause of death, morbidity, delayed treatment, and increased treatment cost. The high morbidity and mortality of VTE raises the need for thromboprophylaxis to reduce the incidence of these clinical conditions. The administration of effective VTE prophylaxis and treatment in cancer patients can improve their survival rate and quality of life. Today, there are several options in medical thromboprophylaxis that include UFH, LMWH, and more recently DOAC also have been validated in several clinical trials involving patients with cancer. The decision to choose one anticoagulant over another was based on clinical ground, type of cancer, risk of bleeding, renal function, patient compliance, social economic religion aspects and finally, patient's preferences.

Recent advances in understanding the mechanism of VTE demonstrate the pivotal role of the immune system and inflammation in its pathogenesis, and show that VTE is an inflammation-related process, instead of merely coagulation-dependent thrombosis. The above paradigm opens new insights for further research on new therapeutic options for VTE prophylaxis by inhibiting immune and inflammatory processes, instead of inhibiting the coagulation factors on the coagulation cascade directly, thereby reducing the risk of bleeding that can occur with the administration of anticoagulants as VTE prophylaxis.

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Molnupiravir and Nirmatrelvir/Ritonavir: The New Available Antiviral Options for COVID-19

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a respiratory tract disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). With the complexity of multimorbidity in Indonesia, it is crucial to find another line of antiviral for COVID-19. This article aims to review two antivirals, molnupiravir and nirmatrelvir/ritonavir, that have been studied extensively in treating COVID-19 with promising results, and their availability in Indonesia. Molnupiravir and nirmatrelvir/ritonavir are two of many repurposed drugs in clinical trials, which have been reported to have a mechanism in quick clearance of SARS-CoV-2, reduction in viral load, and fast symptoms recovery time in phase 1 and 2 clinical trials. Phase 2/3 clinical study in COVID-19 patients without any indication for hospitalization showed that molnupiravir and nirmatrelvir/ritonavir significantly reduced the risk of hospitalization and death.

Keywords**:** *antiviral, COVID-19, molnupiravir, nirmatrelvir/ritonavir, SARS-CoV-2.*

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a respiratory tract disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Up to early September 2022, the disease has caused 613 million cases and 6.5 million deaths globally.[1,2] One of the strategies in eradicating COVID-19 is vaccination, which has reached more than 12 billion doses administered worldwide.[1] Vaccination in Indonesia is underway and reaching over 204 million first dose in September 2022.[3] Although the administration of vaccine has a widespread distribution, the incidence rate of COVID-19 during this past month is still high, reaching 2,344 cases for every 100,000 people.[1,2]

In addition to vaccination as a preventive measure, proper and effective curative treatment is essential to decrease morbidity and mortality caused by COVID-19. Many studies have reported the role of COVID-19 medications, such as the use of inflammatory agents, immunoglobulin, immunomodulator, convalescent plasma, or antibiotics and antivirals. We aim to highlight the role of newly repurposed antiviral agents for COVID-19 treatment, molnupiravir and nirmatrelvir/ritonavir, which are available in Indonesia since early 2022, but have not been widely reviewed.

COVID-19 AND RNA DEPENDENT RNA POLYMERASE (RDRP)

SARS-CoV-2 is a positive single-stranded ribonucleic acid (RNA) coronavirus which is encapsulated by an envelope and a nucleocapsid.[4] It has a 29.9-kb genome with a diameter of 50-200 nm.[5] The virion has many structural proteins, i.e., spike (S), envelope (E), nucleocapsid (N) and membrane (M).[4,5] The S protein facilitates the binding of angiotensin-converting enzyme 2 (ACE2) and the host cell membrane receptor, which then helps the fusion of virus into the host cell. Inside the host cell, the replication of the viral genome occurs in cytoplasm, mediated by RNA-dependent RNA polymerase (RdRp) enzyme.[5–7]

Numerous studies have focused on RdRp as a promising therapy against viral infections.[5] RdRP is an important enzyme in majority of RNA virus' replication process. The enzyme is also relatively stable throughout the evolution of virus, does not have homologous structure in host cells, and has previously been studied. There is already adequate information on its structure and function. We will further discuss the drugs which mechanism of action are principally on the RdRp enzyme, i.e., molnupiravir and nirmatrelvir/ritonavir.

MOLNUPIRAVIR

During COVID-19, there are many preceding drugs which are repurposed as COVID-19 therapy, such as hydroxychloroquine/chloroquine, ivermectin, antibiotics, antivirals, antihypertensives, and immunomodulators. Similar to remdesivir and favipiravir, molnupiravir works as RdRP inhibitor for transcription and replication of viral RNA genome.[5,8] Molnupiravir works by the mechanism of "error catastrophe", where it increases the rate of mutation in the viral genome and eventually become lethal to the virus.[5]

Molnupiravir has a molecular formula C13H19N3O7 and its active form name is Emory Institute for Drug Development (EIDD)-1931-isopropyl ester (EIDD-1931) or β-D-N4-hydroxycytidine-5'-isopropyl ester (NHC).[5,8–11] The active form is converted to NHC-triphosphate that binds to RdRp, instead of binding to cytidine and uridine triphosphates.[12] The RdRp enzyme uses the NHC-triphosphate as a substrate and incorporates into the RdRp active centers to form stable complexes, leading to synthesis of mutated RNA. RdRp synthesizes negative strand genomic RNA (-gRNA) from positive strand genomic RNA template. The +gRNA is also synthesized from M-containing RNA. The M-containing RNA in the -gRNA causes mutation in +gRNA, and subsequently results in mutagenesis which is lethal to the virus.[11,12] The mutagenesis leads to the accumulation of deleterious errors in the genome, hence causing the inability of the virus to replicate. There is some concern that these mutations can also be produced in the host cell (mammalian DNA) and therefore, increasing its potential carcinogenic and teratogenic effects. However, the concern is less likely to happen because of molnupiravir regimens are short-term (5 days).[12,13]

The suggested dose for patients is 800 mg (with 200 mg on each tablet) orally, twice daily for five days in mild, moderate, and severe COVID-19 cases.[14] For patients with comorbidity and with risk of worsening in later stages of COVID-19, the National Institutes of Health (NIH) recommended to use molnupiravir only when nirmatrelvir/ritonavir or remdesivir cannot be used. Some of the comorbidities being studied are type I and II diabetes mellitus, malignancies, cerebrovascular diseases, chronic kidney diseases, chronic liver diseases, chronic pulmonary diseases, cardiovascular diseases, and obese population.[13,14] Molnupiravir should be started within five days of symptoms onset.[13] The contraindication of molnupiravir is pregnancy, breastfeeding, and children younger than 18 years old.[13,14] There are some exception in pregnancy, when other therapies are not available, molnupiravir can be used with risk-benefit assessment and preferably beyond 10 weeks of gestation.[13]

CLINICAL STUDIES OF MOLNUPIRAVIR

The preclinical studies of molnupiravir suggested that it has broader antiviral efficacy toward SARS-CoV-2 compared to remdesivir. A double-blind, randomized, and placebo-controlled (DBRPC) phase 1 clinical study of

molnupiravir reported that the drug is safe and most effective at the dose levels of 50-1600 mg, with half-life between 0.907 and 7.08 hours. The rate of absorption was low during meals, but with longer duration of exposure, the absorption rate of both the fed and unfed states was similar. Another phase 1 clinical study also reported safety and tolerability of 1600 mg daily dose molnupiravir up to 5.5 days, without any serious adverse events.[12] The median time needed for the active form of molnupiravir to reach maximum observed plasma concentration ranges from 1-1.75 hours. Adverse events were more prevalent in the placebo arm. In both studies, the most frequently reported adverse event was headache, without any other safety concern on vital signs, electrocardiogram data, or hematological parameters.[12]

A phase 2 clinical study of molnupiravir reported that the dose of 800 mg twice daily had good efficacy in reducing clearance time of viral RNA compared to placebo (RNA negativity), with median time of 14 days *versus* 27 days in placebo. The most common adverse events were headache, insomnia, and increased levels of alanine aminotransferase, which were reported in both molnupiravir and placebo group.[12] A phase 3 clinical study, MOVe-OUT, reported that the molnupiravir reduced the risk of hospital admission and death by 50% in mild cases of COVID-19; however, this study was criticized due to its inconsistencies in study method and result, implementation of the interim and primary analysis, significant differences between interim and post-interim results, and analysis of the result.[15,16] Bernal AJ et al also reported data from MOVe-OUT that molnupiravir group had a lower risk of hospitalization due to any cause or death until day 29 (**Table 3**). There was no significant benefit to this drug in the later stage of moderate to severe COVID-19. The efficacy of molnupiravir was not affected by the SARS-CoV-2 variant (gamma, delta, or mu), the onset time of symptoms, or the underlying risk factors.[12,16] The most common adverse events in molnupiravir group were COVID-19 pneumonia, diarrhea, bacterial pneumonia, and progressive COVID-19.[16]

A DBRPC phase 3 study (MOVe-AHEAD) is underway to evaluate the safety and efficacy of molnupiravir to prevent the incidence of COVID-19 in non-infected adults living with an infected person. There is still lack of data on molnupiravir clinical trials in vaccinated patients, but it may have lower efficacy or no benefit on this population.[13]

NIRMATRELVIR/RITONAVIR

Another repurposed drug for COVID-19 is nirmatrelvir/ritonavir, with Paxlovid™ as its brand. Unlike molnupiravir, this drug is not associated with the alarming possibilities of mutation induction in human DNA and acceleration of the development of new virus variants. Nirmatrelvir/ritonavir inhibit the main protease (M^{pro}) and 3CL protease of SARS-CoV-2.[7,17] M^{pro}, which is an attractive antiviral target because it is essential in the viral replication cycle.[17] Study of nirmatrelvir in animals had demonstrated its activity to halt the spread of COVID-19 despite the frequent mutations in the viral genomes.[7] Nirmatrelvir shows an effective antiviral effect against recent coronavirus mutants. Working in combination with nirmatrelvir, ritonavir works as a CYP3A4 inactivator and pharmacokinetic enhancer that resulted in boosting the serum concentration of nirmatrelvir. Ritonavir has also previously been used in combination with antiretroviral drugs in human immunodeficiency virus (HIV) infection.[7]

An interim result analysis of phase 2/3 clinical study EPIC-HR (Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients) stated that this drug significantly reduced the risk of hospitalization and death in COVID-19 outpatients, who have at least one comorbidity (diabetes or lung disease), with estimated risk reduction of -6.3%. During 28-day observation, 0.3% of patients in nirmatrelvir/ritonavir group were hospitalized with no mortality case, compared to 6.3% of patients in the placebo group with 12 deaths (**Table 3**).[7]

The suggested dose of nirmatrelvir for patients with normal renal function is 300 mg (with 150 mg on each tablet) and ritonavir 100 mg per tablet orally, twice daily for five days, and should be initiated within five days of symptoms onset.[18,19] The dose adjustment for moderate

renal impairment (estimated glomerular filtration rate (eGFR) ≥ 30 to ≤ 60 mL/min) is 150 mg nirmatrelvir and 100 mg ritonavir twice daily for five days. Nirmatrelvir/ritonavir is not recommended for patients with severe renal impairment (eGFR) < 30 mL/min) or with severe hepatic impairment (Child-Pugh Class C).[14,18,19]

Nirmatrelvir/ritonavir is recommended for children 12 years old and above who weigh at least 40 kg with mild to moderate COVID-19, but it is still contraindicated for children below 12 years old.[14] NIH stated that nirmatrelvir/ritonavir is safe to be used in pregnancy with risk-benefit assessment (medical comorbidities, body mass index, and vaccination status).[19] However, there is still lack of data in Indonesia regarding the safety in pregnancy, breastfeeding, and pediatric population.

Some adverse events of nirmatrelvir/ritonavir are dysgeusia, diarrhea, hypertension, and myalgia, which occur in both nirmatrelvir/ritonavir and placebo groups. The common side effects of ritonavir are nausea, vomiting, diarrhea, changes in taste, fatigue, rash, hyperlipidemia, and lipodystrophy (associated with long-term use).[7,17] The drug may also interact with antiarrhythmics (amiodarone, digoxin), oral antithrombotics (apixaban, rivaroxaban, ticagrelor), statins (atorvastatin, lovastatin, simvastatin), benzodiazepines (diazepam), opioids (methadone, fentanyl), anticonvulsants (carbamazepine), neuropsychiatric drugs, and immunosuppressants; therefore, it should be administered with caution to avoid drug interactions.[18]

WHY MOLNUPIRAVIR AND NIRMATRELVIR/RITONAVIR ESSENTIAL FOR HIGH-RISK PATIENT?

For clinical symptoms outcome, Fisher W et al reported that there are no significantly difference in patients with molnupiravir and placebo. Time to resolution of COVID-19 symptoms was not statistically different between participants.[10] Until now, there is still limited data that discuss about clinical resolutions of COVID-19 symptoms, so there is a need for more studies to learn about symptoms resolutions and preventive effect of antivirals for long COVID-19. Many of the studies have reported clinical data, such as hospitalization rate and death. Even though clinical symptoms outcome data are still lacking, Lai CC et al reported that between three antiviral agents as interventions (molnupiravir, remdesivir or nirmatrelvir/ritonavir) and placebo, with the same study design (**Table 1** and **Table 2**)[20], showed that, Nirmatrelvir/ritonavir is superior than its predecessor and molnupiravir has better outcome than placebo.

High risk patients who are recognized by MOVe-OUT study and CDC are people with >60 years of age, active cancer, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), obesity (body mass index (BMI) ≥ 30 kg/m^2), serious heart conditions (coronary artery diseases (CAD), heart failure, cardiomyopathies), hypertension, diabetes mellitus, immunosuppressive diseases or immunosuppressive treatments, neurodevelopmental disorders (cerebral palsy) or other conditions that confer medical complexity (genetic or metabolic syndromes and severe congenital anomalies), and having a medical-related technological dependence (tracheostomy, gastrostomy).[21] Toussi SS et al reported that implementation of nirmatrelvir/ ritonavir in renal impairment needs dosage adjustment.[22] With current limitation of high-risk patients that could be included in molnupiravir and nirmatrelvir/ritonavir studies, there is already reduced risk of hospitalization and death. In future studies,

Table 1. Results of the pairwise comparisons in the network meta-analysis between antiviral agents for COVID-19.[20]

Antiviral Agents*	Nirmatrelvir Plus Ritonavir	Remdesivir	Molnupiravir	Placebo
Nirmatrelvir Plus Ritonavir		0.89 (0.17-4.69)	0.17 (0.07-0.39)	0.12 (0.06-0.24)
Remdesivir	1.12 (0.21-5.88)		0.19 (0.04-0.89)	0.13 (0.03-0.57)
Molnupiravir	5.85 (2.54-13.46)	5.22 (1.13-24.22)		0.67 (0.46-0.99)
Placebo	8.68 (4.15-18.17)	7.75 (1.76-34.22)	1.48 (1.01-2.18)	

*Odds ratio and 95% confidence interval were presented with drugs on the column as the reference

Table 2. Rank probabilities for treatment by P-score.[20]

Antiviral Agents	***p*-Score***
Nirmatrelvir + ritonavir	0.8510
Remdesivir	0.8087
Molnupiravir	0.3317
Placebo	0.0086

*Higher probability indicates better treatment for COVID-19

we hope that all of the high-risk patients can be included in studies and the effect of these antiviral agents can be more explored.

Table 3. Summary of published phase 3 clinical trials on molnupiravir and nirmatrelvir/ritonavir.

No	Author (year)	Countries	Population (n=patients)	Outcome evaluated	% of outcome in both groups (drugs vs placebo)	Comments (if any)
Molnupiravir						
1.	Fischer W, et al (2021) ClinicalTrials.gov NCT 04405570	Multicountry	N = (204)	Decrease in infectious virus isolation Time to SARS-CoV-2 clearance of viral RNA	*Infectious virus isolation Day 3 : 1.9% (1/53) vs 16.7% (9/54) (p = 0.02) Day 5 : 0% vs 11.1% (6/54) (p = 0.03) *Time to SARS-CoV-2 clearance of viral RNA 14 Days vs 27 Days (p = 0.001)	Randomized, double-blind, placebo-controlled trial
2.	Bernal AJ, et al (2021) MOVe-OUT ClinicalTrials.gov NCT 04575597	Multicountry	N = (1450)	The risk of hospitalization or death until day 29 Adverse events	*The risk of hospitalization or death until day 29 7.3% (28 of 385) vs 14.1% (53 of 377) (p = 0.001) *Adverse events (30.4%) 216 of 710 vs 33.0% (231 of 701)	Randomized, double-blind, placebo-controlled trial
3	Painter WP, et al (2021) NCT04392219	United Kingdom	N = (130)	1. Number of reported adverse events 2.To determine the safety and tolerability of single and mutiple ascending doses of molnupiravir	*Adverse events Headache (12.5% vs 18.8%) Diarrhea (7.1% vs 7.1%) *Single ascending doses Reported adverse events (43.8% vs 35.4%) *Mutiple ascending doses Reported adverse events (50% vs 42.9%)	Randomized, double-blind, placebo-controlled trial
Nirmatrelvir/Ritonavir						
1.	Hammond J, et al (2022) EPIC-HR NCT04960202	Multicountry	N = (2246)	1.The incidence of COVID-19 related hospitalization or death by day 28 2.The incidence of adverse events	*Incidence of COVID-19-related hospitalization or death by day 28 0.77% (3 of 385) vs 7.01% (27 of 385) Deaths : 0 vs 7 6.32% reduction (95% CI, -9.04 to -3.59; p<0.001; relative risk reduction, 89.1%) *Adverse events : 22.6% vs 23.9% Serious adverse events : 1.6% vs 6.6% Adverse events leading to discontinuation : 2.1% vs 4.2%	Randomized, double-blind, placebo-controlled trial

MOLNUPIRAVIR AND NIRMATRELVIR/ RITONAVIR AVAILABILITY AND USE IN INDONESIA

On the 13th of January 2022, the Indonesian National Agency of Drug and Food Control stated that the Emergency Use Authorization (EUA) of molnupiravir has been granted.[23,24] The use of molnupiravir is already registered on Indonesian National Agency of Drug and Food Control. However, nirmatrelvir/ritonavir has not been granted EUA yet in Indonesia. Regarding the availability of molnupiravir in Indonesia, through www.covid19.go.id as the official website for COVID-19 in Indonesia, the Ministry of Health of the Republic of Indonesia reported that they have prepared 400 thousand tablets for the ongoing month since 17th of January 2022. The Indonesian Medical Association, which includes the Indonesian Society of Respirology, the Indonesian Heart Association, the Indonesian Society of Internal Medicine, the Indonesian Society of Anesthesiology and Intensive Therapy, and the Indonesian Pediatric Society, has given their recommendation for use in the fourth edition of guidelines for COVID-19 treatment on January 2022.

CONCLUSION

There has been new insight on the use of antivirals for COVID-19 treatment as molnupiravir and nirmatrelvir/ritonavir has now been made available in Indonesia. Despite the rising number of people who got vaccinated, antiviral treatment is still an important aspect needed to treat COVID-19 infection. In indicated patients, molnupiravir and nirmatrelvir/ritonavir are expected to have a greater impact in the society, i.e., to reduce the risk of hospitalization and death than its predecessor antivirals and placebo. With promising results from preclinical, phase 1, phase 2, and phase 3 studies, both drugs are considered to be safe and tolerable without any serious adverse events.

CONFLICT OF INTEREST

The authors affirm no conflict of interest in this study.

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All named authors have met the criteria for authorship, took responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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Skin Mottling as Clinical Manifestation of Cardiogenic Shock

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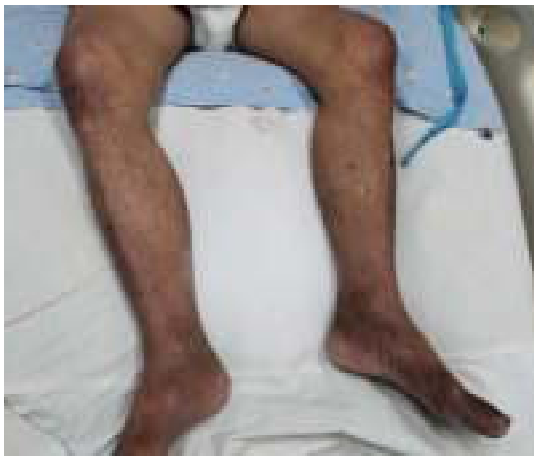

Figure 1. Skin mottling (Mottling Score 2) observed in the lower extremity

According to Van Diepen et al, cardiogenic shock is defined as the inability of the heart as a result of impaired pumping function to deliver adequate amount of blood to tissues to meet resting metabolic demand.[1] According to Society for Cardiovascular Angiography and Interventions (SCAI), the hallmarks of cardiogenic shock are hypotension and hypoperfusion which can be classified into five levels. Based on SCAI consensus, diagnosis and classiciation of cardiogenic shock are dependent on both physical examination and biochemical markers. Prompt recognition of cardinal signs of cardiogenic shock is vital in prompt and appropriate management of said patients.[2]

One of the more easily recognizable signs of CS is the assessment of peripheral circulation. Physical examination of the peripheral is convenient, accessible and were shown to be able to differentiate the stages of shock experienced by the patients. If the patient is at the initial period of shock, there would be systemic vasoconstriction to preserve the vital organs of the patient via decreased perfusion of the tissues. Persisting peripheral vasoconstriction despite stability of hemodynamics may signify worse outcomes in patients with cardiogenic shock.[3]

Skin mottling is defined as a red-violaceous discoloration of the skin that is usually found in the lower extremity. Pathophysiology of mottling skin is believed to be due to peripheral vasoconstriction of the skin. However, the exact patophysiology remain controversial: observations showed that mottling areas are

colder than normally colored skin. Assessment using NIRS showed that mottled areas have lower StO2 compared to the healthy skin tissues. Mottling skin were classified using Mottling Score in which we classified into 5 levels: score 0 found no mottling, score 1 with modest mottling area localized to the center of the knee, score 2 is moderate mottling area that does not exceed superior edge of the kneecap. Score 3 mild mottling area that does not exceed middle thigh. Score 4 is severe mottling area that does not exceed the fold off the groin. Score 5 is extremely severe mottling area that exceeds the fold of the groin.[4] Coudroy et al discusses the impact on the persistence of skin mottling during hospital stay. In this study, we see that a patient assessed with septic shock had 40% skin mottling. Among patient with persistent skin mottling, it is considered as an independent risk factor for in-ICU mortality and also organ dysfunction.[5] Persistent skin mottling is a surrogate marker for poor perfusion to the peripheral tissues.

A 59 years old male came to the emergency department with chief complain of dyspnea. Dyspnea has worsened since 3 days before admission accompanied with dyspnea on effort, orthopnea and paroxysmal nocturnal dyspnea. In the emergency department, patient experienced cardiac arrest after defecating, leading to cardiopulmonary resuscitation for 45 minutes. Administration of vasoactive drugs were done and the patient was intubated.

Post resucitaiton physical examination showed that the patient was sedated, with blood pressure of 72/40 (on dobutamine support). Peripheral circulation examination showed cold and clammy extremities, skin mottling of the lower extremity with mottling score of 2. CRT is more than 2 seconds. Blood gas analysis showed severe metabolic acidosis with blood lactate of 8.1.

Angiographic examination were previously done on the patient during the previous admission with the results of three vessels disease with a chronic total occlusion in the left anterior descending artery. However, patient had refused further intervention regarding the coronary problems. Patient also has longtsanding atrial fibrillation.

Patient was admitted into the intensive care unit for further management. Patient was stabilized during admisison in the intensive care with inotropes, however despite the hemodynamic stablilization the skin remain mottled-regardless. Patient had complicating factors in the form of pneumonia and sepsis. Patient had difficulty in weaning the ventilator and died because of arrythmia complication.

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Revisiting the Overlooked Infection: Rickettsioses

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ABSTRACT

The prevalence of human Rickettsioses cases in Indonesia is unknown and could probably be underestimated. The high prevalence of seropositive Rickettsia sp. was reported in small mammals (as vectors) and humans. In Indonesia, a recent study in patients with acute fever revealed that the prevalence of Rickettsioses is 10%. Many cases of Rickettsioses were often misdiagnosed with dengue fever, enteric fever, or leptospirosis due to their overlapping clinical manifestation. The limitation of point of care testing in Indonesia hindered the adequacy of diagnosis confirmation. Appropriate empirical or definitive treatment with macrolide, mainly doxycycline, is preferable compared to other broad-spectrum antibiotics, such as cephalosporin or quinolones. Moreover, when left untreated, Rickettsioses may deteriorate progressively to fatal outcomes, such as meningitis, sepsis, and even death. The awareness of health care practitioners, the availability of confirmatory rapid diagnostic tests and adequate treatment choices are important in eradicating this disease.

Keywords**:** *infection, Rickettsioses, tropical disease.*

INTRODUCTION

Rickettsial diseases are a group of diseases caused by a genus of obligately intracellular, gram-negative coccobacilli and short bacilli, and non-spore-forming bacteria.[1,2] The family of Rickettsiaceae as the etiology of Rickettsial diseases were originally classified into six genera, i.e., *Rickettsia, Ehrlichia, Anaplasma, Neorickettsia, Candidatus Neoehrlichia,* and *Wolbachia*[1]. However, based on the antigenic and genetic data, it was further classified as *Rickettsia spp.* and *Orientia,* spp. These species are traditionally divided into three Rickettsioses disease groups, i.e., spotted fever group (SFG), typhus group and scrub typhus group.[3,4]

The acute presentations of Rickettsioses are often similar during the first 5 days, i.e., fever, headache, myalgia, nausea, vomiting and cough. It is usually first presented as a fever that may subside without further clinical evolution or it may also evolve along one or more principal clinical lines, i.e., macular or maculopapular rash, eschar, vesicular rash, pneumonitis, meningoencephalitis, and even sepsis and toxic shock syndromes with hypotension and multiorgan failure.[1,2]

Regardless of its potentially fatal outcomes, Rickettsioses are often misdiagnosed in daily clinical practice because the initial signs and symptoms of the disease may overlap with other acute febrile illnesses clinical features, such as dengue fever, leptospirosis, or enteric fever disease.[5,6] Establishing a definitive diagnosis is also challenging as it requires more time to

examine serum samples during the acute and convalescent phases, whereas, the disease course may deteriorate rapidly if they are not promptly treated.[1,2,4,7]

DEFINITION AND CLINICAL FEATURES

Rickettsioses were firstly described as exanthematic typhus in 1760, followed by the first report of scrub typhus in 1879 and the discovery of a pathogen for Rocky Mountain spotted fever (RMSF) by Howard Taylor Ricketts, whose name underlies the etymology of the disease.[1,8,9] In Indonesia, the term typhus (as included in Rickettsioses) or "tifus" (in Indonesian language) is commonly mistaken as typhoid fever or enteric fever which is caused by *Salmonella, sp*. and has a fecal-oral transmission.[10]

The earliest definition of the term "typhus" is difficult to be determined since it was applied to a broad range of infectious diseases before the 20th century. The new era of rickettsiology had been fastened by the discovery of typhus diagnostic test which was able to identify epidemic typhus caused by *Rickettsia spp*. and *Orientia spp*. With the rapid invention of antibiotics, however, the widespread epidemic could be suppressed and nowadays the prevalence rate of the infection has decreased.[9]

Centers for Disease Control and Prevention (CDC) categorized rickettsial diseases as travel-related infectious diseases, in which all travellers from endemic areas are at risk of acquiring rickettsial infections. Since most rickettsial diseases incubate for 5-14 days, tourists may not experience symptoms during their trip and onset may coincide with their return home or within a week after returning.[11] The lists of endemic areas are diverse, for example, RMSF is endemic in south-central United States, Canada, Mexico, and South America; scrub typhus in Asia, Australia, Pacific; murine typhus spreads worldwide; and Mediterranean spotted fever in southern Europe, Africa and Asia.[1,2,7] Table 1 summarizes the groups of Rickettsioses and their features.

SITUATION IN INDONESIA

The epidemiology data of Rickettsioses vary according to geographic distribution and seasonal activities of tick vectors and vertebrate hosts, in addition to the human behaviors that increase the risk of tick exposure, tick attachment, and subsequent infection.[7] The exact prevalence of these diseases is often underestimated since the establishment of definitive diagnosis is still limited.

A study held in 1995 on rickettsial disease risk revealed that Indonesia was an endemic area for murine typhus, scrub typhus, and Q fever, whereas other typhus and SFG endemicity were unknown.[10] Evidence of rickettsial diseases or

Table 1. Group of Rickettsioses and their clinical features.[1,2,11]

Disease*	Organism	Transmission	Incubation (days)	Duration (days)	Rash %	Eschar %	Lymphadenopathy[a]
Spotted fever group (SFG)							
Rocky Mountain spotted fever	*R. rickettsii*	Tick	2-14	2-10	90%	<1	+
Flea-borne spotted fever	*R. felis*	Flea	8-16	8-16	80	15	-
Rickettsialpox	*R. akari*	Mite bite	10-17	3-11	100	90	+++
Mediterranean spotted fever	*R. conori*	Tick bite	5-7	7-14	97	50	+
Typhus group							
Murine typhus	*R. typhi*	Flea feces	8-16	9-18	80	None	-
Epidemic typhus	*R. prowazekii*	Louse feces, fleas, lice	7-14	10-18	80	None	-
Scrub typhus group							
Scrub typhus	*Orientia tsutsugamushi*	Mite bite	9-18	6-21	50	35	+++

*The diseases are not limited to what is listed on the table, however, it conveys the list of the most common diseases.
[a] ++++, severe; +++, marked; ++, moderate; +, present in a small proportion of cases; —, not a noted feature.

Rickettsioses in Indonesia have been described since the early 1900s in western Indonesia (Sumatra and Java) to eastern Indonesia (Papua)[10,12–14] Several cases of underdiagnosed yet fatal clinical features of Rickettsioses also had been reported among travellers returning from Indonesia.[15,16] Another study on ectoparasite and small mammals in Indonesia also showed a high rate of typhus group rickettsiae-specific antibodies, which indicated the high risk for rickettsia infection and the existence of various hosts and vectors across the country.[14,17] This data indicated the potential Rickettsioses endemicity in Indonesia.

In the last decade, many studies have highlighted the importance of Rickettsioses as one important differential diagnosis of fever.[5,6,18–21] In 2020, observational research showed that Rickettsioses were the third most prevalent cause of acute febrile illness in Indonesia. All the cases were misdiagnosed by the standard of care examination.[6] Furthermore, Rickettsioses were also the third most common cause of sepsis in a multinational study held in southeast Asia, therefore, our awareness of this disease is mandatory.[22]

THE EMERGING PROBLEM OF RICKETTSIOSES IN INDONESIA

In the upcoming text, we will elaborate on the emerging problem of Rickettsioses in Indonesia.

1. **Undiscovered facts that Rickettsiosis cases are prevalent**

   During 2013-2016, the Indonesia Research Partnership on Infectious Disease (INA-RESPOND) conducted an observational cohort study (Acute Fever Requiring Hospitalization/AFIRE) on acute febrile illness at eight tertiary healthcare facilities in Indonesia. Among 1,488 enrolled subjects with fever and 1,003 pediatric and adult subjects with identified etiology of disease, Rickettsioses ranked as the third most prevalent infection (103 subjects, 10.3%), following dengue and typhoid fever.[6] The confirmed Rickettsioses were established by one of these examinations: enzyme-linked immunosorbent assay (ELISA) and/or indirect immunofluorescence assay (IFA) for serology examination, followed by polymerase chain reaction (PCR) of bacterial DNA 17-kD outer membrane protein (17-kD *omp*) gene of *Rickettsia sp*., 47-kD *omp* gene of *O. tsutsugamushi*, and 743-bp sequence of *Rickettsia sp. omp*B gene.[5] In this study, IgG of *R. typhi* was detected among 30.8% of total subjects, followed by IgG detection of spotted fever and *O. tsutsugamushi*. Although mere single serology detection is not sufficient in diagnosing Rickettsioses, this high rate of seropositivity reflects the high exposure of *Rickettsia, spp*. in Indonesia, which is higher[13,23–26] or similar[12] compared to the previous studies. One systematic review by Wangdi et al.[20] studied undifferentiated febrile illness cases in south and southeast Asia and revealed that the prevalence of Rickettsioses reached 4%, which comprised of scrub typhus, murine typhus, and spotted fever disease. Compared to this systematic review, data on the prevalence of Rickettsioses in Indonesia is significantly higher and demands further attention.

   Rickettsioses are also one of the most common etiologies of community-acquired sepsis from a multinational multicentre observational study in southeast Asian countries, including Indonesia, together with dengue and leptospiral infection. The prevalence rate of Rickettsioses as the cause of sepsis and severe sepsis reached 6%, comprised of *R. typhi, O. tsutsugamushi,* and spotted fever group.[22]

   Although these studies showed that the prevalence of Rickettsioses was high, all identified cases were initially suspected as leptospirosis, typhoid fever, chikungunya, or dengue fever – none were diagnosed as Rickettsioses.[22] This data may also portray the unawareness of healthcare practitioners to suspect Rickettsioses in the first instance on a patient with acute febrile illness. By reflecting on the rate of misdiagnosis of this disease, healthcare providers need to be conscious and thorough in diagnosing acute febrile illness, and hence can consider Rickettsioses as a differential diagnosis.

2. Inadequacy of diagnostic modalities

One of the possible reasons why Rickettsioses was often misdiagnosed as another disease was because the accompanying symptoms of Rickettsioses, such as fever, nausea, headache, vomiting, lethargy, anorexia, arthralgia, myalgia, chills, and epigastric pain, may overlap with dengue, typhoid, and leptospiral infection.[1,2] The AFIRE study showed that Rickettsioses mostly affected gastrointestinal, lower respiratory tract and systemic organs, and therefore, its manifestation is similar to other differential diagnoses. Signs and symptoms are also often not specific for Rickettsioses, as the Rickettsia Triad (fever, headache, and rash) were only developed in 10% of subjects.[5,6] On 815 adult patients with community-acquired sepsis, Rickettsioses were presented as acute respiratory infection, acute systemic infection, acute diarrhea and acute central nervous system (CNS) infection which overlapped with symptoms of influenza, rhinovirus, hantavirus, leptospirosis, *S. aureus* infection, dengue fever, *E. coli* infection, *Streptococcus,* spp. infection, and other bacterial infection.[22]

A presumptive clinical diagnosis of Rickettsioses can be confirmed by immunohistochemical (IHC) assays in tissue, detection of antibodies by IFA with concomitant seroconversion or a fourfold or greater rise in antibody titre between acute and convalescent samples, or PCR of blood or tissue.[1,2,4] Whilst having high diagnostic value, those examinations are not widely available as point-of-care testing (POCT), expensive, and may cause a delay in empirical treatment (e.g., waiting for the serum convalescent testing). Detection of antibody and antigen for Rickettsioses in Indonesia is still limited for research purposes. The isolation of *Rickettsia, spp.* requires suitable techniques and must be handled as highly pathogenic in a biosafety level 3 laboratory, hence its use for prompt diagnosis is also limited.[4] This lack of diagnostic modalities is concerning because Rickettsioses are most misdiagnosed and potentially lethal, even though they are an easily treatable disease.[6]

In the AFIRE study, less than 50% of microbial etiologies were identified by standard-of-care testing, 23.6% were identified by INA-RESPOND laboratory, while the remaining 32.5% were unidentified. *Rickettsia spp.* (*R. typhi* and *R. felis*) is one of three infectious etiologies which was not captured by standard-of-care examinations.[5]

The previous studies and data highlight the need for a better and more widely accessible POCT for Rickettsioses, preferably based on antigen detection over antibody response to be able to rapidly diagnose and treat Rickettsioses.

3. Choice of therapy is widely available and simple, however, the administration is often delayed

Macrolide, mainly doxycycline, is the drug of choice for the treatment of all tickborne rickettsial diseases in patients of all ages. It should be initiated immediately in persons suggestive of Rickettsioses without waiting for the confirmatory laboratory examination. The drug is given orally or intravenously at a dose of 100 mg twice daily (adult) or 2.2 mg/kg of body weight twice daily (children), until at least 3 days after fever subsides and the patient is clinically improved.[7]

Prior to hospitalization, many patients with Rickettsioses have already consumed antibiotics, including amoxicillin, cefadroxil, cotrimoxazole, or cefixime – none of which are the empiric treatment for Rickettsioses.[6] During hospitalization, broad-spectrum antibiotics, such as ceftriaxone and ciprofloxacin, are effective empiric treatments; however, the routine broad-spectrum administration should be discouraged in the context of antimicrobial stewardship. In cases of acute fever unresponsive to empirical treatment of other acute febrile illnesses, administration of doxycycline should be considered.

4. Fatal cases (sepsis, mortality) in Rickettsioses

The spectrum of Rickettsioses varies from self-limited febrile illnesses to the involvement of CNS, liver, lung, and even death. Among Rickettsioses, *R. prowazeki* and *R. typhi* are known to be able to survive for an extended period outside the reservoir or vector and they are extremely infectious. Due to their high level of infectivity and severe illness after inhalation, *R. prowazekii, R. rickettsii, R. typhi, R. conorii,* and *Coxiella burneti* are known for bioterrorism threats.[2,5,19,27]

According to the AFIRE study, the overall mortality of patients with acute febrile illnesses reached 5.9% and the most common microbiologic etiologies included *R. typhi*, with mortality rate of 6.8%, markedly higher than the previous studies.[5,6] A study in Australia also observed that among 135 cases, 13% required ICU admission, mostly caused by spotted fever group and scrub typhus.[28,29] Another study in India reported a higher fatality rate of 12.2% with evidence of acute respiratory distress syndrome and aseptic meningitis among patients. One retrospective study on acute meningoencephalitis patients also identified *Rickettsia sp.* on cerebrospinal fluid examination, depicting the wide variety of its clinical manifestations. In fact, this underdiagnosed disease can lead to fatal outcomes even though the empirical treatment with doxycycline is relatively simple and widely available. An improvement in presumptive and confirmed diagnosis is important, as it will lead to more appropriate management and reduction of fatal cases and death.

CONCLUSION

Rickettsioses are a group of diseases that are common in Indonesia but often overlooked as a differential diagnosis of acute febrile illness. Previous studies have demonstrated that Rickettsioses are often misdiagnosed as dengue fever, leptospirosis, typhoid fever, and chikungunya due to some possible reasons, i.e., unspecific and overlapping signs and symptoms with other diseases, inadequate modalities of point of care examination to establish the confirmed diagnosis, and unfamiliarity of health care providers and system in suspecting Rickettsioses on patients with acute febrile illness. Therefore, awareness and understanding of the diseases, the adequate point of care diagnosis modalities, and treatment of Rickettsioses in Indonesia need to be established to reduce morbidity and mortality.

COMPETING INTERESTS

The authors declared no conflict of interest.

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Early Recognition of Type 2 Diabetes Complications and Use of SGLT2i in Multidisciplinary Approach: Indonesian Perspective - An Expert Opinion

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ABSTRACT

Indonesia ranks seventh with the highest number of cases of type 2 diabetes mellitus (T2DM). T2DM is associated with major undesirable complications including cardiovascular disease and chronic kidney disease. Kidneys play a major role in maintaining glucose homeostasis, leading the development of sodium glucose transporter inhibitors (SGLT2i). These inhibitors block renal sodium and glucose reabsorption. Several cardiovascular trials proved that SGLT2i have cardioprotective and renoprotective roles and have been suggested as a drug of choice in primary and secondary prevention and management of cardiorenal complications associated with T2DM. This review highlights the need for a multidisciplinary recommendation for T2DM management in Indonesian population. Additionally, it is vital to provide the perspective of Indonesian medical experts in terms of screening, diagnosis and treatment as the outcome differs geographically.

An expert panel of 6 members from Indonesia was convened to review the existing literature and develop an expert-based review/ summary on this topic. Members were chosen for their proficiency in diabetes, kidney disease and cardiovascular disease. The experts opined that the early use of SGLT2i will be effective in preventing and minimising the progression of cardiorenal complications. Moreover, a consistent multidimensional approach is necessary for improved outcomes.

Keywords**:** *Type 2 diabetes mellitus, SGLT2i, cardiorenal complications.*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition that affects 537 million adults worldwide, with the number estimated to increase to 783 million by 2045.[1] DM in Indonesia is a major health problem and has been a cause of serious concern since the 1980s.[2] Indonesia is ranked seventh among the top 10 countries with

the highest number of diabetics.[3] The prevalence of diabetes in Indonesian adults (20–79 years) is estimated to be 10.7 million, increasing to 13.7 and 16.6 million by 2030 and 2045, respectively.[4] According to DiabCare 2008 study in Indonesia, 97.5% of patients had type 2 DM (T2DM), of which 67.9% had poor glycemic control with HbA1c being in the range of 8.1 ± 2.0%.[5] In addition, the DISCOVER study, presented in Lisbon, Portugal during EASD, Indonesia had the highest mean HbA1c levels (9.2± 2%) among other participating countries. Despite the initiation of second-line therapy, the mean HbA1c levels in 70% of patients were >8%.[6]

T2DM is the central factor in the development and progression of CVD and kidney disease, which can lead to atherosclerotic CVD (ASCVD) and heart failure (HF).[7,8] A review by Asian experts from 9 different countries found that the prevalence of HF is approximately identical to global estimates i.e., 1% to 3%. Asian HF patients were observed to spend between 5 (Indonesia) to 12.5 days (Taiwan) in the hospital with a 3 to 15% readmission rate within 30 days due to HF.[8]

A vicious circle exists between T2DM, heart failure, and kidney disease, with the prevalence of HF increasing as CKD progresses.[9] A higher risk of cardiovascular disease (CVD) is associated with T2DM, which is estimated to be 1.6 to 2.6%.[4] Worldwide, CVD affects 32.2% of T2DM patients and it's the leading cause of morbidity and mortality in diabetic population.[10] According to a retrospective cohort study (n=1085) conducted from 2011 -2018, the incidence of cardiovascular events among the prediabetic and diabetic Indonesian population was 9.7% over a six-year period. In addition, age ≥ 45 years and hypertension were the predictors of cardiovascular events.[3]

Following the confluence of CVD and diabetes, CKD adds a layer of complexity.[11] Diabetic kidney disease affects 40% of the diabetic population and is the leading cause of end-stage kidney disease (ESKD). The prevalence of ESKD is 10 times higher in individuals with diabetes that ranges from 10% to 67%.[12] Comorbidities trigger a sudden decline in renal functioning of CKD patients. In Indonesia, the most common underlying disease in CKD patients is hypertension and diabetic kidney disease.[13] As per the Indonesian renal registry 2019, 26% patients with CKD stage 5 patients had an etiological diagnosis of diabetic kidney disease.[13,14] According to the statistics presented at 'the 14th national congress meeting of the Indonesian Society of Nephrology' hypertension (35%) and diabetic nephropathy (29%) are the two key etiological variables observed in CKD 5 patients.[15]

The guidelines of Indonesian Society of Endocrinology 2011, recommend diabetes screening for high-risk groups including individuals with hypertension, dyslipidaemia, coronary artery disease and obese people having sedentary lifestyle. They also recommend that, high risk individuals obtaining a negative result should be tested annually and people >45 years should be screened every 3 years.[16,17] A cross sectional survey of 15,332 urban Indonesians aged between 18-55 years, reported 4.6% of diabetes mellitus prevalence with instances of 1.1% previously diagnosed and 3.5% undiagnosed cases. In addition, prevalence of hypertension and dyslipidemia among previously diagnosed and undiagnosed cases was found to be 41.4% and 50%; 49.4% and 50%, respectively.[18]

To lessen the risk of nephropathy progression, the PERKENI 2021 guidelines recommend, optimising glucose levels and hypertension control. In patients (non-pregnant) with moderate (30-299 mg/24hr) to severe albuminuria (>300/24 hr), therapy with angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers with regular monitoring of serum creatinine and serum potassium levels is suggested, but not to be used as a primary prevention. Furthermore, nephrologist intervention is recommended, if serum creatinine is ≥ 2.0 mg/dL, or difficulty persist in determining the etiology, management, or in advance renal failure cases.[19]

The intersection of diabetes, kidney disease, ASCVD, and HF necessitates the emergence of diabetic treatment modalities that are both safe and effective[7] and simultaneously provide primary prevention from cardiorenal complications associated with T2DM. Newer glucose-lowering

agents have generated a possibility to influence the history of T2DM and cardiorenal complications. Sodium–glucose cotransporter 2 inhibitors (SGLT2i) are the new class of drugs approved for the management of T2DM. Dapagliflozin, empagliflozin, canagliflozin, and ertugliflozin have been investigated in cardiovascular outcome trials (CVOTs) and approved by the European Medicines Agency and Food and Drug Administration (FDA) either as monotherapy or as an adjunct to other antidiabetic agents.[20,21] SGLT2i are a relatively new addition to the armament of T2DM therapeutic modalities. When evaluated in conjunction with other oral medications or insulin, all SGLT2i demonstrate similar reductions in HbA1c.[22–25] Although the short-term HbA1c reduction seen with SGLT2i is comparable to that attained with sulfonylureas and dipeptidyl peptidase-4 inhibitor (DPP-IV), the glycemic effect appears to be more durable with SGLT2i.[26,27] Several meta-analyses reported improvement in glycemic control with the use of SGLT2i.[28,29] In addition to glycemic effects, SGLT2i exerts several extra-glycemic effects, including weight loss, blood pressure reduction, lipid level regulation, CV risk reduction, renoprotective impact, and reduction in macro- and micro-vascular events, as well as lowering the risk of hypoglycaemia.[22] Evidence in line, stating SGLT2i having glycemic and extra glycemic effects, indicates that SGLT2i may be used in primary and secondary prevention of cardiorenal complications in T2DM patients.[20]

This review emphasises on the burden of diabetes in Indonesia and to make recommendations for early screening, diagnosis, and treatment to prevent cardio-renal complications. There is need for a multidisciplinary recommendation for T2DM management in the Indonesian population as there is no data regarding the official guidelines or recommendations. Additionally, the objective of this review is to justify the beneficial role of SGLT2i in primary and secondary prevention of cardiorenal complications associated with T2DM, wherein primary prevention is described as prevention of occurrence of cardiorenal complication and secondary prevention is to reduce the worsening of cardiorenal complication in T2DM patients.

METHODOLOGY

The need for comprehensive review of the early recognition of T2DM complications and its prevention and management using SGLT2i in Indonesian population was identified by the Indonesian medical specialists from various fields. An expert panel of 6 members (four from university hospitals, and two from public sector) from Indonesia was convened to review the existing literature and develop an expert-based review/ summary on this topic. Members were chosen for their proficiency in diabetes, kidney disease and cardiovascular disease. Series of teleconferences and web-based communications were held from June to August 2021. A manuscript outline was developed, with individual sections assigned to the authors as per their expertise. All the authors had continuous access to the working document to provide input and each section was critically reviewed and revised.

In preparation, an extensive literature search was conducted using key words - diabetes mellitus, “type 2 diabetes” “diabetes Indonesia” “chronic kidney disease”, “cardiovascular disease”, “microvascular complications”, “HbA1c”, “SGLT2 inhibitors”, “EMPA”, “DAPA” and “CANA” in, MEDLINE, Cochrane Library and Science Direct databases, to identify relevant articles. In addition, experts recommended articles outside the scope of formal searches, and findings from conference proceedings and relevant online data sources were also reviewed. **(Figure 1)**

A total of 68 articles were identified out of which 54 were shortlisted. A total of 52 full text articles (meta-analysis, reviews, and randomized controlled trials) published in English and in peer-reviewed and indexed journals from 2005-2021 were selected and the studies with only abstract were excluded. Articles published before the start search date provided conceptual content only.

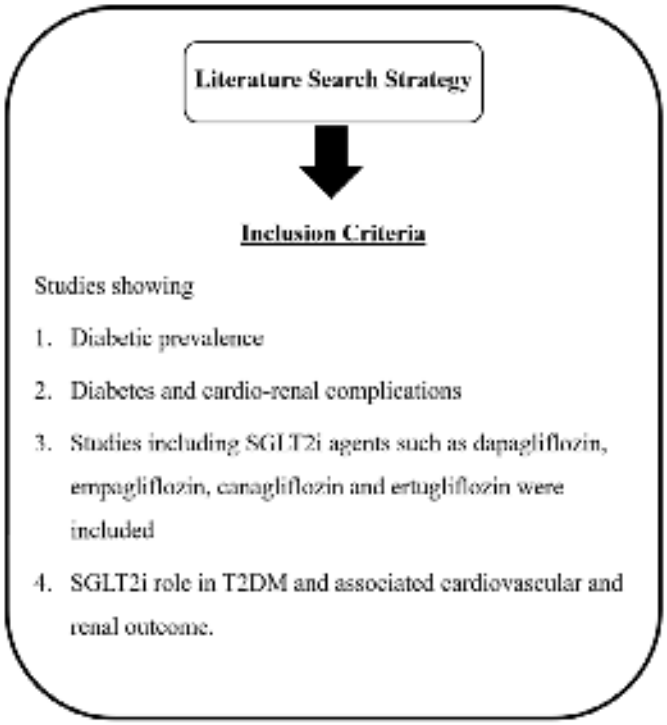

Figure 1. Literature search strategy.

DISCUSSION

Among several SGLT2 inhibitors, empagliflozin has the highest SGLT2 receptor selectivity, and the other agents have intermediate selectivity (dapagliflozin and canagliflozin).[20] The relative specificities of different SGLT2i to various SGLT receptors contribute to modest variances in their clinical characteristics. The mechanism of action of SGLT2i is presented in **Figure 2.**[21]

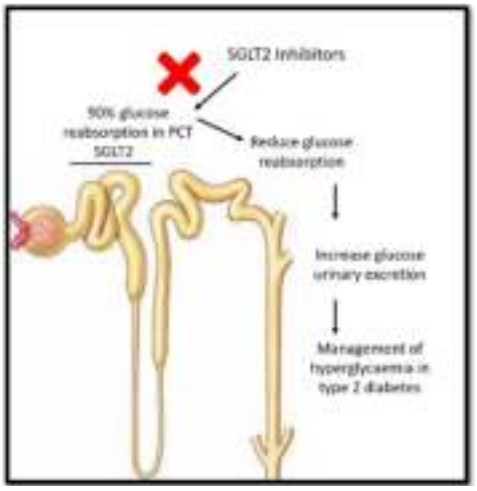

Figure 2. Mechanism of action of sodium glucose transporter 2 inhibitors.

SGLT2i and T2DM

SGLT2i mediates the reabsorption of 90% of filtered glucose and inhibits glucose reabsorption at the level of the proximal convoluted tubule (PCT), resulting in enhanced glucosuria, osmotic diuresis, and natriuresis, thereby managing the hyperglycemia with reduced risk of hypoglycemia.[30] Furthermore, SGLT2i have added benefits of persistent calorie reduction leading to weight loss, reduced β-cell stress, increased rate of insulin secretion, and insulin sensitivity. Sequentially, all these mechanisms regulate blood glucose levels despite β cell dysfunction or insulin resistance. Additionally, they are effective in advanced stages of T2DM due to their insulin-independent mechanism.[21]

Efficacy and safety of SGLT2i in T2DM patients were investigated in a systematic review and meta-analysis that included 38 trials (n=23,997). SGLT2i (canagliflozin, dapagliflozin, and empagliflozin) lowered glycated hemoglobin levels (HbA1C) (–0.6% to –0.9%), fasting blood glucose (FBG) (–1.1 to –1.9 mmol/L), blood pressure (BP) (systolic –4.9 to –2.8 mmHg; diastolic – 2.0 to –1.5 mmHg), and body weight (–1.6 to –2.5 kg) when compared with placebo.[28]

In line with the evidence, a systematic review including three trials of dapagliflozin and two each for canagliflozin and empagliflozin reported that monotherapy with SGLT2i significantly improved glycemic control, induced weight loss, and reduced blood pressure. The common adverse events reported were an increase in urinary tract infections (4–9%) and genital tract infections.[28] Similarly, another systematic review and network meta-analysis including 39 randomized controlled trials (RCTs) (n=25,468) reported that SGLT2i was superior to placebo in terms of glycemic control, weight loss, and reduction of systolic and diastolic BP.[31]

Drug intensification is often required in T2DM patients who are on stable metformin therapy. SGLT2i are potential candidates for combination therapy as they have shown promising outcomes. A meta-analysis, including six RCTs, compared the efficacy and safety of SGLT2i with non-SGLT2 combinations (glimepiride, linagliptin, sitagliptin, glipizide) as an add on to metformin treatment. The study reported that SGLT2i+metformin therapy significantly reduced HbA1c% more than the non-SGLT2i combination after 52 weeks and as well as after 104 weeks of therapy (p<0.00001). Moreover, reduction in FPG, weight, and BP was significantly more in the SGLT2i group (p<0.00001) and the incidence of hypoglycemia was also reported to be lower with SGLT2i.[32–34]

Primary and Secondary Prevention

CVOTs distinguished T2DM patients without established CVD (primary prevention) and patients with established CVD (secondary prevention).[20] Thus, it can be inferred that primary prevention is refers to preventing cardiorenal complications in diabetic patients, whereas secondary prevention refers to the diabetic patients who have experienced an acute ischemic event and to prevent the aforementioned complications from worsening. SGLT2i have shown the possibility of being cardioprotective by demonstrating relative risk reduction of major adverse cardiovascular events (MACE). Several meta-analyses have reported in favour of SGLT2i, highlighting its renoprotective and cardioprotective effects.[35,36] Outcome was reported as significant reduction in MACE in empagliflozin and canagliflozin trials, whereas dapagliflozin showed reduction in CV mortality and hHF.[7,37,38] Studies determining the role of SGLT2i in primary and secondary prevention are presented in **Table 1 and 2** [7,37–43] Glycemic and extraglycemic effects of SGLT2i are presented in **Figure 3.**[21]

SGLT2i and CVOT

T2DM confers a two-to-three-fold increased risk of coronary artery disease, including angina, myocardial infarction (MI), stroke, and HF in patients with or without established cardiovascular illness.[30] SGLT2i has demonstrated the potential of being cardioprotective by exhibiting relative risk (RR) reduction of 3 Point (non-fatal stroke, non-fatal myocardial infarction and CV death)-MACE.[7] The Empagliflozin Cardiovascular Outcome Event Trial (EMPA-REG OUTCOME) was the first trial to demonstrate the CV benefits of SGLT2i. Over a median of 3.1 years. the risk of CV and all-cause mortality reduced in the SGLT2i group by 38% and 32%, respectively, with no significant difference in the risk of non-fatal heart attack or stroke. Empagliflozin was also found to be effective in secondary prevention.[37] Similarly, DECLARE and CANVAS trials have also demonstrated the cardioprotective effects of SGLT2i in multiple risk populations.[7,38,44] DECLARE-TIMI 58, is the largest, longest and broadest SGLT2i trial compared the efficacy and safety of dapagliflozin in 17,160 patients with T2DM over a median of 4.2 years. The study showed risk reduction for both the primary endpoints i.e., MACE and hHf/ CV was insignificant. The renal outcome was 4.3% in dapagliflozin group vs. 5.6% in the placebo group due to the reduced rate of hospitalization for HF, regardless of the previous history of ASCVD and HF.[7] Therefore, it can be stated that dapagliflozin has provided beneficial effects in both primary and secondary prevention.[7,36,45]

SGLT2i and Heart Failure

T2DM is a prevalent co-morbidity in patients with HF and a major prognostic factor in patients with established HF. Chronic HF is the major cause of hospitalization in patients over 65 years, with a 10% 30-day and 50% 1-year mortality.

Table 1. Effect of SGLT2 Inhibitors on Cardiovascular Outcome

Cardiovascular Outcomes	EMPA-REG outcome	CANVAS	DECLARE-TIMI 58	CREDENCE	VERTIS CV	DAPA HF	RWE
	Empagliflozin vs Placebo	Canagliflozin vs Placebo	Dapagliflozin vs Placebo	Canagliflozin vs Placebo Events/1000 patients	Ertugliflozin vs Placebo	Dapagliflozin vs Placebo	Dapagliflozin vs Glucose lowering Drugs Events/1000 patients
Death from CV causes, non-fatal MI or non-fatal stroke HR (95% CI)	10.5% vs 12.1% 0.86 (0.74-0.99)	26.9% vs 31.5% 0.86 (0.75 - 0.97)	8.8% vs 9.4% 0.93 (0.84 - 1.03)	38.7 vs 48.7 0.80(0.67 - 0.95)	11.9% vs 11.9 % 0.97 (0.85-1.11)	----	----
CV death HR (95% CI)	3.7% vs 5.9 % 0.62 (0.49 – 0.77)	11.6% vs 12.8% 0.87 (0.72 – 1.06)	2.9% vs 2.9% 0.98 (0.82 – 1.17)	19.0 vs 24.4 0.78 (0.61 – 1.00)	6.2% vs 6.7% 0.92 (0.77 – 1.11)	9.6% vs 11.5% 0.82 (0.69 – 0.98)	6.1 vs 8.1 0.75 (0.57 – 0.97)
Hospitalization for heart failure HR (95% CI)	2.7% vs 4.1% 0.65 (0.50 – 0.85)	5.5% vs 8.7 % 0.67 (0.52 – 0.87)	2.5% vs 3.3% 0.73 (0.61 – 0.88)	15.7 vs 25.3 0.61 (0.47 – 0.81)	2.5% vs 3.6% 0.70 (0.54 – 0.90)	9.7% vs 13.4% 0.70 (0.59 – 0.83)	15.5 vs 19.6 0.79 (0.67 – 0.93)
Non-fatal MI HR (95% CI)	4.5% vs 5.2% 0.87 (0.70 – 1.09)	9.7% vs 11.6% 0.85 (0.69 – 1.05)	4.6% vs 5.1% 0.89 (0.77 – 1.01)	----	5.6% vs 5.4% 1.04 (0.86 – 1.27)	----	10.3 vs 11.3 0.91 (0.74 – 1.11
Non-fatal stroke HR (95% CI)	3.2% vs 2.6% 1.24 (0.92 – 1.67)	7.1% vs 8.4% 0.90 (0.71 – 1.15)	2.7% vs 2.7% 1.01 (0.84 – 1.21)	----	2.9% vs 2.8% 1.00 (0.76 – 1.32)	----	----

CANVAS: Canagliflozin Cardiovascular Assessment Study Program; CREDENCE: Canagliflozin and Renal Events in Diabetes With Established Nephropathy Clinical Evaluation; CVD: cardiovascular disease; DAPA CKD: Dapagliflozin in patients with chronic kidney disease; DECLARE-TIMI 58: Dapagliflozin Effect on Cardiovascular Events–Thrombolysis in Myocardial Infarction 58; EMPA-REG:, Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients–Removing Excess Glucose; VERTISCV: Cardiovascular Outcomes Following Ertugliflozin Treatment in Type 2 Diabetes Mellitus Participants With Vascular Disease;
eGFR: estimated glomerular filtration rate; ESKD: End stage kidney disease; HR: Hazard Ratio

Table 2. Effect of SGLT2 Inhibitors on Kidney Outcomes.

Renal Outcomes	CANVAS	DECLARE-TIMI 58	CREDENCE	VERTIS CV	DAPA CKD
	CANA vs Placebo	DAPA vs Placebo	CANA vs Placebo	Ertugliflozin vs Placebo	DAPA vs Placebo
Renal composite outcome description	40% reduction in eGFR, renal replacement therapy or renal death	≥ 40% decrease in eGFR to <60 ml/ min/1.73 m2, ESKD, or death from renal cause	Doubling of serum creatinine, ESKD, renal death	Death form renal causes, renal replacement therapy or doubling of serum creatinine level	≥ 50% decline in eGFR, ESKD, renal death
Renal composite outcome HR (95% CI)	5.5% vs 9.0% 0.60 (0.47 – 0.77)	1.5% vs 2.8% 0.53 (0.43 – 0.66)	43.2 vs 61.2 0.70 (0.59 – 0.82)	3.2% vs 3.9% 0.81 (0.63 – 1.04)	9.2% vs 14.5% 0.61 (0.51 – 0.72)

CANVAS: Canagliflozin Cardiovascular Assessment Study Program; CREDENCE: Canagliflozin and Renal Events in Diabetes With Established Nephropathy Clinical Evaluation; CVD: cardiovascular disease; DAPA CKD: Dapagliflozin in patients with chronic kidney disease; DECLARE-TIMI 58: Dapagliflozin Effect on Cardiovascular Events–Thrombolysis in Myocardial Infarction 58; EMPA-REG:, Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients–Removing Excess Glucose; VERTISCV: Cardiovascular Outcomes Following Ertugliflozin Treatment in Type 2 Diabetes Mellitus Participants With Vascular Disease;
eGFR: estimated glomerular filtration rate; ESKD: End stage kidney disease; HR: Hazard Ratio.

Figure 3. Effects of sodium glucose transporter 2 inhibitors.

According to their ejection fraction (EF), patients with T2DM may develop three distinct types i.e., HF with reduced EF (HFrEF), HF with mildly reduced EF (HFmrEF) and HF with preserved EF (HFpEF).[30]

A meta-analysis including six large trials of >46,000 patients with T2DM reported that SGLT2i was associated with a significant reduction of hHF (HR: 0.68; 95%CI: 0.61–0.76), Benefits on the risk of hHF and its related outcomes are independent of baseline ASCVD and prior HF.[46] Further, DAPA HF trial[41], determined the efficacy of dapagliflozin (10 mg/ day) in 4,744 patients with symptomatic HF and reduced EF (<40%). Over a median follow-up of 18.2 months, the primary outcome (worsening HF or cardiovascular death) occurred in 16.3% in the dapagliflozin group vs. 21.2% in the placebo group. Only 42% had significant T2DM. The magnitude of clinical benefits of dapagliflozin on the primary outcome was similar in patients with or without T2DM and with or without ischemic heart disease.[41] The EMPEROR reduced trial,[47] evaluated the efficacy of empagliflozin (10mg/ day) against placebo or indicated therapy in 3730 patients with heart failure and 40% EF. The primary outcome occurred in 19.4% in the empagliflozin group with lowered number of hHF and 24% of the placebo group.[47] These observations provide a strong basis for the guidelines and recommendations supporting the use of SGLT2i.

SGLT2i and CKD

Despite efforts being made to achieve optimal glycemic and blood pressure control, patients with CKD still have a high risk of progressing to ESKD, highlighting the need for additional renoprotective therapies to preserve the estimated glomerular filtration rate (eGFR) and prevent ESKD. The renoprotective effects of SGLT2i were first demonstrated in DECLARE-TIMI 58 trial[7], and CANVAS study[39], although the renal outcomes reported from these studies

were the secondary outcome measures.[7,38] In a meta-analysis of three CVOTs, SGLT2i reduced the composite of worsening kidney function by 45% (0.55 [0.48–0.64]), with a similar effect in those with and without ASCVD.[38] As per European society of cardiology guidelines, treatment with an SGLT2i is recommended at eGFR of 30 - <90 ml/min/1.73 m^2.[45]

The CREDENCE trial[39], evaluated the renoprotective effects of canagliflozin in 4,401 patients with T2DM, CKD, and macroalbuminuria. Patients with eGFR>30 and <90 mL/min/1.73 m^2 and urine albumin-to-creatinine ratio (UACR) >300–5000 mg/g and all patients receiving renin–angiotensin system blockade were included. The primary outcome risk measure was a composite of ESKD, doubling of the serum creatinine level, or renal or cardiovascular deaths which was reduced by 30% in the canagliflozin group relative to the placebo group. The canagliflozin group also reported a lower risk of cardiovascular death, myocardial infarction, or stroke.[39]

Determination of UACR may help in early recognition of renal complications. A prespecified analysis of DAPA-CKD trial,[48] was conducted and the primary outcome was composite of sustained decline in eGFR to at least 50%, ESKD, kidney-related or CV-related death. 68% of patients had T2DM, of which 14% had CKD. The relative risk of primary and secondary outcome with dapagliflozin was consistent in a patient with T2DM, diabetic kidney disease, glomerulonephritis, and ischemic or hypertensive CKD, concluding that dapagliflozin reduces the risk of major adverse kidney and CV events and all-cause mortality in diabetic and non-diabetic CKD.[48]

Indonesian Perspective

The aim in formulating this paper is to emphasise the existing diabetes burden in Indonesia and measures that can be taken to curb its prevalence. Diabetes related consequences are devastating, and it is vital to provide the Indonesian medical experts perspective with regards to screening, diagnosis, and treatment. Thus, timely screening and management of the disease is critical. The root of this article signifies that T2DM care necessitates multidimensional approach including cardiology, endocrinology, and nephrology, as it unfortunately leads to cardiorenal complications affecting the community at large.

For policy makers to consider: The purpose is to focus on improving primary healthcare (PHC) settings in terms of diabetes prevention, screening and early intervention. Enabling these adjustments will help to reduce the ongoing

Table 3. Recommendations for SGLT2 Inhibitors Therapy.

Recommendations for SGLT2i	
American Diabetes Association [50]	Type 2 diabetes patients with established ASCVD or high risk established kidney disease, or heart failure- SGLT2i or GLP 1 r agonist with demonstrated CVD benefit is recommended as part of the glucose lowering regimen independent of HbA1C and in consideration of patient specific factors
Asian Pacific Society of Nephrology (2020) [50]	Recommends SGLT2i in adult patient with type 2 diabetes and eGFR ≥ 30ml/min/1.73m2, who have CVD or diabetic kidney disease
European Society of Cardiology (ESC) and European Association for the Study of Diabetes (2019) [45]	Empagliflozin, Canagliflozin or Dapagliflozin are recommended in patients with type 2 diabetes and CVD, or at very high/high CV risk to reduce CV events
	Empagliflozin is recommended in T2DM and CVD patients to reduce risk of death
	SGLT2i are recommended to lower risk of hospitalization for heart failure in T2DM patients
	SGLT2i is recommended if eGFR is 30 - <90ml/min/1.73 m2 and is associated with lower risk of renal endpoints
Kidney Disease Improving Global Outcomes (2020) [51]	Recommends SGLT2i in treating patients with T2DM, CKD and eGFR ≥ 30ml/min/1.73m2
PERKENI 2021[19]	Recommends SGLT2i for T2DM patients with ASCVD/ high risk, heart failure or CKD

T2DM prevalence and its implementation may prove beneficial given the vast number of people that visit the PHC.

For Doctors and Patients: According to American Diabetes Association (ADA), asymptomatic adults should be screened for prediabetes and type 2 diabetes using an informal assessment of risk factors. Furthermore, annual monitoring of prediabetic patients is recommended. Regardless of medication, urinary ACR and eGFR must be assessed atleast once a year in these patients. Optimal timely referral to nephrologist allows instituting preventive and therapeutic measures designed to retard progression of kidney complications, preparing kidney replacement therapy and enhance the quality of life.[49]

Patients should be referred for evaluation by a nephrologist if they have an eGFR <30 mL/min/1.73m^2 or in the condition of uncertainty in determining etiology of kidney disease, difficult management issues, and rapidly progressing kidney disease. Lastly, all the patients should receive general preventive self-care education.[49] According to PERKENI 2021 guidelines, patients should be referred to an endocrinologist, if they are found to have DM related chronic complications such as diabetic retinopathy/ nephropathy, symptoms of unstable angina, and unresolved hyperglycaemia, persisting even after treatment i.e., FBG > 130mg/dL, post prandial blood glucose >180mg/dL or HbA1c >7% with ≥3 months of therapy. It is also recommended that patients should be educated about their disease condition with the help of educational materials as a part of prevention.[19]

CONCLUSION

Early recognition of T2DM complications and its management with appropriate therapy is the need of the hour. In line of evidence, glycemic and extraglycemic effects of SGLT2i have been thoroughly characterised. They have been found to be beneficial in controlling the HbA1c levels in T2DM patients. According to the facts and literature, it can be stated that SGLT2i are useful in providing primary and secondary prevention of cardiovascular and kidney-related complications in T2DM patients. Dapagliflozin has shown benefit for both primary and secondary prevention, whereas empagliflozin have been proven to be effective in secondary prevention, however its role in primary prevention is yet to be established. Therefore, it can be suggested that the early use of SGLT2i will be effective in preventing and minimising the progression of cardiorenal complications.

According to experts, a coordinated and multidisciplinary management of the patient with T2DM, with earlier implementation of guidelines and clinical recommendations, are the key factors for the comprehensive diabetic care and prevention.

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CONFLICT OF INTEREST

There is no conflict of interest

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